

Effect of Salicylic Acid and Calcium Nitrate Spraying on Qualitative Properties and Storability of Fresh Jujube Fruit (*Ziziphus jujube* Mill.)

Hadi ZERAATGAR¹, Gholam Hossain DAVARYNEJAD^{2*},
Farid MORADINEZHAD³, Bahram ABEDI⁴

¹Ferdowsi University of Mashhad, Faculty of Horticultural Science, Mashhad, Iran / South Khorasan Agricultural and Natural Resources Research and Education Center, AREEO, Birjand, Iran; zeraatgarh@yahoo.com

²Ferdowsi University of Mashhad, Faculty of Horticultural Science, Mashhad, Iran; davarynej@um.ac.ir (*corresponding author)

³Birjand University, Faculty of Horticultural Science, Birjand, Iran; fmoradinezhad@birjand.ac.ir

⁴Ferdowsi University of Mashhad, Faculty of Horticultural Science, Mashhad, Iran; abedy@um.ac.ir

Abstract

Chinese jujube is among the important medicinal plants grown in Iran. Its valuable fruit have a short post-harvest life. Delaying of quality reduction for few days can help maintaining the shelf life of fresh jujube fruit. The current study was conducted to investigate the possible effects of pre-harvest foliar application of salicylic acid (0, 2 and 4 mM) and calcium nitrate (0, 1 and 2%) on physico-chemical characteristics and shelf life of fresh jujube fruit during storage at 10-days intervals, for 40 days. Results indicated that salicylic acid and calcium nitrate played an important role in maintaining and extending post-harvest quality of fresh jujube fruit, as both substances increased fruit firmness, titrable acidity, total phenolic content, antioxidant activity, ascorbic acid and catalase enzyme, but reducing total soluble solids. The highest total phenolic content (2.38 µg gallic acid/gFW), antioxidant activity (76.73 mmol Trolox/g), ascorbic acid (222.4 mg/100g FW) and catalase enzyme (16.67 U. mg⁻¹ protein), as well as the lowest total soluble solids content (23.11%) were observed when salicylic acid 4 mM was used. Furthermore, maximum fruit firmness (4.22 N) was obtained in the treatment containing calcium nitrate 2%. Treatment containing salicylic acid 2 mM and calcium nitrate 2% had the highest amount of titrable acidity TA (0.45 %). Based on the results, reducing trends of some components like ascorbic acid, antioxidant and phenol, were very fast, even when using salicylic acid and calcium nitrate; their application could cause a delay in these processes, but the quality reduction found could not be compensated. In other characteristics that had a slower quality reducing trend, the application of salicylic acid and calcium nitrate could cause at least a 10-day delay in the reduction of the amounts of these attributes.

Keywords: agro-chemical substance, cell wall rigidity, fruit texture, ripening process, storage life

Introduction

Chinese jujube (*Ziziphus jujuba* Mill.), belonging to Rhamnaceae family, has been cultivated in China, the Mediterranean region, southern and eastern parts of Asia, some regions of North Italy, west and south of Turkey (Kirkbride *et al.*, 2006; Ercisli *et al.*, 2007; Ecevit *et al.*, 2008; Gao *et al.*, 2013) and Iran, especially in Southern Khorasan province from long ago.

Being one of the important medicinal plants, Jujube fruit is grown in arid and semi-arid regions, in temperate and subtropical regions (Liu and Zhao, 2009). It is one of the world's most nutritious plants, rich in vitamin C, minerals and amino acids (Li *et al.*, 2007; Boora and Bal, 2008) and contains various types of bioactive substances such as triterpenic acid,

volatileoil, glycosides, saponins and flavonoids that have wide pharmacological effects on humans (Zhao *et al.*, 2008; Ji *et al.*, 2017). Moreover, it is used as table fruit, dry fruit and medicinal plant.

This valuable fruit has a short post-harvest life, characterized by softness and decrease in soluble solids content due to senescence, flesh browning, quality deterioration (Jiang *et al.*, 2004) and fungal diseases (Tian *et al.*, 2005; Cao *et al.*, 2013). Therefore, any efforts that could be made to produce fruits with high quality parameters such as firmness, color intensity, fruit uniformity and increase of ascorbic acid, total phenolic and total antioxidant activity at harvest and during marketing, would be very important for the jujube growers in order to obtain higher return of resources.

The application of agro-chemical substances is considered as one of the most innovative methods to extend the

commercial storage life of vegetables and fruits. Accordingly, some efforts have been made by using particular agro-chemical substances to delay ripening, decrease losses, increase and maintain fruit quality by reducing the speed of metabolic activities at harvest or during storage (Shafiee *et al.*, 2010). Sudha *et al.* (2007) reported that some chemicals could cause an increase in sapota fruit shelf-life and maintain its market ability for a longer term by arresting the growth and spread of micro-organisms. According to John (1987), calcium improves rigidity of cell walls and obstructs enzymes such as poly galacturonase from reaching their active sites, consequently retarding tissue softening and delaying ripening. The movement of calcium in plants is very slow because part of the calcium existing in plants bounded as a building material and other part is in outer surface of the plasma membrane and cell wall as exchangeable form. On the other hand, a large amount of calcium is enclosed in vacuoles and that is the reason for its very low concentration in the cytosol; therefore calcium cannot move in the phloem vessels through symplast and only moves through xylem vessels (Marschner, 2011).

Calcium application maintains cell turgor, membrane integrity and tissue firmness, and delays membrane lipid catabolism, which extend storage life of fresh fruits (Rizk-Alla and Meshreki, 2006). Moreover, calcium existing in fruit tissues usually prevents post-harvest disorders, retards fruit ripening and decreases fruit weight loss and decay (Lara *et al.*, 2004; Hernandez-Munoz *et al.*, 2006). Calcium nitrate sprayed on jujube increased fruit weight and reduced cracking, sugar content and total acidity (Hu *et al.*, 2013). The use of calcium compounds on ber - Indian jujube also reduced the brown spots and increased the amount of calcium in fruit texture (Shamili and Hajiani, 2012).

Salicylic acid is also an endogenous growth regulator with phenolic nature, which participates in the regulation of several physiological processes in plants, such as stomata closure, ion uptake, inhibition of ethylene biosynthesis and transpiration (Khan *et al.*, 2003). It is used as a food additive in handling harvested fruits to delay ripening processes of some fruits as well as enhancing the tolerance of fruits against pathogens, particularly at the early maturity stage (Cao *et al.*, 2006). Furthermore, salicylic acid was reported to reduce fruit weight loss and softening (Shafiee *et al.*, 2010).

Some studies showed that storage at low temperature effectively delayed fruit ripening and extended post-harvest life of fresh jujube, but the beneficial effects may be limited by undesirable physico-chemical changes (Crisosto *et al.*, 2004; Manganaris *et al.*, 2007). The consequences of these changes were the acceleration of quality loss and reduction in consumer acceptability. Thus, there is a need for retarding or inhibiting the physico-chemical changes, as well as improving fruit storability. It was the aim of the present study to evaluate the effects of salicylic acid and calcium nitrate spray on some qualitative characteristics of jujube.

Materials and Methods

Plant material preparation, treatments and storage conditions

The jujube trees were selected from a commercial orchard established in Agricultural Research Center of Southern Khorasan province (Birjand), Iran. All trees were similar regarding age (15 years) and orchard management.

All treatments were sprayed with calcium nitrate (0, 1 and 2%) and salicylic acid (0, 2 and 4 mM) as preharvest treatments, three times [in colour change stage (August 5 - August 22) from green to white till a week before harvesting].

The fruits were harvested at crisp mature stage and transferred to the laboratory; then, uniform fruits in size and free from diseases were selected, packed in perforated polyethylene with a thickness of 30 microns, and kept at 4 ± 1 °C and 85% to 90% R.H in the refrigerator (Wu *et al.*, 2012). The different measurements were performed during storage (0-10-20-30-40) days after harvesting.

Determination of quality attributes

A hand-held refractometer (Atago, Tokyo, Japan) was used to measure total soluble solids (TSS). Titrable acidity (TA) was specified by titration with 0.1 N NaOH to an endpoint of pH 8.1 and expressed as percentage of citric acid (Gao *et al.*, 2011). Fruit firmness (N) was measured on ten fruits from each sample using a TA-XT2i texture analyzer (Stable Micro Systems Ltd, Godalming, UK) fitted with a 2 mm diameter probe operated at a speed of 1 mm s⁻¹.

Determination of ascorbic acid content

Ascorbic acid content was quantified in accordance with the dinitrophenyl hydrazine (DNPH) method, modified by Nunes *et al.* (1995). Freeze dried powder (0.2 g) was homogenized in mortar pestle, with 20 mL of a mixture of 6% (w/v) metaphosphoric acid in 2 mol/L acetic acid. The mixture was centrifuged at 17,600 g for 15 min at 4 °C. The supernatant was filtered through Whatman filter paper (no.1). An aliquot of 0.05 mL of 0.2% (w/v) 2,6-dichlorophenol indophenols (DCPIP) was added to 1 mL of the supernatant and incubated at room temperature for 1 h. Thiourea solution (2%, w/v) in 5% metaphosphoric (w/v) acid and 0.5 mL of 2% (w/v) DNPH in 4.5 mol/L sulphuric acid were added and the solution was incubated at 60 °C for 3 h. Tubes were placed in an ice bath and 2.5 mL of ice cold 90% sulphuric acid was slowly added. Tubes were vortexed and total ascorbic acid was measured by absorbance at 540 nm by spectrophotometry. A standard curve of ascorbic acid was used to calculate the concentration and results were reported as mg/100 gFW.

Determination of total phenolic contents (TPH)

The overall phenolic content was determined by using the method described by Slinkard and Singleton (1977). The reaction began when 1 mL of the supernatant was added into a solution of 1 mL 50% (v/v) Folin-Ciocalteu reagent solution and 2 mL saturated Na₂CO₃ solution. The mixture was left at room temperature for 30 min. The absorbance was recorded at 750 nm. The total phenolic content was expressed as microgram gallic acid per gram fresh weight.

Determination of antioxidant activity (free radical scavenging activity)

DPPH assay was performed on the basis of the measurement of the scavenging ability of antioxidants towards the stable DPPH radical (Brand-Williams *et al.*, 1995). A 3.9 mL aliquot of a 0.0634 mM of DPPH solution, in methanol (95%), was added to 0.1 mL of each extract and shaken vigorously. Change in the absorbance of the sample extract was measured at 515 nm for 30 min until the absorbance reached a steady state.

The percentage inhibition of DPPH of the test sample and known solutions of Trolox were calculated by the following formula: Inhibition (%) = $(A_0 - A) / A_0 \times 100$, where 'A₀' was the beginning absorbance at 515 nm obtained by measuring the same volume of solvent, and 'A' was the final absorbance of the sample extract at 515 nm. Methanol (95%) was used as a blank. Results were reported as mmol Trolox/g.

Assay for enzyme activity

Catalase (CAT) was assayed by using the method of Wang and Tian (2005). Catalase was extracted by using 50 mmol/L sodium phosphate buffer (pH 7.0). The reaction mixture included 2 ml sodium phosphate buffer (50 mmol/L, pH 7.0), 0.5 mL H₂O₂ (40 mmol/L) and 0.5 mL enzyme. The decomposition of H₂O₂ was measured by the reduction in absorbance at 240 nm.

Statistical analysis

The experiment was performed in a factorial split-plot based on randomized complete block design with three replications. Data were analyzed by using SAS statistical software [version 6, 2005], and means comparison was performed by using Duncan's multiple range test. Differences at $P < 0.05$ were considered as significant.

Results and Discussion

Total soluble solids (TSS)

Increase in shelf life duration led to the increase in TSS, so that the highest and the lowest amounts of TSS were obtained at 40th day (26.92%) and zero until the 20th day (22.48-22.63%), respectively (Table 1). Kokabi and Tabatabai (2011) have been reported that an increase in TSS during storage is due to the increase in invertase enzyme that causes a change in sucrose. The reduction in fruit water content and conversion of cell wall components such as starch, protein, pectin and hemicelluloses into simple soluble sugars during storage are responsible for the increase in TSS content. Furthermore, increase in soluble solids and soluble sugar during the period of fruit ripening may be due to the activity of sucrose-phosphate synthase enzyme (SPS), which is an important enzyme in the biosynthesis of sugars (Hubbard *et al.*, 1991).

Enhancement of salicylic acid concentration caused a significant reduction in TSS, so that the highest and the lowest contents were observed in control (25.76%) and concentration of 4 mM (23.11%), respectively (Table 1 and Fig. 1). The reduction found in TSS is in agreement with the data obtained in fresh jujube and palm (Al-Obeed, 2010, 2012). SA reduces enzyme activity of sucrose-phosphate synthase, and therefore reduces ethylene production and sugar synthesis (Aghdam *et al.*, 2010).

Titrateable acidity (TA)

Based on the data obtained, enhancement in the duration of shelf life led to the reduction of TA. The highest value of TA was obtained at day zero (0.55%), while the lowest rate was seen at day 40th of shelf life (Table 1). TA is directly related to the concentration of dominant organic acid, which is an important parameter in maintaining fruit quality. Since organic acids are used as substrates for respiration in enzymatic reactions, it is expected that TA decreases during postharvest (Shokrollahfahm *et al.*, 2012). Organic acids are energy sources that are utilized in fruit ripening phase when metabolism increases (Aguayo *et al.*, 2006).

As the results showed, increase in salicylic acid concentration significantly increase TA, so that the highest and

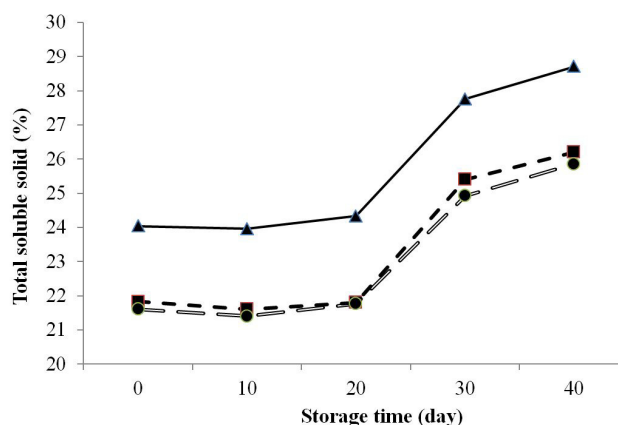


Fig. 1. Effect of salicylic acid on total soluble solid of jujube fruits; (▲) Control; (■) Salicylic acid 2 mM; (●) Salicylic acid 4 mM

Table 1: The results of the main treatments and sub effects comparison on the mean of investigated traits

Treatments	TSS (%)	TA (%)	Fruit firmness (N)	Ascorbic acid (mg/100 g FW)	Total phenolic content (µg gallic acid/g FW)	Antioxidant activity (mmol Trolox/g)	Catalase activity (U.mg ⁻¹ protein)
Salicylic acid (mM)							
0	25.76 a	0.39 b	3.60 b	160.93 c	1.91 b	65.93 c	14.33 b
2	23.36 b	0.45 a	4.08 a	187.33 b	2.20 a	72.93 b	15.95 a
4	23.11 b	0.43 a	4.06 a	222.40 a	2.38 a	76.73 a	16.67 a
Calcium nitrate (%)							
0	23.008 a	0.38 b	3.7 b	175.86 b	1.99 b	67.26 b	14.70 b
1	24.71 a	0.44 a	3.82 b	194.33 a	2.32 a	73.20 a	15.96 a
2	24.52 a	0.45 a	4.22 a	200.46 a	2.18 ab	75.13 a	16.30 a
Storage (day)							
0	22.48 b	0.55 a	4.95 a	379.33 a	3.21 a	97.77 a	16.38 ab
10	22.32 b	0.48 b	4.46 b	248.00 b	2.40 b	81.44 b	17.57 a
20	22.63 b	0.41 c	4.03 c	123.89 c	2.005 c	69.88 c	14.46 ab
30	26.03 b	0.36 d	3.21 d	102.89 d	1.71 cd	57.33 d	14.91 b
40	26.92 a	0.32 d	2.93 d	89.00 d	1.48 d	52.88 d	12.94 c

the lowest contents of TA were observed in 2 mM (0.45%) and control (0.39%), respectively (Table 1 and Fig. 2A). Shokrollahfam *et al.* (2012) reported that any treatments causing a delay in the metabolism and senescence can reduce the rate of TA change during storage. Since the role of salicylic acid has been proved to be delaying fruit ripening and reducing ethylene production and respiration rate, it can decrease the rate of change in TA during shelf life (Han and Li, 1997; Srivastava and Dwivedi, 2000). Sayyari *et al.* (2009) suggested that application of salicylic acid at the concentration of 4.1 mM in pomegranate (Malas-Saveh) increased TA. Moreover, Al-Obeed (2010) stated that the application of salicylic acid on palm increased TA. Reynolds and Dweck (1999) also reported that salicylic acid delayed climacteric peak and respiration rate in banana, peach, kiwi and apple.

Results in Table 1 and Fig. 2B showed that TA increased by the application of calcium nitrate. As it can be seen, the highest amount of TA was due to the treatment of calcium nitrate 2% (0.45%), whereas the lowest rate was seen in control (0.38%). Shokrollahfam *et al.* (2012) reported that calcium compounds decreased TA by expanding strong bands in the cell walls. Besides, Al-Obeed (2012) obtained similar results on jujube fruit. This effect can be explained by the formation of cross links between the carboxyl groups of polyuronide chains found in the middle lamella of the cell wall. Calcium decreases the rate of senescence during commercial and retail fruit storage, with no negative effect on consumer acceptance (Lester and Grusak, 2004).

Flesh firmness

A significant reducing trend was found regarding flesh firmness after harvest, as analysis of data indicated in Table 1. The lowest firmness was observed at 30- and 40- day intervals (Table 1). Fruit texture is often the first of many quality attributes judged by the consumer, and excessive softening is a major factor limiting jujube shelf life. In climacteric fruits softening or reducing fruit texture firmness during storage is the result of the activation of cell wall degrading enzymes such as pectin, methylesterase, polygalacturonase, catalase and cellulase by ethylene (Fischer and Bennett, 1991; Prasanna *et al.*, 2007).

Flesh firmness, as the results showed in Table 1 and Fig. 3 A, was significantly increased as the concentration of salicylic acid enhanced, so that the highest value of firmness was observed in salicylic acid sprayed at concentrations of 2 and 4 mM (4.08 and 4.06 N) and the lowest rate was observed in control (3.6 N). Recently, salicylic acid has been proposed as a new kind of plant hormone that led to the higher firmness of fruits and lower fruit chilling injury and decay incidences (Rao *et al.*, 2011). Leslie and Romarini (1988) stated that salicylic acid as a simple phenolic compound maintains firmness by regulating the expression of genes involved in ACC synthase and ACC oxidase enzyme, and reducing ethylene production and cell wall degrading enzymes such as polygalacturonase, cellulase and pectinase (Shafiee *et al.*, 2010). Zhang *et al.* (2003) reported that salicylic acid affected cell walls and caused fruit to be stronger.

The results obtained on the effect of calcium nitrate application showed that treatment of calcium nitrate produced fruits with higher firmness compared with control and other treatments (Table 1 and Fig. 3 B). Calcium compounds are applied on harvested fruits for the maintenance of quality, prevention of softening and reduction of rottenness rate (Chen *et al.*, 2011). Furthermore, application of calcium maintains cell turgor, membrane integrity and tissue firmness, and delays membrane lipid catabolism, so, it extends the storage life of fresh fruits (Rizk-Alla and Meshreki, 2006). Calcium bands as pectate in the cell walls and tissues are necessary for texture strength. Soft fruit during storage may be dependent on the amount of calcium binding in the cell wall. Calcium ions are linked with phosphate, carboxyl groups, phospholipids and proteins of cell membrane surface, and increase the cell membrane stability (Vicente *et al.*, 2005). In addition, calcium protects cell walls against degrading enzymes (Manganarys *et al.*, 2007). Addition of calcium improves rigidity of cell wall and prevents enzymes such as polygalacturonase from reaching their active sites (John, 1987), therefore increasing firmness, retarding tissue softening and prolonging harvest season (Cheour *et al.*, 1991; Marzouk and Kassem, 2011). This effect can be explained by the formation of cross links between the carboxyl groups of polyuronide chains found in the middle lamella of the cell wall. It reduces the rate of senescence during

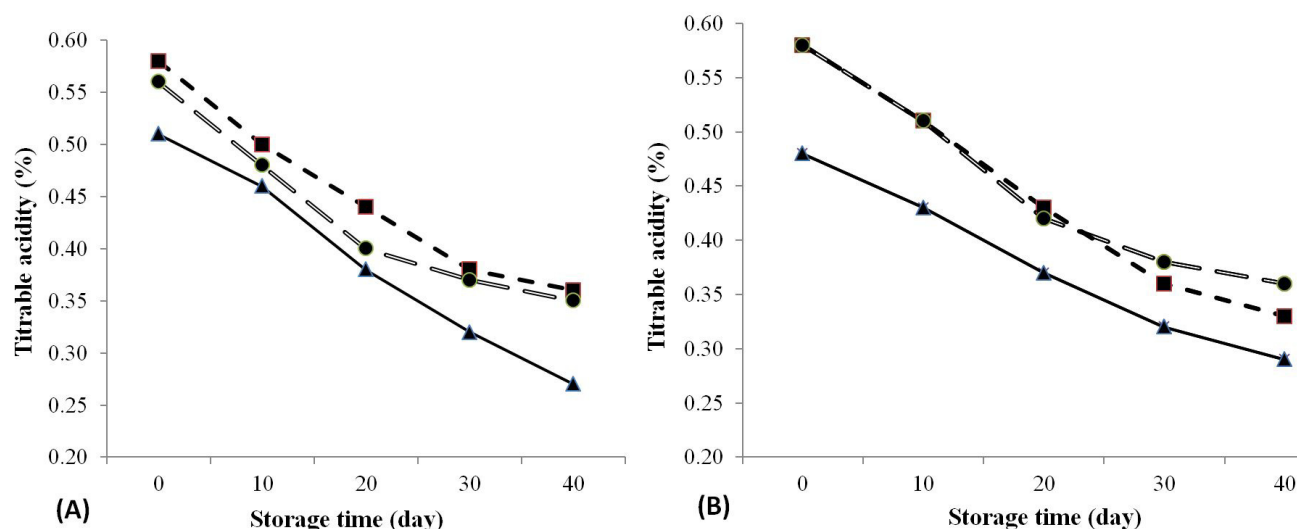
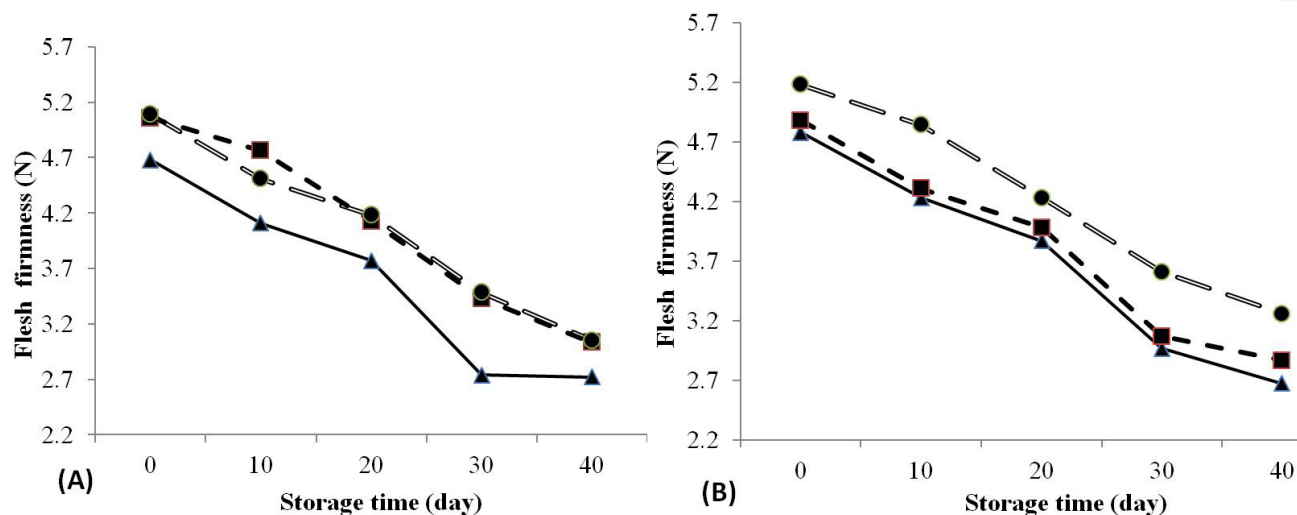


Fig. 2. Effect of salicylic acid and calcium nitrate on titrable acidity of jujube fruits; (A) Salicylic acid: (▲) Control; (■) Salicylic acid 2 Mm; (●) Salicylic acid 4 mM(B) Calcium nitrate: (▲) Control; (◐) Calcium nitrate 1%; (●) Calcium nitrate 2%



commercial and retail storage of fruit, with no undesirable effect on consumer acceptance (Lester and Grusak, 2004). Moreover, Serrano *et al.* (2004) showed that plums treated with calcium compounds had higher firmness than control.

Ascorbic acid (Vitamin C)

Vitamin C content of jujube fresh fruits gradually decreased during storage, as the results showed. Zero-day storage showed the highest content of Vitamin C (379.33) compared to the 30- and 40-day storages with the lowest rates of 102.89 and 89, respectively (Table 1). Ascorbic acid shows sensitivity to destruction when fruits are subjected to adverse postharvest handling and storage conditions (Lee and Kader, 2000). Among the vitamins, ascorbic acid is the least stable and easily destroyed during storage process and is thus very sensitive to degradation (Spinardi, 2005). As an important component of the antioxidative defense mechanism in cells and tissues, vitamin C acts as a reducing and a chelating agent and has been shown to scavenge free radicals.

Furthermore, analysis of data showed that enhancement of salicylic acid concentrations resulted in a significant increase in Vit. C, so that the highest and lowest contents of Vit. C were observed in 4 mM (222.4) and control (160.93), respectively, as can be seen in Table 1 and Fig. 4A. Salicylic acid as an antioxidant activates ascorbate peroxidase, which, in turns, increases antioxidant ability and ascorbic acid amount in fruits

(Dat *et al.*, 1998; Wang *et al.*, 2006). In addition, it has been shown that salicylic acid affects the biosynthesis and action of ethylene (Srivastava and Dwivedi, 2000), prevents vitamin C from being destructed (Wisniewska and Chelcowski, 1999) and acts as an anti-stress power (Elwana and El-Hamahmy, 2009). Reduction in respiration rate in fruits treated with salicylic acid results in the reduction of ethylene biosynthesis and lower activity of ascorbate enzyme by inhibiting ethylene production, thereby maintaining and increasing the level of ascorbic acid (Yahia *et al.*, 2001). Sayyari *et al.* (2009) stated that using salicylic acid at high concentrations (2 mM) maintained ascorbic acid in pomegranate. Application of exogenous salicylic acid on kiwi fruits led to the prevention of softening process, and kept ascorbic acid content and firmness during 5 months of cold storage (Aghdam *et al.*, 2010). Al-Obeed (2012) and Shokrollahfam *et al.* (2012) also reported that application of salicylic acid prevented the reduction of vitamin C in jujube and plum fruits by delaying the deteriorative oxidation reaction.

With respect to the effect of calcium nitrate on Vit. C, the results showed that the highest content of Vit. C was obtained in calcium nitrate 2% (200.46 mg/100 g FW), while the lowest content was observed in control with 175.89 mg/100g FW (Table 1 and Fig. 4B). Shokrollahfam *et al.* (2012) and Veltman *et al.* (2000) reported that calcium compounds bind with membrane and increase its stability, therefore, they

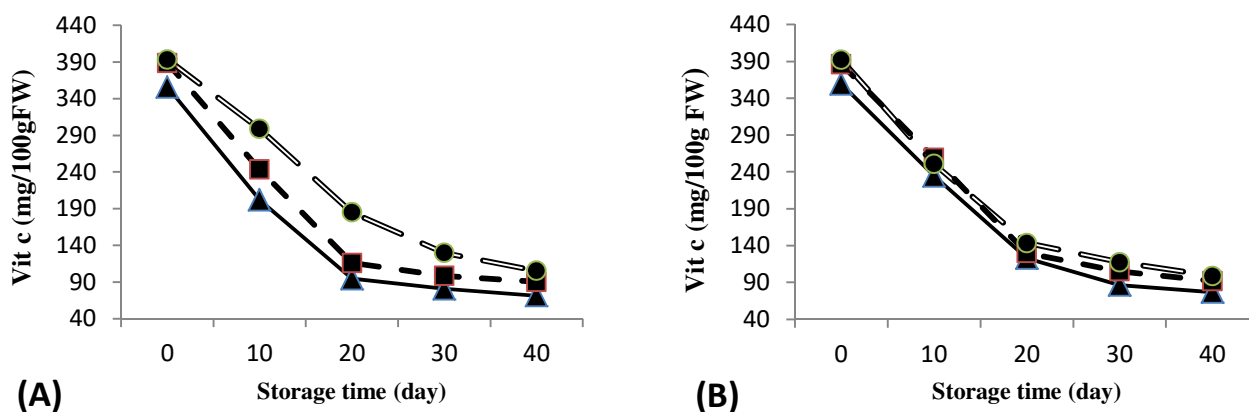


Fig. 4. Effect of salicylic acid and calcium nitrate on vitamin c of jujube fruits; (A) Salicylic acid: (▲) Control; (◻) Salicylic acid 2 mM; (●) Salicylic acid 4 mM; (B) Calcium nitrate: (▲) Control; (◻) Calcium nitrate 1%; (●) Calcium nitrate 2%

prevent free radicals and reactive oxygen species from connecting to membrane and contribute to the maintenance of the health of biological membranes. Additionally, calcium compounds cause a delay in rapid oxidation of ascorbic acid by increasing the activity of APX. Ruoyi *et al.* (2005) stated that ascorbic acid content of peaches was stable for fifty-day storage period with the application of 0.5% CaCl₂. Pooviah (1979) reported that the use of calcium chloride (4%) at harvest time increased ascorbic acid in fruits, and application of calcium before and after harvest improved fruit quality. Al-Obeed (2012) and Shokrolahfam (2012) reported that application of calcium compounds retarded the degradation of Vitamin C content in jujube fruits and plum. The same results on increase of Vitamin C has been reported in apple and kiwi fruit (Paliyath *et al.*, 2009), apple (Sams *et al.*, 1993), pomegranate 'Malas-Yazdi' (Ramezani *et al.*, 2009), papaya (Selvaraj *et al.*, 1982), strawberry (Shafiee *et al.*, 2010) and tomato (Pila *et al.*, 2010).

Total phenolics content (TPH)

Data obtained on the influence of storage intervals on total phenolics content showed that enhancement in duration of shelf life led to the reduction of total phenolic contents, so that the highest phenolic content was obtained at zero-day storage with 3.21 mg GAE/100 g compared to the lowest rate of 1.48 mg GAE/100 g at 40-day storage interval (Table 1). Phenolic compounds are the main contributors to functional quality and have a leading role in counteracting reactive oxygen species (ROS), thus minimizing molecular damage. Based on Remorini *et al.* (2008), decrease in fruit phenolic contents during shelf life is related to the chemical and enzymatic changes occurring during fruit development. Generally, phenolic compounds are reduced during fruit growth and development, which lead to the reduction of astringency. It is suggested that phenolic compounds are incorporated in enzymatic and non-enzymatic reactions during storage, and that may be the reason for lower contents of this chemicals (Sartip and Hajilou, 2015).

Regarding the effect of salicylic acid on total phenolics contents, results showed that an increase in salicylic acid concentration brought about a significant increase in total phenolics content. Based on the results in Table 1 and Fig. 5A, the highest and lowest phenolics contents were observed in SA 2 and 4 mM (2.2 and 2.38 mg GAE/100 g) and control (1.91 mg GAE/100 g), respectively. Salicylic acid is a phenol

promotive in plants that increases its content via effects on specific enzymes. In addition, salicylic acid has been shown to affect the biosynthesis and action of ethylene (Srivastava and Dwivedi, 2000). In kiwi (Du *et al.*, 2009) and peach (Drogoudi and Tsipouridis, 2007), a significant linear relationship was found between total antioxidant capacity of fruits and total polyphenols, because phenolic compounds are biochemical compounds with high antioxidant activity, so one of the reasons for the increase in antioxidant capacity of fruits treated with salicylic acid can be its effects on fruit phenolic materials.

Data showed that calcium nitrate caused a significant increase in the amount of total phenol. Treatment of calcium nitrate 1% showed the highest phenol content (2.32 mg GAE/100 g) in comparison with the control with the lowest rate (1.99 mg GAE/100 g), as the results in Table 1 and Fig. 5B showed. Calcium compounds strengthen cell walls, and maintain and control selective exchange of gas and ion. They also increase the activity of cell wall enzymes, reduce oxygen-free and finally prevent phenol oxidation. The increase in total phenolic in tomato by using calcium compounds was also reported by Pila *et al.* (2010), which is in agreement with the current results. Plant phenolic compounds have multifunctional properties and can function as singlet oxygen quenchers and scavenge free radicals, thus presence of substantial amounts of phenolic compounds in Chinese jujube indicates that they are a significant source of antioxidants that may provide health promoting advantages to the consumers.

Antioxidant activity (AOX)

Data in Table 1 showed that there was significant difference among different periods of fruit storage. Increase in shelf life duration led to the reduction of antioxidant activity, so that the highest value was obtained at zero-day storage (97.7 mmol Trolox/g) and the lowest rates were due to 30- and 40-day storage intervals (57.3 and 52.8 mmol Trolox/g). The parameter shows the existence of efficient oxygen radical scavengers, such as vitamin C and phenolic compounds and their synergistic and/or antagonistic effects. Antioxidant activity varies according to the stage of growth and fruit ripening. Possible reasons for the decrease in total antioxidant in fruits during storage can be chemical changes (enzymatic and non-enzymatic reactions) leading to the destruction of the walls and cell membranes, creation of reactive oxygen and free radicals, and participation of antioxidants themselves in cellular metabolism and their consumption during storage (Sánchez Moreno *et al.*, 1998; Remorini *et al.*, 2008).

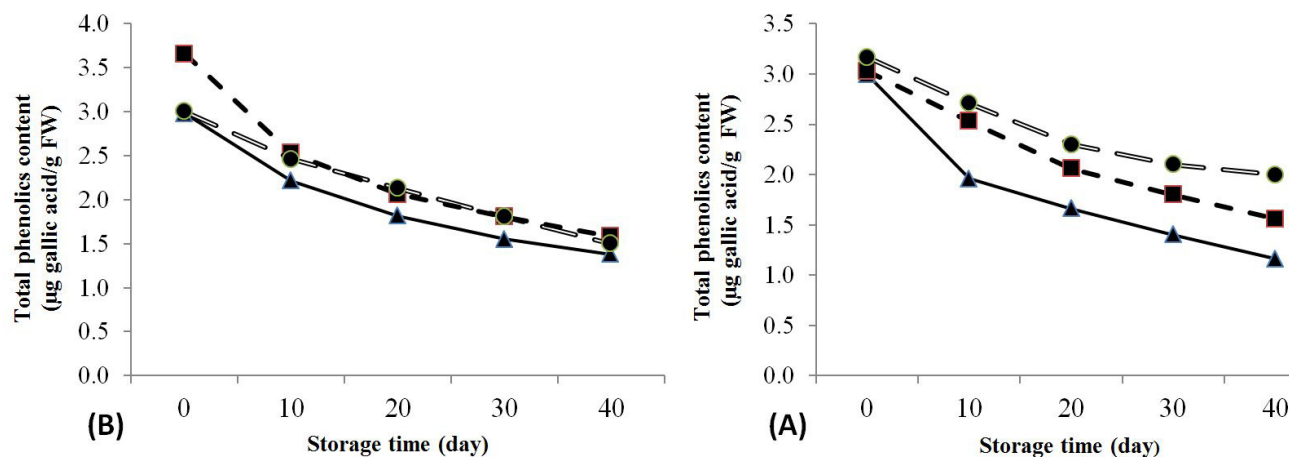


Fig. 5. Effect of salicylic acid and calcium nitrate on total phenolics content of jujube fruits; (A) Salicylic acid: (▲) Control; (■) Salicylic acid 2 mM; (●) Salicylic acid 4 mM; (B) Calcium nitrate: (▲) Control; (■) Calcium nitrate 1%; (●) Calcium nitrate 2%

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Antioxidant activity was also affected by salicylic acid, as the results showed. As can be seen in Table 1 and Fig. 6 A, the highest and lowest rates of antioxidant activity were observed in treatment containing 4 mM salicylic acid (76.76 mmol Trolox/g) and control (65.93 mmol Trolox/g), respectively. The current results were in agreement with the findings reported on strawberry (Shafiee *et al.*, 2010), pear (Cao *et al.*, 2006) and apricot (Ardakani, 2013).

Salicylic acid increases antioxidant activity through the expression of oxidase gene, removes toxic effects of free radicals and protects plant cell tensions against all kinds of stresses (Turnham, 1990; Zhang and Schmidt, 1999). Pila *et al.* (2010) stated that salicylic acid increased the activity of phenylalanine ammonia-lyase enzyme, a key enzyme in the phenylpropanoids metabolism, enhanced the synthesis and accumulation of important phenolic compounds with antioxidant properties, and finally increased tissue resistance to living and non-living stressors.

Results obtained from Ca application showed that calcium nitrate increased the antioxidant activity, so that the highest and lowest rates were observed in calcium nitrate 2% (75.13 mmol Trolox/g) and control (67.26 mmol Trolox/g), respectively (Table 1 and Fig. 6 B). An increase in reactive oxygen species and free radicals (oxidative metabolism) in fruit ripening stages damages cell membrane and increases senescence. Therefore any treatment that reduces the amount of damage to the cell membrane or cell wall strength such as the use of calcium compounds can reduce the amount of reactive oxygen species and free radicals by control and maintenance of

gas exchange and increase of cell wall enzyme activity. Paliyath *et al.* (2009) reported that calcium stimulates and increases tissue resistance to damage by increasing antioxidant activity; therefore use of calcium compounds before and after harvest fruits and vegetables has positive effects on antioxidant activity.

Catalase activity (CAT)

Based on the results obtained, different periods of fruit storage showed significant differences from each other. An increase in the duration of storage led to the reduction of catalase activity, so that 10-day storage duration showed the highest catalase activity (17.57 U. mg⁻¹ protein), a 4.63 U. mg⁻¹ protein increase compared to 40-days storage with the lowest rate (Table 1). Reduction found in catalase activity during higher storage duration is in line with the results reported in some other fruits such as peach (Han *et al.*, 2002), plum (Perez *et al.*, 2003), cherry (Martinez *et al.*, 2006) and banana (Srivastava and Dwivedi, 2000). Catalase is one of the important enzymes that can protect cells from oxidative damage by scavenging reactive oxygen species (ROS) (Scandalios, 1993; Lee and Lee, 2000). ROS accumulation results in oxidative injury, which accelerates the progression of senescence and different senescence-associated disorders (Stadtman, 1992). Studies demonstrated that ROS led to oxidative damage of mitochondria and accelerated senescence processes in peach fruit (Qin *et al.*, 2009). Increased ROS and free radicals due to the increased oxidative metabolism during the ripening process of fruits can cause damage to cell membranes and increase the speed of senescence.

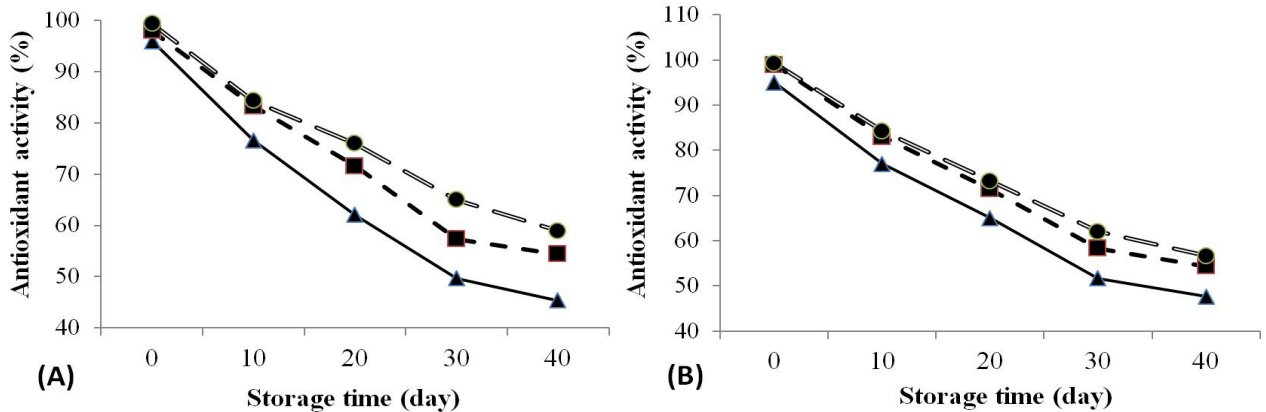


Fig. 6. Effect of salicylic acid and calcium nitrate on antioxidant activity of jujube fruits; (A) Salicylic acid: (▲) Control; (■) Salicylic acid 2 mM; (●) Salicylic acid 4 mM; (B) Calcium nitrate: (▲) Control; (■) Calcium nitrate 1%; (●) Calcium nitrate 2%

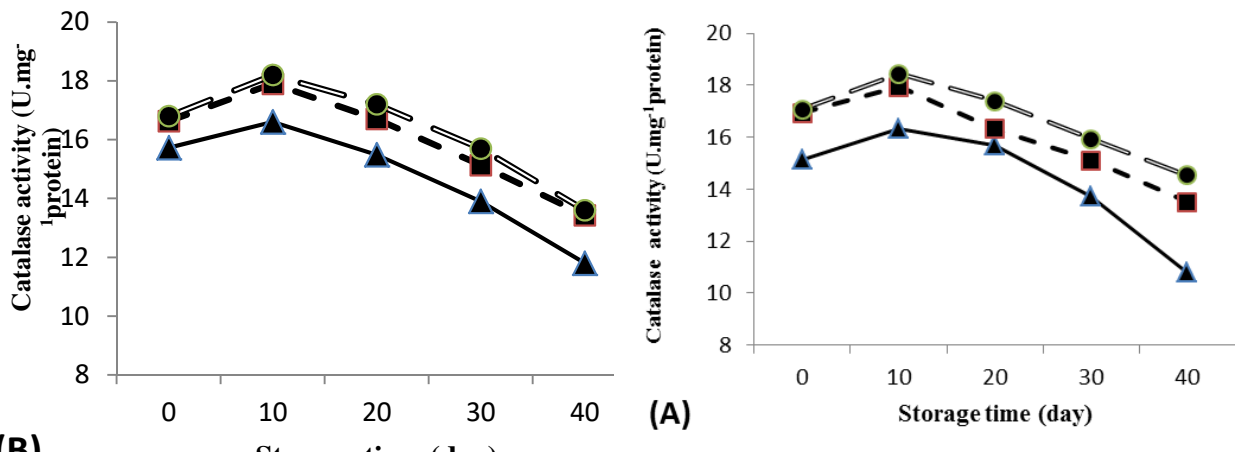


Fig. 7. Effect of salicylic acid and calcium nitrate on catalase activity of jujube fruits; (A) Salicylic acid: (▲) Control; (■) Salicylic acid 2 mM; (●) Salicylic acid 4 mM; (B) Calcium nitrate: (▲) Control; (■) Calcium nitrate 1%; (●) Calcium nitrate 2%

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The results in Table 1 and Fig. 7A showed that increase in salicylic acid concentration significantly increased CAT activity, so that the highest and the lowest catalase activity was observed in 4 mM (16.67 U. mg⁻¹ protein) and control (14.33 U. mg⁻¹ protein), respectively. The increase found in catalase activity by using salicylic acid was also reported by Srivastava and Dwivedi (2000) in bananas, Zainuri and Terry (2001) in mango and Han *et al.* (2002) in peach. The effect of salicylic acid can be associated to its ability in the increase in the activity of antioxidant enzymes, which maintain the nutritional value of vegetables and fruits by preventing the detrimental effects of free radicals (Jing *et al.*, 2008). By expressing the alternative oxidase genes, salicylic acid increases the activity of antioxidant enzymes, and antioxidants together reduce the toxicity of free radicals and protect plant cells against different kinds of stresses (Hogg *et al.*, 1993; Asghari and Aghdam, 2010).

Data indicated that calcium nitrate increased catalase activity. The highest activity was observed in treatment containing calcium nitrate 2% (16.3 U. mg⁻¹ protein) and the lowest was seen in control (14.7 U. mg⁻¹ protein), as the results in Table 1 and Fig. 7 B showed. According to John (1987), calcium compounds improve rigidity of cell wall and prevent enzymes such as polygalacturonase and catalase from reaching their active sites, thereby retarding tissue softening and delaying ripening. Lester and Grusak (2004) reported that the use of calcium compounds maintained the solidity of cell membrane by binding with phospholipids and proteins and caused strength and tightness of the skin and flesh fruit. Strengthening cell wall results in the increase in the activity of antioxidant enzymes, reduces ROS and delays senescence. Furthermore, Zhang and Li (2014) reported that any factor affecting the strength of the cell membrane can control active oxygen metabolism and H₂O₂ by the activation of antioxidant enzymes (Cat, Sod).

Conclusions

The results of this study showed the positive effect of all the sprayed agro-chemicals on the quality of jujube fresh fruit. Based on the results, application of calcium nitrate and salicylic acid improved firmness, Vitamin C content, titratable acidity, total phenolic content, antioxidant activity and catalase enzyme and reduced total soluble solids. Finally, the obtained results suggested that application of salicylic acid at the concentration of 4 mM and calcium nitrate 2% can be a feasible and easy technique for maintaining the fruit quality and extending postharvest life of jujube fresh fruit.

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