Effects of GA$_3$, CaCl$_2$ and Modified Atmosphere Packaging (MAP) Applications on Fruit Quality of Sweet Cherry at Cold Storage

Burhan Ozturk (a), Erdal Aglar (b), Onur Saracoglu (c), Orhan Karakaya (d), and Sefa Gun (e)

Faculty of Agriculture, Department of Horticulture, Ordu University, Ordu, Türkiye; Faculty of Agriculture, Department of Horticulture, Yüzüncü Yıl University, Van, Türkiye; Faculty of Agriculture, Department of Horticulture, Tokat Gaziosmanpaşa University, Tokat, Turkey

**ABSTRACT**

The aim of this study was to investigate the effects of pre-harvest gibberellic acid (GA$_3$) and calcium chloride (CaCl$_2$) and post-harvest modified atmosphere packaging (MAP) applications on fruit quality of sweet cherry during the cold storage. At the end of the cold storage, GA$_3$+CaCl$_2$ applications in MAP-applied fruit and CaCl$_2$ in non-MAP-applied fruit significantly delayed weight loss compared to the control. During the cold storage, the decay of CaCl$_2$-applied fruit has not been observed. At the end of cold storage, the a* and firmness values of the fruit treated with GA$_3$ and CaCl$_2$ were significantly higher than the control. The CaCl$_2$-applied fruit had less soluble solids than the control, but they had significantly higher acidity compared to other applications. In MAP, GA$_3$- and CaCl$_2$-applied fruit had higher vitamin C and antioxidant activity values than the control fruit, but their total phenolics were higher. However, the total flavonoids of CaCl$_2$-applied fruit were higher than the control. On fruit in non-MAP, with GA$_3$ application, less anthocyanin was obtained compared to the control. As a result, GA$_3$-treated fruit had a relatively lower bioactive compound content compared to the fruit of the CaCl$_2$ and control applications both during the harvest and the cold storage. However, it has been revealed that GA$_3$ and CaCl$_2$ applications can be used as significant pre-harvest tools to delay the loss of the fruit firmness.

**KEYWORDS**

Anthocyanin; antioxidant activity; firmness; phenolics

**Introduction**

Sweet cherry is one of the most-accepted fruit species by consumers due to its excellent quality. In sweet cherry, fruit size, appearance, taste, firmness and storability are very significant properties in the marketing of the fresh fruit (Aglar et al., 2019; Serrano et al., 2005). Sweet cherry fruit is more sensitive due to its physical properties, and its losses in the cold storage after harvest are high. Therefore, extending the shelf life of sweet cherry is the most valuable goal for the cherry industry (Aglar et al., 2017; Wani et al., 2014).

Fruit firmness in sweet cherry is one of the most significant factors affecting fruit quality and post-harvest life. Therefore, the main objective of extending the post-harvest life should be to increase the fruit firmness. Plant growth regulators (PGR) such as gibberellic acid (GA$_3$) are widely used for this target (Correia et al., 2019; Ozturk et al., 2019a). GA$_3$, which promotes cell division and elongation, are known as regulators of certain biological processes in the plant and fruit development stage (Choi et al., 2002; Pharis and King, 1985) and are therefore widely used to increase fruit size and quality (Fortes et al., 2015), and contribute to the maintenance of the fruit firmness due to its effect on the cell wall (Choi et al., 2002). In previous studies (Choi et al., 2002; Clayton et al., 2006), it was determined that with pre-harvest GA$_3$ application in sweet cherry, the harder and larger, hence higher-quality
fruits were obtained. Also, Ozkaya et al. (2006) stated that sweet cherry fruit quality during cold storage could be maintained with pre-harvest gibberellic acid treatments (10, 20 and 30 mgL⁻¹).

Calcium application in sweet cherry is one of the most effective methods to prolong the cold storage life by increasing the resistance mechanism and to prevent cracking, which is one of the most significant problems leading to the losses of the fruit quality. Calcium is a significant element in the structure of the cell wall and contributes to the mechanical properties of plant tissues (Correia et al., 2019). Calcium applications, either pre-harvest as a spray or post-harvest as a dip, are a common tool for maintaining or improving the post-harvest quality of sweet cherry fruit (Wang et al., 2014; Zoffoli et al., 2017). Pre-harvest calcium application has been shown to directly improve the fruit quality of sweet cherry (Dong et al., 2019). In some studies (Brown et al., 1995; Wermund et al., 2001), it has been found that pre-harvest calcium application increases fruit firmness and reduces fruit cracking in sweet cherry. In addition, the fruit with lower Ca levels are more susceptible to softening, pitting, and the decay processes that may follow damage to skin integrity (De Freitas and Mitcham, 2012). The pre-harvest bioregulators application is widely used to extend the post-harvest life of fruit species (Correia et al., 2019).

Recently, it has been reported that some post-harvest applications such as MAP in stone fruit have been effective in prolonging the cold storage time and minimizing the fruit quality losses in the cold storage. It has been revealed that MAP, which is applied to sweet cherry to extend the cold storage time and to minimize the fruit quality losses in the cold storage, can be used as a potential tool to maintain post-harvest fruit quality (Ozturk et al., 2019a; Petracek et al., 2002; Serrano et al., 2005).

Studies conducted on the effect of pre-harvest GA₃ and calcium applications on reducing the cracking problem in sweet cherry and on the effect of post-harvest MAP application on maintaining fruit quality properties in the cold storage are limited. Therefore, the aim of this study was to determine the effects of combined GA₃, CaCl₂ and modified atmosphere packing (MAP) applications on the quality properties of sweet cherry fruit during cold storage.

Materials and methods

Plant material

The study was conducted in 2017 on sweet cherry trees planted in Tokat Gaziosmanpasa University Research and Application Center (591 m altitude and 40° 20’ 01.99”N latitude, 36° 28’ 35.62”E longitude). In the study, sweet cherry (Prunus avium) trees (9 years old) belonging to Regina cultivar grafted on MaxMa 14 rootstock were selected as plant material. The trees were planted with 4 × 4 m space and trained by the Vogel Central Leader system. Irrigation, fertilization, pruning and other cultural processes during the development of trees (weed, disease and pest control, etc.) were regularly conducted.

Experimental Design and Spray Treatments

The experiment was established as randomized complete block design with three replications and two trees per replication. For the study, four different applications were determined as control, gibberellic acid (GA₃, ProGibb®, ValentBioScience, USA), calcium chloride (CaCl₂, Sigma-Aldrich, Germany) and GA₃+CaCl₂. In the field studies of the experiment, eight trees with homogeneous product loads were used in each block, so 24 trees in total.

The anticipated harvest date (AHD) (20 June 2017) was determined to be 21 days after the period at the straw-yellow stage of fruit skin color. GA₃ solution was sprayed on trees at a concentration of 30 mg L⁻¹ (Ozkaya et al., 2006) 3 weeks before the AHD, while CaCl₂ solution was sprayed on trees at 0.5% concentration 20 and 10 days before the AHD. Tween 20 (0.1%, Tween 20, Sigma-Aldrich, Germany) was used as the surfactant in all treatments. Prepared solutions were sprayed with a hand priming pump with low pressure in the morning of a windless and rain-free day until the trees were
completely wet. All treatments were separated with two buffer trees to minimize the risk of drift problems.

On the AHD, enough fruit was harvested by hand from two trees belonging to each treatment on each block (replication) and immediately (30 min) transferred to the post-harvest physiology laboratory with a refrigerated vehicle [10°C and 90% relative humidity (RH)].

Firstly, homogeneous in size and color [at commercial maturity of color grade 4 according to the color scale developed by CTIFL (Center Technique Interprofessionnel des Fruit et Legumes, Paris, France), in which 1-light pink and 7-dark mahogany], undamaged, healthy and flawless fruits were selected. Then, 1 kg of fruit was taken from each application in each block for the AHD analysis (June 20, 2017), and analyses were performed. In addition, the fruits of each application harvested from each block to determine the quality characteristics during the cold storage period were divided into 18 groups, each containing approximately 1 kg of fruit. Half of them (nine pieces) were placed in the plastic boxes (39 × 29 × 21 cm, Plastas, Turkey) with modified atmosphere packaging [Xtend® (815-CH97/a, StePac, Tefen, Israel)], and the other half were also placed in the plastic boxes without MAP and the fruits were transferred to the cold storage immediately. The fruits were pre-cooled with cold air in the conditions of 24 h, 4.0 ± 0.5°C and 90 ± 5% RH, and the plastic boxes were closed with plastic clips. Finally, the fruits were kept at 0.0 ± 0.5°C and 90 ± 5% RH for 21 days. The measurements and analyses were made at the end of the 7th, 14th and 21st days of the enclosure. Three boxes of fruit were taken for each application during each measurement period. Each box represented a replication.

**The Change of O2 and CO2 Gas Composition (%) in MAP**

The O2 and CO2 gas composition (%) of MAP during the cold storage was determined by a gas analyzer (Abiss, model legend, France).

**Weight loss**

Firstly, at the beginning of the enclosure, the weights of the fruit (Wi) were determined by a digital scale with a precision of 0.01 g (Radwag, Poland). Then, on days 7, 14 and 21, the fruits (WF) were weighed. The weight loss (WL) that occurs in fruit was based on the weight at the beginning of each measurement period and determined as a percentage through the equality given below (Eq.1).

\[
WL = \frac{Wi - Wf}{Wi} \times 100
\]  

(Eq.1)

**Decay ratio**

Before the cold storage, the fruits (about 1 kg of fruit) were counted in each replication, and the total number of fruit (TF) was determined. Then, during each measurement period, the decayed fruits (DF) in each replication were determined. If the development of mycelium on fruit surface occurred, the fruits were considered rotten. Finally, with the following equation (Eq.2), the decay rate (DR, %) was detected (Selcuk and Erkan, 2014)

\[
DR = \frac{TF - DF}{TF} \times 100
\]  

(Eq.2)

**L* and A* Color Characteristics**

Color data of sweet cherry fruit were presented according to CIE system (Commission Internationale de l’Eclairage) with L* (lightness) and a* color feature (-a* = greenness and +a* = redness). Color measurement was made via a color meter (Konica Minolta, CR-400, Japan). Measurements were
determined at two different points in the equatorial part of the fruit, and 20 fruits were used in each replication (McGuire, 1992).

**Fruit firmness**

Ten fruits from each replication (30 fruits for each treatment) were used for firmness measurements (Ozturk et al., 2019b). The measurements were made on two opposite sides of the equatorial part of the fruit through a portable digital durometer (nondestructive device, Agrostaa 100 Field, France) with a flat cylindrical surface and with a diameter of 10 mm. The tip of the durometer was slightly and longitudinally pressed to the outer skin of the fruit, and the Durofel units on the screen were recorded. If the value is close to 100, the fruit is considered very firm, and close to 0 indicates that the fruit is extremely soft.

**Soluble Solids Content (SSC), titratable acidity and vitamin C**

Twenty fruits taken from each replication were first washed with pure water, and their seeds were separated from the fruit flesh. The fruits were chopped with a stainless steel knife, cut into parts and homogenized by a blender (Model No. Promix HR2653 Philips, Turkey). Then, the homogenate was filtered through a cheesecloth, and the juice was obtained. Soluble solids content (SSC) was measured with a portable digital refractometer (Atago PAL-1, USA) and expressed as a percentage. For titratable acidity measurement, 10 mL juice was taken and 10 mL distilled water was added on. Then, 0.1 N NaOH (sodium hydroxide) was added until the pH of the solution reached 8.2. Based on the amount of NaOH consumed during titration, titratable acidity was determined and expressed as g malic acid 100 mL⁻¹. For vitamin C measurement, 0.5 mL juice was taken, and 5 mL of 0.5% oxalic acid was added to it. The ascorbic acid test strip (Catalog no: 116981, Merck, Germany) was then taken from a collapsible sealed gas-tight tube. Reflectometer (Merck RQflex plus 10) was started. The test strip was plunged into the solution for 2 seconds and then removed from the solution. It was then held for 8 seconds, and reading was done at the end of the 15th second. Results presented as mg 100 mL⁻¹.

**Total Phenolics, Total Flavonoids, Total Monomeric Anthocyanin and Antioxidant Activity**

During each measurement period, 20 fruits were taken from each replication of each application. The fruits were washed with distilled water, and their stones were removed by hand and sliced with a stainless steel knife. Later, the fruit pulp was crumbled by a blender (Model No. Promix HR2653, Philips, Turkey) and homogenized. About 30 mL of homogenate was taken and placed in a 50 mL falcon tube. The prepared tubes were kept at −20°C until analyses were carried out.

Before beginning the analyses, the frozen samples were dissolved below room temperature (21°C). Pulp and juice were separated from each other by a centrifuge at 12,000 × g at 4°C during 35 minutes. The resulting filtrate was used to determine the content of total phenolics, total flavonoids, total monomeric anthocyanin and antioxidant activity.

Spectrophotometric measurements for bioactive compounds were performed at the UV–Vis spectrophotometer (Shimadzu UV-1601PC, Kyoto, Japan). Total phenolics were measured according to the method of Singleton and Rossi (1965) and were expressed as mg GAE (gallic acid equivalent) 100 g⁻¹ fresh weight (fw). Total flavonoids were measured according to the method of Chang et al. (2002) and were expressed as mg QE (quercetin equivalent) 100 g⁻¹ fw. Total monomeric anthocyanin was determined by using pH (4.5 and 7.0) differential principle according to the method described by Giusti et al. (1999). The obtained results were shown as mg cyanidin 3-glucoside (cy-3-glu) 100 g⁻¹ on a fresh weight basis. The antioxidant activity of sweet cherry fruit was determined according to two different procedures of Trolox Equivalent Antioxidant Capacity (TEAC) (Ozgen et al., 2006) and
Ferric Ions (Fe³⁺) Reducing Antioxidant Power (FRAP) (Benzie and Strain, 1999), and the results were expressed in µmol Trolox equivalent (TE) 100 g⁻¹ fw.

**Statistical analysis**

Whether the data were normally distributed was checked by Kolmogorov-Smirnov Test. Homogeneity control of the group/subgroup variances was confirmed by Levene’s test. After the variance analysis of the data was done according to factorial design, Tukey’s multiple-comparison test was used to check whether there were significant differences between treatments. The statistical analyses were performed by using SAS software (SAS 9.1 version, USA).

**Results and discussion**

**The Change of O₂ and CO₂ Gas Composition (%) in MAP**

Data on the change of O₂ and CO₂ gas composition are presented in Table 1. The composition of O₂ varied in the range of 15.20–13.43% in control, 14.83–16.11% in CaCl₂, 15.97–16.80% in GA₃, and 15.46–16.97% in GA₃+CaCl₂. The CO₂ composition varied in the range of 1.40–5.70% in control, 1.13–4.43% in CaCl₂, 0.80–4.90% in GA₃, and 0.80–6.90% in GA₃+CaCl₂.

**Weight Loss and Decay Ratio**

During the cold storage, it was determined that the weight loss on the fruits of GA₃+CaCl₂ application stored in MAP was lower compared to both control and other applications. When the fruit without MAP were evaluated, the weight loss of the fruit treated with CaCl₂ on the 7th day of the cold storage and GA₃ and CaCl₂ on day 14th of the cold storage were significantly lower than the control fruit. At the end of the cold storage, it was observed that only, the weight loss of the CaCl₂–applied fruit was lower than the control (Figure 1).

During the cold storage period, the decay was not observed on the fruit of CaCl₂ application. However, at the end of the cold storage, in the fruit with MAP; GA₃ and CaCl₂ applications had a lower decay ratio compared to the control. In fruit without MAP, the fruit treated with GA₃ had a lower decay ratio than the control fruit (Figure 1).

Weight loss during the cold storage of fruit is a significant problem leading to greater economic losses (Sandhya, 2010). Sometimes, the weight loss can rise to 25–30%. The weight loss in fruit is related to the evaporation of water by transpiration, depending on the surface–volume ratio of the fruit and the relative humidity in the atmosphere surrounding the fresh product (Kader and Yahia, 2011). Technology such as MAP contributes to the extension of the cold storage time and reduction

**Table 1.** The change in MAP gas composition of sweet cherry fruit treated with GA₃ and CaCl₂ during cold storage.

| Treatments   | Storage periods | O₂ concentration (%) |
|--------------|-----------------|-----------------------|
|              | 7 day           | 14 day                | 21 day                |
| Control      | 15.20           | 14.60                 | 13.43                 |
| CaCl₂        | 16.11           | 15.25                 | 14.83                 |
| GA₃          | 16.80           | 16.30                 | 15.97                 |
| GA₃+CaCl₂    | 16.97           | 16.05                 | 15.46                 |

| Treatments   | CO₂ concentration (%) |
|--------------|------------------------|
| Control      | 1.40                   | 2.45                  | 5.70                  |
| CaCl₂        | 1.13                   | 2.20                  | 4.43                  |
| GA₃          | 0.80                   | 2.30                  | 4.90                  |
| GA₃+CaCl₂    | 0.80                   | 3.25                  | 6.90                  |
of weight loss during cold storage by reducing gas exchange and water loss in fruit (Valero et al., 2014). The positive effect of MAP on weight loss reduction has been reported by many researchers (Erkan and Eski, 2012). It has been reported that GA3 and CaCl2 applications in different fruit species have positive effects on delaying weight loss (Sharma and Pratima, 2018). The significant role of PGRs such as calcium (Zoffoli et al., 2017) and gibberellic acid (Fortes et al., 2015) in the formation and structure of the cuticle layer of the fruit may be the reason for the reduction in weight loss in the cold storage.

As in many fruits, in sweet cherry as well, in the cold storage, the decay occurs due to diseases such as Monilinia spp., Botrytis cinerea, Alternaria spp., Rhizopus stolonifer and Mucor piriformis caused by post-harvest damage and excessive moisture, and it causes significant product losses (Cappellini and Ceponis, 1977). It is possible to reduce the losses caused by the decay by increasing the fruit firmness with growth regulators such as CaCl2 and GA3 before harvest and by controlling the atmosphere of gas and moisture around the fruit with post-harvest MAP applications (Rodriguez and Zoffoli, 2016).

MAP successfully prolongs the post-harvest shelf-life of the fruit by reducing their respiration rate, minimizing metabolic activity, delaying enzymatic browning and retaining visual appearance (Waghmare and Annapure, 2013). MAP creates a suitable environment, which is a low level of oxygen and moderate level of carbon dioxide in the package, to maintain the quality characteristics of the packaged product (Mendoza et al., 2016). MAP is a widely used technique for maintaining the natural quality of fruit as well as for extending the cold storage time (Horev et al., 2012). The positive effect of MAP on atmospheric change during the cold storage maintains fruit firmness and reduces respiratory activity, ethylene production, enzymatic reactions and some physiological changes, thus it helps to maintain quality longer (Gorny, 1997). Rodriguez and Zoffoli (2016) reported that the decay rate in the cold storage in “Brigitta” blueberry cultivar was reduced by CaCl2 and MAP applications. CaCl2 is used as a preservative to extend the post-harvest life of fruit and vegetables by increasing fruit firmness (Rico et al., 2007). This effect may be the result of forming a cross-linked polymer network by interacting calcium ions and pectic polymers. Kader (2002) has reported that the high calcium content

Figure 1. Effects of pre-harvest spray treatments (GA3 and CaCl2) on the weight loss and decay ratio of sweet cherry fruit throughout cold storage. The means indicated with the same letter vertically in periods, and above the bars were not significant according to Tukey’s test at P < .05.
in fruit delays the fruit ripening, increases the fruit firmness and reduces physiological disorders and the fruit decay. Gibberellic acid applications, which thicken the cuticle layer in fruit and increase fruit firmness, are widely used to reduce the risk of product losses in sweet cherry.

**Fruit Color Characteristics and Firmness**

At harvest, it was determined that the a* value of the fruit treated with CaCl₂ and GA₃ was significantly lower than the control, but at the end of the cold storage, these fruits had higher a* value. At the harvest, the CaCl₂-applied fruit, and on the 7th day of the cold storage, the fruit treated with CaCl₂ and GA₃ had higher L* than the control fruit. On the 7th day of the cold storage, the L* value of the fruit of CaCl₂ application with MAP was higher than both the control and the other applications. However, on the 14th and 21st days of the cold storage, it was observed that in the fruit without MAP, the L* value of the fruit treated with the GA₃+CaCl₂ was higher than the control fruit (Figure 2).

At harvest, the fruit firmness of the CaCl₂ and GA₃ applied fruit was significantly higher than the control. In the last two measurements of cold storage, the fruit softening was slower in CaCl₂ and GA₃

![Figure 2](image_url)

*Figure 2. Effects of pre-harvest spray treatments (GA₃ and CaCl₂) on the L*, a* and firmness of sweet cherry fruit throughout cold storage. The means indicated with the same letter vertically in periods, and above the bars were not significant according to Tukey’s test at P < .05. * The scale ranges from 0 to 100 for very soft to very firm surfaces.*
treated fruit both with and without MAP. At the end of the cold storage, in the fruit without MAP, the firmness loss of the CaCl$_2$ and GA$_3$-applied fruit was higher compared to the fruit of the GA$_3$+CaCl$_2$ application (Figure 2).

Fruit color, which is a significant criterion in terms of consumer preferences, varies depending on the maturity of the fruit. Patino et al. (2018) reported that there was an increase in red index a* during the cold storage, but the increase was lower with MAP application. Chemical applications such as calcium extend shelf life and reduce discoloration in apples (Rocha et al., 1998). Again, Dong et al. (2019) have determined that GA$_3$ application delays fruit color change during the cold storage in sweet cherry.

Fruit firmness, an important quality attribute, is directly related to enhancing the storability potential and to induce greater resistance to mechanical damage and fruit decay (Esti et al., 2002). The main target is the maintenance of fruit firmness during the cold storage to extend the storage life. It is possible to obtain more resistant fruit by using growth regulators such as pre-harvest calcium (Rico et al., 2007) and GA$_3$ (Einhorn et al., 2013) and to maintain the post-harvest fruit quality by using post-harvest MAP packaging techniques. MAP application contributes to the maintenance of fruit firmness by reducing respiratory activity, ethylene production, enzymatic reactions and some physiological changes during the cold storage and thus helps to maintain quality longer (Gorny, 1997).

CaCl$_2$, which increased the fruit firmness, is used to prolong the post-harvest life of fruits and vegetables (Rico et al., 2007). High calcium content in the fruit also delays ripening and increases fruit firmness (Kader, 2002). In addition, the application of calcium pre- or post-harvest is considered by some to be beneficial (Wang et al., 2014; Zoffoli et al., 2017). The effect of pre-harvest CaCl$_2$ sprays on sweet cherry fruit firmness is inconsistent (Winkler and Knoche, 2019). This effect can be explained by the interaction of calcium ions and pectic polymers and delaying the onset of climacteric elevation in fruit (Ben-Arie et al., 1995).

Growth regulators are widely used to increase fruit size and quality (Fortes et al., 2015), contributing to the preservation of the storage quality due to its effect on the cell wall (Eccher and Hajnaiari, 2006). Studies (Choi et al., 2002; Clayton et al., 2006; Correia et al., 2019) have shown that GA$_3$ applications increase fruit size and fruit firmness in sweet cherry. Again, Cline and Trought (2007), in sweet cherry, GA$_3$ treatments increased fruit firmness by 5% compared with untreated control fruit.

**SSC, Titratable Acidity and Vitamin C**

At the harvest, the highest SSC, titratable acidity and vitamin C were obtained from the fruit belonging to the GA$_3$ application, whereas the lowest SSC, titratable acidity and vitamin C were obtained from GA$_3$+CaCl$_2$ applied fruit. The highest titratable acidity and vitamin C were recorded in the fruit of the CaCl$_2$ application. At the end of the cold storage, in the applications both with and without MAP, the fruit of the CaCl$_2$ application had significantly lower SSC and higher titratable acidity compared to the control. The fruits treated with GA$_3$ and stored without MAP were found to have significantly lower acidity than the control. When the fruits stored in MAP were evaluated, it was determined that the vitamin C content of the fruit treated with GA$_3$ and CaCl$_2$ was significantly lower compared to the control. However, in the fruit stored without MAP, it was observed that the vitamin C content in the fruit of only the GA$_3$+CaCl$_2$ application was significantly lower than the control (Figure 3).

SSC is directly related to the taste of the fruits. The majority of SSC consists of sugars. SCC increases, and titratable acidity and vitamin C decrease due to maturation progresses during the storage (Abd El-Gawad et al., 2019). Similarly, it was reported that during the cold storage in sweet cherry, SSC rate increased and titratable acidity (Gonçalves et al., 2004) and vitamin C content (Tian et al., 2004) decreased. The effect of MAP application at the cold storage on the chemical composition can be explained by the change in physiological phenomena such as ethylene production, respiration and transpiration due to MAP application (Domínguez et al., 2016). It has been reported that MAP applications have caused the decreasing of SSC ratio by slowing down the ripening during the cold storage in kiwifruit (Zhang et al., 2003) and sweet
cherry (Diaz-Mula et al., 2012). However, Aglar et al. (2017) determined that MAP treatment does not cause much change in the SSC value of sweet cherry. Gonçalves et al. (2004) reported that MAP treatment increased SSC rate and decreased titratable acidity rate of sweet cherry fruits throughout the storage. Mohammadi and Hanafi (2014) reported significantly delayed losses in vitamin C content of strawberry fruits treated with MAP treatments.

Beigi et al. (2019) reported that CaCl$_2$ treatment had a positive impact on the fruit chemical composition, and simultaneous application of CaCl$_2$ and GA$_3$ resulted in the maximum content of pH and SSC and the minimum acidity and ascorbic acid. In this study, ripening-retarding effect of GA$_3$ was clearly observed in SSC. In a study by Alrashdi et al. (2017), which was carried out on table grapes, it was determined that SSC content was not affected by the GA$_3$ treatments. In addition, Winkler and Knoche (2019) reported that pre-harvest Ca application reduced losses in soluble solids during storage. However, Correia et al. (2019) determined that CaCl$_2$ application did not have an effect in soluble solids.

**Figure 3.** Effects of pre-harvest spray treatments (GA$_3$ and CaCl$_2$) on the SSC, titratable acidity and vitamin C of sweet cherry fruit throughout cold storage. The means indicated with the same letter vertically in periods, and above the bars were not significant according to Tukey’s test at P < .05.
Total Phenolics, Total Flavonoids, Total Monomeric Anthocyanin and Antioxidant Activity

At the harvest, the total phenolics content of GA3-applied fruit was significantly lower than the control. However, the total flavonoids content of the GA3 and CaCl2 applications and the total anthocyanin of the fruit treated with CaCl2 were significantly higher than the control. According to the FRAP assay, the antioxidant activity of only the CaCl2 application was significantly higher than the control. At the end of the cold storage, the total phenolics of the fruit treated with GA3 and CaCl2 and stored in MAP were significantly higher compared to the control. However, during the cold storage, the total phenolics and total anthocyanin of the GA3-applied fruit and kept without MAP were significantly lower than the control. In the last two measurements of the cold storage, the total flavonoids of CaCl2-applied fruit and stored in MAP were significantly higher than the control. During the cold storage, according to both FRAP and DPPH tests, the antioxidant activity of the fruit treated with GA3+CaCl2-treated and stored in MAP was significantly lower than the control (Figure 4–5).

Sweet cherry is a rich source of dietary phenolics with antioxidant properties that are associated with a wide range of health benefits (Mulabagal et al., 2009). Anthocyanins and flavonoids are

Figure 4. Effects of pre-harvest spray treatments (GA3 and CaCl2) on the total phenolics, total flavonoids and total anthocyanin of sweet cherry fruit throughout cold storage. The means indicated with the same letter vertically in periods, and above the bars were not significant according to Tukey’s test at P < .05.
important phenolic compounds in sweet cherry (Aghdama et al., 2013). The composition and concentration of these compounds may vary depending on genetic and environmental factors and fruit ripening stage (Serra et al., 2011). In sweet cherry, there is a correlation between fruit ripening level and bioactive content of fruit, and phenolic substances, anthocyanins and antioxidants have the highest value at optimal maturity level (Mahmood et al., 2013).

Aglar et al. (2017) determined that an increase in bioactive compound content from the beginning till the end of the cold storage occurred. Ratanchinakorn et al. (1997) and Maul et al. (2000) determined that MAP treatment can delay aroma deterioration and biochemical changes in the postharvest period in sweet cherry. Artes-Hernandez et al. (2006) determined that the MAP treatment delayed carotenoid and anthocyanin formation at cold storage. Guan and Dou (2010) have reported that MAP-treated plum fruits have lower antioxidant capacity. Again, Aglar et al. (2017) reported that MAP treatment caused a difference in the bioactive compound content during cold storage. The reason for this might be due to the effect of MAP treatments on gas composition around the fruit.

Michailidis et al. (2017) have reported that CaCl₂ sprays increased cherries phenol content and antioxidant activity at harvest and post-harvest periods and decreased in post-harvest respiration rate, ascorbic acid and flavonoid loss. Wang et al. (2014) reported that CaCl₂ applications (0.2%, 0.5%) increased total anthocyanin, total flavonoids, total phenolics and total antioxidant capacity after 4 weeks of the cold storage. Aghdama et al. (2013) have shown that post-harvest CaCl₂ application increases the antioxidant capacity by effectively preserving higher total flavonoids, total phenolics and anthocyanin content of the cranberry fruit.

Biosynthesis of phenolics such as anthocyanins and flavonoids in plants is carried out via the shikimate-phenylpropanoid-flavonoid pathways, in which phenylalanine ammonia-lyase (PAL) serves as a key enzyme (Kays and Paull, 2004). It has been postulated that an enhancement of TP and antioxidant activity in fruit by CaCl₂ treatment is due to stimulating the phenylpropanoid pathway by
increasing the PAL activity (Aghdama et al., 2013). Moreover, these results can be explained with the effect of the calcium on several key physiological processes connected to ripening-related phenomena, including those associated with the structure and functionality of cell walls and membranes, or with the activity of particular enzymes (Lara, 2013). In the study, it was determined that GA3-treated fruit had a relatively lower bioactive compound content compared to fruit of calcium and control applications both during harvest and cold storage. The effect of GA3 application had also been effected on the values of CaCl2+GA3 combination application. In accordance with the study results, Diaz-Mula et al. (2009) and Ozkan et al. (2016) reported that GA3 treatments significantly decreased total phenolics, antioxidant capacity and total anthocyanin content of sweet cherry.

**Conclusion**

At the end of the cold storage, with MAP; the weight loss was lower on the fruit treated with GA3 +CaCl2 and on the CaCl2 applied fruit without MAP. During the cold storage, the decay was delayed by CaCl2 application. At the end of the cold storage, the fruit treated with GA3 and CaCl2 had higher a* and firmness values. The SSC in the fruit of the CaCl2 application was lower than the control application. However, significantly higher acidity was determined from CaCl2 application compared to other applications. In fruit stored in MAP, vitamin C content and antioxidant activity of fruit treated with GA3 and CaCl2 were significantly lower. But total phenolics were higher. Also, the total flavonoids of the CaCl2-applied fruit were higher compared to the control. The anthocyanin content of the fruit treated with GA3 and stored without MAP was lower compared to the control.

As a result, it was observed that GA3 treatment retarded fruit ripening and maintained firmness which is a significant quality parameter for sweet cherry. It can be said that pre-harvest GA3 and CaCl2 applications have increased the fruit quality of sweet cherry and have a significant effect on extending the post-harvest life of sweet cherry and pre-harvest GA3 and CaCl2 and post-harvest MAP applications are effective and usable applications to reduce storage losses and maintain fruit quality during the cold storage.

**Acknowledgments**

This study was supported by the Scientific Research Unit (Project number AR-1658) of Ordu University.

**Authors’ Contributions**

Burhan Ozturk, Erdal Aglar, Sefa Gun and Orhan Karakaya. Planning, design, analysis of fruit and data analysis of experiment and writing of the manuscript.

**Disclosure Statement**

No potential conflict of interest was reported by the author(s).

**Compliance With Ethical Standards**

The authors declare that they have no conflict of interest

**ORCID**

Burhan Ozturk [http://orcid.org/0000-0002-0867-3942](http://orcid.org/0000-0002-0867-3942)
Erdal Aglar [http://orcid.org/0000-0002-4199-5716](http://orcid.org/0000-0002-4199-5716)
Onur Saracoglu [http://orcid.org/0000-0001-8434-1782](http://orcid.org/0000-0001-8434-1782)
Orhan Karakaya [http://orcid.org/0000-0003-0783-3120](http://orcid.org/0000-0003-0783-3120)
Sefa Gun [http://orcid.org/0000-0002-9516-386X](http://orcid.org/0000-0002-9516-386X)
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