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Preharvest treatments with salicylates enhance nutrient and antioxidant compounds in plum at harvest and after storage

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Abstract

BACKGROUND: Previous reports have addressed the effectiveness of salicylic acid (SA), acetylsalicylic acid (ASA) and methylsalicylate (MeSA) postharvest treatments on maintaining quality properties during storage in several commodities. However, there is no literature regarding the effect of preharvest treatments with salicylates on plum quality attributes (at harvest or after long-term cold storage), which was evaluated in this research.

RESULTS: At harvest, weight, firmness, individual organic acids, sugars, phenolics, anthocyanins and total carotenoids were found at higher levels in plums from SA-, ASA- and MeSA-treated trees than in those from controls. During storage, softening, colour changes and acidity losses were delayed in treated fruits as compared to controls. In addition, organic acids and antioxidant compounds were still found at higher levels in treated than in control plums after 40 days of storage. Results show a delay in the postharvest ripening process due to salicylate treatments, which could be attributed to their effect in delaying and decreasing ethylene production.

CONCLUSION: Preharvest treatment with salicylates could be a safety, eco-friendly and new tool to improve (at harvest) and maintain (during storage) plum quality and especially its content of bioactive compounds with antioxidant properties, increasing the health effects of plum consumption.

Keywords: salicylic acid; acetylsalicylic acid; methylsalicylic acid; individual phenolics; organic acids; sugars

INTRODUCTION

Japanese plums (*Prunus salicina* Lindl.) are nutritious stone fruits with excellent quality attributes and very appreciated by consumers, but having a limited postharvest storage life due to their climacteric ripening behaviour.[1,2] Storage at low temperature is an appropriate tool to delay the plum postharvest ripening process by decreasing ethylene production, respiration rate, colour changes and loss of firmness and total acidity.[1,3] In addition, greater effects on maintaining plum quality have been reported with the combination of cold storage with other postharvest treatments, such as modified atmosphere packaging,[4] aloe gel[5] or alginate-based-coatings,[6] 1-methylcylcopropene, calcium, heat or polyamines.[1] Nevertheless, in recent years research has been performed regarding the use of preharvest treatments with natural compounds to increase fruit quality at harvest and to maintain it during storage, due to consumers' concerns and legal restrictions concerning postharvest chemical treatments.

In this sense, salicylic acid (SA) and its derivatives acetylsalicylic acid (ASA) and methylsalicylic acid (MeSA) are now considered hormonal compounds with important roles in a wide range of physiological processes, such as inducing systemic acquired resistance, modulation of opening and closing of stomata, flowering, seed germination and providing plant tolerance against different kinds of stress.[7] In addition, postharvest treatments with salicylates have been shown to reduce decay (by increasing fruit resistance to diseases) and chilling injury in numerous commodities as well as to improve other quality properties, such as appearance, texture maintenance and nutritional content.[8,9] Thus postharvest treatments with SA maintained higher quality attributes in apricots,[10] as well as ASA and MeSA treatments on pomegranate,[11,12] SA and ASA treatments on sweet cherry[13] or SA treatments on kiwifruit,[14] among others. Recently, postharvest SA treatments have also been shown to reduce chilling injury, softening and colour evolution, leading to maintenance of fruit quality during storage at 20 °C or low temperature in 'Santa Rosa',[15,16] 'Satluj'[17] and 'Qingnai'[18] plum cultivars. However, to the best of our knowledge, there are no previous reports regarding the effect of preharvest treatments with

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SA, ASA or MeSA on plum quality attributes at harvest or during storage, although some literature exists concerning this issue in other fruits. Thus combination of pre- and postharvest treatments of strawberry with SA decreased fungal decay and maintained overall quality during storage.[19] Similarly, preharvest foliar sprays of jujube plants with 2.0 mmol L^{-1} SA decreased decay caused by *Alternaria alternata* and *Monilinia fructicola* at harvest, and this effect persisted and was also evident during cold storage.[20] Moreover, SA ASA or MeSA treatment of sweet cherry trees, at 0.5, 1.0 and 2.0 mmol L^{-1} concentrations, applied at pit hardening, initial colour changes and onset of ripening, increased fruit quality attributes such as weight, firmness and content of total phenolics, total anthocyanins and antioxidant activity.[21–23]

The aim of this research was to evaluate the effect of SA, ASA and MeSA treatments, applied as foliar spray during on-tree plum development, on fruit quality attributes at harvest, with special emphasis on sugars, organic acids, phenolics and anthocynanins profile because of their impact in organoleptic and antioxidant properties. In addition, these parameters were also evaluated after 40 days of cold storage plus 1 day at 20 °C to discover whether the effects of these treatments persist after long-term storage. This could be a safety, eco-friendly and new tool to improve and maintain plum quality attributes, since no previous papers regarding plum preharvest treatments with salicylates are available in the literature.

MATERIALS AND METHODS

Plant material and experimental design

In this study 'Black Splendor' plum (*Prunus salicina* Lindl.) cultivar was grown on a commercial farm (El Ciruelo) located at Cieza (Murcia, Spain) along the developmental cycle during the 2015 spring–summer period. Plum trees were 6 years old, planted at 5 × 3 m and grafted onto 'Mariana' rootstock. Along the growth cycle, standard cultural practices, such as pruning, thinning, drip irrigation system (in which fertilization was applied) and conventional disease treatments were performed. Date for full blossom was 21 February. The experiment was designed totally at random in triplicate, using three trees per replicate for each treatment: control (distilled water), SA at 0.5 mmol L^{-1}, ASA at 1 mmol L^{-1} and MeSA at 0.5 mmol L^{-1}, which were purchased from Sigma-Aldrich (Madrid, Spain). These doses were chosen according to previous experiments in which 0.5, 1.0 and 2.0 mmol L^{-1} doses of these compounds were applied. Trees were treated with recently prepared SA, ASA or MeSA solutions (plus 0.5% Tween-20 as surfactant) by foliar spray with a mechanical mist sprayer at three dates during the plum growth cycle: T1 (at pit hardening, 61 days after full blossom, DAFB); T2 (initial colour changes, 76 DAFB); and T3 (onset of ripening, 94 DAFB). These dates corresponded to key events of plum fruit development, according to previous experiments.[24] The date of commercial harvest was established based on fruit size (weight ∼90 g, length > 50 mm and diameter > 55 mm), colour (purple skin, with small mahogany-coloured areas on the shoulders) and content of total soluble solids (TSS, more than 10 °Brix, or 100 g kg^{-1}) characteristic of this cultivar. For each treatment and replicate, about 200 plums were transferred to the laboratory, and then six lots of 20 homogeneous fruits were randomly selected, weighed, labelled and stored at 2 °C. Analytical determinations were made in recently harvested fruits (day 0) and in stored fruits for 10, 20, 30, 40 and 50 days at 2 °C + 1 day at 20 °C, using one lot for each replicate taken at random from the cold storage room, except for total carotenoids and individual phenolics, anthocyanins, organic acids and sugars, which were quantified at harvest and after 40 days of cold storage plus 1 day at 20 °C.

Ethylene production and respiration rate

For ethylene production and respiration rate quantification each lot was hermetically sealed in a 3 L jar for 30 min. After that, 1 mL from the holder atmosphere was withdrawn with a syringe and injected into a gas chromatograph with flame ionization detector to quantify ethylene production rate (nmol kg^{-1} s^{-1}). Another 1 mL was used for CO_2 quantification in a gas chromatograph with thermal conductivity detector and respiration rate was expressed as μmol kg^{-1} s^{-1}. Chromatographic conditions and equipment were as previously described.[5]

Firmness, colour, TSS and total acidity (TA)

For firmness, a TX-XT2i texture analyser (Stable Microsystems, Godalming, UK) was used by measuring the force–deformation ratio necessary to achieve a 3% deformation of fruit diameter, and results were expressed as N mm^{-1}. For colour, a colorimeter (model CRC-20, Minolta, Oxaca, Japan) was used by measuring the three colour coordinates (L^*, a^*, b^*) at three equatorial points of the fruit, and colour was expressed as hue angle (arctan b^*/a^*). Both parameters were measured in each individual fruit. The 20 fruits of each lot were peeled and the flesh was cut into small pieces to obtain a homogeneous sample for each replicate. TSS was determined in duplicate in the juice obtained from 5 g of each sample with a digital refractometer (model PR-101, Atago Co. Ltd, Tokyo, Japan) at 20 °C and expressed as g kg^{-1} (mean ± SE). TA was determined in duplicate in the same juice by automatic titration (785 DMP Titrino, Metrohm, Herisau, Switzerland) with 0.1 mol L^{-1} NaOH up to pH 8.1, using 1 mL diluted juice in 25 mL distilled H_2O, and results (mean ± SE) were expressed as g malic acid equivalent kg^{-1} fresh weight (FW). Ripening index (RI) was calculated as the ratio TSS/TA.

Sugars and organic acids

Five grams of plum flesh from each replicate was homogenized with 10 mL deionized water using a polytron homogenizer (IKA Labotechnik, Staufen im Breisgau, Germany) and centrifuged at 10 000 × *g* for 10 min. The supernatant was used to quantify organic acids and sugars in duplicate using a high-performance liquid chromatography (HPLC) system (series 1100, Hewlett-Packard, Waldbrom, Germany) equipped with a SUPELCOGEL C-610H (30 cm × 7.8 mm) column (at 30 °C), an absorbance detector (210 nm UV, for acid analysis) and a refractive index detector (for sugar analysis). The elution system was 0.1% H_3PO_4, running isocratically at a flow rate of 0.5 mL min^{-1}. Organic acids were quantified from the absorbance peaks at 210 nm and using calibration curves performed with malic, citric, ascorbic, succinic and fumaric acid standards from Sigma (Poole, UK). Results were expressed as g kg^{-1} FW. Sugars were quantified by comparison of refractive index peaks with those of standards of glucose, fructose and sucrose from Sigma (Poole, UK) and results were expressed as g kg^{-1} FW.

Individual phenolics and anthocyanins

Individual phenolic compounds were quantified as previously reported.[24] In brief, 0.1 g lyophilized samples from each replicate was mixed with 1 mL methanol–formic acid–water (25:1:24, v/v/v), vortexed, sonicated in an ultrasonic bath for 60 min and

centrifuged at 10 500 × *g* for 5 min. The supernatant was filtered through a 0.45 μm PVDF filter (Millex HV13, Millipore, Bedford, MA, USA) and used for quantification in duplicate of individual phenolics in an HPLC system, which was equipped with a Luna C18 column (25 cm × 0.46 cm i.d., 5 μm particle size; Phenomenex, Macclesfield, UK) and a C18 security guard (4.0 × 3.0 mm) cartridge system (Phenomenex). The mobile phases A and B were water–formic acid (99:5, v/v) and acetonitrile, respectively, with a flow rate of 1 mL min^{-1}. The linear gradient started with 8% of solvent B, reaching 15% of solvent B at 25 min, 22% at 55 and 40% at 60 min, which was maintained up to 70 min. The injection volume was 20 μL. Chromatograms were recorded at 280, 320, 360, and 520 nm. Different phenolics were characterized by chromatographic comparison with analytical standards as well as quantified by the absorbance of their corresponding peaks. Hydroxycinnamic derivatives, *p*-coumaroylquinic acid and hydroxybenzoic acid were characterized by chromatographic comparison according to their retention time and UV–visible spectra. Anthocyanin standards (cyanidin 3-glucoside, cyanidin 3-rutinoside and pelargonidin 3-rutinoside) were purchased from Polyphenols SA (Sandnes, Norway). Anthocyanins were quantified as cyanidin 3-*O*-glucoside at 520 nm, cinnamic acids as 5-*O*-caffeoylquinic acid at 320 nm, and flavonols as quercetin 3-*O*-rutinoside at 360 nm, and expressed as g kg^{-1} FW (mean ± SE).

Total carotenoids

Total carotenoids were quantified as previously reported.[24] In brief, 5 g fresh plum tissue was extracted with acetone (1:3 ratio, w/v) and shaken with 10 mL diethyl ether and 5 mL of 10% NaCl until the two phases were completely separated. The lipophilic phase was washed with 5 mL of 2% Na_2SO_4 and saponified with 5 mL of 10 % KOH in methanol. Then the pigments were subsequently extracted with diethyl ether, which was evaporated, and the residue was dissolved in 25 mL acetone. Total carotenoids were estimated by reading the absorbance at 450 nm in a UNICAM Helios-α spectrophotometer (Cambridge, UK), and expressed as g β-carotene equivalent kg^{-1} FW, taking into account the molar absorption coefficient (ε1% cm) of 2560, and the results were the mean ± SE.

Statistical analysis

Analytical determinations were performed in duplicate (two technical replicates, except colour and firmness, which were measured independently in each fruit) in three lots of 20 fruits (three biological replicates). Data were subjected to analysis of variance (ANOVA), sources of variation being days of storage and treatment. Mean comparisons were performed using Tukey's HSD (honest significant difference) test to examine whether differences were significant at $P < 0.05$. All analyses were performed with SPSS software package 12.0 for Windows.

RESULTS AND DISCUSSION

Ethylene production and respiration rate

Preharvest treatments with SA, ASA and MeSA decreased ethylene production with respect to control fruits, both at harvest and during storage time (Fig. 1). In control plums, ethylene production increased from day 0 to day 20, at which the maximum ethylene production was reached with values of 0.059 ± 0.003 nmol kg^{-1} s^{-1}, whereas in treated plums this peak was reached later: 10 days later in SA- and ASA-treated plums and 20 days later in MeSA-treated ones. In addition, the maximum ethylene production was significantly lower in all treated plums than in controls (Fig. 1). Respiration rate increased during storage time in plums from control and treated trees, although it was also significantly lower in treated plums than in controls, without significant differences among SA-, ASA- and MeSA-treated plums (Fig. 1). Accordingly, reduced respiration rate and ethylene production have been observed after postharvest treatment with salicylates in a wide range of fruits; for example, in custard apples dipped in SA at 0.4, 0.8 and 1.2 mmol L^{-1} for 15 min and stored at 15 °C[25]; in kiwifruit dipped in ASA at 1 mmol L^{-1} for 5 min[14]; in strawberries treated with SA at 1–4 mmol L^{-1},[19] and even in 'Qingnai' plums dipped in SA at 1.5 mmol L^{-1} for 10 min and stored at 1 °C.[18] In addition, it has been shown that fruit response to salicylate treatments on ethylene production depends not only on the concentration used but also on the stage of fruit development. Thus treatment of tomato fruit at green mature stage with MeSA at 1 mmol L^{-1} for 16 h increased ethylene production during storage at 20 °C, whereas it was reduced in tomato treated at breaker stage. This effect of salicylates on inhibiting ethylene production has been attributed to the ability of salicylates to inhibit 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase, the main enzymes involved in ethylene biosynthesis.[14,26] Moreover, Yin *et al.*[14] reported that ASA may also interfere with ethylene perception.

Fruit quality parameters

Fruit quality parameters of plum fruits at harvest, such as weight, TA and firmness, were significantly affect by preharvest salicylate treatment. Thus fruit weight of plums from SA-, ASA- and MeSA-treated trees was higher than those from controls, the main effect being found for SA treatment, with increases of ∼25% (Table 1). This increase in fruit weight led to a significant increase in total yield, with 28.01 ± 1.39 kg per tree in control trees and 34.07 ± 1.71, 32.58 ± 2.09 and 31.42 ± 1.70 kg per tree in those treated with SA, ASA and MeSA, respectively (Table 1), whereas no significant effect was observed on the number of fruit per tree (data not shown). Accordingly, significant increases in cluster weight and yield were observed in vines sprayed with 1.5 or 2.0 mmol L^{-1} SA, compared to controls.[27] These results prove that treatment with salicylates has the effect of increasing net photo-assimilate production of plum trees and/or the sink strength of developing fruits. In this sense, it has been reported that SA foliar application increased chlorophyll content, photosynthetic rate and total dry weight in ginger plants[28]; increased activity of Rubisco and total yield have also been found after SA treatment in maize and mustard plants.[29]

Plums were harvested upon reaching their commercial ripening stage, according to their external colour (purple skin, with small mahogany-coloured areas on the shoulders) and TSS content, which should be higher than 10 °Brix (100 g kg^{-1}) for this cultivar. In this research, plums from control and treated trees were harvested on the same date, and then it could be inferred that salicylate treatment did not affect the on-tree plum ripening process. In fact, colour, expressed as hue angle, and ripening index (TSS/TA) showed similar values in control and treated plums (Fig. 2 and Table 1). However, other quality parameters, such as TA, TSS and firmness, were significantly higher ($P < 0.05$) in plums from treated trees than in controls, although no significant differences were found between SA-, ASA- and MeSA-treated plums (Table 1 and Fig. 2). Taking into account that all trees (control and treated) were located on the same farm, submitted to the same cultural

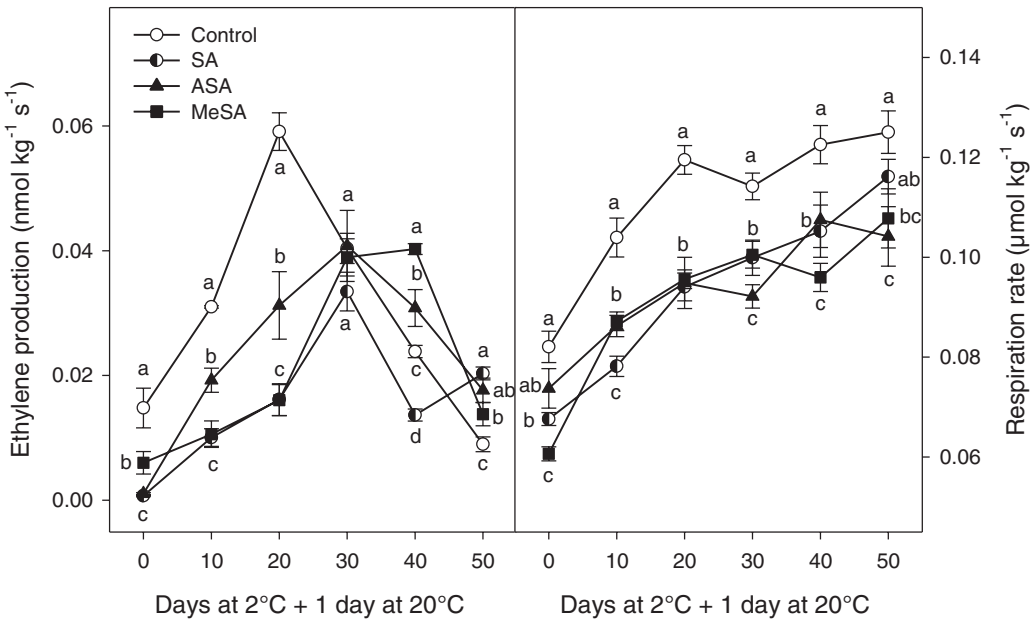

Figure 1. Ethylene production and respiration rate during storage of plums from control trees and those treated with salicylic acid (SA), acetylsalicylic acid (ASA) or methylsalicylic acid (MeSA). Data are the mean ± SE of three replicates of 20 fruits. Different lower-case letters indicate significant differences ($P < 0.05$) among treatments for each sampling date.

Table 1. Yield (kg per tree), fruit weight at harvest and soluble solids (TSS), total acidity (TA) and ripening index (RI) at harvest (Day 0) and after 50 days of storage at 2 °C + 1 day at 20 °C (Day 50) in 'Black Splendor' plums as affected by preharvest treatments with salicylic acid (SA, 0.5 mmol L^{-1}), acetylsalicylic acid (ASA, 1 mmol L^{-1}) and methylsalicylic acid (MeSA, 0.5 mmol L^{-1})

		Control	SA	ASA	MeSA
Yield (kg per tree)		28.01 ± 1.39a	34.07 ± 1.71b	32.58 ± 2.09bc	31.42 ± 1.70c
Fruit weight (g)		92.21 ± 2.24a	114.91 ± 4.35b	102.11 ± 3.21c	99.23 ± 2.61c
TSS (g kg^{-1})	Day 0	117.3 ± 2.1Aa	124.0 ± 1.5Ab	124.9 ± 2.1Ab	123.0 ± 1.1Ab
	Day 50	133.2 ± 1.2Ba	127.0 ± 1.3Ab	127.5 ± 1.0Ab	127.3 ± 1.9Ab
TA (g kg^{-1})	Day 0	15.7 ± 0.4Aa	17.7 ± 0.03Ab	16.4 ± 0.04Ac	17.6 ± 0.07Ab
	Day 50	9.3 ± 0.2Ba	13.4 ± 0.02Bb	13.0 ± 0.03Bb	12.6 ± 0.02Bc
RI (TSS/TA)	Day 0	7.47 ± 0.15Aa	7.56 ± 0.11Aa	7.05 ± 0.31Aa	6.99 ± 0.24Aa
	Day 50	14.32 ± 0.55Ba	9.48 ± 0.14Bb	9.81 ± 0.22Bb	10.10 ± 0.22Bc

For each parameter, different upper-case letters denote significant differences ($P < 0.05$) during storage, whereas lower-case letters denote significant differences ($P < 0.05$) among treatments for each sampling date.

practices (irrigation, fertilization, etc.) and under similar environmental conditions, these differences between plums from control and treated trees should be attributed just to the effect of salicylate treatment.

During storage fruit firmness decreased, this decrease being significantly delayed (by 20 days) in all treated fruits with respect to controls, although no significant differences were observed among SA, ASA and MeSA treatments (Fig. 2). In a similar way, hue angle decreased during storage, which was delayed by 20 days in plums from treated trees as compared with controls (Fig. 2). Hue angle has been reported to be a good colour index to show the occurrence of the typical colour changes from green to purple in plum cultivars with red-purple skin, since it decreases during ripening (either on-tree or during postharvest storage).[4,24] On the other hand, TSS gradually increased and TA gradually decreased during storage, leading to increases in RI, these changes having been also delayed as a consequence of treatment with salicylates (data not shown). Thus the greatest differences between control and treated fruits were found on the last sampling date, in which TSS content was lower in treated than in control fruits, while TA was higher. Values for RI were then higher in control than in treated fruits, although in general no significant differences were observed among SA, ASA and MeSA treatments (Table 1). Decreases in firmness, hue angle and TA, and increases in TSS and RI, show the evolution of the postharvest ripening process of 'Black Splendor' plums as has been reported in a wide range of plum cultivars – either Japanese or European ones.[1,3,6] However, it is worth noting that these changes were significantly delayed ($P < 0.05$) in plums from salicylate-treated trees as compared with those from controls (Table 1 and Fig. 2). According to data from quality parameters, the maximum storage time was established as 30 days for control fruits and 50 days for treated ones, since after this storage period plum fruits were overripened and with poor quality for consumption. Thus the concentrations of individual sugars, organic acids, phenolics and anthocyanins, as well as total carotenoid concentration, were measured at harvest and after 40 days of storage, and at an intermediate period between them. The observed effect of salicylates on delaying changes on quality

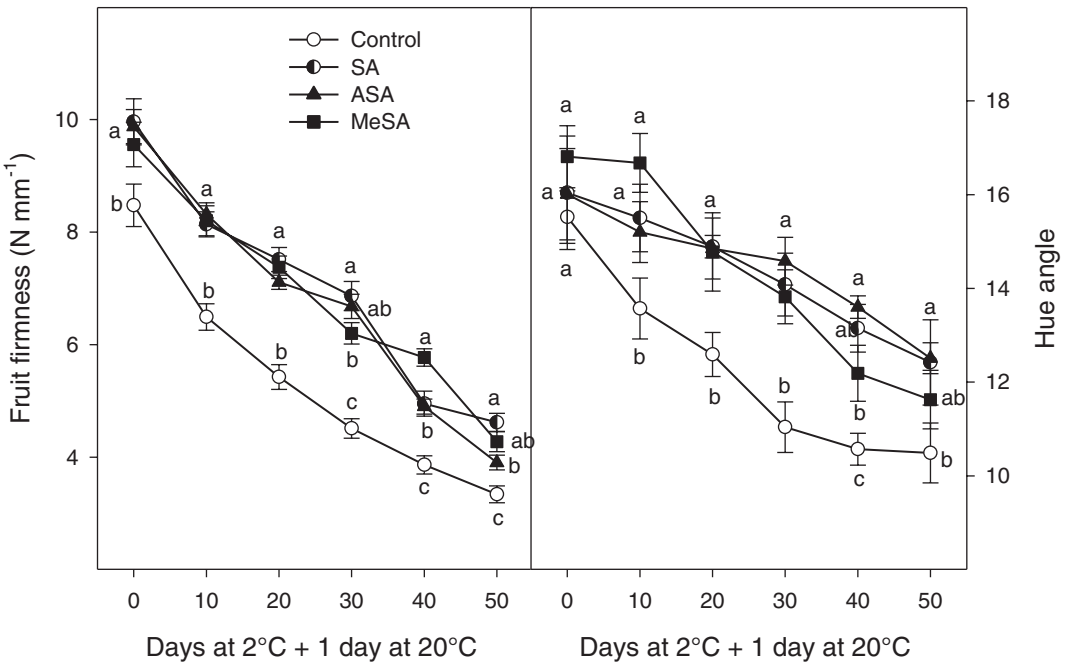

Figure 2. Fruit firmness and skin colour (expressed as hue angle) evolution during storage of plums from control trees and those treated with salicylic acid (SA), acetylsalicylic acid (ASA) or methylsalicylic acid (MeSA). Data are the mean ± SE of three replicates of 20 fruits. Different lower-case letters indicate significant differences ($P < 0.05$) among treatments for each sampling date.

parameters related to plum postharvest ripening could be attributed to their effect on delaying and decreasing ethylene production, given the climacteric behaviour of this plum cultivar.[1,16,24] Similarly, it has been recently reported that postharvest SA treatment maintained quality parameters during storage in other plum cultivars, such as 'Santa Rosa'[15,16] and 'Satluj'[17] throughout a reduction in ethylene production. However, the present results show that preharvest treatment with salicylates not only maintained quality attributes during storage but was also effective in increasing them at harvest time.

Sugar and organic acid concentration

Fruit taste appears to be the main factor in the consumer acceptance of Japanese plum, which is largely dependent upon the concentrations of individual sugars, organic acids and their ratio, since they emit very low levels of aroma volatile compounds.[2] The sugar profile in Japanese plums seems to be dependent on cultivars. Thus, in 'Black Splendor' plums, the major sugars at harvest were fructose and sucrose, followed by glucose either in fruits from control trees or in those from salicylate-treated ones, as well as in 'Amber Jewel' and 'Blackamber' cultivars.[30] However, in 'Red Beaut' plums, fructose presented the highest concentration, followed by glucose, whereas sucrose was found at the lowest concentration.[31] After 40 days of cold storage +1 day at 20 °C, sucrose concentration decreased whereas glucose and fructose concentrations increased with respect to concentration at harvest (Fig. 3), showing the conversion of sucrose into glucose and fructose during cold storage. Similarly, sucrose decreased and fructose and glucose increased in 'Amber Jewel' plums, whereas no changes in sugar concentrations were found in 'Blackamber' plums.[30] In addition, sucrose concentration at harvest was significantly higher ($P < 0.05$) in plums from treated trees than in those from controls, whereas no significant effect attributed to salicylate treatments was observed in fructose or glucose concentration. On the other hand, after 40 days of storage, sucrose, glucose and fructose concentrations were similar in plums from control and treated trees (Fig. 3).

The main organic acid in 'Black Splendor' plum was malic acid, with a concentration at harvest of 13.7 ± 0.4 g kg^{-1} in control plums and significantly higher, between 14.8 and 16.3 g kg^{-1} in plums from SA-, ASA- and MeSA-treated trees (Fig. 4), followed by citric acid, which was also found at higher concentration in plums from treated trees than in those from controls. Minor organic acids were fumaric, ascorbic and succinic acids, although salicylate treatments led also to higher concentrations of fumaric and ascorbic acids with respect to controls, whereas for succinic acid this effect was only evident for SA treatment (Fig. 4). During storage malic acid decreased in control and treated plums, being responsible for the reduction in TA (Table 1). Nevertheless, malic acid concentration was still higher in plums from treated trees than in those from controls after 40 days of storage. A similar trend was observed for ascorbic acid, whereas concentrations of citric and fumaric acids increased from day 0 to day 40 of storage and no changes occurred in succinic acid concentration. The decrease in ascorbic acid during storage could be due to the conversion of dehydroascorbic to diketogulonic acid by oxidation due to ascorbate oxidase.[15] However, it is important to note the effect of preharvest salicylate treatments in maintaining plum ascorbic acid levels during postharvest storage, due to its role as antioxidant with health beneficial effects in human beings.[32] Accordingly, Kazemi *et al.*[26] and Davarynejad *et al.*[15] showed that postharvest salicylic acid treatment had a significant influence on retaining ascorbic acid content during storage in apples and 'Santa Rosa' plums, respectively.

Phenolics, anthocyanins and carotenoids

Plums are a rich source of antioxidant compounds with health beneficial effects, such as phenolics, including anthocyanins,

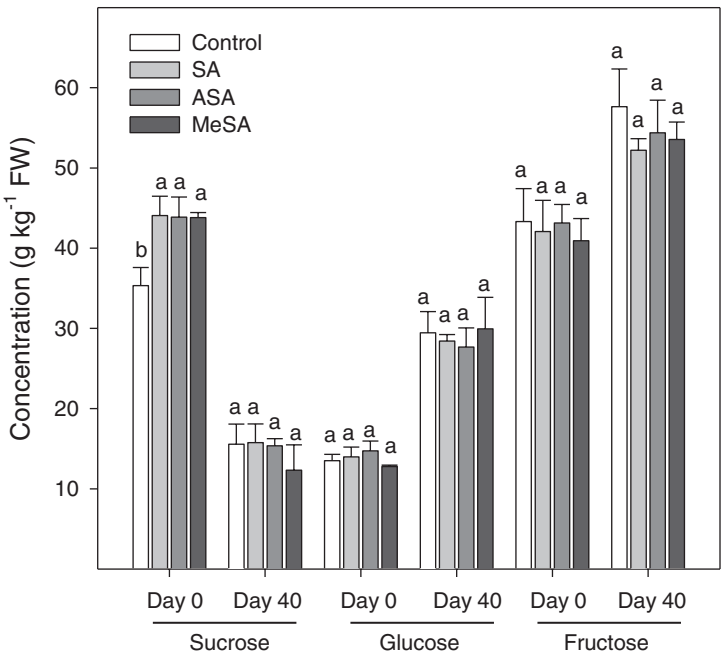

Figure 3. Effects of salicylic acid (SA), acetylsalicylic acid (ASA) and methylsalicylic acid (MeSA) preharvest treatments on concentration of sucrose, glucose and fructose in plums at harvest (Day 0) and after 40 days of storage at 2 °C plus 1 day at 20 °C (Day 40). Data are the mean ± SE of three homogeneous samples or replicates of 20 fruits. Different lower-case letters indicate significant differences ($P < 0.05$) among treatments for each sampling date.

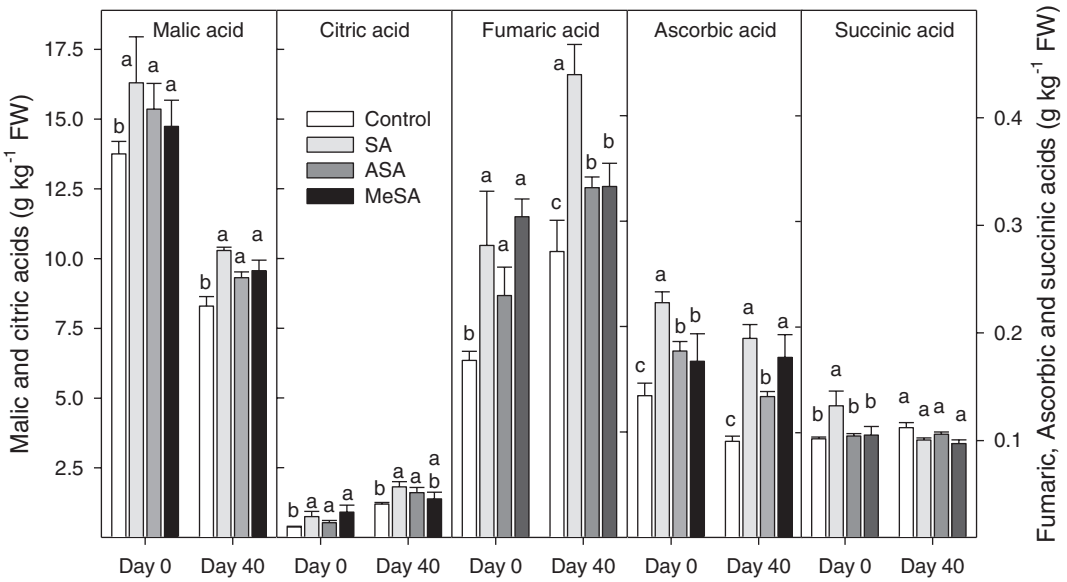

Figure 4. Effects of salicylic acid (SA), acetylsalicylic acid (ASA) and methylsalicylic acid (MeSA) preharvest treatments on concentration of malic, citric, fumaric, ascorbic and succinic acids in plums at harvest (Day 0) and after 40 days of storage at 2 °C plus 1 day at 20 °C (Day 40). Data are the mean ± SE of three homogeneous samples or replicates of 20 fruits. Different lower-case letters indicate significant differences ($P < 0.05$) among treatments for each sampling date.

carotenoids and ascorbic acid, as compared with other fruits of the Mediterranean diet, although important differences in their concentration are found depending on cultivar.[32,33] Eight individual phenolic compounds – one cinnamic acid, five flavonols and two anthocyanins – were identified and quantified using an HPLC–diode array detection–electrospray ionization–mass spectrometry (MS^n) system, at harvest time and after 40 days of storage. Neochlorogenic acid was the major hydroxycinnamic acid in 'Black Splendor' plum; it was found at higher concentrations in plums from salicylate-treated trees than in those from controls, and these differences were still noticeable after 40 days of cold storage. In a similar way, the flavonols quercetin 3-arabinoside, quercetin 3-ruthinoside, quercetin 3-rhamnoside and quercetin 3-xyloside were also increased by preharvest salicylate treatment, whereas no significant differences were observed between control and treated plums for quercetin 3-glucoside (Fig. 5). Most of these flavonols remained at higher concentrations in treated than in control plums after 40 days of storage. A similar profile of hydroxycinnamic acid derivatives and flavonols has been reported in other plum cultivars, such as 'Angeleno', 'Black Beaut' and 'Santa

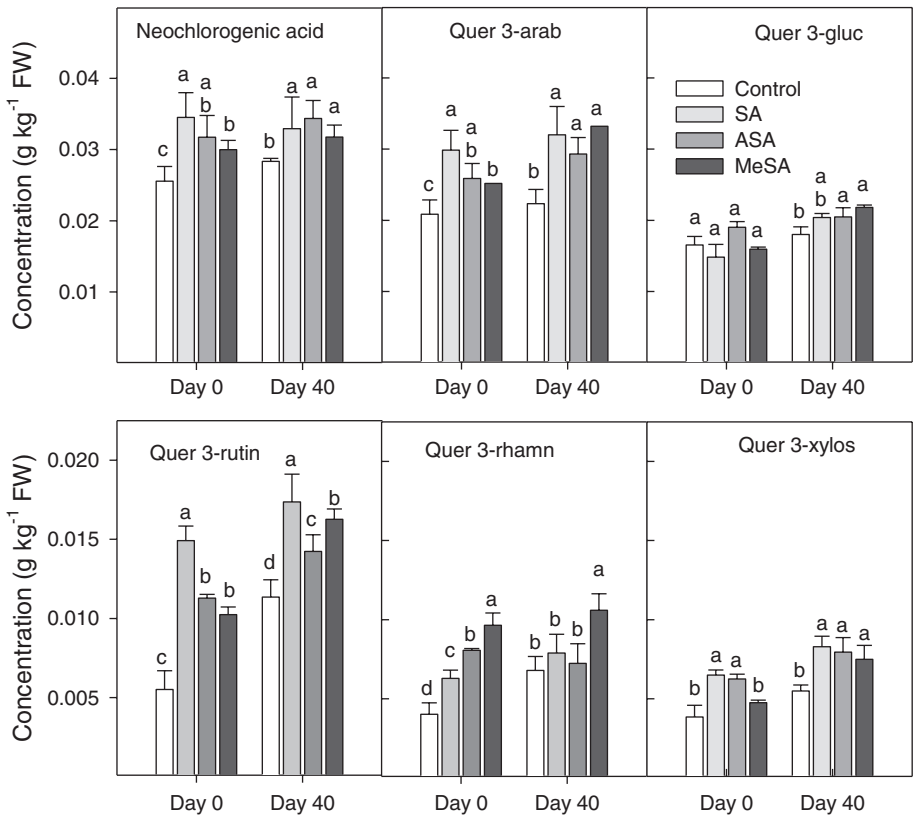

Figure 5. Effects of salicylic acid (SA), acetylsalicylic acid (ASA) and methylsalicylic acid (MeSA) preharvest treatments on concentration of neochlorogenic acid, quercetin 3-arabinoside (Quer 3-arab), quercetin 3-glucoside (Quer 3-gluc), quercetin 3-rutinoside (Quer 3-rutin), quercetin 3-rhamnoside (Quer 3-rhamn) and quercetin 3-xyloside (Quer 3-xylos) in plums at harvest (Day 0) and after 40 days of storage at 2 °C plus 1 day at 20 °C (Day 40). Data are the mean ± SE of three homogeneous samples or replicates of 20 fruits. Different lower-case letters indicate significant differences ($P < 0.05$) among treatments for each sampling date.

Rosa'.[34] Regarding anthocyanins, two individual anthocyanins were identified and quantified, the main one being cyanidin 3-glucoside, followed by cyanidin 3-rhutinoside, according to previous reports in a wide range of purple plums cultivars such as 'Blackamber', 'Larry Ann', 'Laetitia', 'Ruby Red' and 'Saphire', among others.[4,35] However, in other violet and yellow-violet plum cultivars, cyanidin 3-ruthinoside has been found at higher concentrations than cyanidin 3-glucoside.[36] In any case, it is interesting to note that SA, ASA and MeSA preharvest treatments led to increased levels of both anthocyanins in plum flesh, and these levels were maintained at significantly higher concentrations after 40 days of storage (Fig. 6). Moreover, the concentration of total carotenoids at harvest was also found at higher levels in plums from SA-, ASA- and MeSA-treated trees than in those from controls, and these differences were maintained after 40 days of storage (Fig. 6).

No previous reports are available regarding the effect of these treatments on anthocyanin content in plums, and with respect to their effect on phenolic content just one paper has been previously published, in which SA postharvest treatment of 'Santa Rosa' plum led to maintenance of higher total phenolic content during storage with respect to control fruits.[15] Nevertheless, some papers exist on other fruits for comparative purposes. Thus preharvest treatments with SA, ASA and MeSA of sweet cherry trees increased total phenolics and total anthocyanins of fruits at harvest and these differences were maintained during storage, leading to fruits with higher antioxidant activity with respect to controls.[21–23] Accordingly, SA preharvest treatments of table grape led to higher levels of these bioactive compounds at harvest and during postharvest storage.[27] On the other hand, postharvest treatments with SA, ASA or MeSA maintained total phenolics, anthocyanins and antioxidant activity during cold storage in pomegranate,[11,12] sweet cherry,[13] cornelian cherry fruit[37] and apricot,[10] and these enhancements were attributed to an increase in phenylalanine ammonia lyase activity, which is the main enzyme involved in the biosynthetic phenolic pathway. Since carotenoids and phenolics, including anthocyanins and ascorbic acid, have been proved to have beneficial effects against degenerative diseases,[32,38,39] preharvest treatment with salicylates would provide fruit with increased health beneficial effects for human consumption.

CONCLUSIONS

Overall, results show that preharvest treatments of plum trees with salicylates increased plum quality parameters at harvest, since higher fruit weight, firmness, organic acids and sucrose concentration were found in plums from treated trees. In addition, the concentrations of individual phenolics and anthocyanins were also found at higher levels in plums from treated trees than in those from control ones, both at harvest and after prolonged cold storage. Moreover, softening, colour evolution and acidity losses during storage occurred at lower rates in treated than in control plums, which were attributed to the effect of salicylate treatments on delaying and decreasing ethylene production during storage. Thus preharvest treatment with salicylates could be a safety,

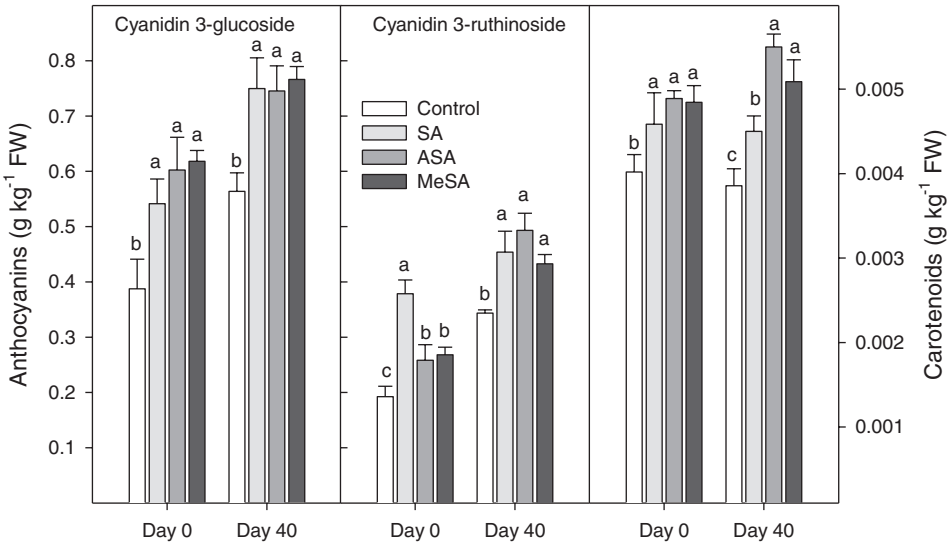

Figure 6. Effects of salicylic acid (SA), acetylsalicylic acid (ASA) and methylsalicylic acid (MeSA) preharvest treatments on concentration of individual anthocyanins and total carotenoids in plums at harvest (Day 0) and after 40 days of storage at 2 °C plus 1 day at 20 °C (Day 40). Data are the mean ± SE of three homogeneous samples or replicates of 20 fruits. Different lower-case letters indicate significant differences ($P < 0.05$) among treatments for each sampling date.

eco-friendly and new tool to improve and maintain plum quality attributes, and especially the content on antioxidant compounds with health beneficial effects.

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