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Effect of Salicylic Acid on Storage Life and Postharvest Quality of Grape (*Vitis vinifera* L. cv. Bidaneh Sefid)

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Postharvest life of table grapes is usually shortened by berry softening, berry drop, stem browning and its desiccation, and also by fungal decay. In the present study, effects of salicylic acid (SA) treatment on grape postharvest storage life and its quality were studied. Harvested clusters of 'Thompson Seedless', or 'Bidaneh Sefid' in Iran, were treated by SA at four concentrations, 0, 1, 2 and 4 mM, stored for 45 days at 0 °C and then exposed for two days at 20 °C to estimate as their shelf life. The SA treatment significantly increased the storage life of the clusters. All of the three SA concentrations were effective to reduce water loss, fungal decay and the softened berry rate. SA used at a concentration of 4 mM effectively inhibited development of decay infection. SA applied at concentrations of 2 and 4 mM significantly delayed rachis browning. The lowest berry shatter was observed in all of the SA–treated clusters. SA used at the high concentration of 4mM obviously maintained berry appearance. In addition, SA treatment also enhanced the increase of total phenolic content of berry skin after the shelf life period. The highest contents of phenolic components were detected in the berries treated by 4 mM SA. The concentrations of quercetin and catechin were higher in SA–treated berries. These results demonstrate that the postharvest treatment of grape berries by SA has potential for increasing storage life of table grapes and maintaining their quality.

Keywords: table grape, salicylic acid, postharvest life, fruit quality, phenolic properties.

INTRODUCTION

Grape is an important fruit crop cultivated on a large scale in Iran as well as in the world. In Iran, grape is mainly consumed as fresh fruit produced from table grape cultivars and as raisin produced from raisin grape cultivars such as 'Thompson Seedless' or 'Sultania'. The postharvest life of table grapes is relatively short due to water loss, skin browning, rachis dehydration and browning, berry shatter, and fungal decay. The use of chemicals such as carbon dioxide, fungicides and growth regulators is necessary to maintain the fruit quality during a long–distance transportation and a long–term storage (Cirami *et al*., 1992; Crisoto *et al*., 2001; Meng *et al*., 2008; Zoffoli *et al*., 2008 and 2009).

The use of SO_2 during cold storage as a fumigant or generator is the most universal method to control fungal decay and also to maintain the table grape quality (Cirami *et al*., 1992; Gao *et al*., 2003; Franck *et al*., 2005; Zoffoli *et al*., 2008). Although SO_2 excellently controls fungal decay and prevents rachis browning, its residues are toxic and dangerous to human health. Therefore, SO_2 application is restricted in several countries (Meng *et al*., 2008). Furthermore, development of SO_2 phytotoxicity symptoms under the high concentration of SO_2 such as berries bleaching, rachis browning (Smilanick *et al*., 1990), hairline cracking (Zoffoli *et al*., 2008) and unfavorable taste (Gao *et al*., 2003) are other problems of using SO_2.

Scientists are going to try to find an alternative method to SO_2 and the ways to reduce its application. Salicylic acid (SA), which was firstly found in *Salix alba* and widely found in horticultural crops with various concentrations (Scotter *et al*., 2007), is a simple phenolic compound with a phytohormone–like function in plant growth and development. Many important roles of SA during a number of regulatory processes of plant growth and development involve ion absorption, heat production, seed germination and sex polarization (Raskin, 1992a and 1992b). SA also activates the expression of several defense–related genes (Loake and Grant, 2007). SA has been showed to inhibit ethylene biosynthesis and action (Leslie and Romani, 1988; Srivastava and Dwivedi, 2000; Zhang *et al*., 2003). On the other hand, SA is known as a medicinal chemical to damage the cells of human in high concentration mainly due to its high acidity and, thus, it has been used to remove a wart and aged horny cells of the skin. SA is also used as a febrifuge and pain–reliever for human in more gentle low acidity form of acetylsalicylic acid or aspirin.

Postharvest application of SA increased storage life of kiwifruit (Zhang *et al*., 2003), strawberry (Babalar *et al*., 2007), Chinese water chestnut (Peng and Jiang, 2006), peach (Han *et al*., 2003; Wang *et al*., 2006), mandarin (Zhang and Zhang, 2004), pomegranate (Sayyari *et al*., 2009) and sweet cherry fruits (Yao and Tian, 2005).

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In harvested fruits, on the other hand, the application of SA delayed ripening (Srivastava and Dwivedi, 2000; Zhang *et al*., 2003; Peng and Jiang, 2006), reduced fruit softening rate (Zhang *et al*., 2003; Wang *et al*., 2006), increased resistance to chilling injury (Wang *et al*., 2006), induced the accumulation of phenolic compounds (Chen *et al*., 2006) and delayed discoloration with the inhibition of browning (Peng and Jiang, 2006). The role of SA in controlling fungi decay may be due to activation of antioxidant defense responses (Xu and Tian, 2008) or its direct antifungal effects on fungus development (Amborabe *et al*., 2002).

Therefore, effective control of rachis browning and decreasing fungi contamination is necessary to control postharvest decay of table grapes. SA, as a natural and relatively safe compound, has been reported to have a high potential in maintaining fruit quality and reducing fungal decay in harvested fruits, as mentioned above. However, there is no report on the possible role of SA during storage of table grapes. The present study was conducted to determine SA effects on postharvest life of seedless grape cultivar Bidane Sefid and its overall quality.

MATERIALS AND METHODS

Plant materials and salicylic acid treatment

The study was performed using mature clusters harvested from 20–year–old 'Bidaneh Sefid' (syn. 'Thompson Seedless') grapevines grown in a commercial vineyard in Hamedan, Iran, in 2009. The clusters were carefully harvested at the time of commercial maturity (TSS>22 °Brix) and selected for the present experiment based on the uniform cluster size, the lacking of mechanical injuries and disease, and the existence of healthy greenish rachises. The selected clusters were randomly divided into four groups so that each group contained 12 clusters, immersed for five minutes in solutions of 0, 1, 2 and 4 mM SA supplemented with 0.05 % tween–20 as a surfactant, and were air–dried at room temperature for 2 h. SA concentrations of 1, 2 and 4 mM were prepared by dissolving SA powder (Merck, Germany) in hot distilled water. Distilled water was used as control. The surface–dried clusters were individually packaged in perforated plastic bags to maintain relative humidity. Each two bags were packaged in a 1L disposable container. All packages were sealed and stored at 0±0.5 °C for 45 days followed by 2 days shelf life at 20 °C. The treated clusters were evaluated in each treatment at 15, 30, 45 and 45+2 days of treatment.

Soluble solid content (SSC) and titratable acidity (TA)

Fruit juice samples obtained randomly from 10 berries in different parts of clusters. Total soluble solid of berry juice was determined by using a refractmeter (Atago, Japan) at 20 °C and the results were expressed as °Brix. The fruit juice was diluted with distilled water (1:10) and then TA was determined by titration with NaOH (0.1 N) up to pH=8.2 and the result were expressed as percentage (g of tartaric acid per 100 ml grape juice).

Berry firmness and weight loss

Firmness was determines using a handheld firmness tester (Wagner, FDK 32, USA) with 2 mm diameter plunger. Determinations were made with 10 berries for each replicate and results were expressed as N/cm^2. Fruit weight was recorded several times during the storage period and expressed as percentage of water loss in comparison to initial weight.

Fungal decay assessment and berry shatter

Berry decay was evaluated by scoring the number of contaminated berries by fungi per cluster, i.e., from 1= no decay to 5= over 20 decayed berries per bunch (Lurie *et al*., 2006). Berry shattering was measured for each cluster by subtracting the weight of detached berries from total cluster weight (Cantin *et al*., 2007) and was expressed as percentage.

Rachis browning and berry appearance

Rachis browning was graded using the scoring system, i.e., 1: healthy, 2: slight, 3: moderate and 4: severe (Crisisto *et al*., 2002). Berry appearance was evaluated from visual inspection of berries and assignment of score, i.e., 1: excellent, 2: good, 3: slightly dull, 4: <50% brownish and soft berries, 5: >50% brownish and soft berries (Xu *et al*., 2007).

Total phenolic content (TPC)

The skin of 20 berries from three clusters was removed and frozen in liquid nitrogen, and ground into a powder. The total phenolic content in grape skin was measured according to the Folin–Ciocalteu method (Slinkard and Singleton, 1977). Gallic acid was used as a standard phenolic compound. Extraction was performed by homogenizing 0.5 g skin powder in 3 ml of 85% MeOH and then the extract was filtered with No.1 filter paper. Fifteen hundred μL diluted Folin–Ciocalteau (Merck) reagent (1:10 with distilled water) was added to 300 μL extract solution and mixed thoroughly. After five minutes, 1200 μL Na_2CO_3 (7%) was added and then the mixture was shake for 1.5 h. The absorbance was measured using UV/Vis spectrophotometer (Carry 100, Varian, USA) at the wavelength of 765 nm. The concentration of total phenolics was expressed as the mg Gallic acid equivalents per gram of fresh weight (FW).

Polyphenol composition

Polyphenol compositions were determined only at end of cold storage using high–performance liquid chromatography (HPLC). For extraction of polyphenols, 2 mL of solvent (methanol/acetic acid, 85:15, v/v) was added to one gram of fine powder of berries fresh tissue. The samples were kept in refrigerator for 24 h and were centrifuged for 10 min at 12000 rpm. The supernatant of centrifuged samples were filtered by disposable 0.45 μm syringe filter. Fifty μL of the filtered sample was injected to HPLC equipped with UV–visible detector (Waters

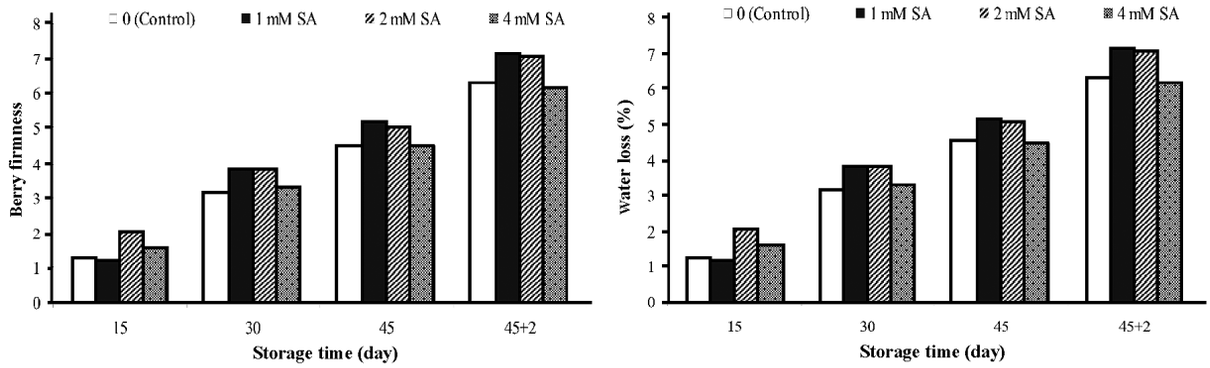

Fig. 1. Effects of SA treatments on berry firmness (left) and weight loss (right) in grape cv. Bidaneh Sefid stored for 15, 30, and 45 days at 0 °C and exposed for 2 days of shelf life.

Dual λ Absorbance 2487), column (Waters Symentery C18 5 μm 4.6×150 mm Column), at 320 and 350 nm (Fig. 1). Polyphenols of injected samples were compared with those of standards: quercetin and catechin (Sigma).

Statistical analysis

Treatments were distributed according to a completely randomized design with three replications of three clusters as experimental units and with one cluster for objective evaluations and water loss monitoring. GLM was performed for the experiment using SAS 9.1 (SAS Institute Inc., Cary, NC, USA). Significance of differences among means of data was statistically estimated with Duncan's Multiple Range Test at confidence levels of 95%.

RESULTS

Soluble solid content (SSC) and titratable acidity (TA)

SSC and TA in berries were not significantly affected by SA treatments (data not shown). The SSC increased with storage in all treated and untreated clusters. Titratable acidity reduced in all clusters during storage and shelf life.

Berry firmness and weight loss

The berry firmness and weight loss were reduced during cold storage and shelf life. The effects of SA treatments on berry firmness and weight loss were not significant (Fig. 1). Analysis showed slightly higher berry firmness in 2 and 4 mM SA–treated clusters at the day of 45+2 in comparison to those of control (Fig. 1).

Fungal decay and berry shatter

Changes for the decay development after postharvest treatments on grapes are summarized in Fig. 2. The incidences of fungal decay were significantly (P=0.05) affected by SA treatment at 4 mM at 45+2 days of postharvest life. There is no difference in decay incidence between grapes treated with SA and those treated without SA during cold storage.

All treatments showed progression in the percentage of berry shattering during cold storage and shelf life. The treatments of clusters by SA significantly reduced the percentage of shattered berries in comparison to non–treated clusters at the day of 45 in cold storage and obviously at the day of the 45 plus 2 days of shelf life (Fig. 2). However, there were no significant differences in the percentage of berry shattering between 1, 2 and 4 mM SA concentrations; the clusters treated with SA showed

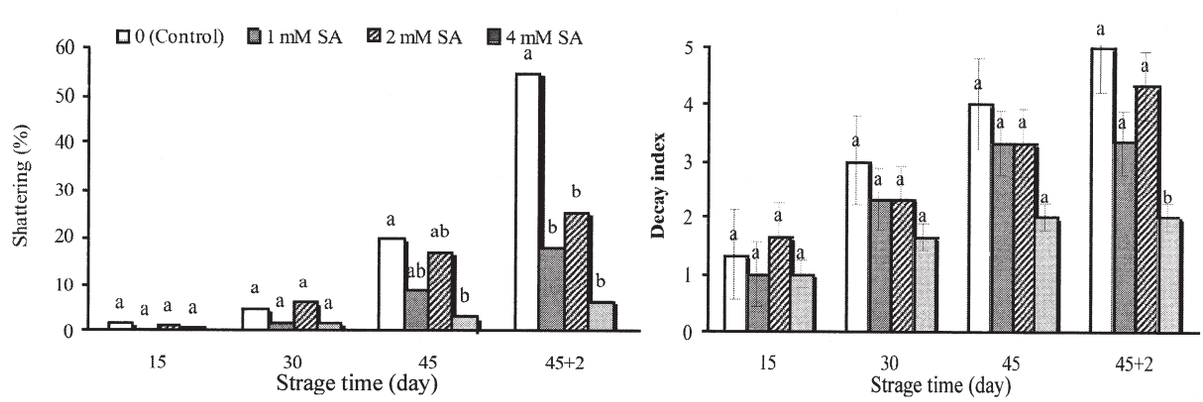

Fig. 2. Effects of SA treatment on berry shattering (left) and decay index (right) of grape cv. Bidaneh Sefid stored for 45 days at 0 °C and exposed for 2 days of shelf life. Bars represent standard errors of the means, and values followed by different letters were significance according to Duncan Multiple Range Test at P<0.05.

suppression for the berry–shattering performances in comparison to control or those treated without SA.

Rachis browning and berry appearance

Browned rachises were scored from 15 days after cold storage. Rachis browning occurred in control as well as SA–treated clusters, as the storage time increased. However, the concentrations of 2 and 4 mM SA suppressed it with significance after 30 days of cold storage (Fig. 3). SA application at a low concentration of 1 mM did not significantly reduced the rachis browning in compared to those of control.

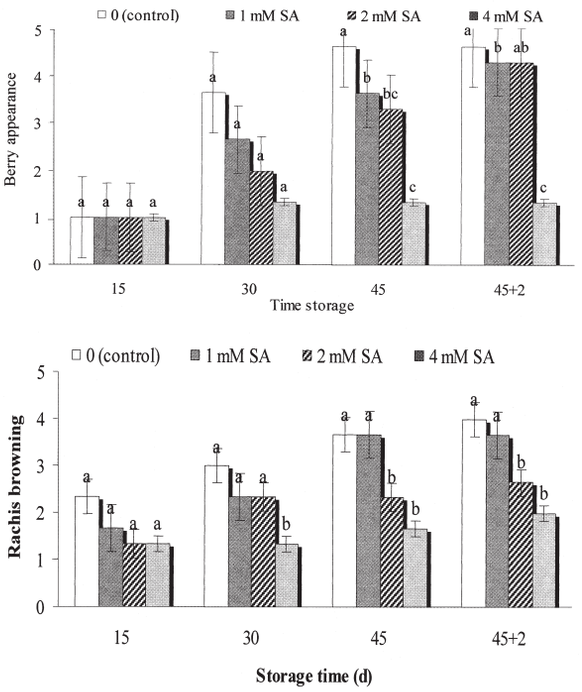

Fig. 3. Effects of SA treatment on berry appearance (upper) and rachis browning (lower) of grape cv. Bidaneh Sefid stored for 45 days at 0 °C and exposed for 2 days of shelf life. Bars represent standard errors of the means, and values followed by different letters were significance according to Duncan Multiple Range Test at $P<0.05$.

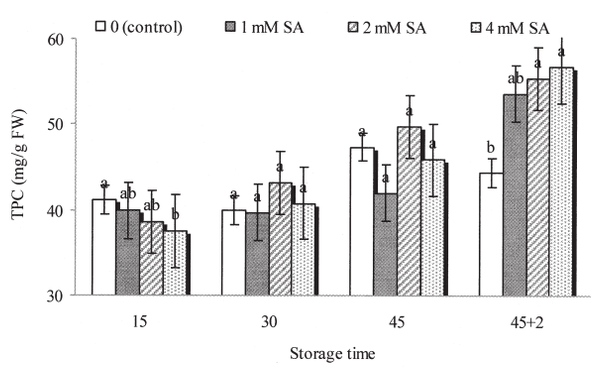

Fig. 4. Effects of SA treatment on total phenolic content (TPC) of grape cv. Bidaneh Sefid stored for 45 days at 0 °C and exposed for 2 days of shelf life. Bars represent standard errors of the means, and values followed by different letters were significance according to Duncan Multiple Range Test at $P<0.05$.

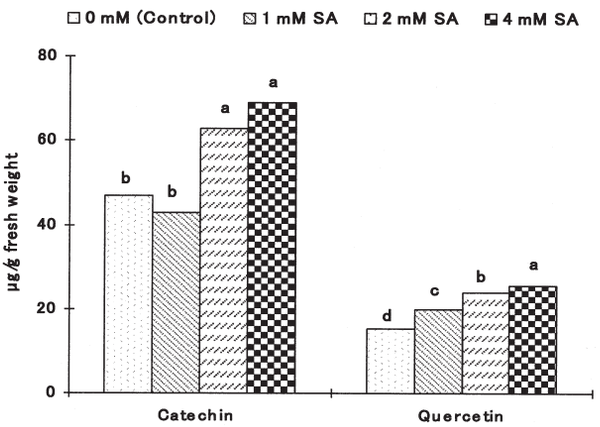

Fig. 5. Influence of SA concentration on quercetin and catechin contents of grape cv. Bidaneh Sefid stored for 45 days at 0 °C. Values followed by different letters were significance according to Duncan Multiple Range Test at $P<0.05$.

Visual appearance of the grapes deteriorated during postharvest storage in control. SA treatment with each of three examined concentrations had significant positive effect on visual appearance of berries at 45 days of cold storage (Fig. 3). At the end of cold storage plus following 2 days of shelf life, the appearance of grapes treated by 4 mM SA was significantly superior to that of the other two SA treatments and control.

Total phenolic content (TPC)

The changes of total phenolic content (TPC) in grape skins during storage are presented in Fig. 4. At the beginning of cold storage, the content of total phenolic was 35.33 mg/g FW. Application of SA had an obvious effect on the phenol accumulation. During cold storage and shelf life period, TPC increased in all SA treatments and control. Analysis showed that SA treatments at high concentrations of 2 and 4 mM significantly enhanced TPC only at the end of shelf life period (45+2 days in Fig. 4) in comparison to that of control.

Phenolic composition

Results indicated that application of SA had an obvious effect on the phenolic composition (Fig. 5). Quercetin content varied from 15.2 μg/g FW for 0 mM SA to 25.5 μg/g FW for 4 mM SA. Similarly, catechin content varied markedly from 42.9 μg/g FW for 1 mM SA to 68.9 μg/g FW for 4 mM SA.

DISCUSSION

Grape berries and clusters are susceptible to postharvest decay during postharvest storage. From the present study, it is clearly demonstrated that SA application can be used for maintaining the quality of harvested table grapes. SA treatments with concentrations of 2 and 4 mM significantly reduced weight loss, delayed rachis discoloration and browning, reduced fungal decay

rate and induced phenolic compounds accumulation in the treated berries.

SSC increased significantly with storage time and coincided with the increase in water loss. It is most likely that the increased SSC attributes to the concentration of juice during storage. Grapes are nonclimacteric type of fruit, and show very low respiration rates (Cirami *et al*., 1992). Therefore, there is low consumption of sugar for respiration during postharvest life in grapes. The main effects of SA, that were reported in some climacteric fruits such as banana (Srivastava and Dwivedi, 2000), are reduction of ethylene production and its function; application of SA to the climacteric fruits significantly results in the SSC reduction rate. In some nonclimacteric fruits such as pomegranate (Sayyari *et al*., 2009) and mango (Ding *et al*., 2007), however, the insignificant effect of SA on SSC in fruits during cold storage has been reported, as has been observed in the present study.

Wang *et al*. (2006) reported that only low concentrations of SA (0.35 and 0.7 mM) were ineffective on softening of peach fruit belonging to the climacteric type. In this study, however, no significant effects of SA treatments with concentrations of more than 1mM were observed on flesh firmness and water loss in the treated grape berries, since flesh firmness decreased and water loss increased with storage term in all SA concentrations as well as control. This means that firmness and water loss of grapes did not change in response to SA treatments. On the other hand, some workers have reported the influence of SA on fruit firmness in fruits such as climacteric kiwifruit (Zhang *et al*., 2003) and climacteric peach (Wang *et al*., 2006). This could be explained that in climacteric fruits SA delays fruit softening by affecting major cell wall degrading enzymes activity such as cellulase, polygalacturonase and xylanase (Srivastava and Dwivedi, 2000) through the reduction of ethylene production. In both climacteric and nonclimacteric fruits, it may be concluded that there is a close relation between water loss and berry firmness during storage.

Endogenous SA affects plant resistance to diseases (Raskin, 1992b). Several reports indicate that exogenous application of SA might induce the expression of many defense genes (Loake and Grant, 2007; Wang *et al*., 2006). Similar results on SA application have been obtained on peach (Wang *et al*., 2006), strawberry (Babalar *et al*., 2007) and sweet cherry (Xu and Tian, 2008). It has been documented that improved resistance in SA–treated fruit against fungal attack can be attributed to the consequence of increases in activities of antioxidant enzymes (Xu and Tian, 2008) and induction of a defense resistance system (Chan and Tian, 2006). The reduction of deterioration rates in SA–treated fruit clusters is due to these reasons.

It is stressed that "Bidaneh Sefid" is susceptible cultivar to berry shatter. The high degree of berry shatter in this cultivar is probably related to rachis dehydration and browning and to high incidence of decayed berries, as has been suggested previously (Xu *et al*., 2007; Zoffoli *et al*., 2009). The effect of SA on berry shatter can be also attributed to a suppression of both ethylene production (Srivastava and Dwivedi, 2000) and its action in the abscission layer of treated berries.

It has been reported that development of rachis browning during grape storage is associated with polyphenol oxidase activity (Carvajal–Millan, *et al*., 2001). The effect of SA on delaying the rachis browning must be through inhibition of polyphenol oxidase activity. In the present experiment, the best scores for rachis condition were given to the SA–treated clusters compared with those of non–treated clusters. It is well known that PPO and POD is involved in browning of fruit and vegetables. The synthesis of brown–colored pigments through the oxidation of phenolics is due to PPO and POD, and results in surface browning; however, SA has the inhibitory effect on their activity (Peng and Jiang, 2006). Phenylalanine ammonia–lyase (PAL) is one of the key phenylpropanoid enzymes, which produces a variety of phenolics (Sanchez–Ballesta *et al*., 2007). Application of SA can suppress the formation of brown substances and reduces browning index through PAL activity prevention (Peng and Jiang, 2006). In this study, fruits treated with high concentrations of SA (2 and 4 mM) showed significantly less rachis browning than the others. This may be due to the similar mechanism indicated by Peng and Jiang (2006).

The application of 2 and 4 mM of SA to berries induced higher total phenolic contents than those in the control and 1 mM of SA, suggesting that the accumulation of phenolic by SA is induced through increase in PAL activity (Chen *et al*., 2006). Phenolic compounds are an important group of secondary metabolites in grape and strongly influence the berry quality such as color, flavor, bitterness, and astringency (Chamkha *et al*., 2003). Furthermore, they are involved in several activities, such as antimicrobial, and antioxidant properties (Frankel *et al*., 1995). The results of the present study indicated that SA treatment significantly enhanced storage life of 'Bidaneh Sefid' grape and its overall quality probably through affecting the secondary metabolites and antimicrobial and antioxidant properties.

Salicylates that occur naturally in plants and those added to food, drinks and oral care products as preservatives and flavourants have clinical significance (Scotter *et al*., 2007). According to Corder and Buckley (1995), does of salicylate as low as 2.6 mg could induce bronchoconstriction in the most sensitive individuals. According to Ingster and Feinleib (1977), on the other hand, salicylates in fruit and vegetables may have contributed to a decline in cardiovascular disease. From the clinical and dietary point of view, Scotter *et al*. (2007) investigated the free SA and acetyl salicylic acid (ASA or aspirin) contents of fruit. They reported that grape contained relatively higher amount of SA (0.6 mg/Kg) and usual amount of ASA (<0.2 mg/Kg) in comparison with other fresh fruits. Since SA and ASA have several merits and demerits for clinical and dietary problems, treated berries with 2 mM and 4 mM SA are necessary to assess for the clinical and dietary problems, although the SA remaining on the surface of treated berries and that absorbed into berries is considered to be very negligible amount

for human health.

In conclusion, the work presented here demonstrates that the postharvest treatments by the concentrations of 2 mM or 4 mM SA increase grape postharvest life through maintaining the rachis conditions, increasing polyphenol content and changing its composition, improving plant defense mechanism, and elimination or reduction of fungi contamination during storage. Thus, the treatments with SA may be an effective alternative to improve the postharvest life and maintaining quality of grapes, with the addition of the benefit of improving phenolic content and composition and reducing the rate of rachis browning during postharvest storage.

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