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Effects of 1-methylcyclopropene (1-MCP) treatment on antioxidant enzymes and fruit quality parameters of cold-stored baby squash

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Abstract: In this study, the effects of 1-methylcyclopropene (1-MCP) treatment on the antioxidant enzymes and fruit quality parameters of baby squash (*Cucurbita pepo* L., cv. Soleil F_1 and İskender F_1) during cold storage was investigated. After being freshly harvested, the İskender F_1 (green-skinned) and Soleil F_1 (yellow-skinned) baby squash were treated with 250 and 500 ppb concentrations of 1-MCP and stored at 10 °C for 14 days. The 250 ppb 1-MCP treatment suppressed the loss of total soluble solid, ascorbic acid, total phenolic, and total flavonoid contents during storage in the Soleil F_1 squash. Similarly, the 250 ppb 1-MCP treatment delayed the loss of ascorbic acids and titratable acidity of İskender F_1 squash. The highest ascorbic acid content and antioxidant activity were determined with the 250 ppb 1-MCP treatment, while the highest total chlorophyll content was detected in the 500 ppb 1-MCP-treated İskender F_1 squash. The initiation of fruit softening and chlorophyll degradation was delayed in the 1-MCP-treated squash. Ethylene emission was also inhibited by the 1-MCP treatments in both types of squash. 1-MCP treatment effectively reduced the weight loss and decay incidence in both squash cultivars. The results indicated that the 250 ppb 1-MCP treatment retained antioxidant enzymes for a longer period and it was found to be more effective for maintaining the fruit quality parameters of the cold-stored baby Soleil F_1 and İskender F_1 squash.

Keywords: Antioxidant, baby squash, 1-methylcyclopropene, quality, storage

1. Introduction

Fruit and vegetables are important sources of proteins, carbohydrates, organic acids, minerals, vitamins, and dietary fibers for human nutrition (Irtwange, 2006) and are seen to be an important part of the dietary system. They are highly beneficial and very important for the prevention of different chronic diseases and the maintenance of human health.

The changes in dietary habits of consumers have shifted from unprocessed to value-added fresh fruits and vegetables with more emphasis on food safety, taste, flavor, freshness, and year-round availability (Manoren, 2013). Furthermore, consumer preferences are changing towards healthy, reliable, high-quality, practical, easy-to-prepare and easy-to-consume, smaller products. Additionally, consumers have increasingly been demanding miniature or baby vegetables in recent years due to their practical uses.

Baby vegetables are smaller versions of the products that are produced in the standard size. Some baby vegetables are the standard varieties harvested at a stage when full maturation has not yet occurred, while some are new plant varieties or cross-bred crops. Baby vegetables are produced from secondary buds and can be sold after the first full-length crop has been harvested. Baby vegetables must be carefully harvested and processed, as they are more sensitive to handling than standard-sized crops. Since their respiration rates are faster than normal-sized products, baby vegetables are particularly susceptible to postharvest decay when compared to the standard-sized vegetables (Maynard, 2006; Kaiser and Ernst, 2017).

There are many techniques used to prolong the storage life and maintain the postharvest quality of horticultural crops. The use 1-methylcyclopropene (1-MCP) is one of the useful techniques to maintain the postharvest quality and prolong the storage life of ethylene-sensitive vegetables and fruit. 1-MCP inhibits the ripening effects of ethylene by blocking ethylene receptors. It is thought that 1-MCP fills the ethylene receptors in a way that prevents ethylene from binding to the site and eliciting activity. 1-MCP has been used to regulate ripening in climacteric and nonclimacteric horticultural crops.

Many researchers have stated that 1-MCP treatments have various impacts on the postharvest quality parameters of horticultural crops. For example, 1-MCP treatments have been found to decrease weight loss in avocados (Jeong

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et al., 2002), maintain flesh firmness in apples (Rupasinghe et al., 2000; Mir et al., 2001) and persimmons (Nakano et al., 2001), reduce disease development in pears (Chen and Spotts, 2005) and avocados (Pesis et al., 2002), delay color changes in peaches (Kluge and Jacomino, 2002), extend storage life in strawberries (Jiang et al., 2001), prolong the shelf-life of broccoli florets (Yuan et al., 2010) and mangos (Hofman et al., 2001), suppress chlorophyll degradation in spinach leaves (Grozeff et al., 2010) and Chinese kale (Sun et al., 2012), and reduce the respiration rate and ethylene emission in plums (Dong et al., 2002) and pears (Villalobos-Acuna, 2011). Although many studies have been conducted on the effects of 1-MCP treatment on the quality parameters of fruits and vegetables, there are not many studies on the influence of 1-MCP on the storage life of baby vegetables. Therefore, the purpose of the present study was to investigate the effects of 1-MCP treatment on the antioxidant enzymes, fruit quality parameters, and storage life of summer-type baby squash.

2. Materials and methods

2.1. Materials

Soleil F_1 (yellow-skinned) and İskender F_1 (green-skinned) baby squash (*Cucurbita pepo* L.) were used as the fruit materials in this study. The squash were harvested at the horticultural maturity stage (color: fully green and yellow, size: approximately 9–10 cm) from a commercial greenhouse and transported to the laboratory of the Department of Horticulture, Akdeniz University in Antalya, Turkey. The baby squash were carefully selected for uniform size and were free of any visual signs of decay or disorders.

2.2. 1-MCP treatments and storage conditions

Freshly harvested baby squash were randomly divided into three lots for different doses of 1-MCP treatment. The first and second lots of squash were treated with 250 and 500 ppb of 1-MCP, respectively. The third lot of squash was not treated with 1-MCP and was considered the control lot. The 1-MCP was obtained from SmartFresh™ powder (AgroFresh Solutions, Inc., Philadelphia, PA, USA) (0.14% active ingredient) and treatments were conducted at 10 °C for 24 h in specially designed 1 m^3 gas-tight containers. After treatment with the 1-MCP, the control and 1-MCP-treated baby squash were placed in plastic boxes, with 24 fruit in each box (per replication), and stored at 10 °C with 90%–95% relative humidity for 14 days. Samples were removed at 7-day intervals and various physical and chemical analyses on the baby squash were performed.

2.3. Physical and chemical analyses

Cumulative weight loss was calculated by weighing the squash at the beginning of the study (day 0) and at 7-day intervals (days 7 and 14) and the results were given as the percentage loss of the initial total weight.

Fruit firmness was measured on three different surfaces of the squash using an Effegi hand-held penetrometer equipped with a 3-mm probe (Facchini, Alfonsine, Italy). In each replication, twenty-four squash were measured on three different parts of their equatorial axes and the results were recorded and defined in Newton (N).

For total soluble solids (TSS) content and titratable acidity (TA) determination, the squash were squeezed using a juice extractor and the juices were centrifuged at 8600 × *g* for 10 min. The supernatants obtained were used to detect the TSS content. The TSS contents of squash juices were measured using a Atago REF 121 digital refractometer (Itabashi, Tokyo, Japan) and the results were given as percent (%). The same supernatants were used to evaluate the amounts of TA. The TA of the samples was detected by titrating the juices with 0.1 mol L^{-1} of NaOH to the endpoint of pH 8.1 and stated as g malic acid kg^{-1} (Erkan and Eski, 2012).

Surface color changes were evaluated using a Minolta CR 400 chromameter (Ramsey, NJ, USA) and the International Commission on Illumination L^*, a^*, and b^* color space values were recorded. Depending on the a^* and b^* values, the chroma values $[C^* = (a^{*2} + b^{*2})^{1/2}]$ and hue angles $[h° = \tan^{-1}(b^* / a^*)]$ were calculated (McGuire, 1992). Measurements were taken on three different parts of each fruit along their equatorial axes and the mean values were determined.

The decay incidence was calculated from the number of fruit that showed signs of any decay over the initial number of fruit and expressed as percent (%).

Squash purees were utilized for the analysis of total chlorophyll, total phenolic, carotenoid, flavonoid, and L-ascorbic acid contents and antioxidant activity. The absorbance of the samples for all of the quality analyses was read using an Analytik Jena AG Specord 40 ST spectrophotometer (Jena, Germany).

The total phenolic contents of squash were evaluated using the method of Spanos and Wrolstad (1990). First, an extract of 0.1 mL was blended with 0.9 mL of distilled water and 5 mL of 0.2 mol L^{-1} N Foline-Ciocalteu reagent. After 3 min, 4 mL of an aqueous solution of Na_2CO_3 (75 g L^{-1}) was added into the blend and the samples were kept in the dark for 2 h at room temperature. Next, the absorbances of the samples were measured at 765 nm against a blank 80% methanol solvent using a spectrophotometer. The total phenolic content was reported as g of gallic acid equivalent (GAE) per kg (g GAE kg^{-1}).

The extraction of the fruit samples was performed with 80% methanol for antioxidant activity, total flavonoid, and total phenolic content analysis. For that purpose, 20 g of squash puree and 20 mL of 80% methanol were homogenized in an ultraturrax homogenizer and the homogenates were centrifuged at 4 °C at 8600 × *g* for 20

min. The antioxidant activity of the squash was detected according to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method explained by Benvenuti et al. (2004). First, 600 µL of 1 mM DPPH* radical solution was placed into 4 test tubes and sample extracts were added into the test tubes in different volumes. Afterwards, 80% methanol was used to bring the final volume in each test tube up to 6 mL. The mixture in the test tubes was vortexed and left to incubate for 15 min in the dark at room temperature. Additionally, the control sample was prepared by placing 5.4 mL of methanol and 600 µL of 1 mM DPPH* radical solution into a test tube and incubating it in the dark for 15 min at room temperature. Following incubation, the absorbances of the samples were read at 517 nm using a spectrophotometer against a blank solvent of 80% methanol and the control. The percent inhibition value of each sample was calculated by the following equation:

% inhibition = $A_{DPPH} - A_{Extract} / A_{DPPH} \times 100$

Here, A_{DPPH} is the DPPH control sample absorbance value and $A_{Extract}$ is the test sample absorbance value.

By applying linear regression analysis, the equation defining the curve was obtained. This equation was used for the calculation of the median effective concentration (EC_{50}) values of the samples. The antioxidant activity (DPPH method) was determined through the EC_{50} value. The EC_{50} value reflects the amount of antioxidant substances present in fruit and vegetable samples that inhibit 50% of the DPPH radical. A decrease in the EC_{50} value exhibits an increase in the antioxidant activity (Cemeroğlu, 2010a). The EC_{50} value was reported in mL fresh weight (FW) DPPH.

The total chlorophyll content was analyzed using the method of Lichtenthaler and Wellburn (1983). First, 3 g of squash puree was homogenized with 80% acetone in an ultraturrax homogenizer. Centrifugation of the homogenized samples was performed at 8600 × *g* for 5 min at 4 °C. After centrifugation, the resulting supernatant was used to measure the chlorophyll content. The supernatant was read using a Specord 40 ST spectrophotometer against a blank 80% acetone solvent at 646 and 663 nm. The total chlorophyll content of the squash was calculated using the equation given below and given as g kg^{-1}.

Chlorophyll a = $12.21 \times A_{663} - 2.81 \times A_{646}$

Chlorophyll b = $20.13 \times A_{646} - 5.03 \times A_{663}$

Total chlorophyll = $(C_a + C_b)$

The total ascorbic acid contents analysis was conducted according to the method of Cemeroğlu (2010b). For that purpose, 5 mL of squash sample was extracted with 6% metaphosphoric acid in a 50-mL test tube, and 5 mL of acetate buffer solution (pH 4.0), 1 mL of 2.6 dichlorophenolindophenol dye solution, and 10 mL of xylene were added. After that, the test tube was stirred for 10 s and centrifugation was performed at 8600 × *g* for 10 min at 4 °C. As a control sample, a second test tube was prepared and 5 mL of 6% metaphosphoric acid was added into the test tube instead of 5 mL of filtrate. The absorbance of the samples was recorded at 500 nm using a spectrophotometer against the xylene and the control sample. The following equation was used to detect the total ascorbic acid contents and the results were given as g kg^{-1}.

$$\text{Ascorbic acid (g kg}^{-1}) = \frac{A_2 - A_1}{a} \times DF$$

Here, A_1 is the extract sample absorbance value, A_2 is the control sample absorbance value, DF is the dilution factor, and a is the ascorbic acid standard curve slope.

The total flavonoid content of the squash was analyzed using the method of Karadeniz et al. (2005). In a 50-mL tube, 1 g of squash puree, 5 mL of distilled water, and 0.3 mL of 5% $NaNO_2$ (Merck, Darmstadt, Germany) were added, respectively. The test tubes were closed and strongly mixed. After 5 min, 0.6 mL of 10% $AlCl_3.6H_2O$ (Merck) was added, and after another 5 min, 2 mL of 1 mol L^{-1} NaOH was added. Distilled water was used to bring the total volume in the tube up to 10 mL. The tubes were then vortexed and the absorbance was assayed at 510 nm using a spectrophotometer against a solvent of blank 80% methanol. The standard calibration curve prepared with catechin was used to calculate the total flavonoid content of the squash and given as g kg^{-1}.

The total carotenoid content was evaluated using the method of Witham et al. (1971). For that purpose, 0.25 g of squash puree was homogenized with 10 mL of 80% acetone for 3–4 min using an ultraturrax homogenizer and 80% acetone solvent was used to bring the total volume of the sample to 15 mL. After that, the samples were centrifuged at 8600 × *g* for 10 min at 4 °C. The carotenoid content was determined using the supernatant fraction. The absorbances used for chlorophyll a, chlorophyll b, and carotenoid were 663, 645, and 440 nm, respectively. The absorbance was read using a spectrophotometer against a control solvent of 80% acetone. The total carotenoid content was calculated using the formulas given below and stated as g kg^{-1}.

Chlorophyll a (g kg^{-1}) = [12.7 (D663) – 2.69 (D645)] × V/1000×W

Chlorophyll b (g kg^{-1}) = [22.9 (D645) – 4.68 (D663)] × V/1000×W

Carotenoids (g kg^{-1}) = [4.69 (D440) – (chlorophyll a + chlorophyll b) × 0.286] × V/1000×W

V = volume of extract

W = sample weight

For ethylene production, replicates of three groups of 8 fruit were placed into 2-L air-tight jars and kept at 20 °C for 1 h. Ethylene emissions were measured using the same squash for 15 days. For that purpose, a 1-mL gas sample,

from the headspace, was taken with a gas-tight syringe and injected into a gas chromatography (GC) equipped with a flame ionization detector. The GC had been calibrated previously with standard ethylene. The results were stated as μL C_2H_4 kg^{-1} h^{-1}. Chromatographic conditions were set: GS-GASPRO, 113-4362 capillary column, 6 FT × ¼ IN, 130 °C oven temperature, 275 °C detector temperature, 35 mL min^{-1} hydrogen flow, 350 mL min^{-1} dry air flow, 1-mL injection, 2-min analysis time.

2.4. Statistical analysis

The experiment was designed according to a completely randomized design (CRD) with three replications. Each replication contained 24 fruit. The data were analyzed using the Statistical Analysis System software 9.0 (SAS Institute, Cary, NC, USA) and the treatments means were statistically compared using the Duncan multiple range test ($P \leq 0.05$).

3. Results and discussion

3.1. Weight loss

The weight losses of the 1-MCP-treated squash were lower than that in the control group. At the end of day 14 of storage, the weight loss of the control, and 250 and 500 ppb 1-MCP-treated squash was 8.00%, 4.26%, and 4.03% in the Soleil F_1 squash, while it was 7.23%, 3.35%, and 3.72% in the İskender F_1 squash, respectively (Table 1).

In this study, the weight loss of the baby squash increased in all of the treatments as the storage prolonged. However, the weight loss of the control group was higher than that of the 1-MCP-treated squash. These findings were supported by the results of Guillen et al. (2007) in tomatoes. They reported that 1-MCP treatments were efficient in decreasing the weight loss of Raf tomatoes. The results were also consistent with the work conducted by Wu et al. (2009) in Chinese chives (garlic chives), by Sun et al. (2012) in Chinese kale, and by Hassan and Mahfouz (2012) in coriander (Chinese parsley) leaves. In these studies, the weight loss in the 1-MCP-treated vegetables was lower than that of untreated control products. It has been suggested that the lower weight loss in 1-MCP-treated fruit may be due to a reduced respiration rate (Mahajan et al., 2010).

3.2. Flesh firmness

Flesh firmness of the Soleil F_1 squash increased with all of the treatments. Flesh firmness of the Soleil F_1 squash at harvest was 12.20 N and increased to 20.97, 14.67 and 15.20 N in the control, and 250 and 500 ppb 1-MCP-treated squash, respectively. Flesh firmness of the İskender F_1 squash at harvest was 16.88 N and it was determined as 16.17, 17.70, and 16.40 N in the control, and 250 ppb and 500 ppb 1-MCP-treated İskender F_1 squash, respectively. However, the impacts of the 1-MCP doses on flesh firmness in the İskender F_1 squash was not significant. In other words, flesh firmness was not influenced by the 1-MCP treatment. These findings were similar to the results of Ilic et al. (2012) in H1530 green peppers. They stated that the impact of 1-MCP treatment on flesh firmness was not significant. In many previous studies, in contrast to the current study, it was reported that the flesh firmness of 1-MCP-treated vegetables was higher than in the control group (Moretti et al., 2002; Guillen et al., 2007; Choi and Huber, 2008; Sun et al., 2012; Han et al., 2015). The reason for higher firmness values in the 1-MCP-treated İskender F_1 may have been the result of the inhibition of polygalacturonase (PG) and pectin methylesterase (PME) activities, and a reduction in the effects of cell wall degrading enzymes (Li et al., 2011). Serna et al. (2012) stated that fruit firmness was associated with cell properties, such as adhesion between neighboring cells, cell fragility, and cell turgor pressure. In addition, they reported that one of the factors affecting the fruit texture was water loss. In this study, the firmness values of the control Soleil F1 baby squash were higher than those of the 1-MCP-treated squash. The higher weight loss due to water loss in the control group may have caused the products to have an elastic structure and resulted in higher firmness values.

3.3. Total soluble solids content (TSS)

During storage, the TSS contents of the Soleil F_1 squash exhibited a decrease in all of the treatment groups. This decrease was slower in the 1-MCP-treated squash when compared to the control group. The TSS content of the Soleil F_1 squash at harvest was 5.47% and, after 14 days of storage, it decreased to 4.20%, 5.30%, and 4.80% in the control, and 250 and 500 ppb 1-MCP-treated squash, respectively (Table 1). The 250 ppb 1-MCP-treated squash had the highest TSS content (5.30%) at the end of 14 days storage.

The TSS content of the İskender F_1 squash exhibited an increase, resulting in the prolongation of storage duration. The TSS content of the İskender F_1 squash at harvest was 5.10%, and it increased to 6.00% in the control group, 5.70% in the 250 ppb 1-MCP-treated squash, and 5.50% in 500 ppb 1-MCP-treated İskender F_1 squash.

These results were consistent with the findings of Moretti et al. (2002) and Wang et al. (2010) in tomatoes, and Liu et al. (2015) in peaches. According to Xu et al. (2016), the reason for the higher TSS content in 1-MCP-treated broccoli was the delay of senescence. In another study, it was stated that the increase in the TSS content with 1-MCP treatment can may have been due to the hydrolysis of starch to sugar or the breakdown of complex organic metabolites into simple molecules and reduced respiration metabolism (Sakhale et al., 2017).

3.4. Titratable acidity (TA)

Herein, the TA of the Soleil F_1 squash increased as the storage time prolonged. The highest TA was in the control

Table 1. Effects of different 1-MCP concentrations on the weight loss, flesh firmness, total soluble solids, titratable acidity, skin color (L^*, C^*, $h°$), and decay incidence of Soleil F_1 and İskender F_1 baby squash.

		Soleil F_1 squash			İskender F_1 squash		
Testing index	Treatments	Storage period (days)					
		0	7	14	0	7	14
Weight loss (%)	Control	0.00e	3.14b	8.00a	0.00e	2.93c	7.23a
	250 ppb	0.00e	1.88d	4.26c	0.00e	1.81d	3.35bc
	500 ppb	0.00e	1.83d	4.03c	0.00e	1.69d	3.72b
	$LSD_{0.05}$	Str. time × Tre.: 1.4885			Str. time × Tre.: 0.4689		
Flesh firmness (N)	Control	12.20d	16.56b	20.97a	16.88a	15.39a	16.17a
	250 ppb	12.20d	16.99b	14.67c	16.88a	17.92a	17.70a
	500 ppb	12.20d	16.37b	15.20c	16.88a	17.45a	16.40a
	$LSD_{0.05}$	Str. time × Tre.: 0.6454			Str. time × Tre.: 2.3149		
Total soluble solids (%)	Control	5.47a	4.85c	4.20d	5.10b	5.60ab	6.00a
	250 ppb	5.47a	5.20abc	5.30ab	5.10b	5.70ab	5.70ab
	500 ppb	5.47a	4.95bc	4.80c	5.10b	5.25b	5.50ab
	$LSD_{0.05}$	Str. time ×Tre.: 0.3941			Str. time × Tre.: 0.6218		
Titratable acidity (g malic acid kg^{-1})	Control	1.33d	1.76b	2.00a	0.46f	2.00b	1.89c
	250 ppb	1.33d	1.74b	1.75b	0.46f	1.67d	2.03a
	500 ppb	1.33d	1.65c	1.60c	0.46f	1.42e	1.90c
	$LSD_{0.05}$	Str. time × Tre.: 0.066			Str. time × Tre.: 0.0231		
L^*	Control	87.20a	73.51bc	74.86b	66.27a	65.05a	64.86a
	250 ppb	87.20a	73.94bc	75.04b	66.27a	57.52b	57.54b
	500 ppb	87.20a	72.16c	75.40b	66.27a	57.96b	57.74b
	$LSD_{0.05}$	Str. time × Tre.: 1.817			Str. time × Tre.: 2.2152		
$h°$	Control	92.26a	85.59ab	77.62b	119.01a	119.01a	117.05b
	250 ppb	92.26a	86.58ab	87.58a	119.01a	113.68c	113.25cd
	500 ppb	92.26a	85.51ab	86.49ab	119.01a	114.09c	112.65d
	$LSD_{0.05}$	Str. time × Tre.: 8.8637			Str. time × Tre.: 0.8686		
C^*	Control	83.67a	70.45bc	69.60cd	39.63a	39.63a	40.76a
	250 ppb	83.67a	70.22bc	68.65d	39.63a	32.69b	31.91b
	500 ppb	83.67a	70.58bc	71.39b	39.63a	32.29b	32.81b
	$LSD_{0.05}$	Str. time × Tre.: 1.4822			Str. time × Tre.: 1.2123		
Decay incidence (%)	Control	0.0c	0.0c	15.72a	0.0e	6.15c	17.94a
	250 ppb	0.0c	0.0c	5.94b	0.0e	1.67d	9.38b
	500 ppb	0.0c	0.0c	6.25b	0.0e	1.39d	9.69b
	$LSD_{0.05}$	Str. time × Tre.: 0.3278			Str. time × Tre.: 0.3174		

group (2.00 g malic acid kg^{-1}), which was followed by the 250 ppb 1-MCP-treated squash (1.75 g malic acid kg^{-1}), while the lowest was in the 500 ppb 1-MCP-treated squash (1.60 g malic acid kg^{-1}), at the end of day 14 of storage (Table 1). The TA of the İskender F_1 squash at harvest was 0.46 g malic acid kg^{-1}, which increased to 1.89 g malic acid kg^{-1} in the control group, 1.90 g malic acid kg^{-1} in the 500 ppb 1-MCP-treated squash, and 2.03 g malic acid kg^{-1} in the 250 ppb 1-MCP-treated squash (Table 1). These results were supported by the findings of Moretti et al. (2002) in tomatoes and Li et al. (2011) in melons. They reported that there were no differences among the control and 1-MCP-

treated tomatoes. On the contrary, in some other studies, it was revealed that 1-MCP treatment maintained higher TA in the fruit (Wills and Ku, 2002; Opiyo and Ying, 2005). Tavallali and Moghadam (2015) expressed that the 1-MCP treatment preserved TA by delaying the ripening process and slowing the respiration rates.

3.5. Skin color

The L^* value of the Soleil F_1 squash at harvest was 87.20 and it was determined as 74.86 in the control group, 75.04 in the 250 ppb 1-MCP-treated squash, and 75.40 in the 500 ppb 1-MCP-treated squash at the end of the 14 days of storage. In the İskender F_1 squash, the highest L^* value was in the control group (64.86), while the lowest was in the 250 ppb 1-MCP-treated squash (57.54) and 500 ppb 1-MCP-treated squash (57.74) squash. Similar results were determined by Fernández-Leon et al. (2013) in broccoli florets. They reported that there were no differences among the control and 1-MCP-treated samples.

The $h°$ values of the Soleil F_1 and İskender F_1 squash exhibited a decrease by extending the storage duration. The $h°$ value of the Soleil F_1' squash at harvest was 92.26°, and after 14 days of storage it decreased to 77.62°, 86.49°, and 87.58° in the control, and 500 and 250 ppb 1-MCP-treated squash, respectively. The $h°$ value of the İskender F_1 squash at harvest was 119.01, and after the storage period it declined to 112.65°, 113.25° and 117.05° in the 500 ppb 1-MCP, 250 ppb 1-MCP, and control group, respectively. The maintenance of $h°$ values in different fruits and vegetables by 1-MCP treatment has also been reported in tomatoes (Choi and Huber, 2008), squash (Massolo et al., 2013), okra (Huang et al., 2012), and broccoli florets (Xu et al., 2016).

The C^* values of both of the squash cultivars decreased during storage in all of the treatment groups, with the exception of the control İskender F_1 squash. The C^* value of the Soleil F_1 squash at harvest was 83.67, which decreased to 69.60, 68.65, and 71.39 in the control, and 250 and 500 ppb squash, respectively, at the end of cold storage. The C^* value of the İskender F_1 squash at harvest was 39.63 and after 14 days of storage, the C^* value of the İskender F_1 squash was 40.76 in the control, 31.91% in 250 ppb 1-MCP-treated squash, and 32.81% in 500 ppb 1-MCP-treated squash. In previous studies, it has been expressed that the C^* values of products were maintained by 1-MCP treatment. For instance, Huang et al. (2012) stated that the C^* value of 1-MCP-treated okra pods was higher than the control fruit. Blankenship and Dole (2003) reported that 1-MCP treatment delayed or prevented chlorophyll breakdown and color changes in many plant species. Moreover, Taye et al. (2019) and Moretti et al. (2002) stated that 1-MCP treatment slowed down carotenoid synthesis. In the current study, similar to the statements of these researchers, changes in the color of the squash were related to changes in the total carotenoid and total chlorophyll concentrations.

3.6. Decay incidence

The amount of decay incidence in the İskender F_1 squash was higher than the Soleil F_1 squash on day 14 of storage (Table 1). The decay incidence of the control, and 250 and 500 ppb 1-MCP-treated squash was 15.72%, 5.94%, and 6.25% in the squash Soleil F_1, and 17.94%, 9.38%, and 9.69% in the İskender F_1 squash, respectively. The decay incidence of both squash varieties exhibited an increase during the storage period. However, the decay incidences of the 1-MCP-treated squash were much lower than those of the control group. These results for the decay incidence were consistent with those published by Cho et al. (2008) in green beans, Massolo et al. (2013) in summer squash, Wu et al. (2009) in Chinese chive, Han et al. (2015) in bitter melon, Guillen et al. (2006) in tomato, Ilic et al. (2012) in green bell pepper, and Su and Gubler (2012) in tomato. They reported that 1-MCP-treated products had a lower decay incidence when compared with the control fruit. Wu et al. (2009) stated that the amount of decay in 1-MCP-treated Chinese chive scapes was lower than in the control group. The lower decay rate in the 1-MCP-treated products may have been due to the 1-MCP treatment slowing the respiration rate and delaying senescence.

3.7. Total phenolic content

The total phenolics content of the Soleil F_1 squash at harvest was 0.104 g GAE kg^{-1}. The highest total phenolics content of the Soleil F_1 squash was recorded in the 250 ppb 1-MCP-treated squash (0.160 g GAE kg^{-1}), which was followed by the 500 ppb-treated squash (0.157 g GAE kg^{-1}), while the lowest was in the control group (0.094 g GAE kg^{-1}) after cold storage (Table 1). The total phenolics content of the İskender F_1 squash was 0.190 g GAE kg^{-1} at harvest and was determined as 0.200, 0.188, and 0.191 g GAE kg^{-1} in the control, and 250 and 500 ppb 1-MCP-treated squash, respectively. The total phenolics contents of the 1-MCP-treated Soleil F_1 squash were higher than that of the control group but in the İskender F_1 squash, the control group had a higher total phenolics content than those of the 1-MCP treated squash. The total phenolics content decreased in the control group, but increased in the 1-MCP-treated Soleil F_1 squash, after 14 days of storage. Similar to the results obtained in the Soleil F_1 squash, Downes et al. (2010), in onion, and Wang et al. (2010), in tomato, expressed that the total phenolics contents of the 1-MCP-treated vegetables were higher than that of control group. On the other hand, similar to the results of the İskender F_1 squash, Sun et al. (2012) stated that the total phenolics content of 1-MCP-treated Chinese kale was lower than that of the control group. Singh et al. (2010) reported that ethylene has a significant effect on the accumulation of phenolic compounds and the amount

of phenolic substances increases due to the increase in ethylene production. In the present study, similar results were obtained in the İskender F_1 squash and the total phenolics content was lower than that in the control group for the 1-MCP-treated squash.

3.8. Antioxidant activity

The antioxidant activity of the Soleil F_1 squash at harvest was 3.185 mL FW DPPH. The total antioxidant activity of the Soleil F_1 squash was 1.720 mL FW DPPH in the control group, 1.713 mL FW DPPH in the 250 ppb 1-MCP-treated squash, and 1.616 mL FW DPPH in the 500 ppb 1-MCP-treated squash, at the end of cold storage. The antioxidant activity of the İskender F_1 squash was 0.927 mL FW DPPH at harvest and determined as 2.866 mL FW DPPH in the control group, 3.224 mL FW DPPH in the 250 ppb 1-MCP-treated squash and 2.967 mL FW DPPH in the 500 ppb 1-MCP-treated squash, after 14 days of storage. The antioxidant activity of the Soleil F_1 squash exhibited an increase, while that of the İskender F_1 squash showed a decrease by prolonging the storage period. Similar to the results determined in the Soleil F_1 squash, previous studies have shown that antioxidant activity was affected by 1-MCP treatment. The antioxidant activity in tomato (Wang et al., 2010), and Chinese kale (Sun et al., 2012) treated with 1-MCP was higher than that in the control. In contrast, in some other studies, researchers stated that the antioxidant activity was lower in 1-MCP-treated broccoli florets (Fernandez-Leon et al., 2013) and summer squash (Massolo et al., 2013) when compared with the control products. In another study, Cao et al. (2012) stated that 1-MCP treatment significantly affected antioxidant activity. According to these researchers, this was based on the reduction of reactive oxygen species (ROS) as a result of the 1-MCP treatment. ROS increase oxidative damage in plants and cause senescence. ROS accumulation reduces the marketability and storage quality of vegetables. Effective destruction of ROS requires the impact of antioxidant enzymes such as peroxidase (POD)**,** superoxide dismutase (SOD), catalase (CAT)and ascorbate peroxidase (APX). It has been reported that 1-MCP treatment results in significantly higher POD, SOD, CAT, and APX activities. Researchers have stated that 1-MCP treatment increases the activity of antioxidant enzymes in bell peppers. In another study, MacLean et al. (2003) claimed that higher antioxidant activities in 1-MCP-treated apples may have been the result of the capability of 1-MCP to prevent the production of free radicals.

3.9. Total chlorophyll content

The total chlorophyll content of the Soleil F_1 squash was 0.0010 g kg^{-1} at harvest. The highest total chlorophyll content of the Soleil F_1 squash was recorded in the 250 ppb 1-MCP-treated squash (0.0022 g kg^{-1}), at the end of cold storage. The total chlorophyll content of the İskender F_1 squash was 0.0321 g kg^{-1} at harvest and was determined as 0.0226, 0.0348, and 0.0386 g kg^{-1} in the control group, and 250 and 500 ppb 1-MCP-treated squash, respectively, on day 14 of the study (Table 2). The total chlorophyll content of the Soleil F_1 squash increased in both the control group and 1-MCP-treated squash after 14 days of storage, whereas the total chlorophyll contents of the 1-MCP-treated İskender F_1 squash increased, while it decreased in the control group. In this study, the total chlorophyll contents of the 1-MCP-treated squash was higher than in the control group of both squash varieties. In the green-skinned fruit, the chloroplast structure broke down during senescence. The mechanism of chlorophyll degradation in the fruit skin may have been influenced by the fruit maturity stage at harvest. These results were consistent with those of Yuan et al. (2010) in broccoli and Huang et al. (2012) in okra, who reported that chlorophyll degradation was delayed in the 1-MCP-treated products. Similar to the results herein, Wu et al. (2009) stated that 1-MCP treatment in Chinese chive reduced chlorophyll degradation. According to these researchers, this could have been due to the decreased activity of chlorophyll-degrading enzymes.

3.10. Total ascorbic acid content

The total ascorbic acid content of the Soleil F_1 squash was 0.164 g kg^{-1} at harvest, and on day 14 of storage, the total ascorbic acid content of the squash was 0.175 g kg^{-1} in the control group, 0.233 g kg^{-1} in the 250 ppb 1-MCP-treated squash, and 0.203 g kg^{-1} in 500 ppb 1-MCP-treated squash. The total ascorbic acid content of the İskender F_1 squash was 0.134 g kg^{-1} at harvest and was determined as 0.140, 0.220, and 0.201g kg^{-1} in the control group, and 250 and 500 ppb 1-MCP-treated squash, respectively, at the end of 14 days of storage. The highest of ascorbic acid content was determined in the 250 ppb 1-MCP-treated squash of both varieties. In addition, the ascorbic acid contents of the 1-MCP-treated squash were higher than those of the control group. These findings were supported by the results of Yuan et al. (2010) in broccoli, Wang et al. (2010) in tomato, and Sun et al. (2012) in Chinese kale. Wang et al. (2010) stated that the ascorbic acid content in tomato was higher than that of the control group. They also reported that the ascorbic acid content in tomato was significantly increased by 1-MCP treatment. Yuan et al. (2010) expressed that the ascorbic acid contents of broccoli in both the 1-MCP-treated and control fruit continuously decreased. In the same study, it was determined that the ascorbic acid contents were better preserved in the 1-MCP-treated broccoli florets. In another study, Sun et al. (2012) stated that the concentrations of ascorbic acid in both the 1-MCP-treated and control fruit decreased progressively. Liu et al. (2015) suggested that the reason for the higher amount of ascorbic acid in 1-MCP-treated

Table 2. Effects of different 1-MCP concentrations on the total phenolic, antioxidant activity, total chlorophyll, ascorbic acid, flavonoid, and carotenoid contents of Soleil F_1 and İskender F_1 baby squash.

		Soleil F_1 squash			İskender F_1 squash		
Testing index	Treatments	Storage period (days)					
		0	7	14	0	7	14
Total phenolic contents (g GAE kg^{-1})	Control	0.104c	0.083g	0.094f	0.190c	0.186e	0.200a
	250 ppb	0.104c	0.099e	0.160a	0.190c	0.183f	0.188d
	500 ppb	0.104c	0.101d	0.157b	0.190c	0.180g	0.191b
	$LSD_{0.05}$	Str. time × Tre.: 0.0001			Str. time ×Tre.: 0.0001		
Antioxidant activity EC_{50} (mL FW DPPH)	Control	3.185a	2.978b	1.720de	0.927g	2.199f	2.866c
	250 ppb	3.185a	1.824d	1.713de	0.927g	2.437d	3.224a
	500 ppb	3.185a	2.737c	1.616e	0.927g	2.318e	2.967b
	$LSD_{0.05}$	Str. time × Tre.: 0.0187			Str. time × Tre.: 0.0089		
Total chlorophyll contents (g kg^{-1})	Control	0.0010b	0.0006b	0.0011b	0.0321c	0.0274e	0.0226f
	250 ppb	0.0010b	0.0010b	0.0022a	0.0321c	0.0175g	0.0348b
	500 ppb	0.0010b	0.0021a	0.0012b	0.0321c	0.0275d	0.0386a
	$LSD_{0.05}$	Str. time × Tre.: 0.0008			Str. time × Tre.: 0.0001		
Total ascorbic acid contents (g kg^{-1})	Control	0.164g	0.179d	0.175f	0.134d	0.114f	0.140c
	250 ppb	0.164g	0.185c	0.233a	0.134d	0.112g	0.220a
	500 ppb	0.164g	0.177e	0.203b	0.134d	0.133e	0.201b
	$LSD_{0.05}$	Str. time × Tre.: 0.0005			Str. time × Tre.: 0.0010		
Total flavonoid contents (g kg^{-1})	Control	0.072f	0.109d	0.070g	0.093g	0.236e	0.378a
	250 ppb	0.072f	0.117c	0.142b	0.093g	0.249d	0.325b
	500 ppb	0.072f	0.083e	0.150a	0.093g	0.186f	0.282c
	$LSD_{0.05}$	Str. time × Tre.: 0.0005			Str. time× Tre.: 0.0025		
Total carotenoid contents (g kg^{-1})	Control	34.69f	48.22d	53.55a	23.35g	41.18b	48.82a
	250 ppb	34.69f	41.76e	48.59c	23.35g	26.37f	37.91d
	500 ppb	34.69f	51.06b	44.81d	23.35g	40.68c	37.57e
	$LSD_{0.05}$	Str. time × Tre.: 0.7155			Str. time × Tre.: 0.0705		

peaches could have been due to the retarding impact of the 1-MCP treatment on senescence.

3.11. Total flavonoid content

The total flavonoid content of the Soleil F_1 squash was determined as 0.070, 0.142, and 0.150 g kg^{-1} in the control group, and 250 and 500 ppb 1-MCP-treated squash, respectively, after 14 days of storage. The total flavonoid content of the İskender F_1 squash at harvest was 0.093 g kg^{-1}. The total flavonoid content of the control group, and 250 ppb and 500 ppb 1-MCP-treated İskender F_1 squash was 0.378, 0.325, and 0.282 g kg^{-1}, respectively, at the end of storage (Table 2). The total flavonoid content of the Soleil F_1 control group increased during the first 7 days of storage, and then decreased on day 14 of storage. However, the total flavonoid content of the 1-MCP-treated Soleil F_1 squash increased as the storage time prolonged. On the other hand, in the İskender F_1 squash, the total flavonoid contents of all of the treated squash increased progressively throughout the storage period. Similar to the results obtained from the İskender F_1 squash, Zhang et al. (2013) reported that 1-MCP treatment remarkably inhibited the accumulation of total flavonoids in avocado. Moreover, they expressed that 1-MCP was more influential at impeding the total flavonoid accumulation at higher concentrations. Honda et al. (2002) showed that ethylene was the main regulator of flavonoid biosynthesis in their study, and the reason for the low flavonoid content in the 1-MCP-treated horticultural crops was the inhibition of ethylene production.

3.12. Total carotenoid content

The highest total carotenoid content in the Soleil F_1 squash was recorded in the control group (53.55 g kg^{-1}), which

was followed by the 250 ppb 1-MCP-treated squash (48.59 g kg^{-1}), while the lowest was in the 500 ppb-treated squash (44.81 g kg^{-1}) on day 14 of storage (Table 2). The total carotenoid content in the İskender F_1 squash at harvest was 23.35 g kg^{-1}, which increased to 48.82, 37.91, and 37.57 g kg^{-1} in the control group, and 250 and 500 ppb 1-MCP-treated squash, respectively, at the end of storage (Table 2). In the present study, the 1-MCP-treated squash had lower carotenoid contents than the control samples in both squash varieties. The 1-MCP treatment significantly inhibited the accumulation of carotenoids. Previous studies have shown that 1-MCP treatment delayed carotenoid accumulation. Ilic et al. (2013), Opiyo and Ying (2005), and Moretti et al. (2002) in tomato, and Ilic et al. (2012) in pepper, reported that the 1-MCP-treated vegetables had lower carotenoid contents than the control samples. The results determined in the current study were in parallel with those found by these researchers. Fabi et al. (2007) stated that 1-MCP treatment in papaya fruit prevented carotenoid accumulation in their study. According to these researchers, 1-MCP delays climacteric peak during ripening by blocking the ethylene effect.

3.13. Ethylene emission

Ethylene emissions of the Soleil F_1 squash at harvest were 2.75, 1.72, and 1.32 μL C_2H_4 kg^{-1} h^{-1} in the control group, and 250 ppb and 500 ppb 1-MCP-treated squash, respectively. The ethylene emission of the control group reached a peak, which was 6.09 μL C_2H_4 kg^{-1} h^{-1} on day 10 of storage, and then decreased to 4.1 μL C_2H_4 kg^{-1} h^{-1} on day 15 of storage (Figure 1). The ethylene emission of the 250 ppb 1-MCP-treated baby squash on day 5 of storage was 3.38 μL C_2H_4 kg^{-1} h^{-1}, which decreased to 1.98 μL C_2H_4 kg^{-1} h^{-1} on day 10 of storage, and then increased to 4.53 μL C_2H_4 kg^{-1} h^{-1} on day 15 of storage. The ethylene emission of the 500 ppb 1-MCP-treated squash reached a peak on day 10 of cold storage (4.71 μL C_2H_4 kg^{-1} h^{-1}) and it was determined as 1.37 μL C_2H_4 kg^{-1} h^{-1} on day 15 of storage.

The ethylene emissions in the İskender F_1 squash at harvest were 4.72, 3.01, and 2.64 μL C_2H_4 kg^{-1} h^{-1} in the control group, and 250 and 500 ppb 1-MCP-treated squash, respectively. The ethylene emissions in the control group were higher than those in the 250 and 500 ppb 1-MCP-treated squash. The ethylene emission in the control group, and 250 and 500 ppb 1-MCP-treated squash reached a peak after day 10 of storage, and thereafter, it decreased (Figure 2). On day 15, the ethylene emission of the İskender F_1 squash was 5.57 μL C_2H_4 kg^{-1} h^{-1} in the control group, 1.62 μL C_2H_4 kg^{-1} h^{-1} in the 250 ppb 1-MCP-treated squash, and 2.65 μL C_2H_4 kg^{-1} h^{-1} in the 500 ppb 1-MCP-treated squash. As shown in Figure 2, the ethylene emission rate in the control group was almost 2-fold higher than that in the 250 and 500 ppb 1-MCP-treated squash.

In this study, in both squash cultivars, the ethylene emissions were suppressed by the 1-MCP treatment. Previous studies have shown that ethylene emission was suppressed by 1-MCP treatment, especially in climacteric fruits. Ma et al. (2010) in broccoli, Choi and Huber (2008), Guillen et al. (2007), and Wills and Ku (2002), in tomato, and Jeong et al. (2002) and Hershkovitz et al. (2005), in avocado, reported that the ethylene emission was inhibited

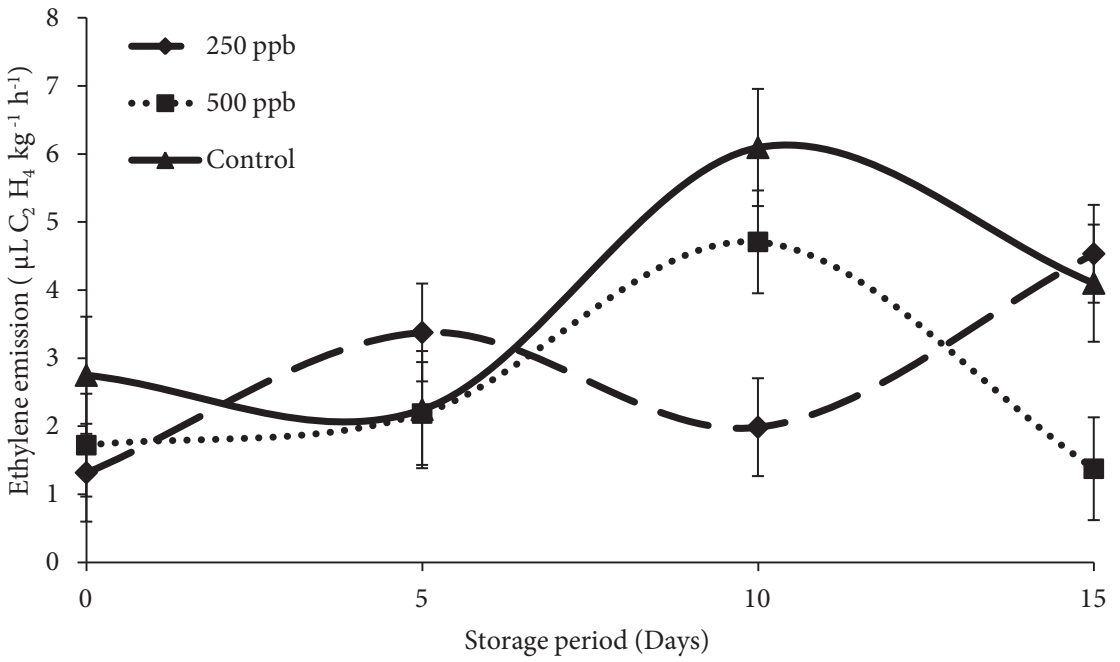

Figure 1. Effects of different 1-MCP concentrations on the ethylene emission of Soleil F_1 baby squash.

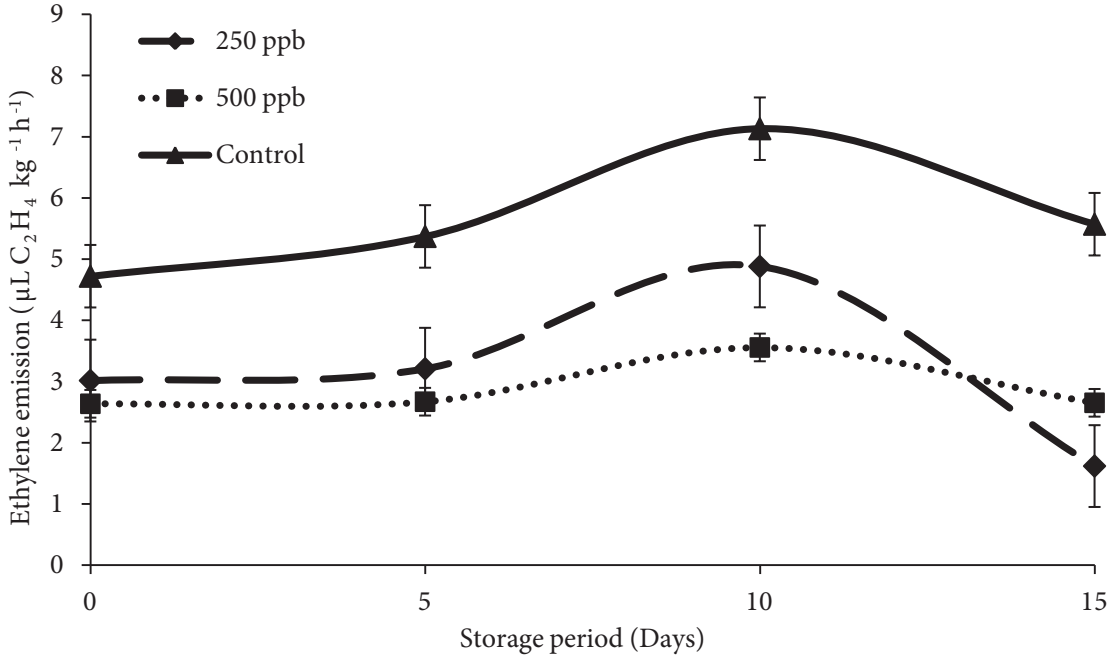

Figure 2. Effects of different 1-MCP concentrations on the ethylene emission of Iskender F_1 baby squash.

by 1-MCP treatment. 1-MCP is thought to fill the ethylene receptors in a way that prevents ethylene from binding to the site and eliciting activity. Similarly, Sisler and Serek (1997) also evaluated the response of 1-MCP to the ethylene receptor in their study. The compatibility of the receptor with 1-MCP was almost 10-fold higher than that of ethylene. Via feedback blockage, 1-MCP also affects ethylene production in some plants and fruit species, including apple, banana, broccoli, pear, plum, and tomato (Blankenship and Dole, 2003).

3.14. Conclusions

1-MCP treatment was able to effectively reduce the rate of weight loss and decay incidence, and inhibited the loss of TSS, and $h°$ and C^* values (500 ppb 1-MCP), total phenolics, total chlorophyll (250 ppb 1-MCP), and ascorbic acid and total flavonoids contents during the storage of Soleil F_1 baby squash. Furthermore, the ethylene emission in the Soleil F_1 squash control group was generally higher than that of the 1-MCP-treated squash. The 1-MCP treatment retarded the loss of TA (250 ppb 1-MCP), total chlorophyll, and ascorbic acid content of the İskender F_1 baby squash. The initiation of fruit softening and chlorophyll degradation was delayed in the 1-MCP-treated squash. In the İskender F_1 squash, 1-MCP restrained the ethylene production, and thereby delayed a series of downstream events in the chlorophyll catabolic pathway. Thus, the fruit retained its green skin for a longer time.

It can be concluded that the Soleil F1 and Iskender F1 baby squash treated with 1-MCP were successfully stored at 10 °C for up to 14 days. The results showed that the 250 ppb 1-MCP treatment was found to be more effective for retaining antioxidant enzymes and maintaining the fruit quality of the Soleil F1 and İskender F1 baby squash.

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