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ORIGINAL ARTICLE

Chinese jujube physicochemical characteristics, storability and marketing in response to preharvest application of salicylic acid and calcium nitrate

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Our study was conducted on Chinese jujube (*Ziziphus jujuba* Mill.) to investigate the effects of preharvest application of salicylic acid and calcium nitrate on total soluble solids, titratable acidity, fruit firmness, total soluble solids / titratable acidity, electrolyte leakage, weight loss, PH, fruit length, fruit width, seed length, and seed diameter. Results indicated that salicylic acid and calcium nitrate increased fruit firmness, titratable acidity and reduced total soluble solids / titratable acidity, ion leakage and weight loss, but fruit width was increased only by Calcium Nitrate and total soluble solids was reduced only by salicylic acid. Fruit length, seed length, seed diameter and PH were not significantly changed. Maximum fruit firmness (4.22 N) was obtained in treatment containing calcium nitrate 2%. Treatment containing 2 mM and calcium nitrate 2% had the highest amount of titratable acidity (0.45 mg/100g FW). The lowest ion leakage (29.26%) and the highest fruit width (16.6 mm), observed in calcium nitrate 2%. Treatment containing salicylic acid 4mM had the lowest amount of total soluble solids / titratable acidity (57.01) and weight loss (3.78%). The lowest TSS content (23.11%) observed in salicylic acid 4mM. Based on the results salicylic acid and calcium nitrate played an important role in maintaining and extending post-harvest quality of fresh jujube fruit and 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 and many countries, especially in Iran from long ago. This valuable fruit has short post-harvest life, 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 to obtain higher return of resources (Tian et al., 2005; Cao et al., 2013). 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 agro-chemical substances to delay ripening, decrease losses, and 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). Calcium application maintains cell turgor, membrane integrity and tissue firmness, and delays membrane lipid catabolism, which extend storage life of fresh fruits (Rizk-Alla, 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). 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).

Materials and methods

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 to 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 at three times in color 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 ºC 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.

Measurement of physicochemical attributes

A hand-held refractometer (Atago, Tokyo, Japan) was used to measure total soluble solids. Titratable acidity 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 was measured on ten fruits from each sample using a TA-XT2i texture analyzer (Stable Micro Systems Ltd, Godalming, UK) fitted with a 2mm diameter probe operated at a speed of 1 mm s^{-1}.

Weight loss was estimated as follows: % Weight loss = [(initial weight − fruit weight after storage intervals)/ initial weight] × 100. Fruit juice acidity was determined using a pH meter (MTT65 Japan). The rate of electrolyte leakage (EL) was determined as described by Mirdehghan et al. (2007b) in duplicate for each replicate, using 6 discs (10mm) of peel tissue (1.50±0.02 g) cut with a cork borer. Conductivity was measured after 4 h of incubation in 25 mL of 0.4M mannitol under constant shaking, using a Crison conductivity meter (Met Rohm, 664). After readings were taken, the vials were autoclaved at 121◦C for 20 min, held for 24 h and conductivity was measured again for total electrolytes.

The rate of electrolyte leak-age was expressed as a percentage of the total (Initial/Total)×100.

Fruit width, fruit length, seed length and seed diameter was determined with digital caliper.

Statistical Analysis

The experiment was performed in a factorial split-plot based on randomized complete block design with 3 replications. Data were analyzed by using SAS 6.0 software (2005), 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 lowest amounts of TSS were obtained at 40th day (26.92%) and 0 until 20th days (22.48-22.63%), respectively (Table 1). Kokabi and Tabatabai (2011) reported that 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 ripening fruit 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 lowest contents were observed in control (25.76%) and concentration of 4 mM (23.11 %), respectively (Table1 and Figure 1). The reduction found in TSS agrees 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).

Titratable acidity (TA)

Based on the data, enhancement in the duration of shelf life led to the reduction of TA. The highest value of TA 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 enzymatic reactions, it is expected that TA decreases during postharvest (Shokrollahfam et al., 2012). Organic acids are energy sources that are utilized in fruit ripening phase when metabolism increases (Aguayoa et al., 2006).

As the results showed, increase in salicylic acid concentration significantly increased TA, so that the highest and the lowest contents of TA were observed in 2 mM (0.45 %) and control (0.39 %), respectively (Table 1 and Fig. 3a).

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 (Srivastava et al., 2000; Han et al., 1997). 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 et al (1999) also reported that salicylic acid delayed climacteric peak and respiration rate in banana, peach, kiwi and apple.

Results in Table 1 and Figure 3 B showed that TA increased by the application of calcium nitrate. As 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 during days 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. 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 (Fisher et al., 1991; Prasanna et al., 2007).

Flesh firmness, as the results showed in Table 1and Fig. 3c, was significantly increased as the concentration of salicylic acid enhanced, so that the highest value of firmness was observed in salicylic acid spray at concentrations of 2 and 4 mM (4.08 and 4.06 N) and the lowest rate 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 et al (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 highest firmness compared with control and other treatments (Table 1 and Figure 3 D). Calcium compounds are applied on harvested fruits for 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, 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 (Viccente 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 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.

Total soluble solids / Titrable acidity (Tss/Ta)

Tss/Ta content of jujube fresh fruits gradually increased during storage, as the results showed. Zero-day storage showed the lowest content of Tss/Ta (42.94) compared to the 40-day storages with the highest rates of 83.95 (Table 1). The ratio of Tss/Ta is one of the important factors in determination of ripening at harvest time, this ratio increase with increase in maturity rate and it shows fruit quality better than each of the others (kader.,1982).

Furthermore, analysis of data showed that enhancement of salicylic acid concentrations resulted in a significant increase in Tss/Ta, so that the highest and lowest contents of Tss/Ta were observed in control (70.3) and 4 mM (57.01), respectively, as can be seen in Table 1and Fig 3 E. Srivastava and Dwivedi (2000) reported that salicylic acid affects the biosynthesis and action of ethylene, and acts as an anti-stress power (Elwana, El-Hamahmy, 2009), and the use of salicylic acid increases value of the taste index(Tss/Ta) by decreasing activity of sucrose-phosphate synthase enzyme and reducing respiration (Aghdam, 2010).

With respect to the effect of calcium nitrate on Tss/Ta, the results showed that the highest content of Tss/Ta was obtained in control with 64.96 while the lowest content was observed in calcium nitrate 2% (58.63) (Table 1 and Fig 3f).

Shokrollahfam et al. (2012) and Veltman et al. (2000) reported that calcium compounds bind with membrane and increase its stability and the fruits treated with calcium compounds, due to the formation of a thin layer on the surface of the fruit, reduce the respiration rate and prevent the organic acids breakdown that act as respiratory substrates and thus reduce the taste index (Sartip, 2015; Shokrollahfam et al., 2012).

Electrolyte leakage (EL)

Data obtained on the influence of storage intervals on electrolyte leakage showed that enhancement in duration of shelf life led to the increase of electrolyte leakage, so that the lowest phenolic content was obtained at zero-day storage with 23.88 % compared to the highest rate of 42.11 % at 40-day storage interval (Table 1).

Aghdam et al. (2012) state that electrolyte leakage is considered as an index of membrane injury for indirect measurement of cell membrane integrity. The amount of electrolyte leakage of the fruit varies in different phase of ripening and increases when fruit ripens completely. According to Asghari et al. (2012), the cause of increment in electrolyte leakage of fruits during the storage period is chemical changes (enzymatic and non-enzymatic reactions) and normal metabolic activity of cells, resulting in the accumulation of Reactive Oxygen Species(ROS) and cell membrane destruction or reduces cell wall integrity.

Regarding the effect of salicylic acid on electrolyte leakage, results showed that increase in salicylic acid concentration brought about a significant decrease in electrolyte leakage. Based on the results in Table 1 and Fig. 3g, the lowest and highest electrolyte leakage were observed in SA 4 mM (31 %) and control (36.12 %), respectively. Sayyari et al. (2011) reported that salicylic acid by increasing the antioxidant activity and accumulating phenolic substances, destroyed free radicals and prevented their harmful effect on cell membrane degradation. The destruction of the cell membrane causes the ions to leak out of the cell (due to the selectivity of the cell membrane) and excessive Reactive Oxygen Species (ROS) production.

Data showed that calcium nitrate caused a significant decrease in the amount of electrolyte leakage. Treatment of calcium nitrate 2% showed the lowest electrolyte leakage (29.26 %) in comparison with the control with the highest rate (35.13 %), as the results in Table 1 and Fig 3 H showed. Shokrollahfam et al. (2012) stated that calcium compounds can stabilize cell membranes through membrane bonding, thereby preventing free radicals and Reactive Oxygen Species (ROS) to membrane and help maintain the health of biological membranes. Lester and Grossack (1999) reported that the immersion of fruits in calcium compounds maintains the integrity of the cell membrane through the strength of the phospholipid and protein bonding and the reduction of ionization.

Weight loss

Data in Table 1 showed that there was significant difference among different periods of fruit storage. Increase in shelf life duration led to the increase of weight loss, so that the lowest obtained at zero-day storage (0 %) and the highest rates were due to 40-day storage intervals (6.59 %). Shafiee et al. (2010) reported that weight loss during storage is due to metabolic activity, transpiration and respiration, and it can lead to wilting and shrinkage, which reduces fruit marketing.

Weight loss was also affected by salicylic acid, as the results showed. As can be seen in Table 1 and Fig 3 I, the lowest and highest rates of weight loss were observed in treatment containing 4 mM salicylic acid (3.78 %) and control (4.94 %), respectively. Salicylic acid can reduce the weight loss of the fruit and the amount of respiration by closing the stomata (Mante et al., 1992), (Shafiee, 2010). Reducing the weight of fresh fruits and vegetables is due to water loss by respiration and transpiration. Reduced weight loss in salicylic acid treatments has been reported in many horticultural products.

Results obtained from Ca application showed that calcium nitrate reduced weight loss, so that the lowhest and highest rates were observed in calcium nitrate 2% (4 %) and control (4.8 %), respectively (Table 1 and Fig. 3j). Levy and Pooviah (1979) and Shokrolahfam et al. (1391) reported that the main factor in weight loss during storage was evaporation and transpiration from the fruit surface and weight loss decreasing in fruits treated with calcium is due to firmness Maintaining of fruit by decreasing the activity of enzymes.

Fruit width

Data obtained on the influence of storage intervals on fruit width showed that enhancement in duration of shelf life led to the reduction of fruit width, so that the highest fruit width was obtained at zero-day storage with 17.47 mm compared to the lowest rate of 14.91 mm at 40-day storage interval (Table 1). Shokrollahfam et al. (2012) reported that the most important factor in reducing the diameter of fruit during storage was tissue metabolism increase and tissue and cellular strength decrease, by increase enzymes activity that destroyed the cell wall structure. The decrease in the width of fruit during storage period is due to normal metabolic activity, transpiration and respiration, which tends to soften or firmness reduction of fruit tissue (Fischer, Benetet, 1991; Prasana et al., 2007).

Data showed that calcium nitrate caused a significant increase in the amount of fruit width. Treatment of calcium nitrate 2% showed the highest fruit width (16.6 mm) in comparison with the control with the lowest rate (15.94 mm), as the results in Table 1 and Fig. 2 showed.

Lester and Grossack (1999) state that the use of calcium compounds helps to maintain the integrity of the cell membrane through the strength of phospholipid and protein bonding, and renders firmness of skin and fruit, and prevents cell plasmolysis and width fruit reduction.

Data indicated that there was not significant difference in effect salicylic acid on width fruit.

Fruit length, Seed length, Seed diameter and pH

Based on the results, salicylic acid, calcium nitrate and storage time had affected on traits: fruit length, seed length, seed diameter and pH, but these changes were not statistically significant.

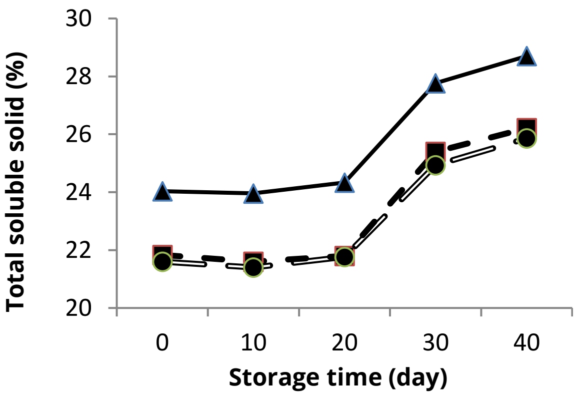

Fig 1. Effect of salicylic acid on total soluble solid of jujube fruits
(▲) Control, (■) Salicylic acid 2 mM (●) Salicylic acid 4 mM

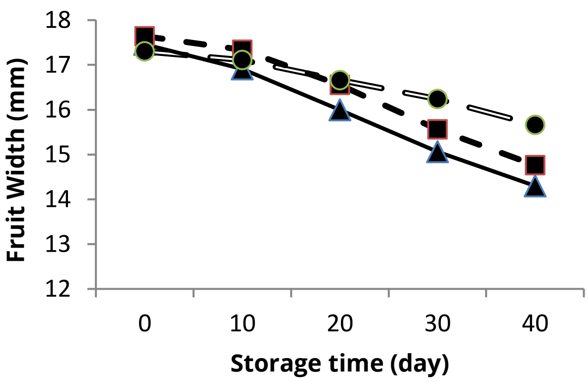

Fig 2. Effect of calcium nitrate on fruit width of jujube fruits Calcium nitrate
(▲) Control, (■) Calcium nitrate 1% (●) Calcium nitrate 2%

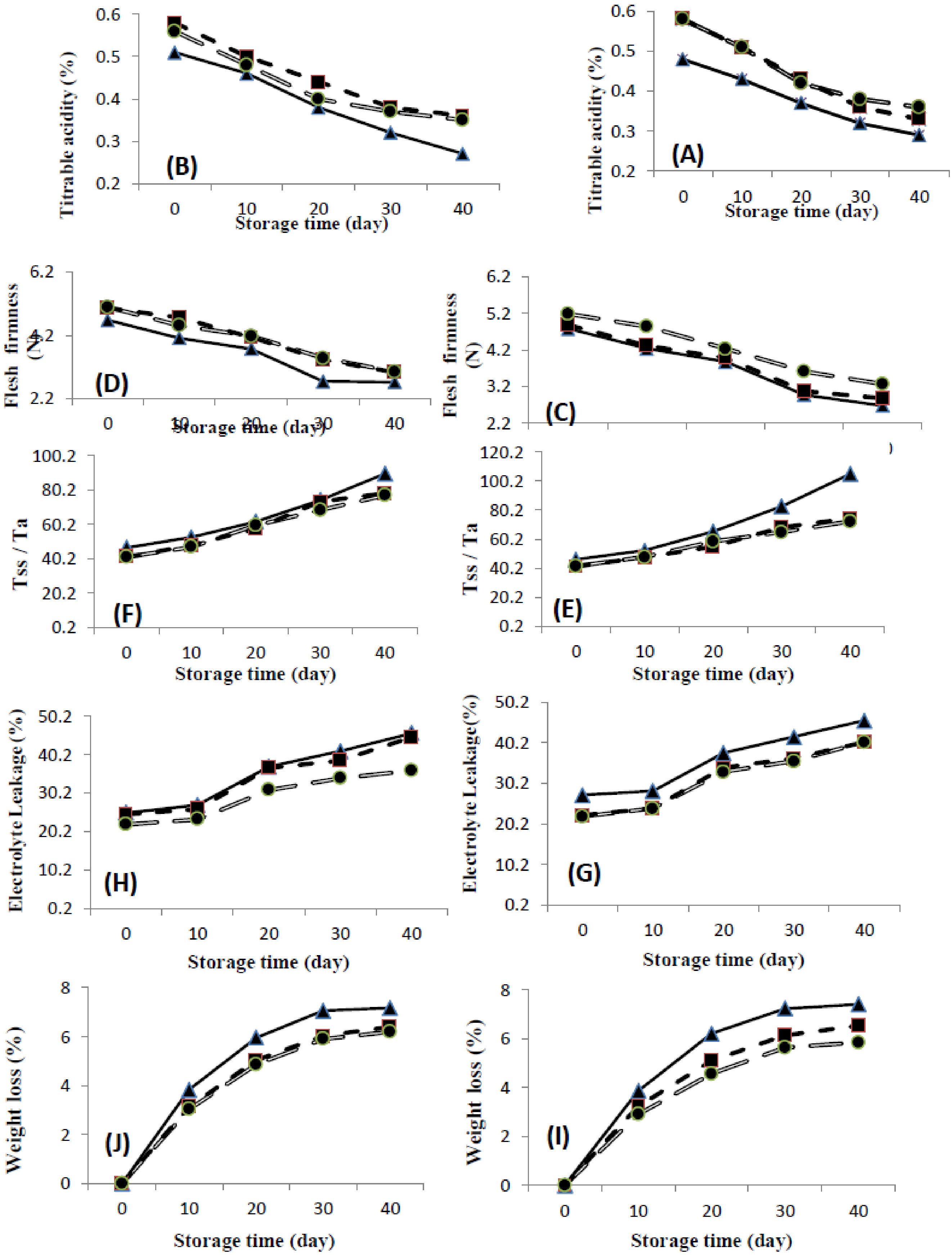

Fig. 3. Effect of salicylic acid and calcium nitrate on titratable acidity, flesh firmness, total soluble solids / titratable acidity, electrolyte leakage, weight loss of jujube fruits; a-g: Salicylic acid: (▲) Control, (■) Salicylic acid 2 mM (●) Salicylic acid 4 Mm. (b-j): Calcium nitrate: (▲) Control, (■) Calcium nitrate 1%, (●) Calcium nitrate 2%.

Table 1. Effect of pre harvest treatments on fruit characteristics of jujube

	TSS (%)	TA (%)	Fruit Firmness (N)	Tss/Ta	Electrolyte leakage (%)	Water Loss (%)	pH	Fruit length (mm)	Fruit width (mm)	Seed length (mm)	Seed diameter (mm)
Salicylic acid(mM)											
0	25.76 a	0.39 b	3.60 b	70.30 a	36.12 a	4.94 a	6.62 a	22.27 a	16.16 a	17.33 a	5.89 a
2	23.36 b	0.45 a	4.08 a	57.28 b	31.40 b	4.20 b	6.58 a	22.23 a	16.39 a	17.32 a	5.83 a
4	23.11 b	0.43 a	4.06 a	57.01 b	31 b	3.78 c	6.68 a	22.48 a	16.37 a	17.47 a	5.79 a
Calcium nitrate(%)											
0	23.008 a	0.38 b	3.7 b	64.96 a	35.13 a	4.80 a	6.59 a	22.01 a	15.94 b	17.59 a	5.87 a
1	24.71 a	0.44 a	3.82 b	61.003 b	34.12 a	4.12 b	6.55 a	22.29 a	16.37 a	17.17 a	5.80 a
2	24.52 a	0.45 a	4.22 a	58.63 b	29.26 b	4 b	6.74 a	22.69 a	16.60 a	17.36 a	5.84 a
Storage(day)											
0	22.48 b	0.55 a	4.95 a	42.94 d	23.88 c	0 d	6.36 a	23.52 a	17.47 a	17.52 a	5.85 a
10	22.32 b	0.48 b	4.46 b	49.19 d	25.44 c	3.33 c	6.51 a	23.13 a	17.12 a	17.46 a	5.93 a
20	22.63 b	0.41 c	4.03 c	59.60 c	34.88 b	5.28 b	6.68 a	22.74 a	16.40 b	17.05 a	5.74 a
30	26.03 b	0.36 d	3.21 d	71.96 b	37.88 b	6.33 a	6.75 a	21.83 a	15.62 c	17.16 a	5.75 a
40	26.92 a	0.32 d	2.93 d	83.95 a	42.11 a	6.59 a	6.85 a	20.42 a	14.91 d	17.66 a	5.91 a

Means in each column not followed by the same letter are significantly different at $P < 0.05$ according to the Duncan's multiple range tests

Conclusions

The results 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 fruit firmness, testable acidity and reduced total soluble solids / testable acidity, ion leakage and weight loss, but fruit width was increased only by Calcium Nitrate. Total soluble solids were reduced only by salicylic acid. Fruit length, seed length, seed diameter and PH were not significantly changed. Finally, our results suggest 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 quality and extending postharvest life of jujube fresh fruit.

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