Postharvest Biology and Technology 109 (2015) 106–113

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Methyl salicylate treatments of sweet cherry trees increase antioxidant systems in fruit at harvest and during storage

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ARTICLE INFO

Article history:
Received 12 March 2015
Received in revised form 17 June 2015
Accepted 21 June 2015
Available online 2 July 2015

Keywords:
Anthocyanins
Ascorbate peroxidase
Catalase
Peroxidase
Phenolics
Postharvest senescence
Superoxide dismutase

ABSTRACT

Sweet cherry trees (*Prunus avium* L.) were treated with methyl salicylate (MeSa) at 1 mM at 3 key events on fruit development on-tree to analyze the effect of MeSa on bioactive compounds, total antioxidant activity (TAA) and antioxidant enzymes at harvest and during 28 days storage. The experiments were performed during two consecutive years, by using two sweet cherry cultivars, 'Sweet Heart' and 'Sweet Late' in 2013 and another more cultivar, 'Lapins', in 2014. Both total phenolics and anthocyanins content were significantly higher in MeSa-treated than in control fruit at harvest and during storage, leading to fruit with higher hydrophilic TAA (H-TAA). The activity of the antioxidant enzymes catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and superoxide dismutase (SOD) decreased along storage for all cultivars and experimental years, although those fruit treated with MeSa exhibited higher activities of these antioxidant enzymes than controls. Thus, MeSa treatment of cherry trees could increase health-promoting properties of cherry fruit consumption, due to its effect on increasing antioxidant and bioactive compounds, with additional effect on delaying the fruit postharvest senescence process by increasing the activity of the enzymes involved in reactive oxygen species (ROS) scavenging.

1. Introduction

Methyl salicylate (MeSa) is a volatile plant compound synthesized from salicylic acid having a role in plant growth and development, plant defense-mechanism, plant responses against several abiotic stresses as well as in the fruit ripening process (Hayat and Ahmad, 2007; Kumar, 2014). Thus, postharvest treatment with exogenous MeSa decreased chilling injury (CI) of tomato (Fung et al., 2006), sweet pepper (Fung et al., 2004) and mango (Han et al., 2006) fruits by protecting cell wall structure and cell membranes from dysfunction caused by lipid peroxidative injury. Accordingly, in pomegranate fruit, postharvest treatment with MeSa reduced significantly the CI symptoms, by maintaining membrane structure and its selective permeability leading to lower values of electrolyte leakage. In addition, other parameters related to fruit quality, such as fruit firmness, total soluble solids and total acidity were also maintained in MeSa treated fruit while significant losses occurred in control pomegranates (Sayyari et al., 2011a). Moreover, the content of total phenolics and total anthocyanins as well as the antioxidant activity in the arils increased along storage, although these increases were significantly higher in MeSa-treated than in control pomegranates, showing that MeSa has potential postharvest applications for reducing CI, maintaining quality, and improving the health benefits of pomegranate fruit consumption by increasing its antioxidant capacity and bioactive compounds (Sayyari et al., 2011a; Valero et al., 2015).

Reactive oxygen species (ROS), such as superoxide radical ($O_2^{\bullet -}$), peroxide radical ($O_2^{\bullet 2-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet -}$), are inevitably generated in plant cells as a consequence of normal metabolism, mainly in reactions catalyzed by oxidase and lipoxygenase and in β-oxidation of fatty acids. The ROS content in plant cell is dependent on their producing systems and scavenging mechanism, both enzymatic and non-enzymatic ones (Apel and Hirt, 2004). Non-enzymatic antioxidant compounds are reduced forms of ascorbate and glutathione, tocopherols, phenolics, alkaloids and carotenoids, while enzymatic scavenging mechanisms include mainly superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX). SOD detoxifies $O_2^{\bullet -}$ free radicals by converting them to O_2 and H_2O_2, which is further converted to H_2O and O_2 by CAT, APX and POD. CAT catalyzes the decomposition of hydrogen peroxide to

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http://dx.doi.org/10.1016/j.postharvbio.2015.06.011
0925-5214/

water and oxygen, while APX uses ascorbate and H_2O_2 as substrates producing water and dehydroascorbate as products, the last one being converted to ascorbate by glutathione reductase enzyme. In addition, H_2O_2 can be also reduced to water by POD, by using organic molecules such as phenols as electron donor (Tareen et al., 2012). However, in spite of the presence of these efficient antioxidant systems, oxidative damages still occur in plant cells either due to uncontrolled production or inefficient scavenging of ROS. Thus, since the overall process of fruit ripening is considered as a functionally modified protracted form of senescence, associated with ROS accumulation (Hodges et al., 2004), the presence of high content of antioxidant compounds and high activity of antioxidant enzymes could lead to a delay of the fruit postharvest ripening process and to maintain fruit quality attributes for longer periods (Mondal et al., 2009; Kumar et al., 2014).

In this sense, in fruits such as mango and sugar apple, it has been shown that postharvest salicylic acid (SA) treatments led to lower superoxide free radical production and lipoxygenase activity and increases in the activity of SOD, CAT, and APX antioxidant enzymes during storage as compared with control fruit (Ding et al., 2007; Mo et al., 2008). Similar increases in these antioxidant enzymes were observed in peach fruit after postharvest SA treatment (Tareen et al., 2012). Even in sweet cherry, treatments with SA three days before harvesting or after harvesting increased POD activity and its transcript levels, which was related to the reduced fungal infection found in SA-treated fruit, especially when SA was applied to fruit at earlier maturity stages (Yao and Tian, 2005; Chan et al., 2008).

In previous reports, we have found that postharvest treatments of sweet cherry with SA or acetylsalicylic acid (ASA), which are close analogues of MeSa, delayed the postharvest ripening process, manifested by lower color changes and acidity and firmness losses, and maintained higher content of bioactive compounds and antioxidant activity during storage as compared with control fruit (Valero et al., 2011). Moreover, treatments of sweet cherry trees with SA or ASA, by foliar spray, during on-tree cherry growth and ripening, increased fruit weight and quality attributes (such as color and firmness) at commercial harvest, as well as the concentration of total phenolics and anthocyanins, which led to fruits with higher antioxidant activity, in both hydrophilic and lipophilic fractions (Giménez et al., 2014). By other hand, similar MeSa treatments of sweet cherry trees also increased fruit size and quality properties, such as firmness and TSS, at time of harvesting, showing also a significant effect on maintaining sweet cherry organoleptic properties along storage (Giménez et al., unpublished data). However, as far as we know, there is no scientific literature about the possible effect of MeSa treatment, either at pre- or postharvest application, on sweet cherry antioxidant systems. Then, the aim of this research was to evaluate for the first time the effect of preharvest MeSa treatments of sweet cherry trees on antioxidant compounds and the activity of the antioxidant enzymes SOD, CAT, POD and APX, at harvest and during prolonged cold storage. To achieve these objectives, the experiments were performed in two consecutive years, 2013 and 2014, by using two and three cherry cultivars, respectively.

2. Materials and methods

2.1. Plant material and experimental design

The experiments were performed in two consecutive years, 2013 and 2014 spring–summer periods. In 2013, two sweet cherry cultivars, 'Sweet Heart' and 'Sweet Late', cultivated in a commercial plot from "Fincas Toli S.L." located at Jumilla (38.473800 N, −1.323861 W, Murcia, Spain) were used and in 2014 another cultivar, 'Lapins', cultivated in a commercial plot for "Cerezas Aitana" located at Alcoy (38.780634 N, −0.443124 W, Alicante, Spain) was also used. Both locations are close each other with similar environmental and climatic conditions, and cherry tress grown under similar cultural practices. All cultivars are grafted on the 'Santa Lucía 64' rootstock and planted at 3 × 4 m. Three trees were selected completely at random for each cultivar and treatment: control (distilled water), and MeSa at 1.0 mM. This MeSa concentration was shown as appropriate in terms of increased fruit quality attributes (Giménez et al., unpublished data). Freshly prepared solutions (containing 0.5% of Tween 20) were foliar sprayed with a mechanical mist sprayer (7.5 L/tree) and repeated at three dates of the growth cycle, which corresponded to key events in fruit developmental process, according to previous experiments (Díaz-Mula et al., 2009; Giménez et al., 2014): T1 (at pit hardening), T2 (initial color changes) and T3 (onset of ripening). Sweet cherry fruit were harvested at commercial ripening stage which corresponded to S2 stage according to Serrano et al. (2009), and immediately transferred to the laboratory. Then, 90 fruits, homogeneous in color and size and without visual defects were selected from each tree or replicate, cultivar, year and treatment and randomly grouped in 3 lots of 30 fruits, which were stored in a cold room at normal atmosphere at 2 °C and RH of 85%. One lot was taken at random from each replicate after 0, 14 and 28 days of cold storage and transferred for 1 day at 20 °C and RH of 70%, afterward the analytical determinations were performed. The edible portion of the fruit from each lot was cut in small pieces to obtain a homogeneous sample, frozen in liquid N_2, mixed and stored at −20 °C until total anthocyanins, total phenolics, antioxidant activity, in both hydrophilic and lipophilic fractions and SOD, CAT, POD and APX activities were determined in duplicate for each sample.

2.2. Total phenolics, total anthocyanins, total carotenoids and total antioxidant activity determination

Phenolic extraction was performed by using 2 g of frozen tissue and 10 mL of water:methanol (2:8) containing 2 mM NaF (to inactivate polyphenol oxidase activity and prevent phenolic degradation) as previously described (Serrano et al., 2009) and quantified using the Folin–Ciocalteu reagent and results (mean ± SE) were expressed as mg gallic acid equivalent 100 g^{-1} fresh weight (FW). Total anthocyanins were extracted and determined according to previously reported (Serrano et al., 2005) and calculated as cyanidin 3-glucoside equivalent (molar absorption coefficient of 23,900 L cm^{-1} mol^{-1} and molecular weight of 449.2 g mol^{-1}) and results (mean ± SE) expressed as mg 100 g^{-1} FW. Total carotenoids were extracted according to Valero et al. (2011). Briefly, 2 g of sweet cherry fruit were 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%), 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 and expressed as mg of β-carotene equivalent 100 $^{-1}$ FW, taking into account the $\varepsilon^{1\%}$cm = 2,560 and results were the ±SE.

TAA was quantified as previously described (Serrano et al., 2009) by homogenizing 2 g of tissue in 10 mL of 50 mM Na-phosphate buffer at pH 7.8 and 3 mL of ethyl acetate. After centrifugation at 10,000 g for 15 min at 4 °C, rhe upper fraction was used to quantify total antioxidant activity due to lipophilic compounds (L-TAA) and the lower for total antioxidant activity due to hydrophilic compounds (H-TAA), in a reaction medium containing 50 μL of the extract, 2 mM of the chromophore 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium