AGRICULTURAL AND FOOD CHEMISTRY

Postharvest Treatments with Salicylic Acid, Acetylsalicylic Acid or Oxalic Acid Delayed Ripening and Enhanced Bioactive Compounds and Antioxidant Capacity in Sweet Cherry

Daniel Valero,[†] Huertas M. Díaz-Mula,[‡] Pedro Javier Zapata,[†] Salvador Castillo,[†] Fabián Guillén,[†] Domingo Martínez-Romero,[†] and María Serrano^{*,‡}

[†]Department of Food Technology and [†]Department of Applied Biology, EPSO, University Miguel Hernández, Ctra. Beniel km. 3.2, 03312, Orihuela, Alicante, Spain

ABSTRACT: Sweet cherry cultivars ('Cristalina' and 'Prime Giant') harvested at commercial ripening stage were treated with salicylic acid (SA), acetylsalicylic acid (ASA) or oxalic acid (OA) at 1 mM and then stored for 20 days under cold temperature. Results showed that all treatments delayed the postharvest ripening process, manifested by lower acidity, color changes and firmness losses, and maintained quality attributes for longer periods than controls. In addition, total phenolics, anthocyanins and antioxidant activity increased in untreated fruit during the first 10 days of storage and then decreased, while in fruits of all treatments, these parameters increased continuously during storage without significant differences among treatments. Thus, postharvest treatments with natural compounds, such as SA, ASA or OA, could be innovative tools to extend the storability of sweet cherry with higher content of bioactive compounds and antioxidant activity as compared with control fruits.

KEYWORDS: phenolics, anthocyanins, carotenoids, total antioxidant activity, salicylic acid, acetylsalicylic acid, oxalic acid, postharvest

INTRODUCTION

Sweet cherry quality is determined by attributes affecting fruit marketing appeal and consumer satisfaction. Among the attributes related to consumer purchase decision are visual appearance, fruit size and color, firmness, and especially sweetness and flavor. However, these parameters change with cultivar¹ and are associated with the ripening process.² On the other hand, sweet cherry has been reported to contain several phenolic compounds and anthocyanins which contribute to the antioxidant capacity.^{3,4} The two dominant polyphenols in cherries are caffeoyltartaric acid and 3-*p*-coumaroylquinic acid, while the main anthocyanins are cyanidin-3-rutinoside and cyanidin-3-glucoside followed by pelargonidin-3-rutinoside, peonidin-3-rutinoside and peonidin-3-glucoside.^{5,6}

Cherry fruits deteriorate rapidly during postharvest storage, and in some cases do not reach consumers with optimal quality after transport and marketing. The main causes of sweet cherry deterioration are weight loss, color changes, softening, surface pitting, stem browning, loss of acidity and slight increases in TSS.^{6–8} The storage period also affects the content of bioactive compounds, with general increases in phenolic and anthocyanin concentrations associated with the postharvest ripening process.^{5,6}

Consumers demand food preservation systems with absence of chemicals or pesticide residues, and thus there is increasing interest in the use of natural compounds. In the case of sweet cherry the combined use of modified atmosphere packaging and essential oils⁷ or *Aloe vera* as edible coating⁸ resulted in a reduction of spoilage microorganisms, maintenance of fruit quality and extension of shelf life. Other natural compounds, such as salicylic acid (SA), acetylsalicylic acid (ASA) and oxalic acid (OA), are present in fruits and vegetables, and have shown important roles in delaying the ripening process when applied as postharvest treatment.

SA and ASA retarded the ripening process in banana,⁹ sugar apple fruit,¹⁰ kiwifruit,¹¹ mango¹² and peach,¹³ through an induction of antioxidant enzymes, such as peroxidase, catalase and superoxidodismutase and reduction of lipoxygenase activity. In pomegranate, ASA treatment reduced occurrence of chilling injury (CI) and maintained higher content in nutritive and bioactive compounds.¹⁴ OA has been involved in controlling litchi browning,¹⁵ and delaying the ripening process in some climacteric fruits such as mango,¹⁶ peach,¹⁷ and jujube fruit,¹⁸ through an inhibition of the ethylene biosynthesis. More recently, Sayyari et al.¹⁹ reported that application of oxalic acid alleviated CI symptoms of pomegranate, a nonclimacteric fruit.

As far as we know, few reports exist on the role of SA in increasing resistance against cherry decay,^{20,21} but there is no literature about the effect of postharvest treatments with SA, ASA or OA on the sweet cherry ripening process and parameters related to fruit quality. Thus, the objective of this research was to analyze the effect of these treatments on the organoleptic parameters related to sweet cherry quality and the content of bioactive compounds and antioxidant activity during storage.

MATERIALS AND METHODS

Plant Material and Experimental Design. Sweet cherry cultivars ('Cristalina' and 'Prime Giant') were harvested at commercial ripening stage, S2 according to Serrano et al.,⁶ from a commercial plot

Received:	March 3, 2011
Accepted:	April 20, 2011
Revised:	April 18, 2011
Published:	April 20, 2011



Figure 1. Color, firmness, total acidity and total soluble solids at harvest (day 0) and after 10 or 20 days of storage at 2 °C in control fruit, and after 20 days of storage in sweet cherry 'Cristalina' and 'Prime Giant' treated with salicylic acid (SA), acetylsalicylic acid (ASA) or oxalic acid (OA). Data are the mean \pm SE. LSD value for each cultivar is shown inside figures.

(Finca Los Frutales, Villena Alicante, Spain), with scores of 5 and 4 for 'Cristalina' and 'Prime Giant', respectively, according to the color chart from Centre Technique Interprofessionel de Fruits et Légumes (CTIFL, Paris). For each cultivar, about 12 kg of cherries were picked and immediately transferred to the laboratory. Then, 1020 homogeneous fruits in color and size and without visual defects were selected for each cultivar and randomly grouped in 51 lots of 20 fruits. Three lots were used to analyze the properties at harvest and 48 lots for the following treatments in triplicate: control (distilled water), 1 mM salicylic acid (SA), 1 mM acetylsalicylic acid (ASA), and 1 mM oxalic acid (OA). These concentrations were chosen based on a preliminary experiment, in which higher concentrations did not show delays in the ripening process additional to the effect found with 1 mM (data not shown). Treatments were performed by dipping fruits in 10 L of solution for 10 min, and then they were left to dry at room temperature before cold storage at 2 °C and RH of 85% in darkness for up to 20 days. After 5, 10, 15, and 20 days, 3 lots from each cultivar and treatment were sampled at random from cold chambers for analytical determinations. In these fruits, color and firmness were individually measured and then the edible portion of each lot was cut in small pieces to obtain a homogeneous sample. For each sample 5 g was used to determine in duplicate total soluble solids (TSS) and total acidity (TA) and the remaining sample was frozen in liquid N2, mixed and stored at -20 °C until total anthocyanins, total phenolics, total carotenoids, and

antioxidant activity in both hydrophilic and lipophilic fractions were determined.

Ripening Parameters. Color was determined in a Minolta colorimeter (CRC200, Minolta Camera Co., Japan), using the CIELab coordinates and expressed as chroma $[(a^2 + b^2)^{1/2}]$. Fruit firmness was determined using a TX-XT2i Texture Analyzer (Stable Microsystems, Godalming, U.K.) interfaced to a personal computer, with a flat steel plate mounted on the machine. For each fruit, the cheek diameter was measured and then a force that achieved a 3% deformation of the fruit diameter was applied. Results were expressed as the mean \pm SE of the force-deformation ratio (N mm⁻¹). TSS was determined from the juice obtained for each subsample with a digital refractometer (model PR-101, Atago Co. Ltd., Tokyo, Japan) at 20 °C, and results (mean \pm SE) were expressed as % (°Brix). Total acidity (TA) was determined by automatic titration (785 DMP Titrino, Metrohm, Herisau, Switzerland) with 0.1 N NaOH up to pH 8.1, using 1 mL of diluted juice in 25 mL of distilled H_2O_1 and results (mean \pm SE) were expressed as g of malic acid equivalent per 100 g fresh weight.

Total Antioxidant Activity Determination. Total antioxidant activity (TAA) of hydrophilic and lipophilic compounds was quantified according to Arnao et al.²² which enables determination of TAA due to both hydrophilic and lipophilic compounds in the same extraction. Briefly, for each subsample, five grams of tissue was homogenized in 5 mL of 50 mM Na-phosphate buffer (pH = 7.8) and 3 mL of ethyl



Figure 2. Total anthocyanin concentration in sweet cherry 'Cristalina' and 'Prime Giant' during storage at 2 °C in control fruits and fruits treated with salicylic acid (SA), acetylsalicylic acid (ASA) or oxalic acid (OA). Data are the mean \pm SE.

acetate, and then the mixture was centrifuged at 10000g for 15 min at 4 °C. The upper fraction was used for total antioxidant activity due to lipophilic compounds (L-TAA) and the lower for total antioxidant activity due to hydrophilic compounds (H-TAA). In both cases, TAA was determined using the enzymatic system composed of the chromophore 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), the horse radish peroxidase enzyme (HRP) and its oxidant substrate (hydrogen peroxide, H_2O_2), in which ABTS^{•+} radicals are generated and monitored at 730 nm. The reaction mixture contained 2 mM ABTS, 15 μ M H₂O₂ and 25 μ M HRP in 50 mM Na-phosphate buffer (pH = 7.8) in a total volume of 1 mL. The decrease in absorbance after adding the extract was proportional to TAA of the sample. A calibration curve was performed with Trolox ((R)-(+)-6-hydroxy-2,5,7,8-tetramethyl-croman-2-carboxylic acid) (0-20 nmol) from Sigma (Madrid, Spain), and results are expressed as mg of Trolox equivalent per 100 g.

Bioactive Compound Determination. Total phenolics were extracted according to Tomás-Barberán et al.²³ using water:methanol (2:8) containing 2 mM NaF (to inactivate polyphenol oxidase activity and prevent phenolic degradation) and quantified using the Folin-Ciocalteu reagent, and results (mean \pm SE) were expressed as mg of gallic acid equivalent per 100 g fresh weight. Total anthocyanins were determined according to García-Viguera et al.²⁴ adapted as previously reported⁶ and calculated as cyanidin 3-glucoside equivalent (molar absorption coefficient of 23900 L cm⁻¹ mol⁻¹ and molecular weight of 449.2 g mol⁻¹), and results were expressed as mg per 100 g fresh weight and were the mean \pm SE. Total carotenoids were extracted according to Mínguez-Mosquera and Hornero-Méndez.²⁵ Briefly, 2 g of sweet cherry fruit was extracted with acetone and shaken with diethyl ether and 10% NaCl for separation of the two phases. The lipophilic phase was washed with Na_2SO_4 (2%) and saponified with 10% KOH in methanol, and the pigments were subsequently extracted with diethyl ether, evaporated and then made up to 25 mL with acetone. Total carotenoids were estimated by reading the absorbance at 450 nm according to Díaz-Mula et al. 2008²⁶ and expressed as mg of β -carotene equivalent per 100 g, taking into account the $\varepsilon^{1\%}_{cm}$ = 2560. Analytical reagents were purchased from Sigma-Aldrich (Madrid, Spain).

Statistical Analysis. Data for the analytical determinations were subjected to analysis of variance (ANOVA). Sources of variation were storage time and treatment. Mean comparisons were performed using HSD Tukey's test to examine if differences were significant at P < 0.05.

Linear regressions were performed between the color chroma parameter and anthocyanin concentration, as well as among the bioactive compounds and H-TAA or L-TAA taking into account data from both for cultivars and all sampling dates. All analyses were performed with SPSS software package v. 12.0 for Windows.

RESULTS AND DISCUSSION

Sweet Cherry Ripening Parameters. During storage of control fruits, color (chroma index), fruit firmness and total acidity (TA) decreased in both cultivars while total soluble solids (TSS) increased. These changes were significantly delayed in 'Cristalina' and 'Prime Giant' treated with SA, ASA and OA (Figure 1). Interestingly, the observed values after 20 days of storage in treated fruits were similar to those of control fruit just after 10 days. Thus, for both cultivars shelf life of control cherries was established at 10 days while it was extended up to 20 days in treated fruits. The loss of firmness and TA and the increase of color to dark-red have been associated with ripening of sweet cherry and loss of quality attributes.^{4–6} OA and SA delayed the ripening process in climacteric fruit, such as mango,¹⁶ peach¹⁷ and jujube fruit, ¹⁸ as well as SA and ASA on kiwifruit ¹¹ due to the inhibition of ethylene production. In addition, retention of fruit firmness after SA treatment has been reported in several crops due to inhibition of cell-wall degrading enzymes, such as polygalacturonase, cellulase, and pectinmethylesterase.²⁷ Although sweet cherry is a nonclimacteric fruit, the results show a clear effect of these treatments on delaying the ripening process and maintaining fruit quality in this fruit, as has been reported for other nonclimacteric fruit such as pomegranate treated with SA,²⁸ OA¹⁹ and ASA.¹⁴

Bioactive Compounds and Total Antioxidant Activity. At harvest, the anthocyanin concentration differed between cultivars with values of 81.89 ± 1.62 and 18.56 ± 0.62 mg per 100 g, for 'Cristalina' and 'Prime Giant', respectively (Figure 2). For both cultivars a similar pattern was found over storage in control fruits, that is, increases until day 10 of storage followed by a decrease. The application of SA, ASA and OA led to a continuous increase in anthocyanin concentration until the end of the experiment, the values being higher for OA-treated cherries



Figure 3. Total phenolics concentration in sweet cherry 'Cristalina' and 'Prime Giant' during storage at 2 °C in control and treated fruits with salicylic acid (SA), acetylsalicylic acid (ASA) or oxalic acid (OA). Data are the mean \pm SE. LSD is shown inside figures.



Figure 4. Total carotenoid concentration in sweet cherry 'Cristalina' and 'Prime Giant' during storage at 2 °C in control and treated fruits with salicylic acid (SA), acetylsalicylic acid (ASA) or oxalic acid (OA). Data are the mean \pm SE. LSD is shown inside figures.

followed by ASA and SA treated fruit. The main anthocyanins in 'Cristalina' and 'Prime Giant' cultivars are cyanidin-3-rutinoside followed by cyanidin-3-glucoside and pelargonidin-3-rutinoside, and their changes have been related to the advancement of the ripening process, either on-tree^{1,2} or during storage,⁶ and negatively correlated with color parameters.^{3,6} Thus, control fruits exhibited the lowest chroma index, acquiring a dark-red color after 10 days, at which the highest anthocyanin concentration was found. After this period, control cherries became even darker and however loss of anthocyanins was detected. Discrepancies exist on the changes of anthocyanins during postharvest storage. Thus, in 'Sciazza' and 'Ferrovia' cherry cultivars, over 50% of the anthocyanin concentration was lost after 15 days at 1 °C, while in 'Burlat', 'Saco', 'Summit' and 'Van' total anthocyanin concentration increased during 14 days and no changes have been found in 'Lambert Compact' cherries.^{5,29} In our work, the storage period was extended up to 20 days and the fruit could be

considered as over-ripe and in senescence phase, at which a diminution of total anthocyanins occurred. Accordingly, in 'Bing' cultivar the cyanidin-3-rutinoside, which is the major anthocyanin in cherries, decreased from day 15 to day 30 of storage, and the increase in color intensity was attributed to minor anthocyanins that impart dark-red color.³⁰ On the contrary, the clear effect of SA, ASA and OA on delaying the ripening process was reflected in the increase in anthocyanins over storage.

A similar pattern to that of total anthocyanins was found for total phenolic compounds, that, is increases in control fruits up to day 10 of storage and further decreases, while in all treated fruits total phenolics increased throughout the experiment (Figure 3). Among treatments, the highest levels of total phenolics were found in those cherries treated with OA and ASA followed by SA. In our and other sweet cherry cultivars nechlorogenic acid was the predominant hydroxycinnamic acid followed by 3-*p*coumaroylquinic acid.^{5,6,29} Phenolic compounds increased their



Figure 5. Lipophilic total antioxidant activity (L-TAA) in sweet cherry 'Cristalina' and 'Prime Giant' during storage at 2 °C in control and treated fruits with salicylic acid (SA), acetylsalicylic acid (ASA) or oxalic acid (OA). Data are the mean \pm SE. LSD is shown inside figures.



Figure 6. Hydrophilic total antioxidant activity (H-TAA) in sweet cherry 'Cristalina' and 'Prime Giant' during storage at 2 °C in control and treated fruits with salicylic acid (SA), acetylsalicylic acid (ASA) or oxalic acid (OA). Data are the mean \pm SE. LSD is shown inside figures.

concentration (40-60%) during cold storage and also were related to the advancement of the ripening process. However, the change of total phenolics during postharvest storage could be affected by several factors including ripening stage at harvest, cultivar, season growth and duration of storage time, since a general increase occurred in a short period of storage for a wide range of cherry cultivars,⁶ although decreases or increases were reported for 'Summit' and 'Van' cherries depending on year and season growth.⁵ The data reported herein suggest that increases occurred during the first days of storage and the prolongation of storage led to significant decreases in phenolic content. Linear regression was performed between total polyphenols and anthocyanins taking into account all data (cultivar, treatment and storage), and a high correlation was found (y = 1.30x + 57.23; $R^2 = 0.978$), which suggests that anthocyanins are the main phenolic compounds as has been shown in other cherry cultivars.^{3,4,6} No literature exists on the effect of SA, ASA or

OA on the content of total phenolics in fruits for comparative purposes, apart from our previous papers on pomegranate treated with OA or ASA, which alleviated CI and reduced the phenolic losses found in control fruits.^{14,19}

Although carotenoids are other important bioactive constituents in fruits,³¹ no evidence exist on their occurrence in sweet cherry. In both cultivars carotenoids were present but at different concentrations, with 'Prime Giant' having significantly higher total carotenoids (1.06 ± 0.07 mg per 100 g) than 'Cristalina' (0.61 ± 0.04 mg per 100 g). Along storage, an accumulation of total carotenoids was observed in both control and treated cherries for both cultivars (Figure 4). This increase was retarded in treated fruits with respect to controls, although at the end of the experiment the carotenoid concentration was similar for all cases, with final values ≈ 1.3 and ≈ 2.2 mg per 100 g, for 'Cristalina' and 'Prime Giant', respectively. In other stone fruits, such as plums, an increase of carotenoid levels was also observed along storage and related to the advancement of the ripening process in both yellow and red-purple cultivars.^{26,32}

Total antioxidant activity (TAA) was measured in hydrophilic (H-TAA) and lipophilic (L-TAA) fractions separately, since early reports demonstrated that the contribution of L-TAA supposed about 20–30% of the TAA in a wide range of sweet cherry cultivars.^{1,6} The change of L-TAA was similar to that obtained for carotenoids, that is, a continuous increase alongside the storage, although the application of SA, ASA or OA induced a significant delay in the increase of L-TAA (Figure 5). In fact, a high correlation was found between L-TAA and carotenoids (y = 29.63x - 6.71; $R^2 = 0.824$), which would indicate that carotenoids are the main lipophilic bioactive compounds contributing to L-TAA, although other lipophilic compounds such as tocopherols could also be present in sweet cherry and having a role as antioxidant moieties.³³

During storage, continuous increases occurred in H-TAA for those cherries treated with SA, ASA or OA, while in control fruits H-TAA peaked at day 10 and showed decreases after that (Figure 6). The pattern of H-TAA was similar to that observed for total phenolics, and therefore high correlation was found between total phenolic and H-TAA (y = 0.51x + 16.70; $R^2 =$ 0.975), which is in agreement with previous papers reporting that phenolic compounds are the main compounds responsible for antioxidant capacity of sweet cherry,^{2,4} although ascorbic acid can also contribute to this activity.² Early reports have demonstrated that these treatments have also effects on antioxidant enzymes. For example, increases in catalase, peroxidase, superoxide dismutase and ascorbate peroxidase have been found in fruits such as banana, sugar apple fruit and mango after SA, ASA or OA treatment.^{9,10,12} Moreover, in kiwifruit and loquat, ASA application delayed the increase in lipoxygenase associated with senescence leading to a lower production of superoxide free radicals.^{11,34} During storage of these fruits, a decline in the content of free SA occurred, and thus exogenous treatments with SA or ASA led to increase its endogenous concentration and delaying of the ripening process.

Given the relationship between L-TAA-carotenoids and H-TAA-phenolics, it could be concluded that phenolic compounds, including anthocyanins, and carotenoids could be responsible for the health-beneficial effects after sweet cherry consumption in relation to its reported effect on reducing the risk of several diseases, such as cancer, diabetes, Alzheimer's and cardiovascular diseases.³⁵ The application of natural compounds as postharvest tools, such as SA, ASA or OA, resulted in delayed ripening rates of sweet cherry during storage, and maintained higher contents of bioactive compounds and antioxidant activity as compared with control fruit. Thus, control fruit could be stored for 10 days while this period was extended up to 20 days in treated cherries, without significant differences among treatments.

AUTHOR INFORMATION

Corresponding Author

*Fax: 34-96-6749678. E-mail: m.serrano@umh.es.

Funding Sources

This work has been funded by Spanish Ministry of Science and Technology through Project AGL2006/004359/ALI and European Commission with FEDER Funds. H.M.D.-M. is granted by a research fellowship (BES-2007-15415) of the Spanish Ministry of Science and Technology.

ACKNOWLEDGMENT

Authors thank "Finca Los Frutales" for permission to use their plots, for provision of the cherries and for the technical support received.

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