doi: 10.17221/177/2015-HORTSCI

Salicylic acid treatment saves quality and enhances antioxidant properties of apricot fruit

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Abstract

Ezzat A., Ammar A., Szabó Z., Holb I. J. (2017): Salicylic acid treatment saves quality and enhances antioxidant properties of apricot fruit. Hort. Sci. (Prague), 44: 73–81.

The aim of this study was to investigate the effect of three salicylic acid (SA) concentrations (0.5, 1 and 2 mmol/l) on 7 fruit quality attributes of three apricot cultivars ('Flavor Cot', 'Jumbo Cot' and 'Bergeron') during cold storage (at 1°C for 7, 14, 21 and 28 days). Applications of 1 or 2 mmol/l SA significantly reduced chilling injury and fruit decay of apricot fruit as well as membrane electrolyte leakage and ascorbic acid content. Fruits treated with SA resulted in high total polyphenolic content, antioxidant capacity and carotenoids content while these parameters significantly decreased in non-treated control fruits. Overall, our results showed that SA prolonged the storability of fruits of three different apricot cultivars during cold storage.

Keywords: postharvest quality; physico-chemical parameters; antioxidant activity; carotenoids; chilling injury; fruit decay

Apricots as climacteric stone fruits have a limited post-harvest life and they remain fresh only for 2–4 weeks stored at 0°C depending on cultivar (Stanley 1991; Crisosto et al. 1995). Apricot fruits during cold storage were reported to show chilling injury symptoms and a high percentage of fruit decay (Stanley 1991). It is well known that apricot fruit starts to lose its physical and chemical quality (e.g. fruit firmness, increased fruit acidity and reduction in soluble solid content) directly after harvest and through the storage period (Stanley 1991; Ezzat et al. 2012).

Previous research argued the role of salicylic acid (SA) in physiological or biochemical processes including ion uptake, membrane permeability, enzyme activity, heat production, growth development (Arberg 1981). SA was extensively used for quality improvement in a number of crops (Peng, Jiang 2006). SA significantly reduced the quality loss and/or chilling injury in fruits such as peach (Li, Han 1999; Wang et al. 2006), banana (Srivastava, Dwivedi 2000), loquat (Cai et al. 2006), and apricot fruits (Satraj et al. 2013). SA and its derivatives are widely used to enhance pre- and postharvest quality of fruit by controlling firmness of harvested peaches and strawberry during storage (Li, Han 1999; Wang et al. 2006; Shafiee et al. 2010; Valero et al. 2011) and banana fruits during

Supported partly by the Hungarian Research Science Fund, Grant Nos K78399 and K108333.

doi: 10.17221/177/2015-HORTSCI

ripening (Srivastava, Dwivedi 2000). The postharvest quality properties were not widely investigated in apricot fruit and no detailed information is available for specific cultivars.

The aim of this study was to investigate the effect of three salicylic acid (SA) concentrations (0.5, 1 and 2 mmol/l) on 7 fruit quality attributes of three apricot cultivars ('Flavor Cot', 'Jambo Cot' and 'Bergeron') during cold storage (at 1°C for 7, 14, 21 and 28 days). The 7 attributes included chilling injury, fruit decay, membrane electrolyte leakage, ascorbic acid content and antioxidant capacity parameters such as total antioxidant capacity, total soluble phenol and carotenoid content.

MATERIALS AND METHODS

Plant material and chemical treatment. Fruits of apricot cultivars 'Flavor Cot', 'Jumbo Cot' and 'Bergeron' were hand harvested in a commercial orchard in Boldogkőváralja, Hungary at ripe stage and selected for uniform size, colour and examined to exclude all visual defects. The harvested fruits of each cultivar were divided into three groups (each group containing 150 fruits, altogether 3 × 150 = 450 fruits). Fruits were dipped into solutions of 0.5, 1 and 2 mmol/l SA for 15 minutes as well as control fruits were submerged into distilled water. Then fruits were placed in cold storage treatment, stored at 1°C and 90% humidity. The examinations were done on days 7, 14, 21 and 28. Each treatment was replicated three times and experiments were repeated twice.

Chilling injury, fruit decay index and membrane electrolyte leakage. For quality loss evaluation, parameters of chilling injury (CI), fruit decay (FD) and membrane electrolyte leakage were performed at each assessment date using 30 fruits per replicates.

Apricot fruit CI symptoms were manifested as flesh browning. The degree of CI was visually investigated on the fruit flesh following a double cut parallel to the axial diameter. The extent of flesh browning was divided into the following classes according to Wang et al. (2006): (0) no browning; (1) extensive browning covering <25% of the cut surface; (2) extensive browning covering ≥25% but <50% of cut surface; (3) extensive browning covering ≥50% but <75% of cut surface; (4) extensive browning covering ≥75% of cut surface. From this, a CI index was expressed as CI index = Σ[(CI level) × (number of fruit at the CI level)]/(4 × total number of fruit in the treatment).

Symptoms of FD were manifest as superficial browning on the fruit surface. The severity of the symptoms was assessed visually according to the five-stage scale of Wang et al. (2006): (0) no browning; (1) browning ≥25% of the fruit surface; (2) browning ≥25% but <50% of the fruit surface; (3) browning ≥50% but <75% of the fruit surface; (4) browning ≥75% of the fruit surface. From this, FD index was expressed as FD index = Σ[(FD level) × (number of fruit at the FD level)]/(4 × total number of fruit in the treatment).

Membrane electrolyte leakage was measured according to the method of Zhao et al. (2009). Three mm thick of mesocarp tissue were excised from the equatorial part of 5 fruits. Disks were put into aqueous 0.1M mannitol under constant shaking. The conductivity of the solution ($L1$) was measured with a conductivity meter (Hanna DiST, Sigma Aldrich, St. Louis, USA). Solutions were boiled for 10 min and then cooled to 20°C. The conductivity of tissues ($L2$) was measured. The percentage of electrolyte leakage was calculated using the following formula: % electrolyte leakage ($L1/L2$) × 100.

Ascorbic acid, total antioxidant capacity, total soluble phenol and carotenoid content

Ascorbic acid content (from 100 g fresh fruit) was estimated spectrophotometrically by dinitrophenylhydrazine (DNPH) method (Terada et al. 1978); the ascorbic acid content was expressed as ascorbic acid on fresh weight basis, mg per 100 g fresh weight. Results from the tests were means of 30 recorded fruits in three replicates at each assessment date.

The total antioxidant capacity related to ascorbic acid was determined spectrophotometrically using the FRAP (Ferric Reducing Antioxidant Power) (Benzie, Strain 1996). It is based on the reduction of the Fe^{3+}-TPTZ complex to the ferrous form at low pH. This reduction is monitored by measuring the absorption change at 593nm. Results are expressed as mg equivalents of ascorbic acid (mg AA/g FW).

Total amount of soluble phenols was determined using the Folin-Ciocalteu's (FC) reagent (Sigma-Aldrich, St. Louis, USA). Total phenolic contents of the fruit extracts were determined using the FC assay, which was described by Singleton and Rossi (1965). 40 µl of properly diluted fruit extract solu-

doi: 10.17221/177/2015-HORTSCI

tion were mixed with 1.8 ml of FC reagent. The reagent was pre-diluted, 10 times, with distilled water. After standing for 5 min at room temperature, 1.2 ml of (7.5% w/v) sodium carbonate solution was added. The solutions were mixed and allowed to stand for 1 h at room temperature. Then, the absorbance was measured at 760 nm, using a UV-visible spectrophotometer (Hitachi UV2800; Tokyo, Japan). A calibration curve was prepared, using a standard solution of gallic acid (20, 40, 60, 80 and 100 mg/l, R^2 = 0.995). Results were expressed on fresh weight basis (FW) as mg gallic acid equivalents for 100 g of sample (GAE/100 g FW).

Total carotenoids were extracted according to Akin et al. (2008) with some modifications. Briefly, five grams of sample were extracted with 100 ml of methanol/petroleum ether (1:9, v/v) using a high speed homogenizer, and the homogenized sample was transferred to a separating funnel. Petroleum ether layer was filtrated through sodium sulphate, transferred to volumetric flask and to a volume of 100 ml with petroleum ether. Finally, total carotenoid content was measured spectrophotometrically (Hitachi UV2800; Tokyo, Japan) at 450 nm. Carotenoid content was evaluated by using an extinction coefficient of 2,500, and the results were expressed as β-carotene equivalents (milligrams of β-carotene per 100 g fresh mass; Gross 1987).

Statistical analysis. Experiments were performed using a completely randomized design. The data were subjected to the analysis of variance using SPSS program (SPSS Inc., Chicago, USA). The effects of SA treatment (control, 0.5, 1 and 2 mmol/l SA), cultivar ('Jumbo Cot', 'Flavor Cot' and 'Bergeron'), assessment date (days 7, 14, 21 and 28) and their interactions on each parameter were evaluated. Means separation was performed by the Duncan's multiple range tests. Differences at $P < 0.05$ were considered as significant.

RESULTS AND DISCUSSION

Chilling injury, fruit decay index and membrane electrolyte leakage

Treatments of 1 and 2 mmol/l SA decreased significantly CI and FD values at all assessment dates

Table 1. Effect of treatments of 0.5, 1, and 2 mmol/l salicylic acid (SA) on chilling injury (CI) and fruit decay (FD) index of cvs 'Flavor Cot', 'Jumbo Cot' and 'Bergeron' apricot fruit in a cold storage treatment at days 7, 14, 21 and 28 at 1°C

Cultivar	SA concentration (mmol/l)	CI index				FD (%) index			
		day 7	day 14	day 21	day 28	day 7	day 14	day 21	day 28
'Flavor Cot'	water	5.26^{A}	18.12^{A}	29.12^{A}	38.24^{A}	17.25^{A}	22.35^{A}	30.25^{A}	45.58^{A}
	0.5	4.21^{A}	16.24^{A}	25.26^{A}	35.12^{A}	17.52^{A}	21.23^{A}	32.25^{A}	40.25^{A}
	1	2.01^{B}	10.26^{B}	16.24^{B}	29.22^{B}	13.22^{A}	15.15^{B}	20.52^{B}	28.26^{B}
	2	2.51^{B}	05.62^{B}	10.25^{C}	26.25^{B}	9.27^{A}	12.35^{B}	18.12^{B}	20.01^{B}
	$LSD_{0.05}$	1.71	5.24	5.67	5.52	8.48	5.89	8.35	9.87
'Jumbo Cot'	water	4.23^{A}	17.25^{A}	30.25^{A}	39.24^{A}	16.58^{A}	25.24^{A}	38.23^{A}	55.21^{A}
	0.5	4.25^{A}	17.21^{A}	25.23^{A}	36.12^{A}	16.25^{A}	23.27^{A}	38.01^{A}	55.68^{A}
	1	3.12^{B}	9.15^{B}	10.57^{B}	26.15^{B}	10.12^{B}	12.21^{B}	22.52^{B}	40.68^{B}
	2	2.01^{C}	5.36^{B}	13.25^{B}	20.15^{B}	8.12^{B}	11.15^{B}	19.24^{B}	31.26^{B}
	$LSD_{0.05}$	1.07	4.01	6.48	7.96	4.52	5.16	6.38	10.84
'Bergeron'	water	5.12^{A}	16.57^{A}	28.24^{A}	36.25^{A}	18.25^{A}	30.25^{A}	42.25^{A}	53.24^{A}
	0.5	5.05^{A}	14.28^{B}	18.35^{B}	28.54^{A}	17.27^{A}	32.21^{A}	34.58^{A}	40.22^{B}
	1	4.20^{B}	7.64^{B}	15.24^{B}	16.22^{B}	11.02^{B}	15.24^{B}	21.35^{B}	30.25^{C}
	2	2.13^{C}	3.15^{C}	10.25^{C}	13.45^{B}	07.58^{B}	10.25^{B}	19.25^{B}	29.28^{C}
	$LSD_{0.05}$	0.78	3.14	4.92	8.07	4.24	6.78	9.06	7.83

CI index – chilling injury index, CI index at day 0 was zero; FD index – fruit decay index percentage of the fruit, FD index at day 0 was zero; values within a column followed by different letters are significantly different at $P < 0.05$ according to the Duncan's multiple range tests; the results represent the means ± SD of triplicate assay; $LSD_{0.05}$ – least significant differences at $P = 0.05$

doi: 10.17221/177/2015-HORTSCI

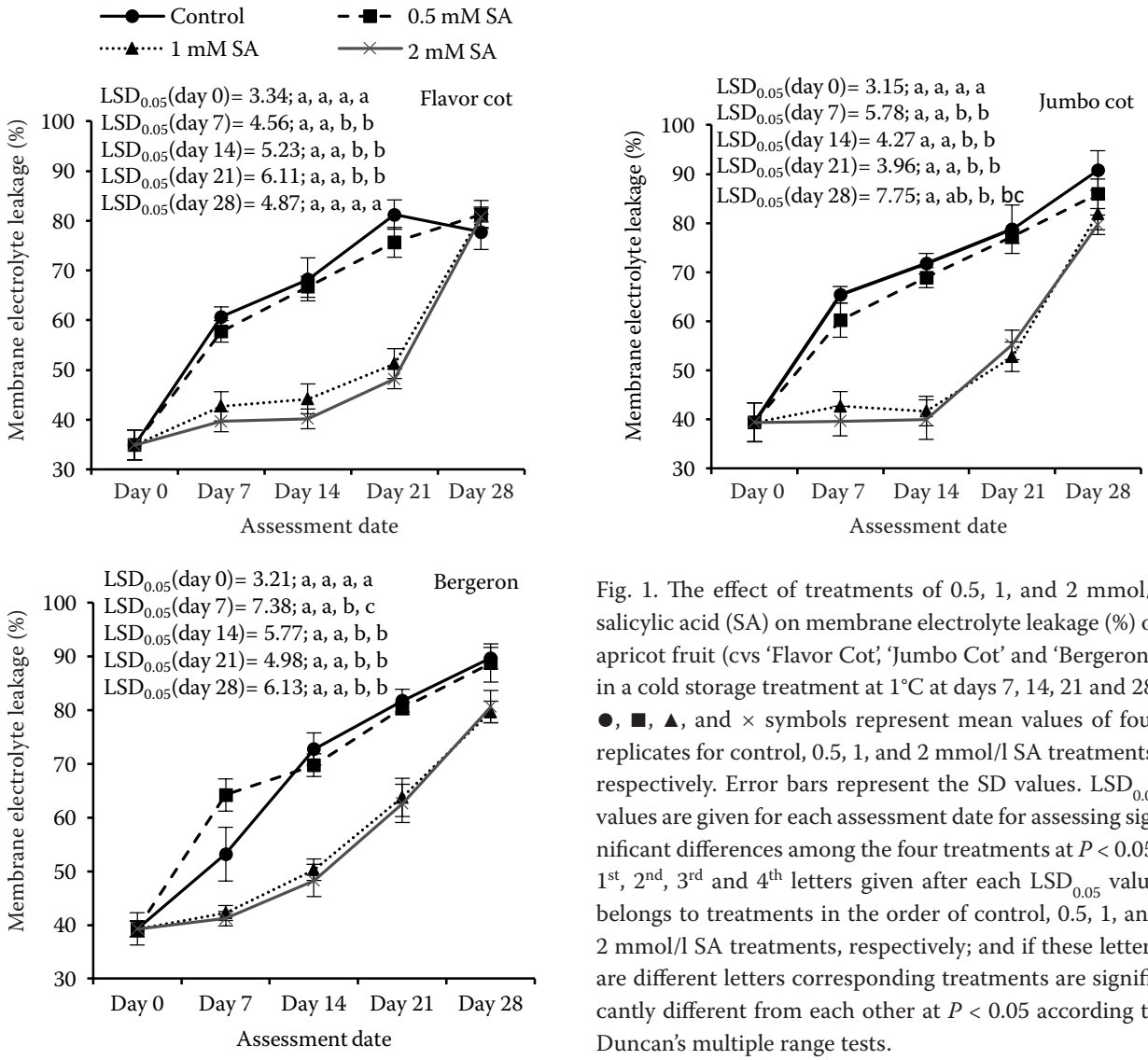

Fig. 1. The effect of treatments of 0.5, 1, and 2 mmol/l salicylic acid (SA) on membrane electrolyte leakage (%) of apricot fruit (cvs 'Flavor Cot', 'Jumbo Cot' and 'Bergeron') in a cold storage treatment at 1°C at days 7, 14, 21 and 28. ●, ■, ▲, and × symbols represent mean values of four replicates for control, 0.5, 1, and 2 mmol/l SA treatments, respectively. Error bars represent the SD values. $LSD_{0.05}$ values are given for each assessment date for assessing significant differences among the four treatments at $P < 0.05$. 1st, 2nd, 3rd and 4th letters given after each $LSD_{0.05}$ value belongs to treatments in the order of control, 0.5, 1, and 2 mmol/l SA treatments, respectively; and if these letters are different letters corresponding treatments are significantly different from each other at $P < 0.05$ according to Duncan's multiple range tests.

on all the three cultivars compared to water-treated fruits (Table 1). In addition, 0.5 mmol/l SA treatments decreased significantly CI on cv. 'Bergeron' at assessment days 14 and 21 but this effect was not seen for FD or for other cultivars (Table 1). Fruit treated with 1 and 2 mmol/l SA exhibited a significantly lower ($P < 0.01$) membrane electrolyte leakage at assessment days 7, 14 and 21 for all cultivars compared to untreated control or 0.5 mmol/l SA (Fig. 1).

Exogenous application of SA could enhance resistance to pathogens and control postharvest decay on fruits and vegetables (Asgharia, Aghdam 2010) as was also shown in this study on apricot (Table 1). In previous studies, SA treatments showed direct antifungal activity against plant pathogens (Yao, Tian 2005; Wang et al. 2006; Ezzat et al. 2013). 2 mmol/l SA showed direct toxicity to *Monilinia fructicola* and *M. fructigena* and significantly inhibited the growth of mycelium and spore germination of the pathogen *in vitro* (Yao, Tian 2005; Ezzat et al. 2013).

Wang et al. (2006) documented that SA treatment reduced chilling injury of peach fruits due to its ability to induce antioxidant activity, which our study confirmed. Sayyari et al. (2009) showed that 2 mmol/l SA were effective in reducing CI and electrolyte leakage in the husk of pomegranate which was similar to our results (Table 1, Fig. 1). Generally, CI occurs primarily at the cell membrane with changes in the fatty acid phospholipids composition (Lurie et al. 1987; Stanley 1991)

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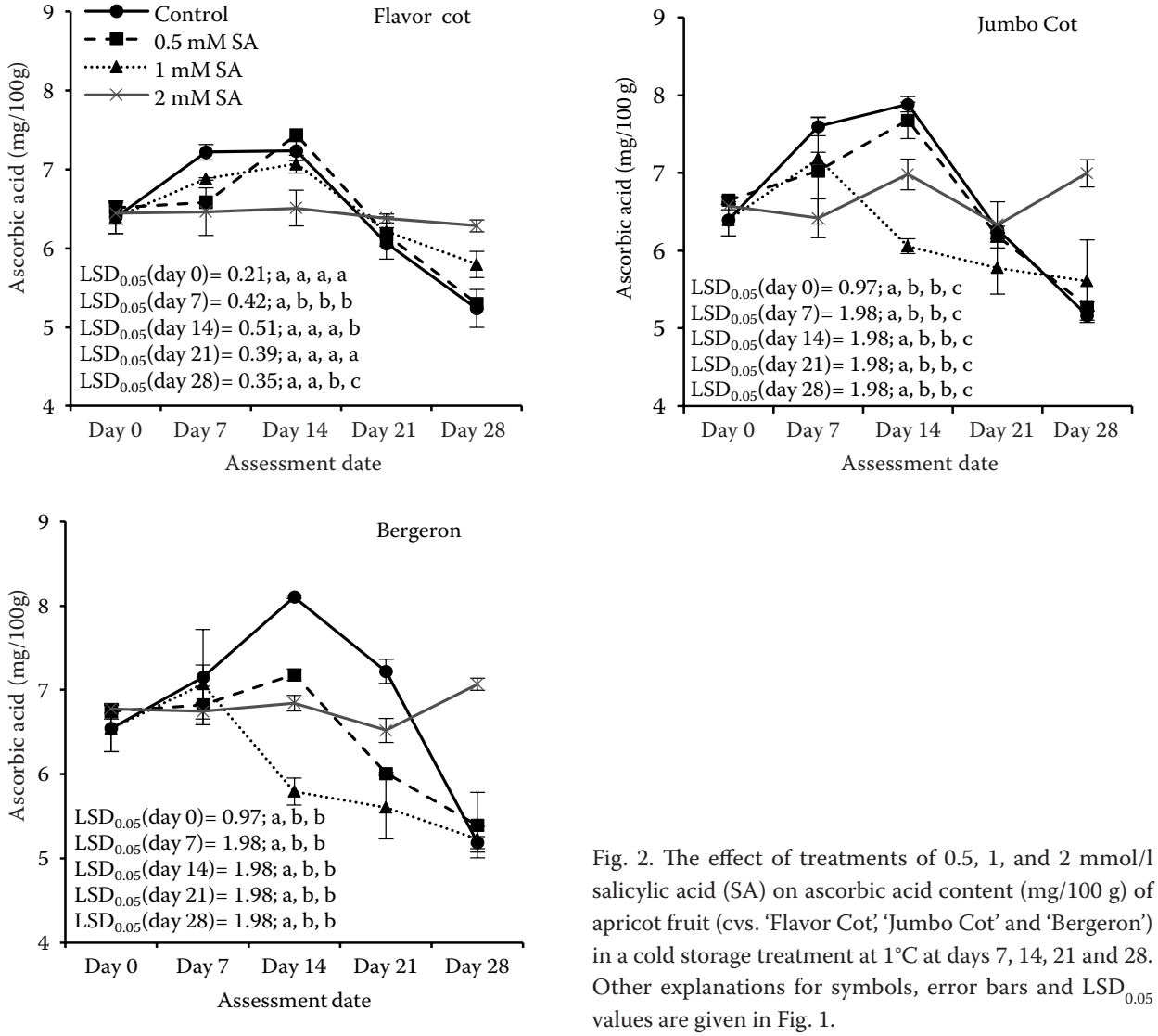

Fig. 2. The effect of treatments of 0.5, 1, and 2 mmol/l salicylic acid (SA) on ascorbic acid content (mg/100 g) of apricot fruit (cvs. 'Flavor Cot', 'Jumbo Cot' and 'Bergeron') in a cold storage treatment at 1°C at days 7, 14, 21 and 28. Other explanations for symbols, error bars and $LSD_{0.05}$ values are given in Fig. 1.

and the membrane damages initiate a cascade of secondary reactions leading to disruption of cell structures. This membrane damage was measured by the electrolyte leakage, which was significantly higher in the control fruit in this study compared to SA treated fruit, especially at the concentration of 2 mmol/l (Fig. 1). Our results showed the capability of SA in maintaining membrane integrity in agreement with studies on loquat (Cai et al. 2006) and pomegranate fruit (Sayyari et al. 2009).

Ascorbic acid content

Ascorbic acid content increased at assessment days 7 and 14 then decreased until day 28 for all the three cultivars in the water treatment (Fig. 2). Ascorbic acid content was lower in all SA treatments for all cultivars at assessment days 7 and 14 compared to water-treated control except for 0.5 mmol/l SA for cv. 'Flavor Cot'. Cultivar 'Flavor Cot' showed lower treatment effect compared to cvs 'Jumbo Cot' and 'Bergeron' (Fig. 2).

Ascorbic acid content of apricot gradually increases through the ripening stages as ascorbic acid is synthetized from uronic acid components of pectin degradation during ripening (Hegedűs et al. 2011). Our data showed that SA treatment reduced ascorbic acid content (Fig. 2); due to that SA may have delayed pectin degradation by delaying cell wall degrading enzymes (Li, Han 1999; Srivastava, Dwivedi 2000), and as a consequence,

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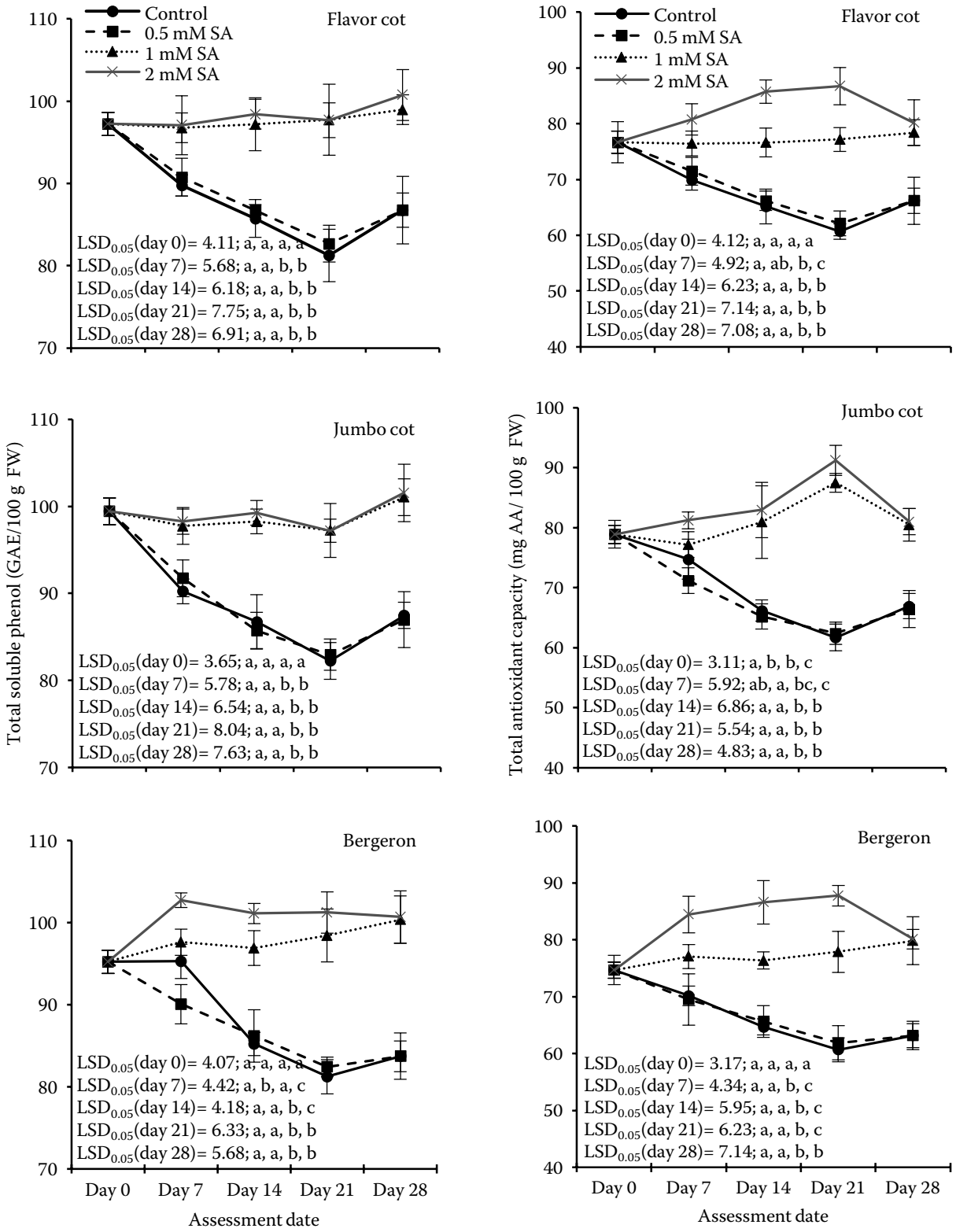

Fig. 3. The effect of treatments of 0.5, 1, and 2 mmol/l salicylic acid (SA) on total soluble phenol content (GAE/100 g FW) and total antioxidant capacity (mg AA/100 g FW) of apricot fruit (cvs. 'Flavor Cot', 'Jumbo Cot' and 'Bergeron') in a cold storage treatment at 1°C at days 7, 14, 21 and 28. Other explanations for symbols, error bars and $LSD_{0.05}$ values are given in Fig. 1.

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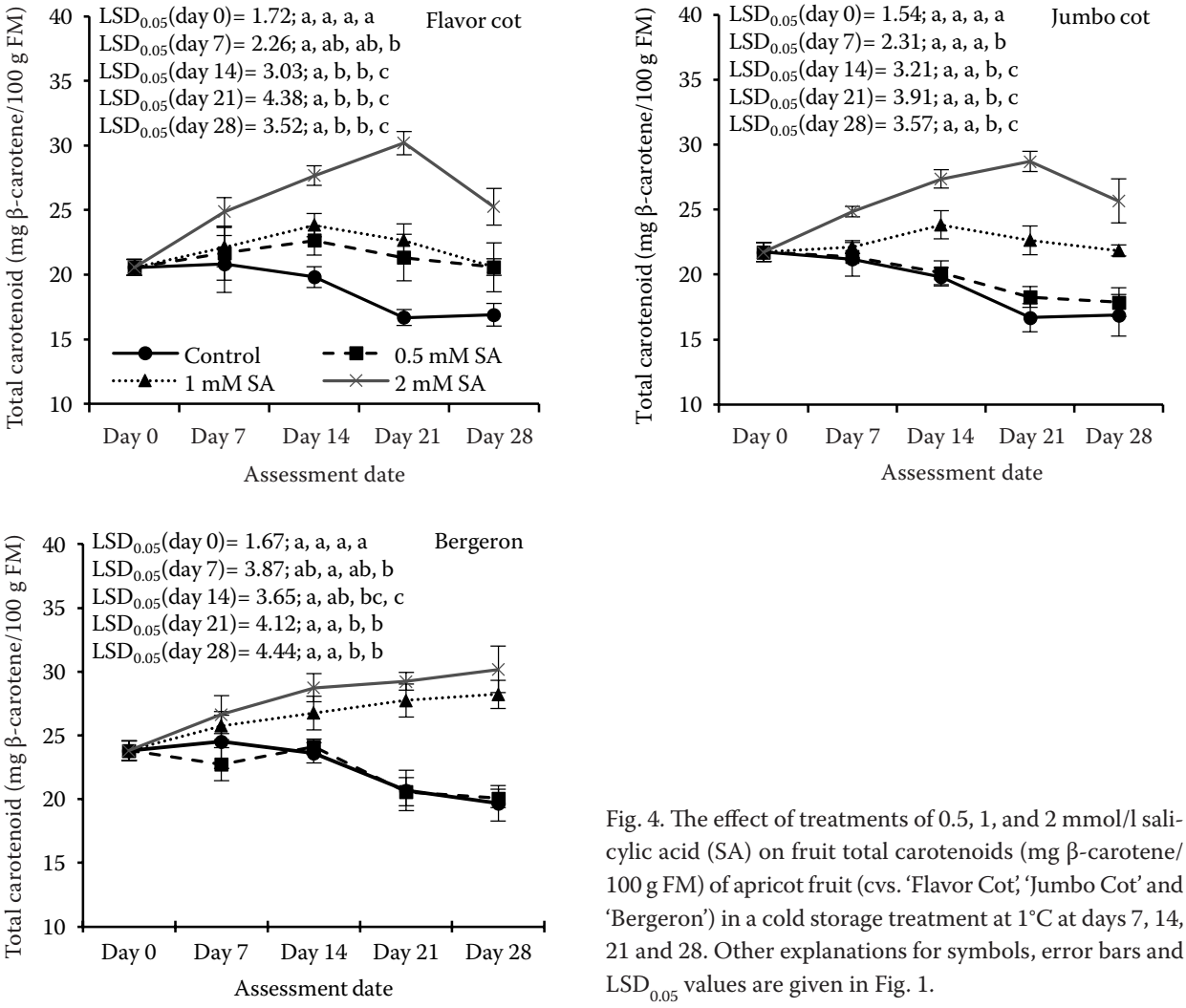

Fig. 4. The effect of treatments of 0.5, 1, and 2 mmol/l salicylic acid (SA) on fruit total carotenoids (mg β-carotene/100 g FM) of apricot fruit (cvs. 'Flavor Cot', 'Jumbo Cot' and 'Bergeron') in a cold storage treatment at 1°C at days 7, 14, 21 and 28. Other explanations for symbols, error bars and $LSD_{0.05}$ values are given in Fig. 1.

ascorbic acid content also decreased with increasing SA concentration. Differences in ascorbic acid contents among cultivars were in agreement with Hegedűs et al. (2011) though they did not investigated the effect of SA treatments on cultivars.

Total soluble phenol content and antioxidant capacity

Total soluble phenol content of the fruits remained at similar level (approx. 100 GAE/100 g FW) in the treatments of 1 and 2 mmol/l SA for all the three cultivars at all assessment dates (Fig. 3) and was significantly higher compared to either water-treated control or 0.5 mmol/l SA treatment.

Antioxidant capacity for the three cultivars was significantly higher in the treatments of 1 and 2 mmol/l SA at assessment days 7, 14, 21 and 28 compared to either water-treated control or 0.5 mmol/l SA treatment (Fig. 3). The antioxidant capacity increased in fruits treated with 2 mmol/l SA until day 21 for all cultivars, and then it started to decrease (Fig. 3). The antioxidant capacity remained unchanged in the treatment of 1 mmol/l SA for all cultivars except for cv. 'Jumbo Cot' (Fig. 3). Both the antioxidant capacity and the total soluble phenol content continuously decreased in water-treated control and in the 0.5 mmol/l SA treatment for all cultivars until the assessment day 21 (Fig. 3).

In previous research, Hayat et al. (2005) showed that exogenous application of SA enhanced the activity of antioxidant system in plants and this increased

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antioxidant activity which helped to protect SA treated fruits against chilling injury during storage. This finding was in agreement with our results in Fig. 3 and Table 1. The results of our study are in agreement with findings presented by Sayyari et al. (2011) showing that MeSA treatments increased total phenol and anthocyanin contents of stored pomegranates fruits compared to control treatments.

Carotenoid content

For all cultivars, total carotenoid content was significantly higher in the treatment of 2 mmol/l SA at assessment days 7, 14, 21 and 28 compared to either water-treated control or 0.5 mmol/l SA treatment (Fig. 4). Total carotenoid content increased in fruits treated with 2 mmol/l SA until day 21 for all cultivars, and then it started to decrease except for cv. 'Bergeron' (Fig. 4). Our investigation was in agreement with the findings of Hayat et al. (2005), who reported that SA may lower the level of oxidative stress in plants, which acts as a hardening process, improving the antioxidant capacity of the plants and helping to induce the synthesis of protective compounds (such as carotenoids).

CONCLUSION

Positive effects of 1 and 2 mmol/l SA treatments were reported in this study on fruit quality of three apricot cultivars during cold storage. These treatments reduced fruit decay and chilling injury, and increased total phenol and carotenoid contents as well as antioxidant capacity of stored fruit. Our results suggest that SA maintains postharvest quality and improves the health benefits of apricot fruit consumption by increasing the antioxidant capacity. However, further studies are necessary to understand more deeply the mechanism of action by which SA enhance the phytochemical contents of apricot fruit.

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Received for publication August 17, 2015
Accepted after corrections July 7, 2016