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Research Report

Impact of Postharvest Hot Salicylic Acid Treatment on Aril Browning and Nutritional Quality in Fresh-Cut Pomegranate

Afsaneh Yousefpour Dokhanieh[1*], Morteza Soleimani Aghdam[2], and Mohammad Ali Askari Sarcheshmeh[3]

[1]*Department of Horticultural Science, Tabriz Branch, Islamic Azad University, Tabriz, Iran*
[2]*Young Researchers and Elite Club, Ahar Branch, Islamic Azad University, Ahar, Iran*
[3]*Department of Horticultural Science, University College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran*

*Corresponding author: a.yousefpour@iaut.ac.ir

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Abstract. In this study, we investigated the impact of hot salicylic acid treatment on the browning and nutritional quality of fresh-cut pomegranate arils during 12 days of storage at 4°C. Aril browning was concurrent with malondialdehyde (MDA) and H_2O_2 accumulation. Due to reduced polyphenol oxidase (PPO) activity in conjunction with higher phenylalanine ammonia-lyase (PAL) activity, pomegranate arils treated with hot salicylic acid exhibited higher total phenolic and anthocyanin contents during storage at 4°C for 12 days, leading to arils with higher DPPH radical scavenging capacity. Pomegranate arils treated with hot salicylic acid also exhibited lower H_2O_2 accumulation, which was caused by higher activity of the antioxidant enzymes catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD), and glutathione reductase (GR) during the storage period. The higher ascorbic acid content in pomegranate arils treated with hot salicylic acid can be attributed to higher APX/GR system activity. Thus, hot salicylic acid treatment not only enhances the health-promoting attributes of arils due to increased antioxidant molecule accumulation, but it also delays aril browning by increasing ROS scavenging enzyme activity, which helps maintain membrane integrity, as revealed by reduced MDA accumulation.

Additional key words: antioxidant enzymes, bioactive molecules, membrane integrity, polyphenol oxidase

Introduction

Despite its high nutritional quality, the difficulty in peeling and extracting arils from pomegranate has restricted the consumption of fresh pomegranate. Therefore, providing consumers with fresh-cut pomegranate arils could increase the consumption of fresh pomegranates (Maghoumi et al., 2013). As fresh-cut pomegranate arils are highly prone to browning and the loss of nutritional quality, their shelf life is short. Preventing aril browning while maintaining their nutritional quality would be economically beneficial (Gil et al., 1996; Ghasemnezhad et al., 2013; Maghoumi et al., 2013).

Storing fresh-cut pomegranate arils at 4°C results in increased production of reactive oxygen species (ROS), which reduce membrane integrity and disrupt cellular compartmentalization. This causes polyphenol oxidase (PPO), which is located in plastids, to come in contact with phenolic substrates produced by phenylalanine ammonia-lyase (PAL) enzyme activity in the vacuole, leading to the formation of brown polymers, which in turn reduce the storage quality and marketability of pomegranate arils (Ghasemnezhad et al., 2013; Maghoumi et al., 2013). A higher PAL/PPO ratio would lead to higher phenol accumulation which, along with higher antioxidant system activity due to lower ROS accumulation, would help maintain membrane integrity due to lower membrane unsaturated fatty acid peroxidation, all of which would result in reduced aril browning. Therefore, a postharvest treatment aimed at enhancing antioxidant system activity while increasing the PAL/PPO activity ratio could be employed to reduce enzymatic browning of pomegranate arils during storage at 4°C.

Postharvest heat treatment (38-60°C) is a practical strategy for commercial use to maintain postharvest quality while minimizing postharvest losses in fruits and vegetables during postharvest life. However, heat treatment may have unfavorable

effects, such as flesh reddening and mealiness (Jin et al., 2009). Therefore, it would be useful to determine if the combination of heat and other non-toxic treatments could counteract the unfavorable effects of heat treatment alone. Salicylic acid (SA) is a natural signaling molecule that can be used during postharvest as an environmentally friendly treatment. SA has high commercial potential for enhancing nutritional quality while extending the shelf life of fruits and vegetables (Asghari and Aghdam, 2010; Dokhanieh et al., 2013). Therefore, SA can be used in combination with heat treatment, which act synergistically to delay losses in postharvest nutritional quality and to ameliorate postharvest stress.

In this study, we evaluated the effects of postharvest hot salicylic acid treatment on pomegranate aril browning and nutritional quality during storage at 4°C for 12 days.

Materials and Methods

Plant Material and Treatments

Sweet-tasting 'Malase Yazd' pomegranate fruits were harvested at the commercial mature stage from a commercial orchard in Yazd Province, Iran and immediately transported to the horticulture laboratory at the University of Tehran, Karaj, Iran. The arils were manually extracted as described by Gil et al. (1996). The arils were washed and disinfected (5°C, 2 min, 100 $\mu L \cdot L^{-1}$ NaOCl, pH 6.5), followed by rinsing with tap water (5°C, 1 min). The clean arils were randomly distributed into four treatments: Control: rinsed arils were dipped in water at 25°C for 30 s. Hot water: rinsed arils were dipped in water at 45°C for 30 s. SA: rinsed arils were dipped in 250 μM SA at 25°C for 30 s. Hot salicylic acid: rinsed arils were dipped in 250 μM SA at 45°C for 30 s. After air-drying, the arils were packed directly in polyethylene boxes (10 cm × 6 cm × 5 cm). All boxes were stored at 4°C and 95% relative humidity for 12 days. The experiment was conducted twice with similar results; therefore, only the results of the first experiment are presented.

Aril Browning

To assess aril browning, the surface color of 20 g of arils in a 4-cm-diameter plate was evaluated based on CIE color parameters: L* (light/dark), a* (red/ green), and b*(yellow/blue) values, which were obtained with a Minolta spectrophotometer (CR-400); and browning index, which was calculated as follow: Browning Index (BI) = [100 (x - 0.31)]/0.17, where x = (a* + 1.75L*)/(5.645L* + a* – 0.3012b*), according to Ding and Ling (2014).

MDA Content

Malondialdehyde (MDA) content was measured using the thiobarbituric acid (TBA) method, as described by Hodges et al. (1999). MDA content was expressed as $nmol \cdot g^{-1}$ fresh weight (FW).

PAL and PPO Anzyme Activity

PAL and PPO were extracted and assayed according to Nguyen et al. (2003). PAL and PPO activities were expressed as $U \cdot mg^{-1}$ protein.

Total Phenol, Anthocyanin, and Ascorbic Acid Contents

Total phenol content was assayed according to the Folin-Ciocalteu procedure (Chen et al., 2008). Total phenol content was expressed as mg of gallic acid equivalent (GAE) per 100 g of FW. Total anthocyanin content was assayed according to the pH differential method, as described by Lako et al. (2007). Total anthocyanin content was expressed as mg cyanidin-3-glucoside per 100 g of FW. Total ascorbic acid content was determined using the dinitrophenyl hydrazine (DNPH) method (Terada et al., 1978). Total ascorbic acid content was expressed as mg per 100 g of FW.

Antioxidant System Activity

To analyze antioxidant enzyme activities, 5 g of aril tissue was homogenized in 50 mM phosphate buffer (pH 7.8) containing 0.2·mM EDTA and 2% PVP. The homogenate was centrifuged at 12,000 × g for 20 min at 4°C, and the supernatant was used for CAT, APX, SOD, and GR activity measurements. CAT, APX, and SOD activity was measured according to Zhang et al. (2013). One unit of CAT activity was defined as a decrease in absorbance at 240 nm of 0.01 per min. One unit of APX activity was defined as the amount of enzyme that oxidizes 1 μmol of ascorbate per minute. One unit of SOD activity was defined as the amount of enzyme that causes a 50% inhibition of nitro blue tetrazolium (NBT) reduction under assay conditions. GR activity was measured by following the decrease in absorbance at 340 nm due to NADPH oxidation according to Sofo et al. (2005). One unit of GR activity was defined as the amount of enzyme that oxidizes 1 nmol of NADPH per min at 25°C. CAT, APX, SOD, and GR activity was expressed as $U \cdot mg^{-1}$ protein. Protein content was estimated according to Bradford (1976) using bovine serum albumin (BSA) as a standard. H_2O_2 content was measured according to Patterson et al. (1984). H_2O_2 content was expressed as μmol per g of FW. Free radical $DPPH^{\bullet}$ scavenging activity was measured according to Nakajima et al. (2004). The percent of reduction of $DPPH^{\bullet}$ was calculated according to the following equation, where Abs control is the absorbance of $DPPH^{\bullet}$ solution without extract.

$$\% \text{ inhibition of DPPH}^{\bullet} = (\text{Abs control} - \text{Abs sample})/\text{Abs control} \times 100$$

Statistical Analysis

The experiments were performed using a completely randomized design. Analysis of variance (ANOVA) was carried out with SPSS software version 18 (SPSS Inc., Chicago, IL, USA). Differences between means were assessed by Tukey multiple range test, with differences considered significant at $p < 0.05$.

Results and Discussion

Postharvest treatments delayed aril browning during storage at 4°C for 12 days ($p < 0.01$; Fig. 1). Hot water or SA treatment alone significantly reduced aril browning during storage at 4°C for 12 days. However, hot salicylic acid treatment was more efficient at delaying aril browning during the storage period ($p < 0.01$; Fig. 1). Aril browning during storage at 4°C for 12 days was concurrent with MDA accumulation (Fig. 2). Compared to the controls, pomegranate arils treated with hot salicylic acid exhibited lower MDA accumulation ($p < 0.01$; Fig. 2). During the storage period, hot salicylic acid treatment ameliorated the browning of pomegranate arils, as indicated by the delay in MDA accumulation. Treatment with hot salicylic acid resulted in reduced MDA content, i.e., inhibited lipid peroxidation, under storage at 4°C, which clearly indicates that hot salicylic acid treatment strongly protects pomegranate arils from oxidative damage and thus reduces aril browning.

The total phenol and anthocyanin contents in the pomegranate arils decreased during storage at 4°C for 12 days (Table 1). Treatment with heat or SA alone delayed the decrease in total phenol and anthocyanin contents, which were significantly ($p < 0.05$) higher than that in control arils during storage (Table 1). The total phenol and anthocyanin contents were higher in arils treated with hot salicylic acid than in hot water- or SA -treated arils (Table 1). PPO and PAL in control arils decreased during storage at 4°C for 12 days (Table 2). PPO activity was significantly lower ($p < 0.01$), and PAL activity was significantly higher ($p < 0.01$), in hot salicylic acid-treated pomegranate arils than in hot water- or SA -treated arils. Also, DPPH scavenging activity decreased during storage at 4°C. Treatment with hot water or SA alone delayed the decrease in DPPH scavenging activity, which was significantly ($p < 0.01$) higher than that in control arils during storage (Table 1). Higher DPPH scavenging activity was detected in arils treated with hot salicylic acid than in hot water- or SA treated arils due to higher DPPH scavenging activity during storage.

High PAL activity in pomegranate arils during storage at 4°C for 12 days leads to high phenol accumulation, can considerably reduce browning. Zhou et al. (2015) reported that SA treatment delays external and internal enzymatic browning of fresh-cut chestnut via competitive inhibition of PPO by SA. In the current study, hot salicylic acid treatment promoted an increase in PAL activity, leading to higher total phenol and anthocyanin contents in pomegranate arils during storage at 4°C for 12 days. The accumulation of total phenols and anthocyanins in pomegranate arils treated with hot salicylic acid was associated with lower PPO enzyme activity, which led to reduced browning. Phenols and anthocyanins have antioxidant activity, and their oxidation by PPO decreases the antioxidant capacity of arils and increases the susceptibility of arils to browning (Tomás-Barberán and Espín, 2001). The reduction in pomegranate aril browning by hot salicylic acid treatment could have be due to the increased DPPH scavenging activity of the arils, which led to decreased membrane lipid peroxidation, as revealed by

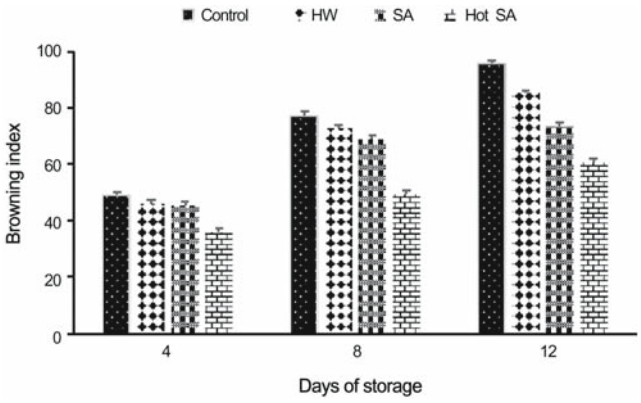

Fig. 1. Effects of postharvest salicylic acid (SA), hot water (HW), and hot salicylic acid (Hot SA) treatment on the browning of pomegranate arils stored at 4 ± 0.5°C for 12 days. The values are the means ± SE of three replicates. Tukey test at $p = 0.05$ level.

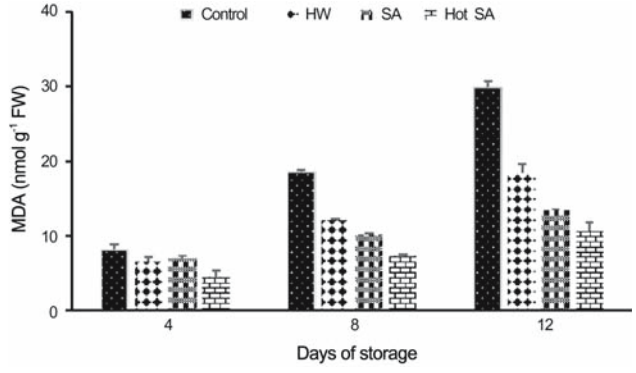

Fig. 2. Effects of postharvest salicylic acid (SA), hot water (HW), and hot salicylic acid (Hot SA) treatment on malondialdehyde content in pomegranate arils stored at 4 ± 0.5°C for 12 days. The values are the means ± SE of three replicates. Tukey test at $p = 0.05$ level.

Table 1. Effects of postharvest salicylic acid (SA), hot water (HW), and hot salicylic acid (Hot SA) treatment on total phenol, anthocyanin, and ascorbic acid contents and DPPH scavenging capacity in pomegranate arils stored at 4 ± 0.5°C for 12 days

Time (days)	Treatment	Nutritional quality attributes			
		TP (mg·100 g^{-1} FW)	TA (mg·100 g^{-1} FW)	AA (mg·100 g^{-1} FW)	DPPH (%)
0	-	262.62 ± 1.52	178.25 ± 1.24	165.32 ± 1.05	86.52 ± 0.74
4	Control	191.67 ± 3.28 ef [z]	129.27 ± 4.04 bc	95.89 ± 1.09 d	82.71 ± 0.34 a
	HW	219.67 ± 3.46 bc	142.05 ± 1.32 bc	108.31 ± 0.59 c	83.82 ± 0.29 a
	SA	228.67 ± 3.14 b	157.05 ± 3.56 a	119.48 ± 0.45 b	84.46 ± 1.16 a
	Hot SA	251.21 ± 5.13 a	161.78 ± 4.57 a	128.58 ± 0.55 a	85.06 ± 0.34 a
8	Control	171.42 ± 4.57 gh	94.07 ± 2.49 de	57.12 ± 2.52 g	68.46 ± 1.28 c
	HW	199.65 ± 4.54 def	105.53 ± 3.50 de	69.25 ± 0.78 f	72.48 ± 0.36 b
	SA	208.42 ± 5.25 cde	119.45 ± 0.61 c	83.77 ± 0.82 e	71.30 ± 0.40 b
	Hot SA	217.72 ± 2.65 bcd	128.99 ± 1.90 bc	97.68 ± 0.72 d	71.82 ± 1.05 b
12	Control	127.47 ± 1.54 i	66.38 ± 1.03 f	26.49 ± 0.18 j	55.19 ± 0.55 e
	HW	155.47 ± 2.57 h	86.18 ± 1.25 e	44.32 ± 0.55 i	61.65 ± 0.33 d
	SA	164.45 ± 2.68 gh	90.9 ± 0.32 de	48.42 ± 0.65 h	63.35 ± 0.50 d
	Hot SA	181.94 ± 1.22 fg	97.59 ± 1.12 de	60.27 ± 0.84 g	66.87 ± 0.43 c

[z]The values are the means ± SE of three replicates. Different letters indicate significant differences at a significance level of $p = 0.05$ using Tukey test.

Table 2. Effect of postharvest salicylic acid (SA), hot water (HW), and hot salicylic acid (Hot SA) treatment on PAL and PPO activity in pomegranate arils stored at 4 ± 0.5°C for 12 days

Time (days)	Treatment	Browning enzymes	
		PAL (U·mg^{-1} protein)	PPO (U·mg^{-1} protein)
0	-	105.21 ± 0.84	10.25 ± 0.42
4	Control	68.97 ± 0.80 f[z]	25.31 ± 0.59 i
	HW	76.85 ± 0.92 d	20.96 ± 0.32 j
	SA	88.68 ± 0.58 b	17.86 ± 0.19 k
	Hot SA	98.52 ± 0.44 a	12.75 ± 0.27 l
8	Control	45.28 ± 0.61 i	58.55 ± 0.66 d
	HW	58.72 ± 0.33 g	45.42 ± 0.50 f
	SA	78.42 ± 0.50 d	40.42 ± 0.45 g
	Hot SA	83.05 ± 0.42 c	28.85 ± 0.24 h
12	Control	24.74 ± 0.43 k	97.15 ± 0.96 a
	HW	36.72 ± 0.64 j	83.41 ± 0.49 b
	SA	50.96 ± 0.62 h	78.28 ± 0.53 c
	Hot SA	69.76 ± 0.05 e	53.91 ± 0.33 e

[z]The values are the means ± SE of three replicates. Different letters indicate significant differences at a significance level of $p = 0.05$ using Tukey test.

the reduced MDA content. Hot salicylic acid treatment increased the total phenol and anthocyanin contents in pomegranate arils due to the higher PAL/PPO enzymatic activity ratio. This, in turn, increased DPPH scavenging capacity, which may have contributed to the reduced browning in pomegranate arils stored for 12 days at 4°C.

As shown in Table 1, ascorbic acid content decreased during storage at 4°C for 12 days, but pomegranate arils treated with hot salicylic acid exhibited higher ascorbic acid contents during the storage period ($p < 0.01$). Rao et al. (2011) suggested that reducing ascorbic acid oxidase (AAO) enzyme activity using SA and $CaCl_2$ treatments helps maintain the nutritional quality of sweet pepper by reducing ascorbic acid oxidation, as this compound is not only a powerful

Table 3. Effect of postharvest salicylic acid (SA), hot water (HW), and hot salicylic acid (Hot SA) treatment on antioxidant system activity in pomegranate arils stored at 4 ± 0.5°C for 12 days

Time (days)	Treatment	Antioxidant system activity				
		APX ($U \cdot mg^{-1}$ protein)	CAT ($U \cdot mg^{-1}$ protein)	SOD ($U \cdot mg^{-1}$ protein)	GR ($U \cdot mg^{-1}$ protein)	H_2O_2 ($\mu mol \cdot g^{-1}$ FW)
0	-	265.32 ± 0.32	279.32 ± 0.35	54.21 ± 0.21	4.95 ± 0.04	22.32 ± 0.78
4	Control	195.37 ± 0.53 d[z]	225.75 ± 0.53 cd	39.87 ± 0.10 d	2.59 ± 0.01 f	30.37 ± 0.42 g
	HW	203.52 ± 0.63 c	230.52 ± 0.63 c	43.49 ± 0.12 b	2.87 ± 0.02 d	30.33 ± 1.01 g
	SA	215.68 ± 0.55 b	242.68 ± 0.55 b	44.81 ± 0.11 b	3.02 ± 0.01 c	30.20 ± 0.20 g
	Hot SA	241.08 ± 0.21 a	268.08 ± 0.21 a	49.65 ± 0.04 a	4.32 ± 0.02 a	29.31 ± 1.45 g
8	Control	143.73 ± 0.67 g	180.10 ± 0.67 g	30.02 ± 0.13 g	2.08 ± 0.02 h	63.07 ± 0.56 c
	HW	165.48 ± 0.62 f	192.48 ± 0.62 f	36.24 ± 0.12 ef	2.48 ± 0.01 g	53.98 ± 0.87 e
	SA	179.06 ± 0.27 e	206.06 ± 0.37 e	37.83 ± 0.05 e	2.62 ± 0.03 e	49.61 ± 0.51 f
	Hot SA	201.83 ± 0.25 c	228.83 ± 0.25 c	42.17 ± 0.05 c	3.79 ± 0.02 b	46.93 ± 1.10 f
12	Control	57.68 ± 0.43 k	79.06 ± 0.43 k	13.62 ± 0.08 j	1.24 ± 0.02 k	77.53 ± 1.01 a
	HW	67.02 ± 1.43 j	98.02 ± 1.43 ij	17.47 ± 0.27 i	1.46 ± 0.02 j	69.27 ± 0.58 b
	SA	77.92 ± 0.37 i	104.92 ± 0.37 i	18.55 ± 0.07 i	1.58 ± 0.03 i	65.53 ± 0.80 c
	Hot SA	134.9 ± 0.19 h	161.90 ± 0.19 h	29.41 ± 0.04 h	2.87 ± 0.03 d	58.89 ± 0.39 d

[z]The values are the means ± SE of three replicates. Different letters indicate significant differences at a significance level of $p = 0.05$ using Tukey test.

antioxidant molecule, but it also exhibits anti-browning activity (Rao et al., 2011). Huang et al. (2008) reported that SA treatment maintains ascorbic acid content in navel orange fruit. Huang et al. (2008) suggested that SA treatment increases cytosolic Ca^{+2} concentrations, which, by enhancing GR enzyme activity, could increase GR/APX system activity, leading to higher ascorbate/dehydroascorbate (AA/DHA) and glutathione/glutathione disulfide (GSH/GSSG) redox ratios in navel orange fruit. Shao et al. (2013) reported that the amelioration of chilling injury in loquat fruit treated with hot air (45°C for 3 h) results from higher APX and GR activities along with higher ascorbic acid and glutathione contents, which contribute to lower H_2O_2 accumulation along with higher reducing sugar (glucose and fructose) accumulation. Higher reducing sugar (glucose and fructose) contents result in higher accumulation of ascorbic acid and glutathione, which are crucial for overcoming chilling oxidative stress (Couée et al., 2006). Higher levels of glucose accumulate in loquat fruit treated with hot air due to the enhanced oxidative pentose phosphate pathway, resulting in higher production of NADPH, which can be consumed by GR in the AA-GSH cycle (Cruz de Carvalho, 2008). In addition, glucose can be used in ascorbic acid biosynthesis (Smirnoff et al., 2001) and in the carbon skeleton of glutamic acid, which can be consumed for GSH biosynthesis (Noctor and Foyer, 1998). Therefore, higher glucose accumulation in loquat fruit treated with hot air may contribute to its higher ascorbic acid and glutathione levels, higher APX and GR activity, higher AA/GSH cycle activity, and reduced oxidative chilling stress. Thus, the higher ascorbic acid content in pomegranate arils treated with hot salicylic acid may be attributed to higher GR/APX system activity, lower AAO enzyme activity, and/or higher reducing sugar (glucose and fructose) accumulation.

As shown in Table 3, the activities of the antioxidant enzymes CAT, APX, SOD, and GR decreased during storage at 4°C for 12 days, which coincided with increased H_2O_2 contents. Treatment with hot water or SA alone delayed the decrease in CAT, APX, SOD, and GR activity and H_2O_2 accumulation. CAT, APX, SOD, and GR activity was significantly ($p < 0.05$) higher and H_2O_2 content was significantly ($p < 0.05$) lower in pomegranate arils treated with hot water or SA alone compared to the control (Table 3). There was a marked increase in CAT, APX, SOD, and GR activity ($p < 0.01$) and a decrease in H_2O_2 content ($p < 0.01$) in arils treated with hot salicylic acid (Table 3). Plants ameliorate oxidative stress and minimize ROS accumulation by employing antioxidant enzymes such as SOD, CAT, APX, and GR (Foyer and Noctor, 2005). Higher SOD, CAT, APX, and GR activity could result in lower H_2O_2 contents along with higher DPPH scavenging capacity in hot salicylic acid-treated pomegranate arils. Cao et al. (2010) reported that treatment with hot air in combination with SA (1 mM for 5 min) ameliorated chilling injury in peach fruit by increasing antioxidant enzyme activity (SOD, CAT, APX, and GR) and reducing lipoxygenase (LOX) activity. LOX is responsible for O_2^- production, which can be converted into H_2O_2 by SOD activity. H_2O_2 can be scavenged by CAT, APX, and GR (Mittler, 2002). In peach fruits treated with hot air in

combination with SA, higher SOD/LOX ratios were concurrent with lower O_2^- accumulation, and higher CAT, APX, and GR activity was concurrent with lower H_2O_2 accumulation (Cao et al., 2010). In the current study, pomegranate arils treated with hot salicylic acid showed significantly higher CAT, APX, SOD, and GR activity and lower MDA and H_2O_2 accumulation than the control, suggesting that hot salicylic acid treatment minimizes membrane lipid peroxidation by promoting antioxidant system activity, resulting in reduced aril browning.

Conclusion

In conclusion, the amelioration of pomegranate aril browning by hot salicylic acid treatment may be attributed to higher antioxidant enzyme activity and higher antioxidant molecule accumulation. Therefore, hot salicylic acid treatment could be a useful strategy for ameliorating browning in pomegranate arils. Hot salicylic acid treatment led to higher antioxidant enzymes activity, along with higher antioxidant molecule accumulation, in pomegranate arils during storage at 4°C, which could help increase ROS scavenging during storage, in turn delaying aril browning. Hot salicylic acid enhanced antioxidant system activity by scavenging ROS, which led to a decrease in oxidative stress during storage at 4°C and ultimately maintained postharvest quality by preventing the unfavorable impacts of ROS on aril nutritional quality.

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