

MAINTENANCE OF POSTHARVEST QUALITY OF BLACKBERRY (*Rubus fruticosus* L.) FRUITS THROUGH SALICYLIC ACID AND CaCl₂ IMMERSIONS

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ABSTRACT

Blackberries (*Rubus* spp.) have significant nutraceutical properties, providing phenolic compounds for the consumers. These compounds are useful to human health due to their high antioxidant activity. However, most blackberries destined for fresh markets become unmarketable in 2 to 3 days due to decay and leakage, because they have thin and fragile skin. The present study was aimed to investigate the effect of salicylic acid (SA) and calcium chloride (CaCl₂) on extending the postharvest quality and bioactive compounds of blackberry during refrigerated storage. Blackberry fruits cv. ‘Jumbo’ were dipped in 2% CaCl₂, or in 2.0 mM salicylic acid (SA) for 10 min. Total soluble solids contents, titratable acidity, pH, ascorbic acid contents, total phenolics contents, and total antioxidant activity were investigated initially on 4th, 7th, or 10th day (d) of storage. Changes in fresh weight, titratable acidity, ascorbic acid and total phenol contents were markedly delayed by both treatments. Berries treated with 2 mM SA or 2% CaCl₂ exhibited markedly better visual quality during the storage period. Considering the overall findings, this study revealed that postharvest SA or CaCl₂ applications maintained the storage-life and conserved the valuable marketing features of blackberries over 10 d in cold storage, presumably due to their inhibitory effects on fruit senescence.

Key words: *Rubus fruticosus* L., cold storage, postharvest physiology

INTRODUCTION

Blackberry (*Rubus* L.) is a naturally growing fruit in Turkey and consumption of fresh blackberries has markedly increased in recent years [Tosun et al. 2008]. Blackberry fruit is an important dietary source of fiber, anthocyanins and essential vitamins [Wu et al. 2010], all of which are essential for human health [Beattie et al. 2005]. Blackberry is a highly perishable fruit because of its thin and fragile skin, high respiration and transpiration rates. Rapid changes in physicochemical properties and fast ripening along with postharvest period of blackberry hamper the storage and marketing [Han et al. 2004]. Besides, postharvest life of black-

berries is also limited by their sensitivity to water loss, softening, physical injuries and postharvest diseases such as gray mold and Rhizopus rot [Perkins-Veazie et al. 2000]. Therefore, blackberry fruits destined for fresh markets became unmarketable after 2 or 3 days when stored at 0°C due to fruit rot and leakage [Hardenburg et al. 1986]. As the low temperature slows down metabolism related with physicochemical changes and fungal deteriorations in blackberries [Antunes et al. 2003], producers generally conserve blackberries in cold storage looking for their quality extension to gain a marketing and profitability edge [Basiouny 1995].

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Salicylic acid (SA), classified as a phenylpropanoid compound, is naturally synthesized by plants to generate a certain metabolic and physiological responses in plants involved in defense [Supapvanich and Promyou 2013]. Many previous works have proved that exogenous SA application could delay senescence process [Gerailoo and Ghasemnezhad 2011], maintain the fruit quality [Tareen et al. 2012], decrease incidence of phyto-pathogen fungi, enhance physio-biochemical features [Noreen et al. 2017] and increase the bioactive compounds including antioxidant activity [Wei et al. 2011] in plants. Currently, SA has been successfully used for the postharvest conservation of perishable fruits such as papaya [Promyou and Supapvanich 2016], apricots [Hajilou and Fakhimrezaei 2013], kivi [Kemi et al. 2011] and grapes [Ranjbaran et al. 2011]. Similarly, CaCl₂ was also tested to exert a favorable influence on physiological processes in apples [Conway et al. 1994], apricots [Hajilou and Fakhimrezaei 2013] and tomatoes [Kucukbasmaci-Sabir 2008]. Thus, they were proven as good candidates to be used commercially to maintain the shelf-life of fruits.

Because of relatively short growth season and limited shelf-life, fresh blackberries are generally processed [Hager et al. 2008] and the phenols are destructed by processing method [Wu et al. 2010]. The market demand for blackberries has expanded significantly with the production of organic blackberry in the United States increased by 173% from 2005 to 2008 [Strik et al. 2007, USDA 2010]. Sustainable treatments to extend postharvest quality of blackberries are critical for successful commercialization and long-distance shipping. However, few studies have investigated the impact of postharvest treatments on the physicochemical and antioxidant properties of fresh blackberry fruit during the storage. Therefore, the current study was aimed to investigate the effect of salicylic acid and CaCl₂ on the postharvest maintenance of quality and bioactive compounds of blackberry fruit (cv. 'Jumbo') during refrigerated storage.

MATERIAL AND METHODS

Blackberries (*Rubus fruticosus* L.) of 'Jumbo' cultivar, with large fruits [Gerçekcioglu and Esmek 2005], was harvested at full maturity stage and selected for uniformity of shape size and peel color (100% of

the surface with black color). They were cleaned with tap water and dipped in 100 µL L⁻¹ sodium hypochlorite to control fruit rot and allowed to air-dried before treatments. The pedicels of all the blackberries were cut with a sharp scissor leaving 1–2 mm of cap stem. The fruits were randomly distributed into three groups for different treatments: (a) untreated fruit were used as control which did not receive SA or CaCl₂ (b) immersion to 2 mM SA for 5 min, then dried at 23 ±1°C for 30 min and (c) immersed to 2% CaCl₂ for 5 min, then dried at 23 ±1°C for 30 min. After dipping, about 250 g of each fruit sample was put in a 12 cm × 15 cm rigid polypropylene cup and wrapped with monolayer film polyesters (thickness 18 µm). A total of 48 cups were used, 12 of which belonged to each treatment (apart from initial analysis). Three replicates per treatment (cups) were used. The filled cups, with a surface area of about 100 cm², were hermetically sealed and kept at 1 ±0.5°C and 85% relative humidity for 10 d [Sabir and Sabir 2013]. Fruit were sampled at the initial day (day 0) and at 4th, 7th and 10th days of storage.

Visual quality and weight loss. Visual attributes of the fruit was evaluated by five panelists for the general acceptability with the following scales: 5 = extremely like, 3 = neither like nor dislike, the limit of acceptance, and 1 = extremely dislike. The weight of each blackberry package was determined on day 0 and on each sampling day (4th, 7th and 10th) using a precision balance (Adventurer™ precision balance, OHAUS, Pine Brook, NJ, USA). Weight losses are recorded in percent of the initial berry weight.

Sample preparation. For ascorbic acid, total antioxidant capacity and total phenol determination, whole fruits (~250 g including peel, pulp, and seeds) were thawed at room temperature for 30 min and then processed in juice extractor in order to obtain natural fruit juice. Three replicates of juice (20 ml each) were centrifuged at 7000 rpm for 10 min. All juices were analyzed the same day when they were prepared.

Soluble solid and titratable acidity content. SSC of blackberry samples of each package were blended for 30 s. SSC in the juice were determined using a refractometer (ATAGO MNL-1125, Japan). The refractometer was calibrated prior to measurements. Three measurements were performed for each sample and recorded as percent. For TA analysis, a 5–10 g sample of the blackberry puree was diluted with 50 mL of

distilled water. The TA of the juice was determined by titration with 0.1 N NaOH to an end-point of pH 8.2 by a PHB-212 pH meter (Omega Engineering Inc., Stamford, CT, USA). The pH meter was calibrated before the measurements. The acidity was reported as citric acid (%). Three cups per treatment were used at each sampling day, and the values were averaged.

Ascorbic acid content assay. Blackberries were ground with a warring blender and 5 g sample was mixed with 45 mL 0.4% oxalic acid and then filtered. One milliliter of filtrate and 9 mL 2,6-dichlorophenolindophenol sodium salt solution (C₁₂H₆C₁₂NO₂-Na) were mixed and then reading of transmittance values at 520 nm were performed in a spectrophotometer. Data were recorded as mg 100 g⁻¹ [Ozdemir and Dundar 2006].

Total antioxidant capacity (TAC). The antioxidant capacity of the sample was found using a ferric reducing antioxidant potential (FRAP) assay according to the method defined by Benzie and Strain [1996]. The FRAP reagent was a mixture of 25 mL acetate buffer pH 3.0, 2.5 mL 10 mM 2,4,6-trioryridyl-1,3,5-triazine (TPTZ) and 2.5 mL 20 mM ferric chloride hexahydrate. The mixture reaction started when 150 µL of the supernatant was added into 2850 µL of FRAP solution. The reaction solution was incubated at ambient temperature for 30 min and then the absorbance was measured at 593 nm. The antioxidant capacity was expressed as micro moles of Trolox equivalents per gram fresh weight (µmole Trolox equivalent g⁻¹ FW).

Total phenolic content (TPC). Folin–Ciocalteu method, as defined by Singleton et al. [1999], was used to find TPC with minor changes. A 5 mL fruit juice was homogenized in methanol for 1 min and then centrifuged at 4000 × g for 30 min at 5°C. A 100 µL aliquot of each extract was mixed with 1.58 mL of water, 100 µL of Folin–Ciocalteu’s reagent and 300 µL of sodium carbonate solution (200 g L⁻¹). The absorbance was read at 760 nm after 2 h. The content of total phenols was calculated on the basis of the calibration curve of gallic acid and was expressed as mg gallic acid 100 g⁻¹ FW.

Statistical analysis. Statistical tests were performed in triplicate (replication) on three different treatments. The averages and standard deviation were calculated. The findings displayed in the figures are the average of all repetitions, where the error bars are the stan-

dard deviations. Experimental data were submitted to one-way analysis of variance (ANOVA) and Student’s t-test (P < 0.05) using the software SPSS 13.0 for Windows. Averages of the applications were compared by Tukey’s LSD (least significant differences).

RESULTS AND DISCUSSION

As depicted in Fig. 1, ‘Jumbo’ blackberries underwent a weight loss along with the storage duration, but the reduction was more pronounced during the first four days of storage. At the end of the storage, significant differences were found between the treatments and the greatest loss in weight occurred in control fruits (2.70%). The lowest change in weight was determined in fruits treated with SA (2.17%), which was followed by CaCl₂ (2.34%). Similar loss in weight (3.4%) in blackberry cultivars were found by Perkins-Veazie et al. [1993], while Meneghel et al. [2008], studying the blackberries stored at 0°C, reported an average weight loss of 9.0% after 18 days of storage. Literature reveals that great differences in blackberry weight loss can occur depending on the cultivars, treatments, packaging systems and storage conditions. Loss of berry moisture is the main reason for fruit weight loss and it results from the transpiration and respiration processes occurring during postharvest.

Variation in SSC, TA and pH of the blackberries during the storage are depicted in Table 1. Changes of

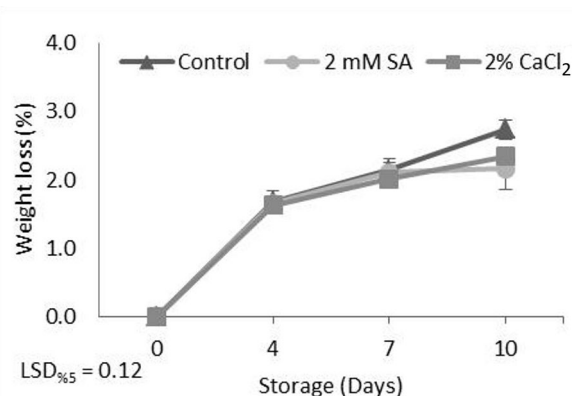


Fig. 1. Changes in weight losses (%) of blackberries during the prolonged storage. Mean values of three replications ± standard deviation

Table 1. Changes in SSC (°Brix), TA (%) and pH values of blackberry during the prolonged cold storage. Means (three replications) not connected by same letter are significantly different ($P < 0.05$) by LSD (\pm standard deviation)

Quality features	Postharvest applications	Storage duration (days)			
		0	4	7	10
SSC	Control	9.27 \pm 0.31	8.67 \pm 0.58	10.80 \pm 0.35	11.20 \pm 0.80
	2 mM SA	9.27 \pm 0.31	7.67 \pm 0.58	9.27 \pm 0.46	10.40 \pm 0.69
	2% CaCl ₂	9.27 \pm 0.31	7.10 \pm 0.70	9.20 \pm 0.53	10.40 \pm 0.53
TA	Control	1.35 \pm 0.04 ^a	0.82 \pm 0.03 ^c	0.70 \pm 0.04 ^{de}	0.44 \pm 0.00 ^g
	2 mM SA	1.35 \pm 0.04 ^a	0.91 \pm 0.07 ^b	0.73 \pm 0.05 ^d	0.62 \pm 0.00 ^f
	2% CaCl ₂	1.35 \pm 0.04 ^a	0.95 \pm 0.03 ^b	0.83 \pm 0.02 ^c	0.64 \pm 0.04 ^{ef}
pH	Control	4.36 \pm 0.07 ^b	4.51 \pm 0.27 ^b	4.07 \pm 0.16 ^c	4.58 \pm 0.03 ^b
	2 mM SA	4.36 \pm 0.07 ^b	4.47 \pm 0.04 ^b	4.53 \pm 0.09 ^b	4.89 \pm 0.10 ^a
	2% CaCl ₂	4.36 \pm 0.07 ^b	4.44 \pm 0.08 ^b	4.47 \pm 0.35 ^b	4.59 \pm 0.02 ^b

SSC– soluble solid content, TA – titratable acidity, SA – salicylic acid, LSD for SSC – not significant, TA = 0.07, pH = 0.25

the SSC of blackberry fruits along with the storage period are shown in Fig. 2. SSC declined during the four days of storage and then progressively increased with the highest increase in control fruits. Differences in SSC values were statistically insignificant at the end of the storage. Very little or no change in the SSC value has been demonstrated for blackberries after packaging and storage at low temperature [Moggia-Lucchini 1990]. As it is commonly known, blackberries store plentiful amount of organic acids in their vacuoles [Green 1971], and these acids are converted back to sugars [a pathway called gluconeogenesis] that results in an increase of SSC [Famiani et al. 2009]. The SSC

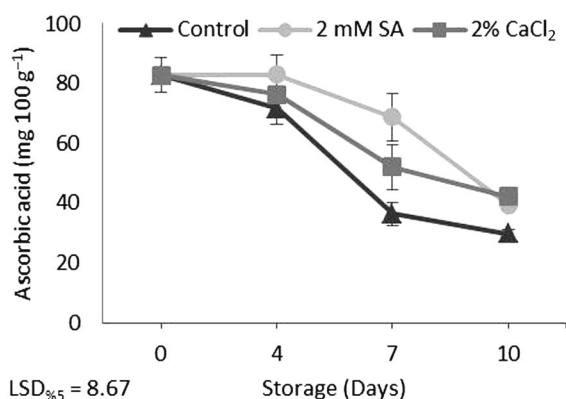


Fig. 2. Changes in ascorbic acid content of blackberries during the prolonged storage (%). Mean values of three replications \pm standard deviation

increment may also lead to gradual increase in soluble solutes resulting from the water loss during the storage [Sabir et al. 2011]. TA markedly decreased during the storage. This decrease has previously been demonstrated for various blackberry cultivars by different researchers [Perkins-Veazie et al., 1999, Joo et al. 2011] and this has been related to the ripening physiology. As it is known, organic acids generally decrease during ripening as they are respired or converted to sugars. A TA decline around 60% has been revealed between mottled and shiny black stages of ripeness, and about 40% between shiny and dull black states [Perkins-Veazie and Collins 1993]. TA decrease was significantly affected by treatments. Decrease in TA was greater in untreated control blackberries than those treated with SA or CaCl₂. Acidity is inversely correlated to pH. The pH value of blackberries slightly increased along with the prolonged storage and the changes were significant among the treatments. SA treatment led to the increase in pH.

There was no remarkable change in ascorbic acid amount during the first four days of storage period in blackberry fruits untreated with calcium chloride (Fig. 2). This may be most probably due to a very short period of storage (4 days), which is insufficient to the oxidation process of ascorbic acid in darkness. Afterwards, ascorbic acid content drastically decreased with the significant differences among the applications. SA or CaCl₂ treatment considerably retarded the loss in

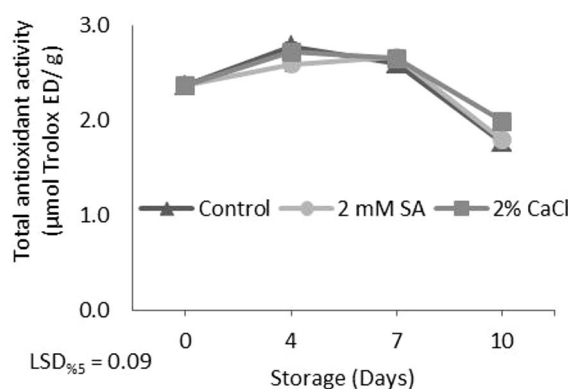


Fig. 3. Changes in total antioxidant activity of blackberries during the prolonged storage (%). Mean values of three replications ± standard deviation

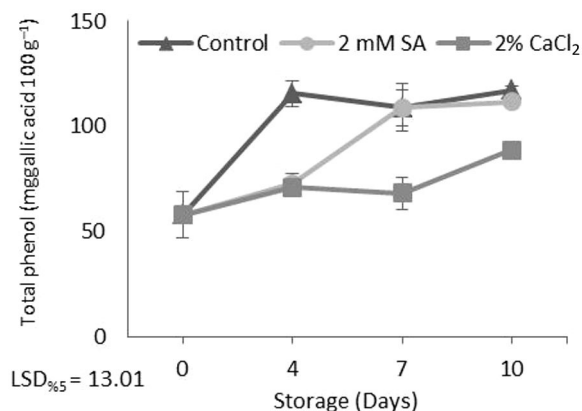


Fig. 4. Changes in total phenol content of blackberries during the prolonged storage (%). Mean values of three replications ± standard deviation

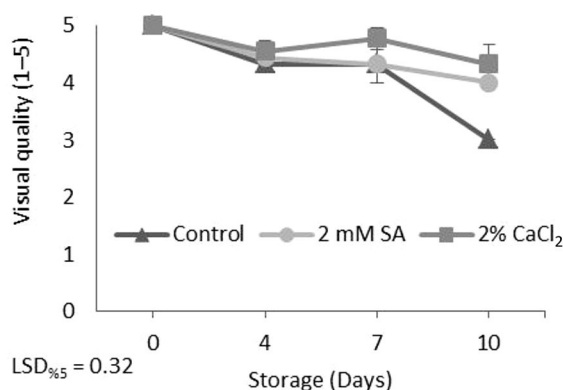


Fig. 5. Changes in visual quality (5 points = extremely like, 1 point = extremely dislike) of blackberries during the prolonged storage (points). Mean values of three replications ± standard deviation

ascorbic acid in comparison to control fruits. Ascorbic acid is quite sensitive to chemical and enzymatic oxidation during the storage of fruits. Therefore, fruits and vegetables display a general decline in ascorbic acid content as the storage time increases [Adisa 1986]. Mehlhorn [1990] reported an increase in ascorbate peroxidase activity in response to ethylene.

During the first four days of the storage, total antioxidant activity of blackberry fruits increased regardless of the treatments (Fig. 3). Afterwards, it declined gradually and the magnitude of such decrease was more pronounced between the 7th and 10th days.

At the end of the storage, the greatest total antioxidant activity was determined in fruits treated with CaCl₂ with statistically significant differences. As previously mentioned by Antunes et al. [2014], cold storage alone cannot efficiently maintain bioactive components in berries, and a significant antioxidant activity decrease is proven following the storage. Thus, postharvest treatments together with cold storage is required to keep the quality of blackberry after harvest. In this study, CaCl₂ significantly delayed the loss in antioxidant activity of blackberries. Madani et al. [2015] revealed that calcium-treated (1.5 and 2.0%) mango

fruits resulted in higher total antioxidant activity relative to untreated fruits after 9 d in ambient storage. As it is known, antioxidant activity of fresh fruits depends on several factors, such as cultivation conditions and postharvest treatments [Lurie 1998]. Postharvest calcium application increased antioxidant capacity in fresh cut sweet leaf bush including total phenols, and ascorbic acid contents [Supapvanich et al. 2012].

Blackberries are an absolute source of phenols [Zadernowski et al. 2005] and thus have an extremely high antioxidant capacity [Hager et al. 2008, Wu et al. 2010]. Therefore, preventing the loss in phenols along with the storage is one of main challenges in preservation of fruits. Figure 4 shows that CaCl₂ treatment had a remarkable positive effect on retaining the total phenols of the blackberry fruits during storage. Up to the 4th day of storage, phenol content of control fruits drastically increased, while both of the treatments effectively retarded the phenol increase. Afterwards, total phenol contents in the fruits subjected to SA underwent a significant increase. At the end of the ten-day storage, observations revealed that CaCl₂ treatment significantly maintained the total phenol content of blackberries. This might be due to the immersion of blackberries in CaCl₂, which strengthened the cell wall, enhanced the formation of egg-box structure and minimized syneresis/leaching of water soluble compounds such as phenols [Turmanidzea et al. 2016].

Visual quality of the blackberries was significantly affected by treatments (Fig. 5). Little changes occurred in visual quality with insignificant variations between the treatments until the 7th d of storage. However, visual quality sharply declined in control fruits after the 7th d. At the end of the storage, there was a large decrease in visual quality of control fruits in concomitant with a substantial increase in the weight loss. Control fruits had the lowest visual quality score (3.0) with significant difference from both of SA (4.0) and CaCl₂ (4.3) treatments. This may indicate the useful influence of SA and CaCl₂ on extension of initial quality of the commodity. Appearance is a major criterion for determining the acceptability of products. Along with the prolonged storage duration, respiration rate, water loss, and enzyme activities are accelerated in commodities [Meneghel et al., 2008, Sabir et al. 2011]. Such increases generally cause rapid senescence, decreased visual quality and shortened storage life.

CONCLUSION

Blackberry fruits cv. 'Jumbo' were stored in 250 g packages at 1°C for 10 d after dipping in 2% CaCl₂ or in 2.0 mM SA, while control fruits were stored under the same condition without any treatment. Both the treatments significantly and positively affected the fresh weight, titratable acidity and ascorbic acid contents. CaCl₂ provided better maintenance of total phenol with lower changes from the beginning of the storage till the end.

Fruit immersed into SA or CaCl₂ exhibited better visual quality during the storage. Overall investigations revealed that postharvest treatment with SA or CaCl₂ extended the storage-life and conserved the valuable marketing features of blackberries. For healthy, attractive and marketable fruits, it is recommended that cold storage of blackberry fruits cv. 'Jumbo' can be achieved with 2 mM SA or 2% CaCl₂ treatments up to 10 days.

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