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Effects of Postharvest Salicylic Acid Treatment on Some Quality Parameters of Sour Cherry (Prunus cerasus) Fruits during Cold Storage

Bonaventure Ufitinema[1&2*], Jean De Dieu Niyomugabo[1], Modeste Hakizimana[3]

[1]Department of Agricultural Engineering, Rwanda Polytechnic- IPRC Karongi, P.Box. 85 Karongi, Rwanda;
[2]Department of Horticulture, Faculty of Agriculture, Ankara University, Ankara, Türkiye,
[3]Department of Crops, Horticulture and Soils, Egerton University, P.O. Box 536-20115, Nakuru, Kenya,

*Corresponding author: ufbona@gmail.com

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Abstract

The rising demand for sour cherry fruit is attributed to its nutritional benefits and economic significance. Given its perishable nature, preserving its quality and extending storage life are crucial for ensuring a sustainable supply of fresh fruits to consumers and processors. The current study examined effects of post-harvest salicylic acid (SA) treatments on quality parameters (respiration rate, soluble solid contents (SSC), titratable acidity (TA), total bioactive compounds such as phenolic contents, anthocyanin contents and antioxidant activity, and weight loss) of sour cherry 'Kütahya' variety during cold storage and shelf life period. In this research, three treatments (0, 1 & 2 mM salicylic acid concentrations) and three replications in completely randomized design were used. Mature harvested sour cherries were treated for 10 minutes at room temperature, thereafter stored at 0±1°C and 85-90% relative humidity for 42 days. Fruit quality parameters were assessed on weekly basis in the fruit taken at each analysis date and retained at 20 ± 1°C for 1 and 4 days. SA resulted in lower respiration rate (25.41 mL CO2 kg−1 h−1 for 1 mM SA), decreased TA (2.204±0.028 % malic acid mL for 2 mM SA). Both 1 and 2 mM SA significantly increased the fruit total phenolic contents (279.80±10.50 and 288.78±9.71 mg GAE/100 mL, 1 and 2 mM SA respectively) until 35th day, total anthocyanin contents (26.53±0.60 and 28.87±0.56 mg C-3-RE/ 100 mL, 1 and 2 mM SA respectively) and antioxidant activity (90.89±0.17 and 89.80±0.34 % inhibition, 1 and 2 mM SA respectively) until 28th day. Since SA applications, especially 1 mM SA, reduced respiration rate, decreased TA, and increased bioactive compounds, it could be considered as promising practice.

Keywords: Sour cherry, Quality, Storage, Shelf life, Salicylic acid, bioactive compound

Introduction

Sour cherry (*Prunus cerasus)* is one of important fruit with high commercial and human health potential benefits (Ferretti et al., 2010; Serradilla et al., 2017). It is well recognized for being good source of nutrients and bioactive compounds, including organic and inorganic substances, vitamins, dietary fibers, aromatic ad phenolic compounds substances particularly anthocyanins, quercetin, carotenes, and melatonin hormone (Pedisic et al., 2007; McCune et al., 2011).

These bioactive substances provide antioxidant capacities that aid in scavenging oxygen free radicals and lowering the risk of various chronic inflammatory illnesses, including Alzheimer's disease, cancer, cardiovascular disease and diabetes (Ferretti et al., 2010; McCune et al., 2011; Kelley et al., 2018; Blando and Oomah, 2019). The cherry consumption is also thought to enhance sleep, cognitive function, and pain relief following energetic activity (Kelley et al., 2018). The acidity and flavour of sour cherries make them highly appreciated and more appealing cherry products, and they are consumed dried, frozen, in liquor, wine, juice, and fresh in combination with other different fruit. (Aslantas et al., 2016). Thus, to ensure the

sustainable supply of fresh fruit, the producers need not only to increase the production but also to use appropriate postharvest technology to preserve the nutrient content of their product.

Nowadays, preharvest and postharvest application of the plant growth regulators are being used to facilitate in keeping postharvest quality of different fruits (Giménez et al., 2016). One of these growth regulators, salicylic acid, is a naturally occurring substance produced by plants that is safe for human health and plays a variety of crucial physiological roles, including controlling plant growth and development and boosting plant tolerance and resistance to different biotic and abiotic stress conditions (Hayat et al., 2010). Several studies showed that the pre and postharvest application of salicylic acid contribute to preserving quality parameters with prolonged storage and shelf life of different fruits (Valero et al., 2011; Cao et al., 2013; Giménez et al., 2015; 2016). However, there is no or limited information on effects of post-harvest salicylic acid treatment on extending post-harvest life and maintaining quality parameters of sour cherry fruit.

Hence, this study was designed to investigate the potency post-harvest application of various concentrations of salicylic acid on shelf life and some quality parameters (respiration rate, soluble solid contents, and titratable acidity, total phenolic content, total anthocyanin content and antioxidant capacity, and weight loss) of sour cherry 'Kütahya' variety under cold storage.

Material and Methods

Study Site

This study was conducted at Postharvest Physiology Laboratory of the Department of Horticulture, Faculty of Agriculture, Ankara University located in Ankara province, Türkiye at 39.950° E longitude, 32.860° N latitude, and an altitude of 857 meters with weather and climate characterized by hot, dry summers that may be fairly warm, with temperatures often exceeding 30°C and cold winters that can be chilly, with temperatures frequently falling below zero.

Fruit sampling, Treatments and Storage

In the study, "Kütahya" variety of sour cherry fruit (*Prunus cerasus*) harvested at the commercial harvesting time were used. The fruit were taken from the farmer's garden located at Çubuk district of Ankara province and brought right away to the Postharvest Physiology Laboratory. After selecting the fruit suitable for the experiment, salicylic acid (SA %0.01 Tween-20) was applied at different (0, 1 & 2 mM) concentrations with three replications by dipping method for 10 minutes at room temperature (20±1°C). The control groups were stored without any treatment. Thereafter, the fruit were preserved at 0±1°C temperature and 85-90% Relative Humidity (RH) for the period of 42 days.

Assessment of quality parameters

In this study, analysis was done on a weekly basis. On each analysis date, the quality parameters of the fruit taken from cold storage and retained at room temperature for the period of 1 and 4 days were assessed as follow:

Respiration rate

The respiration rate (mL CO_2 kg^{-1} h^{-1}) was calculated using 25 fruit samples kept at 20°C in a gas-tight jar for an hour and the amount of CO_2 in the jar was determined in percentage with a Servomex brand CO_2 analyzer.

Total soluble solid content (SSC) and titratable acidity (TA)

Total soluble solid content (SSC) was determined using a digital table refractometer and expressed as a percentage of Brix, whereas titratable acidity (TA) was determined by titrating a 50 mL mixture of distilled water and 1 mL of fruit juice with 0.1 N NaOH solution until pH equals 8.1 in an automatic titrator (Mettler Toledo DL 50 Graphix) and expressed as a percentage of malic acid. (AOAC 2012).

Bioactive compounds (phenolic contents, anthocyanin content and antioxidant)

The fruit total phenolic content was determined by spectrophotometer apparatus using the Folin-Ciocalteu reagent according to the method used by Rimpara et al. (2007) and Singleton and Rossi (1965) with minor modifications and the results were represented in mg gallic acid equivalent (GAE)/100 mL of fruit extract. The fruit extract was prepared by squeezing the cherries and thereafter 4 mL of distilled water was added to 1mL of obtained extract. The cherry extract was diluted again at 1/10 ratio and 1mL of this solution and 5mL of diluted Folin-Ciocalteu reagent at 1/10 ratio were mixed. This mixture was left for about 10 minutes and then 4 mL of Na2CO3 (75 g / L) was added and kept at room temperature. After an hour, the absorbance was determined at 765 nm wavelength. Total phenolic content was measured as gallic acid equivalent by comparing it with a standard curve created using 0, 25, 50, 100, 150, 200 and 250 ppm of gallic acid.

The total anthocyanin level was determined using the pH differential technique according to the methods used by Rimpara et al. (2007) and Proctor (1974) with minor modifications. The results were expressed as mg cyanidine-3-rutunozide (C-*3*-RE)/100 mL fruit extract. Briefly, the buffer solutions of 0.025 M potassium chloride (KCl) at pH 1 and 0.4 M sodium acetate ($CH_3CO_2Na.{}_3H_2O$) at pH 4.5 were used. The mixtures of 1mL diluted fruit extract and 4 mL of KCl or $CH_3CO_2Na.{}_3H_2O$ were kept at room temperature for approximately 30 minutes and the absorbance of the mixtures were recorded by reading the spectrophotometer at 520 and 700 nm wavelengths and Total anthocyanin was calculated as follows:

Total anthocyanin= (A x MW x Df x 1000)/ MA x C and A= (Amax-A700) at pH 1- (Amax-A700) at pH 4.5, where, Amax: Absorbance values at 520 nm as maximum wavelengths of cyanidin-3-rutinoside; A700: absorbance value at 700 nm is subtracted as there are other phenolic compounds present (Filimon et al., 2011); MW: Molecular weight of anthocyanin to be taken as a base (595.2 for cyanidin-3-rutinoside); Df: Dilution Factor; MA: Molar Absorption coefficient (28800 L/cm mg for cyanidin-3-rutinoside) and C: Thickness of the used cuvette (1 cm).

The antioxidant capacity was determined using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity according to Brand-Williams et al. (1995). The 60 µL of diluted fruit extract (1/5 ratio) and 1940 µL DPPH solution were mixed and absorbance of sample and control (methanol in place of fruit extract) at 517 nm wavelength were recorded and the antioxidant capacity was determined as follows:

Inhibition (%) = (Absorbance of control- absorbance of sample x 100)/Absorbance of control.

Fruit weight loss

The fruit weight loss was calculated using the method determined by Vasylyshyna (2018) whereby the percentage (%) of weight losses in fruit were calculated by porportioning the weight of the fruit at each analysis date to the initial fruit weight at the beginning of the storage.

Experimental design and statistical analysis

The experiment was performed in completely randomized design with three replications. Three way analysis of variance (ANOVA) of the obtained results at $P \leq 0.05$ was performed using MINITAB 17 package program. Significant differences were checked with Tukey test in MSTAT-C package program.

Results

Respiration rate

In this study, the storage period (SP), shelf life (SL), SA treatments (T), SP x SL and SP x T interactions were significantly affected in the sour cherry respiration rate (Table 1)

Table 1: Results of variance analyzes.

Assessments	Factors						
	SP	SL	T	SPxSL	SPxT	SLxT	SPxSLxT
Respiration rate (CO_2 kg^{-1} h^{-1})	0.000***	0.000***	0.000***	0.000***	0.046*	0.489 ns	0.643 ns
SSC (%)	0.000***	0.000***	0.000***	0.000***	0.000***	0.067 ns	0.000***
TA (malic acid %)	0.000***	0.000***	0.000***	0.000***	0.000***	0.038*	0.430 ns
Total phenolic content (mg GAE / 100 mL)	0.000***	0.000***	0.000***	0.000***	0.000***	0.007**	0.000***
Total anthocyanin content (mg C-3-RE/ 100 mL)	0.000***	0.968 ns	0.000***	0.038*	0.000***	0.130 ns	0.000***
Antioxidant capacity (% DPPH inhibition)	0.000***	0.028*	0.000***	0.351 ns	0.000***	0.006**	0.054 ns
Fruit weight loss (% and angle)	0.000***	-	0.580 ns	-	0.889 ns	-	-

[1]SP: storage period, SL: Shelf life, T: treatments (control, 0, 1, 2 mM SA), SP x SL: Storage period x shelf life interactions, SP x T: Storage period x treatments interactions, SL x T: Shelf life x treatments interactions, SP x SL x T: Storage period x shelf life x treatments interactions [2]*: P≤0.05; **: P≤0.01:***, P≤0.001; [3]ns, non-significant at P≤0.05.

The Respiration rate increased from the 7th to the 35th days of storage period and decreased in parallel with the prolongation of shelf life. The fruit treated with 1 mM SA had the lowest average respiration rate (25.41 mL CO2 kg^{-1} h^{-1}), whereas the highest average respiration rate (30.43 mL CO2 kg^{-1} h^{-1}) was recorded in the fruit treated with 2 mM SA (Table 2).

Total soluble solid content (SSC) and titratable acidity (TA)

In storage and shelf life processes, the SSC values changed depending on the effects of all factors (storage period, shelf life period and SA applications), except for the SL x T interactions (P=0.000). The TA values varied depending on the individual and interactive effects of all factors (P=0.000) (Table 1). While the SSC values increased in the first 7 days, it decreased as the storage period progressed after the 7th day of storage. The lowest SSC value was recorded in the controls and the highest SSC values were measured in other treatments. However, with the prolongation of storage and shelf life period, a decline in the TA values was recorded. Among the treatments, the highest mean TA value was measured in controls (2.408% malic acid) and the lowest value (2.204% malic acid) was measured in 2 mM SA application (Table 2).

Table 2: The changes in fruit respiration rate SSC, TA and bioactive compounds.

Factors	Respiration rate (mL CO2 kg^{-1} h^{-1})	SSC (%)	TA (%malic acid)	Total phenolic content (mg GAE/100 mL)	Total anthocyanin content (mg C-3-RE/ 100 mL)	Antioxidant capacity (% inhibition)
SP (day)[1]	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000
0	26.06 ± 1.11 bc[2]	20.18 ± 0.18 c[2]	2.652 ± 0.037 a[2]	169.58 ± 3.15 d[2]	21.10 ± 0.32 d[2]	87.80 ± 0.52 c[2]
7	22.73 ± 1.04 e	23.06 ± 0.26 a	2.509 ± 0.019 b	220.95 ± 6.46 c	23.46 ± 0.54 c	88.55 ± 0.40 bc
14	25.02 ± 0.86 cd	22.90 ± 0.13 a	2.433 ± 0.022 c	227.41 ± 7.46 c	24.50 ± 0.79 c	89.51 ± 0.42 ab
21	22.97 ± 0.73 de	22.24 ± 0.09 b	2.439 ± 0.021 bc	231.20 ± 6.74 c	24.54 ± 0.66 c	89.82 ± 0.54 ab
28	27.94 ± 0.74 b	21.98 ± 0.12 b	2.265 ± 0.021 d	311.30 ± 13.00 b	31.18 ± 0.64 a	90.20 ± 0.38 a
35	35.45 ± 1.59 a	22.11 ± 0.08 b	2.102 ± 0.015 e	347.30 ± 12.50 a	28.01 ± 0.76 b	89.87 ± 0.30 ab
42	35.36 ± 0.82 a	22.22 ± 0.09 b	1.882 ± 0.014 f	316.01 ± 8.16 b	27.70 ± 0.59 b	90.30 ± 0.15 a
SL (day)	P=0.000	P=0.000	P=0.000	P=0.000	P=0.968	P=0.028
1	30.24 ± 0.88 a[3]	21.73 ± 0.09 b[3]	2.293 ± 0.018 b[3]	223.88 ± 4.18 b[3]	25.79 ± 0.44 ns	89.14 ± 0.22 b[3]
4	25.63 ± 0.53 b	22.49 ± 0.11 a	2.356 ± 0.020 a	297.18 ± 7.27 a	25.74 ± 0.39 ns	89.73 ± 0.23 a
T	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000
Control	28.27 ± 1.10 b[4]	21.62 ± 0.17 b[4]	2.408 ± 0.028 a[4]	229.05 ± 7.53 c[4]	23.60 ± 0.51 c[4]	88.50 ± 0.38 c[4]
0 mM SA	27.62 ± 1.00 b	22.24 ± 0.15 a	2.335 ± 0.027 b	244.50 ± 7.55 b	24.00 ± 0.48 c	88.54 ± 0.27 c
1 mM SA	25.41 ± 1.02 c	22.20 ± 0.13 a	2.353 ± 0.023 b	279.80 ± 10.50 a	26.53 ± 0.60 b	90.89 ± 0.17 a
2 mM SA	30.43 ± 1.11 a	22.38 ± 0.13 a	2.204 ± 0.028 c	288.78 ± 9.71 a	28.87 ± 0.56 a	89.80 ± 0.34 b

[1]SP (day): Storage period, SL (day): Shelf life, T: Salicylic acid (SA) Treatments, [2] Range differences among storage periods,[3]Range differences among shelf life periods, [4]Range differences among treatments at P≤0.05.

Bioactive compounds (phenolic contents, anthocyanin content and antioxidant)

The total phenolic contents changed depending on individual and interactive effects of all factors (P≤ 0.05), whereas total anthocyanin contents changed depending on individual and interactive effects of other factors except SL and SL x T interaction (P≤ 0.05) and antioxidant capacity were significantly affected by individual and interactive effects except SP x SL and SP x SL x T interactions (Table 1). There was an increase in total phenolic compound contents from the beginning of storage to 35th day and on the 4th day of shelf life. The highest total phenolic contents were measured in the fruit treated with 2 mM SA (288.78 mg GAE/100 mL) and 1 mMSA (279.80 mg GAE/100 mL), and the lowest values were determined in controls (229.05 mg GAE/100 mL) (Table 2).

Total anthocyanin content increased from the beginning of storage until the 28th day of storage followed by a decreasing trend in the further storage period. Among the treatments, the highest fruit total anthocyanin content (28.87 mg C-*3*-RE/ 100 mL) was recorded in the fruit treated with 2 mM SA while the lowest values were measured in the control (23.60 mg C-*3*-RE/ 100 mL) and 0 mM SA (24.00 mL mg C-*3*-RE/ 100 mL) groups (Table 2).

The fruit antioxidant capacity showed an increasing trend during the storage period starting from the 7th day of storage and the highest values were found in the samples from the 28th (90.20%) and 42nd (90.30%) days. There was also an increase in the average antioxidant activity values depending on the prolongation of the shelf life period. Among the treatments, the highest average antioxidant value was found in fruit treated with 1 mM SA (90.89%), while the lowest values were recorded in the control (88.50%) and 0 mM SA (88.54%) groups (Table 2).

Fruit weight loss

The fruit weight loss had significantly affected by only storage period (P=0.000) (Table 1). It was increased with prolongation of storage period. Weight loss values were determined as 2.99% on the 7th day and 13.07% on the 42nd day of storage. None of the SA applications had a positive or negative effect on fruit weight loss (Table 3).

Table 3: The changes in fruit weight loss

Factors	Fruit weight loss (%)
SP (day)[1]	P= 0.000
0	0.00 ± 0.00 (0.00 ± 0.00) [2] g
7	2.99 ± 0.12 (9.93 ± 0.21) f
14	5.37 ± 0.11 (13.39 ± 0.15) e
21	7.53 ± 0.42 (15.87 ± 0.43) d
28	9.28 ± 0.54 (17.67 ± 0.49) c
35	11.07 ± 0.55 (19.38 ± 0.47) b
42	13.07 ± 0.57 (21.15 ± 0.46) a
T	P= 0.580
Control	7.51 ± 1.21 (14.19 ± 1.66) ns
0 mM SA	6.93 ± 0.95 (13.81 ± 1.49) ns
1 mM SA	6.69 ± 0.86 (13.65 ± 1.42) ns
2 mM SA	7.05 ± 0.92 (14.00 ± 1.47) ns

[1]SP (day): Storage period, T: Salicylic acid (SA) Treatments

[2] Range differences among storage periods, ns: non-significant at P≤0.05.

Discussion

Respiration Rate

Our results presented that the use of 1 mM SA delays the increase in sour cherry fruit respiration rate during cold storage and shelf life period. However, the high SA dose is not effective in reducing sour cherry

respiration rate. Different other studies in sweet cherries (Giménez et al., 2016); plums (Luo et al., 2011) and pomegranates (Sayyari et al., 2011) showed the similar results whereby SA application significantly reduced fruit respiration rate during cold storage. The low respiration rate in fruit means slow physiological mechanism and delayed fruit aging and senescence which leads to the preservation of organic compounds (Giménez et al., 2016). During the storage period salicylic acid, as a potential inhibitor of ethylene production and activity, can prevent the dramatic increase in fruit respiration rate by delaying the breakdown of starch into glucose (Asghari and Aghdam, 2010).

Total soluble solid content (SSC) and titratable acidity (TA)

The SSC/TA ratio determines the flavour and taste of fruits (Blando and Oomah, 2019). In the postharvest period, the fruit total soluble solid increases and titratable acidity decreases leading to an increase in fruit sweetness (Ferretti et al., 2010; Giménez et al., 2016). SA applications increase the levels of sugar, organic acid and total soluble solid content in various fruit (Sayyari et al., 2011; Razavi et al., 2014; Giménez et al., 2017; Sabır et al., 2019). Valero et al. (2011) also stated that sweet cherry fruit treated with 1 mM SA, ASA or oxalic acid (OA) had the lower titratable acidity than controls during cold storage. Our study showed the similar results whereby the fruit treated with 2 mM SA and 1 mM SA recorded higher SSC and lower TA values than the fruit in other treatments.

Bioactive compounds (phenolic contents, anthocyanin content and antioxidant)

The increase in the phenolic compounds of the fruit during cold storage is linked to the stress conditions from low temperature. Shah et al. (2018) stated that the polyphenol compounds, chlorophyll breakdown and color change of sour cherry fruit are likely to increase during postharvest period. The phenolic and anthocyanin compounds are recognised as beneficial antioxidants and have a scavenging effect on reactive oxygen species (ROS) (Asghari and Aghdam, 2010). According to different studies (Yao and Tian, 2005; Luo et al., 2011), salicylic acid boosts the fruit's ability to fight off reactive oxygen species (ROS) during storage by promoting the activity of antioxidant enzymes like phenylalanine ammonia-lyase (PAL), glutathione reductase (GR), peroxidase (POD) and polyphenol oxidase (PPO). The increased activity of PAL enzyme enhances the production of phenolic compounds (Guardo et al., 2013).

The high antioxidant capacity and high total phenolic and anthocyanin level in the fruit treated with salicylic acid (SA) are thought to be linked to the enhanced activities of these enzymes by the applied salicylic acid. Our findings agreed with the findings of Giménez et al. (2017) on ‘Sweet Late’ ‘Sweet Heart’, and ‘Lapins’ cherries and Giménez et al. (2014) on ‘Sweet Heart’ and ’Sweet Late’ cherry varieties stating that Acetyl salicylic acid (ASA) and salicylic acid (SA) applications increase antioxidant activity, total phenolics and anthocyanin contents. Valero et al. (2011) also found the increase in total phenolic and anthocyanin contents in the first 10 days of storage in the controls and the decrease in the first 10 days followed by gradually increase in sweet cherry fruit treated with 1 mM SA, ASA or or oxalic acid (OA) throughout the storage period.

Different other postharvest studies showed that SA applications increased antioxidant activity, total phenolic content and total anthocyanin levels in apricots (Ezzat et al., 2017), nectarines (Bal, 2016); plums (Davarynejad et al., 2015); peaches (Awad, 2013; Razavi et al., 2014) and kiwi (Fatemi et al., 2013). The same result was reported by Giménez et al. (2016) who reported that MeSA applications in 'Early Lory' cherry variety fruit increased the antioxidant capacity of the fruit while preserving the concentrations of total phenolic compounds and anthocyanins during storage period. García-Pastor et al. (2020) and Sayyari et al. (2011) also stated that postharvest ASA applications increase the total anthocyanin levels in pomegranates. Similarly, Koyuncu et al. (2019) also stated that pomegranate fruit treated with SA and putrescine had higher antioxidant activity and total phenolic compound content compared to controls.

Fruit weight loss

The increase in fruit weight loss in cherries during storage brings along with the physiological, biochemical and sensory changes (Shah et al., 2018). Our findings on weight loss are parallel to the findings of Baninaiem et al. (2016), who argued that SA applications do not have a substantial influence on tomato weight loss during storage. However, they are contrasted with the findings of the studies carried out on the nectarines (Bal, 2016); plums (Davarynejad et al., 2015); peaches (Razavi et al., 2014) and apples (Kazemi et al., 2011), The authors reported that SA applications during storage reduced fruit weight loss. This shows that the effect of SA applications on fruit weight loss in postharvest period varies by species. In addition, sour cherry fruit are very sensitive to water loss even under high relative humidity conditions and the use of packaging materials during storage should be taken into account while dealing with sour cherry fruit water loss.

Conclusion

The postharvest application of 1 mM SA delayed the increase in respiration rate and decreased TA levels. While the SSC increased in all applications during the cold storage, SA application significantly delayed this increase till 28 days of storage. Both SA concentrations (1 and 2 mM) increased total phenolic content in the first 35 days and total anthocyanin content and antioxidant capacity in the first 28 days of storage. Therefore, the postharvest SA applications, especially 1 mM SA application, can be used to preserve the quality of the sour cherry fruit 'Kütahya' variety for 28 days in the cold storage with 4 days of shelf life after the fruit are removed from the cold storage.

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