

Research Article

Effects of preharvest GA₃, CaCl₂ and modified atmosphere packaging treatments on specific phenolic compounds of sweet cherryBurhan Ozturk^{1*}, Erdal Aglar², Orhan Karakaya^{1*}, Onur Saracoglu³, Sefa Gun¹¹ Ordu University, Faculty of Agriculture, Department of Horticulture, Ordu-Turkey² Sivas Cumhuriyet University, Suşehri Timur Karabal Vocational School, Sivas-Turkey³ Tokat Gaziosmanpaşa University, Faculty of Agriculture, Department of Horticulture, Tokat-Turkey**ABSTRACT**

The fruit of sweet cherry are more sensitive against external factors than many fruit species so post harvest quality loss on sweet cherry is quite higher. For this reason, it is very significant to reduce the postharvest losses and to extend the storage period in sweet cherry, and this is one of the main objectives for producers and consumers. The aim of the study was to determine the effects of GA₃, CaCl₂ and modified atmosphere packaging (MAP) applications on individual phenolics compounds in postharvest storage on sweet cherry. In the study, GA₃ was applied at 30 mg L⁻¹ concentration when fruit skin was yellow-straw, and CaCl₂ was applied at 0.5% concentration 20 and 10 days before the estimated harvest date. The study consisted of 4 different spray (Control (water only), GA₃, CaCl₂, GA₃+CaCl₂) and 2 packaging applications (without and with MAP). MAP was applied to the fruit after the harvest. In our study, individual phenolics such as catechin, 4-hydroxybenzoic acid, epicatechin, caffeic acid, *p*-coumaric acid, 4-aminobenzoic acid and protocatechuic acid were determined. It has been determined that pre-harvested GA₃, and CaCl₂ applications have increased the content of individual phenolics in fruit. The concentration of individual phenolics generally decreased with increasing cold storage time, whereas epicatechin and 4-hydroxybenzoic acid concentrations increased. 4-hydroxybenzoic acid and caffeic acid concentrations were higher CaCl₂, and GA₃ treatments than in control in all measured period. The MAP application had a positive effect on the losses of other phenolic compounds except catechin during cold storage.

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4-hydroxybenzoic acid*** CORRESPONDING**burhanozturk55@gmail.com
orhankarakaya7@gmail.com**1. Introduction**

With the increasing interest in human health in the recent years, the using of the fruits and vegetables in a balanced diet and proper diet is very significant. While there is a positive correlation between the daily use of fruits and vegetables and the reduction of the risk of disease, especially clinical tests and chemical analyzes emphasize the role of fruits and vegetables in preventing diseases (cardiovascular disorder, cancer, rheumatoid arthritis, lung emphysema, skin inflammation, cataract, neurological degenerative, endothelial cell disorder) caused by oxidative stress (Kaur and Kapoor, 2001; Benzie, 2003). Fruits are considered a natural source of antioxidants such as anthocyanins and polyphenols, which can reduce the risk of stress-induced degenerative disease such as cancer, heart disease, stroke (Ames et al., 1993; Robards et al., 1999; Kaur and Kapoor, 2001; Ma and Kinner, 2002). It has been reported the phenolic antioxidants have different positive effects such as anti-inflammatory and anti-carcinogenic on human healthy (Garcia-Closas et al., 1999; Kroon and Williamson, 1999;

Mamani-Matsuda et al., 2006), and are significant in human nutrition (Usenik et al., 2008).

Sweet cherry is greatly rich in terms of the bioactive compounds such as anthocyanins, quercetin, hydroxycinnamates (Gao and Mazza, 1995; Chaovanalikit and Wrolstad, 2004; González-Gómez et al., 2009; Gimenez et al., 2016; Aglar et al., 2019). The prominent anthocyanins present in sweet cherry are cyanidin 3-rutinoside and cyanidine 3-glucoside (Mozetic et al., 2002; Gonçalves et al., 2007; Usenik et al., 2008; González-Gómez et al., 2010; Liu et al., 2011; Kelebek and Selli, 2011) while hydroxycinnamates, neochlorogenic acid and *p*-coumaric acid have been found in adequate quantities (Kim, et al., 2005; Usenik et al., 2010; Liu et al., 2011). Matilla et al. (2006) have reported that there are small amounts of chlorogenic acid, hydroxybenzoic acids and ferulic acid on sweet cherry. The polyphenol compounds such as anthocyanins and hydroxycinnamic esters on sweet cherry have a positive effect cancer, cardiovascular disease, diabetes, inflammatory diseases (McCune et al., 2011; Ballistreri et al., 2013), and these compounds are significant

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due to their potential contribution to the color of sweet cherry fruit (Mazza and Miniatti, 1993; Gao and Mazza, 1995; Mozetic et al., 2002).

However, the amounts of nutrients and bioactive components on sweet cherry can be altered depending on factors such as the ripening of the fruit, postharvest storage conditions, and processing (Serrano et al., 2009, Valero et al., 2011; Díaz-Mula et al., 2012). Sweet cherry is a non-climacteric fruit species with high respiratory rate and susceptible to fungal decay (Alique and Zamorano, 2005), and sweet cherries are by nature more perishable and postharvest quality loss is consequently higher. For this reason, it is very important to reduce the postharvest losses and to extend the storage period in sweet cherry, and this is one of the main objectives for producers and consumers (Meheriuk et al., 1995). In recent years, in order to preserve the quality of fruits after harvest and to reduce the losses at cold storage, the pre-harvest plant growth regulators such as gibberellic acid (Amarante et al., 2005; Steffens et al., 2011; Huang et al., 2014; Souza et al., 2016; Zaho et al., 2018; Ozturk et al., 2018, Dong et al., 2019), calcium (Martín-Diana et al., 2007; Tsantili et al., 2007; Wójcik et al., 2013; Wójcik and Wawrzyńczak, 2014; Michailidis et al., 2017; Dong et al., 2019), methyl jasmonate (Kucuker and Ozturk, 2015; Venkatachalam and Meenune, 2015; Gomez et al., 2017), methylcyclopropene (Baswal et al., 2020), salicylic acid (Lu et al., 2011; El-Razek et al., 2013; El-Shazly et al., 2013; Fatemi et al., 2013; Baswal et al., 2020), and the post-harvest coating materials such as Aleo vera (Carrillo-Lopez et al., 2000; Martinez-Romero et al., 2006), chitosan (Romanazzi et al., 2003; Valero et al., 2014), modified atmosphere packaging (Guilbert et al., 1996; Petracek et al., 2002; Cantín et al., 2008; Kaynas et al., 2010; Diaz-Mula et al., 2012; Giacalone and Chiabrande 2013; Guillen et al., 2013; Naserzaeim et al., 2015; Aglar et al., 2017) and alginate (Diaz-Mula et al., 2012) were applied.

Gibberellic acid (GA₃), which plays significant role in breaking seed dormancy, promoting flower bud differentiation and stem elongation, and delaying the senescence of plant organs (Achar et al., 2009; Sun, 2010; Zaho et al., 2018), has been used in commercial horticultural cultivation as a plant growth regulator to improve fruit size and quality (Khalil and Aly, 2013; Zang et al., 2016; Gundogdu et al., 2017), to delay ripening and to maintain fruit quality of postharvest crops (Steffens et al., 2011; Huang et al., 2014; Souza et al., 2016; Zaho et al., 2018). Basak et al. (1998), Clayton et al. (2003), Lenahan et al. (2008), Zhang and Whiting (2011) Einhorn et al. (2013) and Canli et al. (2015), have reported that pre-harvest GA₃ application has a significant effect on quality of sweet cherry fruit. GA₃ have a significant effect on fruit coloration and anthocyanin synthesis capacity, and normal phenolic metabolism on sweet cherry (Li et al., 2019). Dong et al. (2019) have determined that GA₃ application significantly reduced phenolic compounds, anthocyanin accumulation and antioxidant capacity on sweet cherry. Pre-harvest GA₃ applications in sweet cherry have significant effects on preservation of the quality and chemical composition of the fruit at postharvest cold storage (Kappel and Macdonald, 2002; Einhorn et al., 2013).

Calcium, which is highly effective against fruit's physiological disorders (Hernandez-Munoz et al., 2006; Korkmaz et al., 2016) and has many significant functions both structurally as a stabilizer of plant cell wall and membrane integrity (Hocking et al., 2016; Hosein-Beige et al., 2019), is used to improve the fruit quality of sweet cherry (Martín-Diana et al., 2007; Tsantili et al., 2007; Wójcik et al., 2013; Wójcik and Wawrzyńczak, 2014; Michailidis et al., 2017). Hosein-Beigi et al. (2019) have determined that pre-harvest Ca application has a significant effect on the fruit chemical composition on sweet cherry. Wójcik and Wawrzyńczak (2014) and Michailidis et al. (2017) reported that the pre-harvest Ca applications in sweet cherry have significant effects on the preservation of the quality and chemical composition of the fruit at postharvest cold storage

It has been reported the storage techniques such as MAP are effective in extending the storage period and on minimizing the losses the fruit quality at cold storage in stone fruits (Zhang et al., 2003 Naserzaeim et al., 2015; Aglar et al., 2017). Petracek et al. (2002), Remon et al. (2000), Spotts et al. (2002) and Tian et al. (2004) have reported that the application of the MAP, which is used to delay the physicochemical changes, to retard microbial spoilage and to retain color by reducing the oxidation, extending the shelf life of the fruit species (Singh et al., 2010), has a significant effect in delaying of the physico-chemical changes on sweet cherry. The MAP applications that balance the CO₂ concentrations and inhibits enzyme activity favoring stability of color (Rocha and Morais, 2001; Remon et al., 2004) has slightly increased the total anthocyanin content of sweet cherry during the cold storage (Conte et al., 2009; Padilla-Zakour et al., 2007; Remon et al., 2000). However, Remon et al. (2003) have determined that MAP application has not a significant effect on the total anthocyanin content of sweet cherry at postharvest.

Many studies on the effects of GA₃, CaCl₂ and MAP applications on the quality and chemical composition of the fruit of sweet cherry at harvest and at postharvest, has been carried out. However, there is no study on the effect of GA₃, CaCl₂ and MAP applications on individual phenolic in sweet cherry at harvest and at postharvest storage. The aim of the study was to determine the effects of GA₃, CaCl₂ and modified atmosphere packaging (MAP) applications on the composition and concentration of individual phenolic in postharvest storage on sweet cherry.

2. Materials and methods

2.1. Material

In the research, the trees belonging to Regina cultivar grafted on MaxMa 14 rootstock and were planted in 2008 in Gaziosmanpaşa University Faculty of Agriculture Horticulture Application Orchard, were used as plant material.

2.2. Methods

In the study, GA₃ was applied at 30 mg L⁻¹ concentration when fruit skin was yellow-straw, and CaCl₂ was applied at 0.5% concentration 20 and 10 days before the estimated harvest date. The research was planned in a randomized block design as 2 trees at per replication with 3 replications.

The study consisted of 4 different applications (Control (water only), GA₃, CaCl₂, GA₃+CaCl₂) for both MAP and non-MAP treatment. The solutions prepared for each application were sprayed to the trees in the morning of a windless and rainless day with a pressure back pump until the trees were completely wet. Tween 20 (0.1%) was used as the spreading adhesive in the applications. The harvest of the fruit was carried out on 20 June. The fruit, homogeneously colored, uniformly sized, undamaged healthy and perfect ones, had been selected and stacked in cardboard packages. These fruits were immediately transferred to Ordu University, Faculty of Agriculture, Department of Horticulture, and Fruit Laboratory by means of refrigerated vehicle. Fruit was divided into 8 different groups as control, GA₃, CaCl₂, GA₃ + CaCl₂, control + MAP, GA₃ + MAP, CaCl₂ + MAP and GA₃ + CaCl₂ + MAP. In addition, each application was arranged as 3 replicates (each replicate containing approximately 1 kg of fruit). Analyzes were carried out to determine the composition and concentration of individual phenolic in fruit during the harvesting period. After these analyzes, the fruit were stored for 21 days in 0 °C and 90 ± 5% relative humidity conditions in the cold storage of Ordu University Faculty of Agriculture. On the 7th, 14th and 21st days of the cold storage, measurements and analyzes of individual phenolic were carried out in Ordu University Faculty of Agriculture Department of Horticulture. Modified atmosphere packaging (MAP) application: Fruit were transferred to cold storage immediately after the separation and packaging. Subsequently, they were exposed to pre-cooling at about 1 °C for 24 hours until the fruit temperature dropped to 3-4 °C. After the pre-cooling, the fruit were placed in 22 µm LDPE based 5 kg MAP package (Xtend, Stepac, Israel) and their mouths were closed with plastic clips. In the study, the concentrations of the individual phenolic acids in MAP package were determined with 7 day intervals for 21 days. Individual phenolic acids were analyzed as follows. Homogeneously selected fresh fruit samples were weighed as 1 gram and extracted with methyl alcohol (5 mL) in a test tube for 6 hours. The extract was analyzed by high pressure liquid chromatography (HPLC) (Perkin-Elmer series 200, Norwalk, USA). The HPLC system was equipped with UV detector (Series 200, UV / Vis detector) and quaternary solvent dispensing system (Series 200, analytical pump) and used at 280 nm. Analytes were separated by a Phenomenex Kromasil (Phenomenex, Torrance, USA) 100A C18 (250 mm × 4.60 mm, 5 µm) column. The column temperature was maintained at 26 °C using a water bath (Wisebath, WB-22, Daihan Scientific, Seoul, Korea). The mobile phase was formed from water and acetonitrile (A) containing 2.5% formic acid (B). The mobile phase flow rate was maintained at 1 mL per minute and 20 µL of the sample was injected and expressed in mg kg⁻¹ in light of the results of the peak areas obtained.

2.13. Statistical analysis

The Kolmogorov-Smirnov test was used to confirm and the homogeneity of variances was tested the Levene's test. After the data were analyzed by analysis of variance, the significance level between the treatments was determined by Duncan multiple comparison test. Statistical analyzes were

performed in SAS program (SAS 9.1 version, USA). The significance level was considered as $\alpha = 5\%$ in statistical analysis and interpretation of results.

3. Results

3.1. Catechin

The data relative to the effects of pre-harvest gibberellic acid and calcium chloride and post-harvest modified atmosphere packaging applications on catechin was shown in Table 1.

The highest catechin content during a harvest period was measured in GA₃ treated fruit and the lowest in control fruit. Catechin content decreased in all applications during cold storage. When the fruit applied with MAP were examined, the catechin concentration was significantly higher in the fruit treated with CaCl₂ + MAP and GA₃ + MAP on day 7th than the control, whereas on day 14th and 21th it was determined that GA₃ + MAP the application had higher catechin concentration than the other applications. When the averages of application were examined, the catechin content of fruit treated with MAP in all applications was higher than control fruit, whereas in non-MAP treated fruit, the highest concentration was recorded with the application of GA₃ while the difference between the applications of GA₃ and CaCl₂ was not significant at harvest and in 7th of storage. When the measurement periods were compared, it was seen that MAP treated fruit had significantly lower catechin content than fruit without MAP at all periods (Table 1).

3.2. 4-hydroxybenzoic acid

When the effect of pre-harvest CaCl₂ and GA₃ applications on the 4-hydroxybenzoic acid in fruit was evaluated, the highest amount of 4-hydroxybenzoic acid was recorded with GA₃ applied fruit and the lowest hydroxybenzoic acid concentration was obtained with control application in without MAP fruit at harvest, 7th and 14th days. The cold storage period had a significantly effect on hydroxybenzoic acid concentration. Thus, the concentration of hydroxybenzoic acid, which was 14.08 mg kg⁻¹ at the harvest the MAP application, reached 19.17 mg kg⁻¹ at the end of storage the MAP application. In addition to, the increase in MAP application was observed to be higher. When the effect of CaCl₂ and GA₃ applications during cold storage was evaluated, it was determined that the highest value was obtained with GA₃+ CaCl₂ application at all measurement periods, while the fruit of the control application had the lowest hydroxybenzoic acid concentration. On the 7th day of the cold storage, the significant differences between all applications have occurred. On the 14th day, there was no difference between CaCl₂ and control application, and the difference between CaCl₂ or GA₃ and control applications at the end of the cold storage was not statistically significant. The highest values were obtained with GA₃ + CaCl₂ application while no significant difference between CaCl₂ and control application in all measurement periods in MAP treated fruit was observed. On the 7th day of the cold storage, higher hydroxybenzoic acid concentration with application of GA₃ were recorded compared to control and CaCl₂ applications in fruit not treated with MAP, but there was no significant difference between three applications at the end of the cold storage.

Table 1. Effects of pre-harvest spray treatments (GA₃ and CaCl₂) and MAP on catechin contents of sweet cherry fruit throughout cold storage

Treatments	Catechin (mg kg ⁻¹)			
	Harvest	7	14	21
Results by packaging				
MAP	636	285 b	222 b	188 b
non-MAP	636	382 a	296 a	231 a
<i>Significance</i>		**	*	*
Results by spraying				
Control	382 c	287 c	225 b	209 ab
CaCl ₂	739 a	256 c	205 b	195 ab
GA ₃	893 a	433 a	341 a	266 a
GA ₃ +CaCl ₂	530 b	358 b	263 b	168 b
<i>Significance</i>	*	*	**	*
MAP				
Control	382 c	343 b	271 b	253 b
CaCl ₂	739 a	205 b	199 c	191 c
GA ₃	893 a	502 a	399 a	304 a
GA ₃ +CaCl ₂	530 b	480 a	314 b	176 c
non-MAP				
Control	382 c	232 b	179 c	166 c
CaCl ₂	739 a	307 a	212 b	199 b
GA ₃	893 a	365 a	283 a	227 a
GA ₃ +CaCl ₂	530 b	237 b	213 b	161 c
<i>Significance (interaction)</i>	*	*	**	*

*: $p < 0.05$, **: $p < 0.01$. The differences among the means indicated with the same lowercase letter in the same column were not significant

There was no statistically significant difference between CaCl₂, GA₃ and CaCl₂ + GA₃ applications in fruit not treated with MAP in the 7th, 14th and 21th days of the cold storage. There was no statistically significant difference between CaCl₂, GA₃ and CaCl₂ + GA₃ applications in fruit without MAP (Table 2).

3.3. Epicatechin

When the effects of pre-harvest CaCl₂ and GA₃ applications on epicatechin content in fruit were evaluated, it was found that CaCl₂ application had no effect on epicatechin in the study, whereas GA₃ application increased epicatechin in fruit. An increase in the rate of epicatechin was observed in proportion to the storage time. When the effects of CaCl₂, GA₃ and MAP applications at postharvest were evaluated, it was determined that in MAP-treated fruit, the epicatechin concentration of the fruit of CaCl₂ + MAP application was significantly lower than control's on the 7th day, whereas the epicatechin concentration of the fruit treated with GA₃ on 14th day and the fruit of all applications on the 21st day higher epicatechin content than control fruit. In fruit not treated with MAP, the highest amount of epicatechin during cold storage was recorded in GA₃-treated fruit while there was a significant difference between GA₃ and CaCl₂ applications and control application on the 7th and 14th days of cold storage; however, no difference was found between the epicatechin concentrations of GA₃, CaCl₂ and control applications at the end of cold storage (Table 3)

3.4. Caffeic acid

When the effect of pre-harvest CaCl₂ and GA₃ applications

on fruit caffeic acid content was evaluated, it was determined that the increase in caffeic acid concentration occurred with CaCl₂ and GA₃ applications. However, there was no difference between caffeic acid concentrations of the fruit of CaCl₂ and GA₃ applications. It was determined that caffeic acid concentration decreased with the extended cold storage time and this decrease was lower with MAP application. On the 7th day of the cold storage, there was no significant difference between CaCl₂, GA₃ and CaCl₂+GA₃ applications, whereas caffeic acid concentrations of CaCl₂ and GA₃ were similar at 14th day. On the 21st day of the cold storage, the significant differences between the all applications were observed and the highest value was recorded with CaCl₂ application. However, it was found that the fruit of the control were found to have the lowest caffeic acid concentration at all measurement periods (Table 4). The statistically significant differences between CaCl₂ and GA₃ applications in MAP-treated fruit in terms of caffeic acid concentration on the 7th and 21st days of the cold storage occurred. The highest value in 7th day of the cold storage was obtained with GA₃ application, but on the 21st day, the highest value was recorded with CaCl₂ application. On the 14th day of the cold storage, significant differences between the caffeic acid content of the fruit of CaCl₂ and GA₃ applications were found. The statistically significant differences between CaCl₂ and GA₃ applications in caffeic acid concentration in fruit without MAP on the 7th and 21st days of the cold storage were observed. The highest value on 7th day of the cold storage was obtained with GA₃ application, but on 21st day, the highest value was recorded

with CaCl₂ application. On the 14th day of the cold storage, the difference between the amount of caffeic acid of the fruit

of CaCl₂ and GA₃ applications was not significant, the lowest value was recorded with control application (Table 4).

Table 2. Effects of pre-harvest spray treatments (GA₃ and CaCl₂) and MAP on 4-hydroxybenzoic acid contents of sweet cherry fruit throughout cold storage

Treatments	4-hydroxybenzoic acid (mg kg ⁻¹)			
	Harvest	7	14	21
Results by packaging				
MAP	14.08	16.95 a	17.69 a	19.17 a
non-MAP	14.08	15.76 b	16.72 b	18.08 a
<i>Significance</i>		*	*	**
Results by spraying				
Control	11.36 c	14.21 c	16.15 b	17.23 b
CaCl ₂	13.83 b	15.85 b	16.17 b	17.91 b
GA ₃	15.73 a	17.38 a	17.68 a	18.25 b
GA ₃ +CaCl ₂	15.38 a	17.97 a	18.83 a	21.11 a
<i>Significance</i>	*	**	**	**
MAP				
Control	11.36 c	15.40 c	16.80 b	17.65 b
CaCl ₂	13.83 b	15.70 c	15.81 b	17.91 b
GA ₃	15.73 a	17.38 b	17.69 b	18.20 b
GA ₃ +CaCl ₂	15.38 a	19.31 a	20.46 a	22.90 a
non-MAP				
Control	11.36 c	13.02 b	15.50 b	16.80 b
CaCl ₂	13.83 b	16.00 a	16.53 a	17.91 a
GA ₃	15.73 a	17.38 a	17.67a	18.29 a
GA ₃ +CaCl ₂	15.38 a	16.62 a	17.19a	19.31 a
<i>Significance (interaction)</i>	*	**	*	*

*: $p < 0.05$, **: $p < 0.01$. The differences among the means indicated with the same lowercase letter in the same column were not significant.

3.5. *p*-coumaric acid

When the effect of pre-harvest CaCl₂ and GA₃ applications on *p*-coumaric acid content in fruit was evaluated, the difference between the applications was significant, the highest amount of 4-hydroxybenzoic acid was obtained from fruit applied with GA₃ + CaCl₂, and the lowest *p*-coumaric acid concentration was recorded with control application. It was determined that the cold storage period had a positive effect on *p*-coumaric acid concentration. Such that, the *p*-coumaric acid concentration, which was 9.30 mg kg⁻¹ at the harvest, decreased to 6.20 mg kg⁻¹ at the end of the cold storage. In addition to, the amount of reduction during the cold storage was lower with MAP application. When the effects of CaCl₂ and GA₃ applications at the end of the cold storage was evaluated, the highest value was obtained with CaCl₂+GA₃ application. At the end of the cold storage, the difference between CaCl₂ and GA₃ applications was not statistically significant. While the highest *p*-coumaric acid concentration was recorded in all fruit applied with GA₃ + CaCl₂ at all measurement periods in MAP-treated fruit, there was no statistically significant between the other three applications. There was no statistically significant difference

between CaCl₂ and GA₃ applications in fruit without MAP. The lowest value was obtained with control application while the highest value was recorded with CaCl₂+GA₃ application (Table 5).

3.6. 4-aminobenzoic acid

The effects of pre-harvest CaCl₂ and GA₃ applications and post-harvest MAP application in 4-aminobenzoic acid content of fruit at post-harvest cold storage was shown in Table 6. When the data were evaluated, CaCl₂ and GA₃ decreased to the 4-aminobenzoic acid concentration while the lowest amount of 4-aminobenzoic acid was recorded in fruit applied with GA₃. It was determined that 4-aminobenzoic acid concentration decreased as the cold storage time increased, but CaCl₂ and GA₃ applications had no effect on this decrease. Considering the data of CaCl₂ and GA₃ and MAP combinations, significant differences between the applications on the 14th and 21st days of the cold storage were not observed while on the 7th day of the cold storage, the lowest value was obtained with the application of GA₃ + CaCl₂. There were no significant differences in terms of 4-aminobenzoic acid concentration after the cold storage in fruit without MAP application (Table 6).

Table 3. Effects of pre-harvest spray treatments (GA₃ and CaCl₂) and MAP on epicatechin contents of sweet cherry fruit throughout cold storage

Treatments	Epicatechin (mg kg ⁻¹)			
	Harvest	7	14	21
Results by packaging				
MAP	13.54	15.47	18.07 a	19.91
non-MAP	13.54	15.71	16.93 b	19.59
<i>Significance</i>		ns.	*	ns.
Results by spraying				
Control	12.26 b	15.71 a	16.61 b	19.22 b
CaCl ₂	12.15 b	14.71 b	17.61 ab	21.29 a
GA ₃	14.41 a	16.29 a	18.51 a	20.14 ab
GA ₃ +CaCl ₂	15.33 a	15.67 a	17.28 ab	18.33 c
<i>Significance</i>	*	*	*	**
MAP				
Control	12.26 b	16.25 a	17.21 b	17.86 c
CaCl ₂	12.15 b	14.42 c	17.57 b	21.81 a
GA ₃	14.41 a	15.63 b	18.88 a	19.51 b
GA ₃ +CaCl ₂	15.33 a	15.59 b	18.63 a	20.44 a
non-MAP				
Control	12.26 b	15.17 c	16.01 b	20.58 a
CaCl ₂	12.15 b	14.99 c	17.64 a	20.77 a
GA ₃	14.41 a	16.94 a	18.13 a	20.77 a
GA ₃ +CaCl ₂	15.33 a	15.74 b	15.92 b	16.22 b
<i>Significance (interaction)</i>	*	**	*	**

*: p < 0.05, **: p < 0.01, ns: non-significant. The differences among the means indicated with the same lowercase letter in the same column were not significant.

Table 4. Effects of pre-harvest spray treatments (GA₃ and CaCl₂) and MAP on caffeic acid contents of sweet cherry fruit throughout cold storage

Treatments	Caffeic acid (mg kg ⁻¹)			
	Harvest	7	14	21
Results by packaging				
MAP	9.93	8.81 a	8.46 a	7.28 a
non-MAP	9.93	8.40 b	8.25 b	7.03 b
<i>Significance</i>		*	*	*
Results by spraying				
Control	7.61 b	6.87 b	6.69 c	6.09 c
CaCl ₂	10.49 a	9.31 a	8.75 b	8.00 a
GA ₃	10.82 a	8.71 a	8.12 b	7.40 b
GA ₃ +CaCl ₂	10.79 a	9.54 a	9.86 a	7.13 b
<i>Significance</i>	**	*	*	**
MAP				
Control	7.61 b	6.95 c	6.78 c	6.49 c
CaCl ₂	10.49 a	10.17 a	9.13 ab	7.37 b
GA ₃	10.82 a	8.21 b	8.12 b	8.10 a
GA ₃ +CaCl ₂	10.79 a	9.90 a	9.80 a	7.14 b
non-MAP				
Control	7.61 b	6.78 c	6.60 c	5.69 c
CaCl ₂	10.49 a	8.44 b	8.36 b	8.62 a
GA ₃	10.82 a	9.21 a	8.12 b	6.70 b
GA ₃ +CaCl ₂	10.79 a	9.17 a	9.92 a	7.12 b
<i>Significance (interaction)</i>	**	**	*	**

*: p < 0.05, **: p < 0.01. The differences among the means indicated with the same lowercase letter in the same column were not significant.

Table 5 . Effects of pre-harvest spray treatments (GA₃ and CaCl₂) and MAP on *p*-coumaric acid contents of sweet cherry fruit throughout cold storage

Treatments	<i>p</i> -coumaric acid (mg kg ⁻¹)			
	Harvest	7	14	21
Results by packaging				
MAP	9.30	6.84	6.68 a	6.20 a
non-MAP	9.30	6.59	6.38 b	5.65 b
<i>Significance</i>		<i>ns.</i>	*	**
Results by spraying				
Control	6.18 d	6.10 c	6.03 c	5.42 c
CaCl ₂	7.30 c	6.78 b	6.54 b	5.91 b
GA ₃	10.94 b	6.45 bc	6.22 c	5.85 b
GA ₃ +CaCl ₂	12.77 a	7.54 a	7.35 a	6.53 a
<i>Significance</i>	**	*	*	*
MAP				
Control	6.18 d	6.07 b	5.96 b	6.07 b
CaCl ₂	7.30 c	6.67 b	6.43 b	5.94 b
GA ₃	10.94 b	6.17 b	6.12 b	5.82 b
GA ₃ +CaCl ₂	12.77 a	8.45 a	8.22 a	6.97 a
non-MAP				
Control	6.18 d	6.13 b	6.09 b	4.77 c
CaCl ₂	7.30 c	6.89 a	6.65 a	5.88 b
GA ₃	10.94 b	6.72 a	6.31 ab	5.87 b
GA ₃ +CaCl ₂	12.77 a	6.63 a	6.48 a	6.09 a
<i>Significance (interaction)</i>	**	*	*	*

*: $p < 0.05$, **: $p < 0.01$, *ns.*: non-significant. The differences among the means indicated with the same lowercase letter in the same column were not significant.

3.7. Protocatechuic acid

In the study, it was observed that the concentration of the protocatechuic acid increased with CaCl₂ application and there was no significant difference between GA₃ and control application. It was determined that a decrease in the amount of protocatechuic acid in proportion to the cold storage time occurred, in this decrease, MAP application had no effect on the 7th and 14th days of the cold storage, but MAP -treated fruit had higher the protocatechuic acid concentration at the end of the cold storage. When the effect of GA₃ and CaCl₂ applications on protocatechuic acid concentration during the cold storage was evaluated, the highest value was obtained from the fruit of the CaCl₂ application and there were no statistically significant differences between GA₃ and control applications (Table 7).

4. Discussion

In our study, individual phenolics identified in sweet cherry samples were catechin, 4-hydroxybenzoic acid, epicatechin, caffeic acid, *p*-coumaric acid, 4-aminobenzoic acid, protocatechuic acid. Catechin was the individual phenolic, which had the highest concentration (636 mg kg⁻¹), while the individual phenolic with the lowest concentration (3.77 mg kg⁻¹) was protocatechuic acid. Jakobek et al. (2009) and Gonçalves et al. (2019) have reported that gallic (0.73–10.64 mg 100g⁻¹ fw), *p*-hydroxybenzoic (0.73–10.64 mg 100 g⁻¹), and 2,5-dihydroxybenzoic acids (0.46–1.64 mg 100g⁻¹) are the most prevalent phenolic compounds on sweet cherry.

Han et al. (2007) have reported that caffeic, coumaric, ferulic, and sinapic acids are the major hydroxycinnamic acids on sweet cherry. The fact that Hayaloglu and Demir (2016), have determined that the phenolics on sweet cherry are neochlorogenic and *p*-coumaroylquinic acids, epicatechin, chlorogenic acid, and rutin on sweet cherry. In addition to Serrano et al. (2005) and Usenik et al. (2008) reported that *p*-coumaroylquinic acid was principal phenolic on sweet cherry.

Pre-harvest GA₃ application has a significant effect on fruit quality of sweet cherry (Basak et al., 1998; Clayton et al., 2003; Lenahan et al., 2008; Zhang and Whiting, 2011; Einhorn et al., 2013; Canli et al., 2015). In the study, it was found that the application of GA₃, which is applied before harvest, generally increases the content of individual phenolics in fruit. In accordance with the results of the study, Diaz-Mula et al. (2009) reported that a significant increasing occurred in total phenolic, anthocyanin and antioxidant capacity in fruit by application of GA₃, while Ozkan et al. (2016) found that total anthocyanin content and total antioxidant capacity of GA₃ application in Regina and Sweetheart sweet cherry cultivar were significantly lower than control. With the application of GA₃, anthocyanin accumulation in strawberry (Martinez et al., 1994) and antioxidant capacity in plum (Eroglu and Sen, 2015) decreased. This can be explained by means of delaying the maturity of the fruit with GA₃ application (Kappel and Mac Donald, 2002; Amarente et al., 2005; Lenahan et al., 2006; Çetinbaş and Koyuncu, 2013).

Table 6. Effects of pre-harvest spray treatments (GA₃ and CaCl₂) and MAP on 4-aminobenzoic acid contents of sweet cherry fruit throughout cold storage

Treatments	4-aminobenzoic acid (mg kg ⁻¹)			
	Harvest	7	14	21
Results by packaging				
MAP	4.80	4.36	4.25	4.15
non-MAP	4.80	4.31	4.22	4.11
<i>Significance</i>		<i>ns.</i>	<i>ns.</i>	<i>ns.</i>
Results by spraying				
Control	5.49 a	4.32	4.20	4.15
CaCl ₂	4.99 b	4.47	4.23	4.03
GA ₃	4.38 c	4.34	4.32	4.21
GA ₃ +CaCl ₂	4.32 c	4.21	4.19	4.12
<i>Significance</i>	*	<i>ns.</i>	<i>ns.</i>	<i>ns.</i>
MAP				
Control	5.49 a	4.41 ab	4.22	4.16 a
CaCl ₂	4.99 b	4.56 a	4.31	4.12 a
GA ₃	4.38 c	4.30 b	4.30	4.18 a
GA ₃ +CaCl ₂	4.32 c	4.16 c	4.16	4.12 a
non-MAP				
Control	5.49 a	4.22 a	4.17	4.14 b
CaCl ₂	4.99 b	4.37 a	4.15	3.93 c
GA ₃	4.38 c	4.37 a	4.33	4.24 a
GA ₃ +CaCl ₂	4.32 c	4.26 a	4.22	4.11 b
<i>Significance (interaction)</i>	*	*	<i>ns.</i>	*

*: $p < 0.05$, **: $p < 0.01$, ns: non-significant. The differences among the means indicated with the same lowercase letter in the same column were not significant.

Pre-harvest Ca application has a significant effect on fruit quality (Dong et al., 2019). The fruit chemical composition (Hosein-Beigi et al., 2019) on sweet cherry. Many studies were carried out (Lidster et al., 1979; Facticeau et al., 1987; Tsantili et al., 2007; Wójcik et al., 2013; Eroglu, 2014; Michailidis et al., 2017) relative to the using of calcium to reduce cracking and improve fruit quality in sweet cherry. However, the inconsistent results relative to the increasing of the calcium content and the improving of the fruit quality of these applications in the plant have been obtained (Val et al., 2008; Sotiropoulos et al., 2010). In our study, which was relative to the effect of applications of CaCl₂ on the content of individual phenolic in fruit during harvest and post-harvest storage on sweet cherry, it was found that the application of CaCl₂ increased the individual phenolic content in fruit. Chen et al. (2011) reported that the using of calcium at low concentration increases the nutritional quality of fruit and vegetables.

The preharvest foliar sprayed trees with plant growth regulators such as salicylic acid and GA₃ induced also enhancement of phenolic content at harvest in Navel oranges (Huang et al., 2008) and jujube (Cao et al., 2013) fruits (Gimenez et al., 2014). In our study, the concentration of the individual phenolics generally decreased during the cold storage, whereas epicatechin and 4-hydroxybenzoic acid concentrations increased with increasing cold storage time. Gonçalves et al. (2004) have determined that that phenolic

acid contents decreased with processing and storage at 1–2°C and increased with storage at 1–5°C, and the epicatechin concentration decreased at 22°C. In the study, although the percentage of losses in individual phenolic during cold storage was higher in CaCl₂ and GA₃ applications than in control, it was found that the amount of individual phenolic at the end of the cold storage was higher on CaCl₂ and GA₃ applications. The increasing of the individual phenolic concentrations of the fruit at harvest by pre-harvest CaCl₂ and GA₃ applications caused by the occurrence of this result. Calcium treatments represent a safe and potentially effective technology for enhancing the postharvest life and nutritional quality of fruits and vegetables (Martin-Diana et al., 2007; Aghdam et al., 2013). Supapvanich et al. (2012) determined that CaCl₂ application maintained to total phenolics, total flavonoids, ascorbic acid content at cold storage (Aghdam et al., 2013). In addition to, Aghdam et al. (2013) have determined that the postharvest CaCl₂ application has increased total phenolics, flavonoids, antocyanins and ascorbic acid contents of cornelian cherry fruit, and they have suggested that the CaCl₂ enhanced antioxidant potential could be due to the activation of phenylpropanoid-flavonoids pathways in the cornelian cherry fruits.

Gibberellic acid (GA₃), which plays significant role in breaking seed dormancy, promoting flower bud differentiation and stem elongation, and delaying the senescence of plant organs (Achard et al., 2009; Sun, 2010;

Zaho et al., 2018), has been used in commercial horticultural cultivation as a plant growth regulator to improve fruit size and quality (Khalil and Aly, 2013; Zang et al., 2016;

Gundogdu et al., 2017), to delay ripening and to maintain fruit quality of postharvest crops (Steffens et al., 2011; Huang et al., 2014; Souza et al., 2016; Zaho et al., 2018).

Table 7. Effects of pre-harvest spray treatments (GA₃ and CaCl₂) and MAP on protocatechuic acid contents of sweet cherry fruit throughout cold storage

Treatments	Protocatechuic acid (mg kg ⁻¹)			
	Harvest	7	14	21
Results by packaging				
MAP	3.77	3.63	3.53	3.37 a
non-MAP	3.77	3.55	3.46	3.19 b
<i>Significance</i>		<i>ns.</i>	<i>ns.</i>	**
Results by spraying				
Control	3.72 b	3.54 b	3.37 b	3.27 b
CaCl ₂	4.33 a	4.12 a	4.06 a	3.70 a
GA ₃	3.57 b	3.35 b	3.29 b	3.13 b
GA ₃ +CaCl ₂	3.45 b	3.34 b	3.27 b	3.02 b
<i>Significance</i>	*	*	**	*
MAP				
Control	3.72 b	3.56 b	3.38 b	3.17 b
CaCl ₂	4.33 a	4.25 a	4.18 a	3.99 a
GA ₃	3.57 b	3.43 b	3.32 b	3.28 b
GA ₃ +CaCl ₂	3.45 b	3.26 b	3.25 b	3.02 b
non-MAP				
Control	3.72 b	3.51 a	3.35 b	3.36 a
CaCl ₂	4.33 a	3.99 a	3.94 a	3.41 a
GA ₃	3.57 b	3.27 a	3.26 b	2.98 a
GA ₃ +CaCl ₂	3.45 b	3.41 a	3.28 b	3.02 a
<i>Significance (interaction)</i>	*	*	**	*

*: $p < 0.05$, **: $p < 0.01$, ns: non-significant. The differences among the means indicated with the same lowercase letter in the same column were not significant.

Dong et al. (2019) have reported that GA₃ combined with Ca application may have high potential for improving storage or shipping quality of commercial sweet cherry. The fact that, in our study, it was observed that CaCl and GA₃ combination has occurred to the differences on the effect. Petracek et al. (2002), Remon et al. (2000), Spotts et al. (2002) and Tian et al. (2004) have reported that the application of the MAP, which is used to delay the physicochemical changes, to retard microbial spoilage and to retain color by reducing the oxidation to extend the shelf life of the fruit species (Singh et al., 2010), has a significant effect in delaying of the physico-chemical changes on sweet cherry. The MAP applications that balance the CO₂ concentrations and inhibits enzyme activity favoring stability of color (Rocha and Morais, 2001; Remon et al., 2004) has slightly increased the total anthocyanin content of sweet cherry during the cold storage (Conte et al., 2009; Padilla-Zakour et al., 2007; Remon et al., 2000). However, Remon et al. (2003) have determined that MAP application has not a significant effect on the total anthocyanin content of sweet cherry at postharvest. In our study, MAP application had a positive effect on the losses of other phenolic compounds except catechin during cold storage.

The fact that, Serrano et al. (2006) reported decreased losses in antioxidant activity, total phenolics and vitamin C contents of broccoli with MAP treatments. However Aglar

et al. (2017) have reported that MAP-treated fruits had significantly lower antioxidant activity than the control fruits in cold storage. In addition to Giacalone and Chiabrando (2013) indicated that MAP treatments did not have any negative impacts on phenolic compounds and antioxidant activity. Khan and Singh (2008) reported that MAP-treated plums had lower antioxidant activity than the control fruit. Similarly, Artes-Hernandez et al. (2006) reported that MAP treatments retarded the formation of carotenoids and anthocyanin-like color pigments.

5. Conclusion

Pre-harvest GA₃ and CaCl₂ applications caused an increase in the content of individual phenolic in fruit. The concentration of individual phenolic generally decreased with increasing cold storage time. The percentage of losses in individual phenolic during cold storage was proportionally higher in CaCl₂ and GA₃ applications. However, at the end of the cold storage, the amount of the individual phenolic of CaCl₂ and GA₃ applications was higher. The increasing of the individual phenolic concentrations of the fruit at harvest by pre-harvest CaCl₂ and GA₃ applications caused by the occurrence of this result. MAP application had a positive effect on the losses of other phenolic compounds except catechin during cold storage. In conclusion, pre-harvest GA₃ and CaCl₂ applications are significant applications to

increase the percentage of individual phenolic in fruit. MAP can be used to reduce the losses of individual phenolic in cold storage.

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