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Effect of postharvest calcium chloride treatment on the storage life and quality of persimmon fruits (Diospyros kaki Thunb.) cv. 'Karaj'

Maryam Bagheri, Mahmoud Esna-Ashari*, Ahmad Ershadi
Department of Horticultural Sciences, College of Agriculture, Bu-Ali Sina University, Hamedan, Iran

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Abstract
The objective of this study was to evaluate the storage life and quality of persimmon (*Diospyros kaki* Thunb.) fruit cv. 'Karaj' stored at 0°C for 4 months after postharvest calcium chloride treatment. Fruit weight loss, fruit firmness, total soluble solids, titratable acidity, total phenolic content, soluble tannin content, chilling injury, antioxidant activity, electrolyte leakage, and malondialdehyde content were measured in 0, 15, 30, 45, 60, 75, 90, 105, and 120 days after storage. Peroxidase and catalase enzyme activities were also determined at the end of the storage. Fruits were dipped in the solutions containing 0.5, 1, and 2% (w/v) $CaCl_2$ as well as distilled water only as the control. Results showed that the fruit weight loss and chilling injury significantly decreased by $CaCl_2$ treatments compared with the control. $CaCl_2$ treatments also increased fruit firmness and catalase and antioxidant activities, whereas they reduced MDA content, EL, and peroxidase activity during 4 months of cold storage. The results indicated that $CaCl_2$ application influenced TPC and soluble tannin compared with the control but had no significant effect on TA. The best result was obtained from the 2% $CaCl_2$ treatment in almost all the studied parameters. In general, postharvest $CaCl_2$ treatment of persimmon could decrease chilling symptoms during the cold storage.

Keywords: antioxidant activity, chilling injury, electrolyte leakage, soluble tannin content, total phenolic content.

Abbreviations: $CaCl_2$, calcium chloride; **CAT**, catalase; **EL**, electrolyte leakage; **MDA**, malondialdehyde; **min**, minute(s); **POD**, peroxidase; **TA**, titratable acidity; **TPC**, total phenolic content; **TSS**, total soluble solids.

Introduction

Persimmon (*Diospyros kaki* Thunb.) is one of the most important climacteric fruit crop in Iran. According to statistics published by Food and Agriculture Organization of the United Nations (FAO), Iran ranked 12th in terms of world production of persimmon in 2012. Persimmons are divided into two groups, astringent and non-astringent fruits (Zeng *et al*., 2006). The Iranian persimmons are in the first group. Poor postharvest storage life has been identified as the major problem for the Iranian persimmon cultivars, including 'Karaj' cultivar. In order to maximize the storage period of persimmons, keeping fruits in the cold storage (0–1°C) has been suggested by some scholars (Krammes *et al*., 2006; de Souza *et al*., 2011).

Internal browning, softening, and gelatinization are the most important physiological disorders of persimmons that

* Corresponding author Email: m.esnaashari@basu.ac.ir

extend quickly within 2–3 days after transferring from the cold into the physiological temperatures (15–30°C) (Krammes *et al*., 2006). A direct relationship between fruit calcium (Ca^{2+}) levels and some of the physiological disorders has been confirmed (Softner *et al*., 1998). Fertilization regimes and pre- and postharvest treatments have been applied to increase the amount of calcium in fruit tissues, thereby fruit quality is maintained during storage (Conway *et al*., 2002). Depending on the salt type and concentration, postharvest calcium treatment can increase fruit calcium content without causing injury. Calcium chloride has a good effect on cell wall integrity and membrane turgor pressure, being an intermolecular bonding agent that stabilizes pectin compounds in the septum (Hussain *et al*., 2012). Calcium also acts as a link between two chains of galacturonic acid in pectin polymers (Canway *et al*., 2002).

Fruit softening occurred through the activity of hydrolytic enzymes in the cell wall during ripening (Nakano *et al*., 2003). Exogenously applied calcium chloride stabilizes the plant cell wall and protects it from cell wall– degrading enzymes (White and Brodley, 2003). However, there is a little information regarding the enzymatic behavior of persimmon fruit after cold storage (de Souza *et al*., 2011). Postharvest calcium chloride treatment decreases fruit decay, storage disorders, respiration, and ethylene production (Kader and Rolle, 2004; Ali *et al*. 2013a, b). It also delays ripening of some fruits, such as tomatoes (Subbiah and Perumal, 1990), strawberries (Garcia *et al*., 1996), pears (Rosen and Kader, 1989), apples (Softner and Conway, 1998; Hussain *et al*., 2012), peaches (Mahajan and Sharma, 2000), pineapples (Goncalves *et al*., 2000), and loquats (Akhtar *et al*., 2010).

The present study contributed to the evaluation of storage life and quality of persimmon (*D. kaki* Thunb., cv. 'Karaj') fruits treated with $CaCl_2$ and kept at 0°C for 4 months.

Materials and Methods

Plant material and $CaCl_2$ treatment

Persimmon fruits cv. 'Karaj' was picked from an orchard near Karaj, Alborz province, Iran. They were harvested at their commercial maturity stage (dark orange and completely hard), transferred to the laboratory immediately, selected based on uniformity, size, color, firmness, and absence of disease symptoms, and randomly distributed into boxes. Persimmons were then dipped into solutions containing 0.5, 1, or 2% $CaCl_2$, all with 0.05% Tween-20, and distilled water (as control) for 5 min (20°C). All treated fruits left air dried at room temperature (25°C) for 1 hour before being stored. Fruit quality was determined every 2 weeks during 4 months of cold storage at 0–1°C.

Fruit firmness and weight loss

Flesh firmness was measured at three points of fruits' equatorial region using a penetrometer (FDK, Wanger Instrument, Greenwich, CT, USA) fitted with a 5-mm diameter flat probe. Five fruits were used for replication, and results were expressed as Newton per square centimeter. In order to determine fruit weight loss, eight fruits per treatment were weighed weekly and the water loss percentage was calculated as compared with the initial weight.

Total soluble solids (TSS) and titratable acidity (TA)

Fruit samples from each treatment were pooled off and juiced for the determination of TSS and TA. TSS was measured using a handheld refractometer (Atago, Japan) at 20°C, and the results were reported as ^{o}Brix. TA was determined through titration of fruit juice with 0.1 N NaOH up to pH 8.2, using 1 ml of juice diluted to 10 ml with distilled water, and expressed as the percentage of malic acid.

Total phenolic content (TPC)

TPC was extracted from pooled-off fruits based on the Folin-Ciocalteu method (Slinkard and Singleton, 1977). Samples were ground in liquid nitrogen and stored at −20°C until further analysis. One-half gram of ground flesh was extracted with 3 ml of 85% MeOH/HCl (99:1 v/v) filtered through a Whatman No. 1 filter paper. Then, 300 µl of filtered extract was thoroughly mixed with 1.5 ml of Folin-Ciocalteu reagent. After 5 min of incubation at room temperature (25°C), 1.2 ml of 7% sodium carbonate was added. This was shaken at room temperature and held in darkness for 1 h. The absorbance was measured at 765 nm with a UV/Vis spectrophotometer (Carry 100; Varian Analytical Instruments, Walnut Creek, CA, USA). TPC was finally calculated using the standard curve generated with different concentrations of tannic acid and expressed as milligrams of tannic acid equivalents per gram of fresh weight.

Soluble tannin

Soluble tannin content was measured through the Folin-Denis method (Taira, 1996) with a minor change. The absorbance was read against a blank at 760 nm; the intensity of the solution is proportional to the amount of tannins and can be estimated against standard tannic acid, expressed as milligrams of tannic acid equivalents per 100 g of the sample dry weight.

Chilling injury

Fruit chilling injury was assessed by measuring the extent of browning area on a weekly basis as described by Wang *et al*. (2005), considering the following scales: 0 = no browning, 1 = less than ¼ browning, 2 = ¼ to ½ browning, 3 = ½ to ¾ browning, and 4 = more than ¾ browning. Chilling injury was calculated using the following formula:

Chilling injury = $[(1 \times N_1 + 2 \times N_2 + 3 \times N_3 + 4 \times N_4)/(4 \times N)]$ 100,

where N = total number of observed fruits and N_1, N_2, N_3, and N_4 are the number of fruits showing different degrees of browning.

Antioxidant activity

Antioxidant activity of the samples was determined through the DPPH (2,2-diphenyl-1-picrylhydrazyl, free radical) assay. The DPPH assay was carried out following the method reported by Leong and Shui (2002) with a minor change. Various amounts of samples dissolved in methanol were added to 5 ml of a 0.004% methanol solution of DPPH and incubated at 25°C for 30 min. The absorbance was then read against a blank at 517 nm with a UV/Vis spectrophotometer (Carry 100; Varian Analytical Instruments, Walnut Creek, CA, USA). The percentage of inhibition of free-radical DPPH was calculated by the following formula:

Inhibition Percentage = $[(A_{Control} - A_{Sample})/A_{Control}]$ 100.

Determination of Malondialdehyde (MDA)

Determination of MDA was carried out according to the method described by Li (2000). Persimmon pericarp (1 g) from 10 fruits was homogenized in 15 ml of 10% trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000*g* for 20 min, and then 2 ml supernatant of the sample reacted with 2 ml of 0.6% 2-thiobarbituric acid. The absorbance was read at 600, 532, and 450 nm, respectively. MDA was calculated using the following formula:

MDA content ($nmol \cdot g^{-1}$ F.W.) = 6.45 (A532 − A600) − 0.56 A450.

Electrolyte leakage measurement

To determine the amount of electrolyte leakage, the method explained by McCollum and McDonald (1991) was used. For each replication, six discs (10 mm each) of tissue were randomly cut with a cork borer. Discs were placed into a solution containing 25 ml of 0.4 M

mannitol for 4 h on a shaker at room temperature. The initial electrical conduction of this solution was measured using EC meter (Basic 30; Crison Instruments, S.A.). After the first reading (initial EC), the vials were autoclaved at 121°C for 20 min and left overnight (25°C), and the conductivity was read again (second reading for total EC). The amount of electrolyte leakage was then calculated as (initial/total) × 100.

Extraction of enzyme

To extract the total enzyme content of the tissues, 1 g of sample was homogenized in 3 ml of 50 mM phosphate buffer (pH, 7.2) containing 1 mM EDTA, 10 mM $Na_2S_2O_5$, and 1% polyvinyl pyrrolidone (PVP) at 4°C. The homogenate was centrifuged at 14,000*g* for 15 min, and the supernatant was used to determine the enzyme activity. Enzyme activity was measured immediately at the end of storage time.

Peroxidase (POD) activity assay

POD was evaluated by the use of guaiacol by measuring the absorption of tetra guaiacol–containing guaiacol as the peroxidase activity. Tetra guaiacol absorption read at 470 nm 1 min after addition of extract enzyme in reaction was recorded. The amount of tetra guaiacol was calculated by absorbance change per minute at 470 nm, tetra guaiacol extinction coefficient ($\varepsilon = 25.5$ $mMol \cdot cm^{-1}$), and related formula of $A = \varepsilon bc$ (Plewa *et al*., 1991).

Catalase (CAT) activity assay

CAT was measured using H_2O_2 reduced absorption at 240 nm according to the method of Dhindsa and Motowe (1981). The amount of H_2O_2 in the reaction mixture was calculated by absorbance change per minute at 240 nm, H_2O_2 extinction coefficient ($\varepsilon = 28$ $mMol \cdot cm^{-1}$), and related formula of $A = \varepsilon bc$.

Data analysis

The study was conducted as a factorial experiment based on a completely randomized design with two factors ($CaCl_2$ concentration and time of storage, "time of sampling") and three replications. All data were analyzed using one-way analysis of variance (PROC GLM; SAS Institute, Cary, NC), and the means were compared using Duncan's multiple range test ($a \leq 0.05$).

Results and Discussion

Fruit firmness and weight loss

$CaCl_2$ treatments decreased weight loss compared with the control (Table 1), and in particular, fruits treated with 1 and 2% (w/v) $CaCl_2$ concentrations showed less weight loss during the periods of evaluation. The highest weight loss was observed in the control fruits, whereas the lowest weight loss was obtained in fruits treated with 2% calcium chloride throughout 120 days of cold storage (Table 1). Calcium treatments have been suggested to be effective in maintaining membrane integrity and lower rate of weight loss. Shirzadeh *et al*. (2011) reported that apple fruits treated with calcium chloride proved to be the most impressive in reducing weight loss compared with nontreated samples during the 5-month storage. Calcium could decrease the rate of respiration followed by delayed senescence.

Flesh firmness was reduced during storage as shown in Table 1. Tissue firmness was significantly maintained by $CaCl_2$ treatments, so that the treated fruits were similarly firmer than the nontreated samples. Markedly, flesh firmness of the treated fruits with 2% $CaCl_2$ was higher than the other fruits. Positive effects of $CaCl_2$ on fruit firmness have previously been reported (Akhtar *et al*., 2010 and Hussain *et al.*, 2012). Calcium seems to serve as an intermolecular binding agent that stabilizes protein-pectin complexes of middle lamella (Dey, 1984), thus plays a role in holding cell wall structure by interacting with pectic acid in the cell wall to form calcium pectate. High calcium uptake in fruits has been shown to

reduce respiration rates and ethylene production, to delay ripening, increase firmness, and reduce the incidence of physiological disorders and decay, all of which result in increased postharvest shelf life (Kader and Rolle, 2004).

Table 1. Mean comparison of weight loss, tissue firmness, total soluble solid content, titratable acidity, chilling injury and soluble tannin of persimmon fruit cv. 'Karaj' treated with different concentrations of $CaCl_2$ during 120 days of storage at 0°C

Storage period (d)	$CaCl_2$ treatment (%)	Weight loss (%)	Firmness (N. cm^{-2})	TSS (%)	TA (%)	Chilling injury (%)	Soluble tannin (ppm)
0	-	0.0	8.13	11.20	0.58	0.0	6956.3
15	0.0	4.72[a†]	8.06[a]	12.80[a]	0.63[a]	6.25[a]	6547.0[b]
	0.5	3.77[b]	8.10[a]	11.86[a]	0.60[a]	4.16[a]	6883.7[a]
	1.0	3.05[bc]	8.13[a]	11.80[a]	0.56[a]	2.08[a]	6899.0[a]
	2.0	2.58[c]	8.16[a]	11.73[a]	0.53[a]	2.08[a]	6927.7[a]
0	0.0	5.4[a]	7.50[b]	14.33[a]	0.53[a]	8.33[a]	6178.0[b]
	0.5	4.55[b]	7.56[b]	13.06[b]	0.53[a]	6.25[a]	6469.0[a]
	1.0	3.37[c]	7.63[ab]	13.00[b]	0.53[a]	6.25[a]	6500.3[a]
	2.0	3.18[c]	7.90[a]	12.86[b]	0.50[a]	4.16[a]	6519.0[a]
45	0.0	6.16[a]	6.96[a]	16.13[a]	0.43[a]	12.5[a]	5629.6[b]
	0.5	5.27[b]	7.23[a]	14.73[b]	0.43[a]	8.33[b]	5930.3[a]
	1.0	3.92[c]	7.30[a]	14.66[b]	0.40[a]	6.25[b]	5891.3[a]
	2.0	3.28[c]	7.33[a]	14.60[a]	0.40[a]	6.25[b]	5859.0[a]
60	0.0	6.61[a]	6.16[b]	17.06[a]	0.36[a]	14.58[a]	4941.0[b]
	0.5	5.91[b]	6.26[b]	15.80[b]	0.33[a]	19.41[a]	5558.6[a]
	1.0	4.76[c]	6.90[a]	15.53[b]	0.33[a]	10.41[a]	5602.0[a]
	2.0	3.64[d]	7.03[a]	15.26[b]	0.33[a]	8.33[a]	5602.3[a]
75	0.0	8.03[a]	5.53[c]	18.46[a]	0.26[a]	20.83[a]	4226.3[b]
	0.5	7.01[b]	5.73[bc]	17.06[b]	0.26[a]	14.58[b]	4938.3[a]
	1.0	5.67[c]	6.16[ab]	16.86[b]	0.23[a]	12.5[b]	4996.7[a]
	2.0	4.13[d]	6.36[a]	16.80[b]	0.20[a]	10.41[b]	5035.3[a]
90	0.0	9.04[a]	4.23[c]	18.93[a]	0.23[a]	25[a]	3135.3[b]
	0.5	7.67[b]	4.96[b]	17.26[b]	0.23[a]	16.66[b]	4179.0[a]
	1.0	6.47[c]	5.66[a]	17.26[b]	0.20[a]	16.66[b]	4210.3[a]
	2.0	4.59[d]	6.16[a]	17.20[b]	0.20[a]	12.5[c]	4238.3[a]
105	0.0	9.78[a]	3.70[c]	19.93[a]	0.23[a]	31.25[a]	2122.6[b]
	0.5	8.18[b]	4.26[b]	17.73[b]	0.23[a]	27.08[b]	3074.3[a]
	1.0	6.96[c]	5.6[a]	17.73[b]	0.20[b]	20.83[bc]	3124.6[a]
	2.0	5.71[d]	5.83[a]	17.53[a]	0.20[a]	16.66[c]	3245.6[a]
120	0.0	10.17[a]	3.36[c]	21.06[a]	0.16[a]	39.58[a]	1910.6[b]
	0.5	8.95[b]	3.90[b]	18.26[b]	0.16[a]	33.33[b]	2300.6[a]
	1.0	7.90[c]	5.00[a]	18.20[b]	0.16[b]	27.08[c]	2350.6[a]
	2.0	6.64[d]	5.30[a]	17.86[b]	0.13[a]	25[c]	2433.0[a]

† Similar letters in each column indicate nonsignificant differences among treatments at $P \leq 0.05$.

Total soluble solids and titratable acidity

Total soluble solids of the samples increased slowly as the storage period increased. Although the differences between the calcium chloride–treated fruits in terms of TSS were not significant, their values were still higher than the controls (Table 1). Our results were in agreement with Shirzadeh *et al.* (2011) who reported that apple fruits treated with $CaCl_2$ had lower TSS compared with nontreated fruits during storage. This increase in TSS during storage is probably because of the hydrolysis of polysaccharides, such as starch, and the concentrated juice content as a result of dehydration (Akhtar *et al*., 2010). Calcium application increased fruit tissue calcium content and influenced postharvest changes and senescence process involving sugars, acids, anthocyanins, and texture (Ali *et al*., 2013a).

Titratable acidity of the samples decreased slightly in all treatments and did not seem to be impressed by the postharvest calcium treatments. Manganaris *et al*. (2005) and Akhtar *et al*. (2010) have also reported that postharvest calcium chloride treatments did not affect TA in peaches and loquats during storage. Titratable acidity is related to the concentration of organic acids in the fruit extract, which is an important parameter in maintaining the quality of fruits. It seems that $CaCl_2$ treatments had no significant effect on maintaining TA (Table 1).

Chilling injury

Chilling injury in 'Karaj' persimmon was observed as wrinkled peel, flesh browning, gelling, and softening. Low temperature induces oxidative stress in the cell, and chilling temperatures alter the equivalency between reactive oxygen species (ROS) generation and oxidative stress defense mechanisms. These mechanisms cause development of the chilling symptoms.

The highest chilling injury was seen in the controls and the lowest in 2% $CaCl_2$-treated fruits (Table 1). The results obtained from 0.5 and 1% calcium chloride treatments were statistically similar. Chilling injury increased during storage in all treatments (Table 1). Flesh browning is a symptom of chilling, and it often occurs because of the effect of enzyme polyphenol oxidase on phenolic compounds released from the vacuole after chilling (Arnal and del Rio, 2004). It seems that $CaCl_2$ prevents leakage of phenolic compounds in the intercellular space by consolidation of cell wall reduces the flesh browning as a consequence of polyphenol oxidase activity. The effect of $CaCl_2$ treatments on reducing chilling symptoms has previously been reported by Safizadeh *et al.* (2007) and Akhtar *et al*. (2010). Calcium ions link up peptic molecules in the middle lamella and retard disintegration of cell walls (Ali *et al.*, 2103a); so, it seems that $CaCl_2$ reduces chilling damages through cell wall protection.

Total phenolic content and soluble tannin

The greatest values of total phenolic content were obtained from the fruits treated with 2% $CaCl_2$ compared with the control (Fig. 1c). Fruits treated with 0.5 and 1% $CaCl_2$ were found to have more TPC than the control during 2 months of storage, whereas they showed no significant difference with the control after the storage period. Phenolic compounds in fruits and vegetables are capable of protecting cells against oxidative injury through scavenging free radicals (Wada and Ou, 2002; Chun *et al*., 2003). However, phenolic constituents decrease with the advancement in storage time, but the maximum retention of phenolic compounds can be inferred by reduced respiration, softening, and acidity loss in calcium-treated fruits (Ali *et al*., 2013b).

The main characteristic associated with the quality of ripe persimmon fruit is tannin content. Results showed that the soluble tannin content of fruit was affected by storage time and $CaCl_2$ treatments, which is also confirmed by El-badawy (2007). Soluble tannin content decreased during storage in

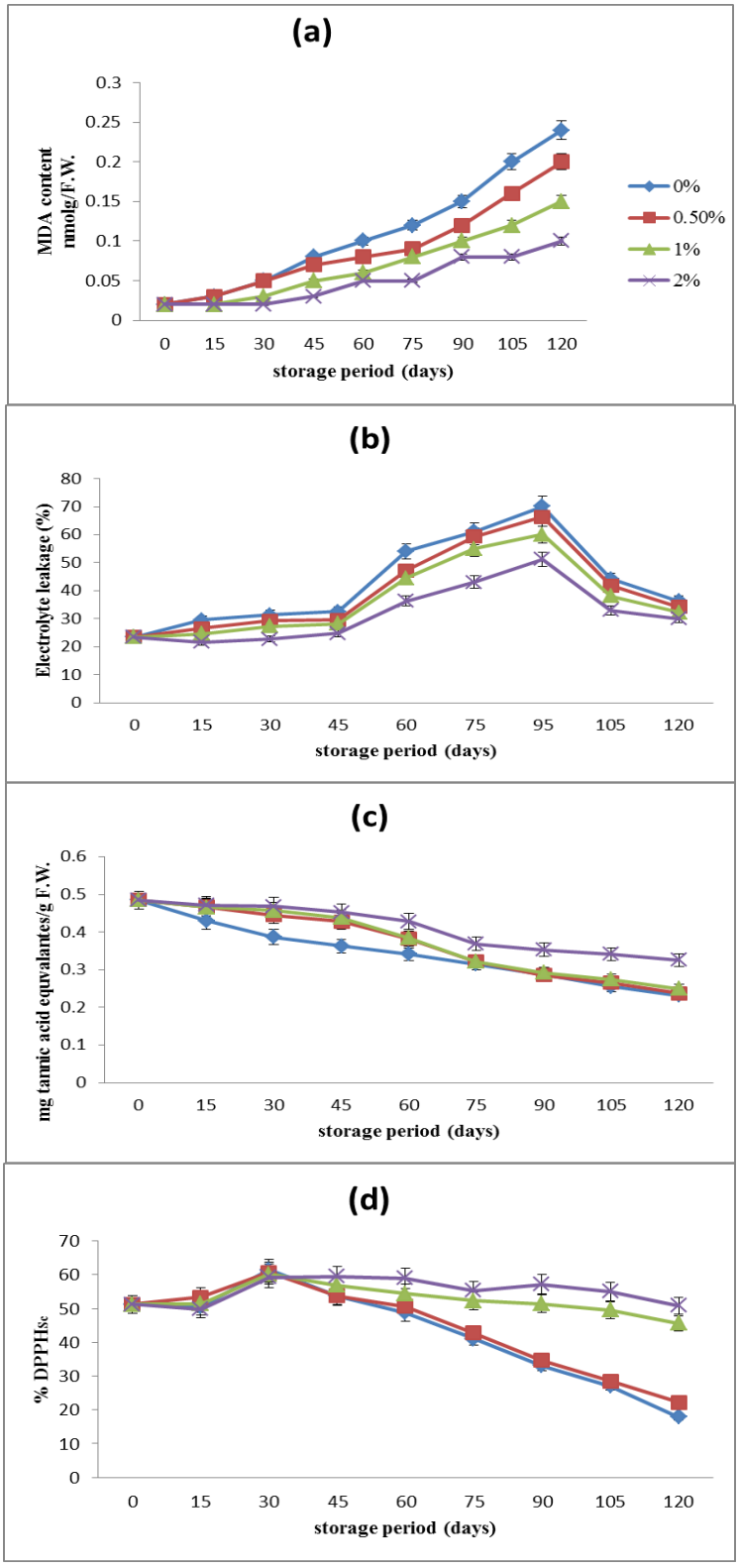

Fig. 1. Effects of different $CaCl_2$ concentrations on a) changes in malondialdehyde (MDA) content, b) electrolyte leakage, c) TPC, and d) percent DPPH inhibition of persimmon fruit cv. 'Karaj' during 120 days of storage at 0°C. Values are the means of the treatments (n = 3), and comparison of values has been made through the Duncan's multiple range test ($P \leq 0.05$). Vertical bars represent standard errors of the mean.

control fruits, and no significant difference was found among the $CaCl_2$-treated fruits. Reduction of soluble tannin content during storage is related to the complex formation between pectin released from cell walls and tannins (Taira *et al*. 1997). It has been suggested that calcium delays the onset of ethylene climacteric period and climacteric peak (Ben-Arie *et al*., 1995). As ethylene polymerizes tannins, it seems that Ca treatments can maintain soluble tannin for a longer period in persimmon.

Malondialdehyde

MDA content was very low at the harvesting time but increased continuously with the storage time in all treatments, whereas the control and 0.5% $CaCl_2$-treated fruits showed a higher MDA content compared with the other treatments. In contrast, a relatively low MDA content was observed in the 2% $CaCl_2$ treatment (Fig. 1a). MDA is formed by peroxidation of fatty acids resulting in developing free radicals during fruit senescence (Voisine *et al*., 1993), which is a major product of membrane fatty acid oxidation (Su *et al.*, 2005). MDA has been recognized as a proper indicator of membrane integrity (Bailly *et al.*, 1996). Greater conservation of specific membrane lipid components in the Ca^{2+} infiltrated fruit, both during and after low-temperature storage, may contribute to the well-known beneficial effects of Ca^{2+} infiltration in maintaining fruit quality (Picchioni *et al*. 1998). Also, the results of this study showed that $CaCl_2$ could be a good treatment to increase membrane integrity and fruit storage time.

Electrolyte leakage

Electrolyte leakage is a good indicator to evaluate the loss of cell membranes' semipermeability. It is also considered as an index for membrane damage and has widely been used as an indicator of chilling injury (Maccollum and Macdonalds, 1991). The extent of electrolyte leakage depends on fruit tissue integrity, and increase in this parameter should be expected at the end of ripening or when the fruit is exposed to severe stress conditions like low temperatures (Vicente *et al*., 2006).

Electrolyte leakage in persimmon reduced during the initial 45 days of cold storage but increased rapidly thereafter (Fig. 1b). Fruits treated with $CaCl_2$ showed a similar trend, but $CaCl_2$ treatment reduced the leakage, being about 17% of that in control on day 120 ($P < 0.05$). Calcium is considered to be efficient in maintaining membrane integrity by reducing ion leakage, phospholipids, and protein losses in the cellular network (Lester and Grusak, 1999).

Antioxidant activity

The DPPH method used to evaluate the antioxidant capacity of fruits was dependent on the ability of antioxidants in the samples to scavenge specific radicals. Antioxidants are known to play a key role in preventing oxidative damage in cells. Results showed a significant lower antioxidant capacity for the control fruits compared with the $CaCl_2$-treated fruits. There is a positive correlation between antioxidant activity and total phenolic compound (Wang and Lin, 2000). In accordance with TPC, maximum antioxidant capacity was seen in $CaCl_2$-treated fruits than in controls (Fig. 1d). According to our findings, phenolic compound reduced with increase in storage period, through enzymatic activity and oxidation, and as a result, antioxidant activity decreased in fruits (Kevers *et al*., 2007).

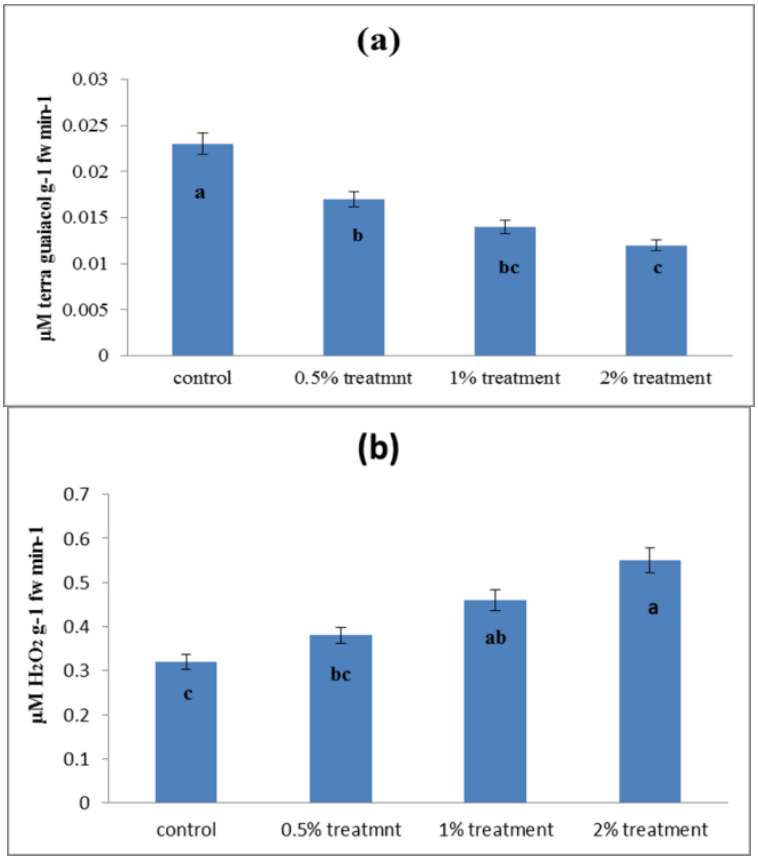

Fig. 2. Effects of different $CaCl_2$ concentrations on a) peroxidase and b) catalase activities of persimmon fruit cv. 'Karaj' after 120 days of storage at 0°C. Values are the means of the treatments (n = 3), and their comparison has been made through the Duncan's multiple range test (P ≤ 0.05). Vertical bars represent standard errors of the mean.

Peroxidase and catalase activities

The results indicated that maximum POD activity was obtained from the controls, whereas a significant difference was observed with other treated fruits. POD activity reduced with increasing $CaCl_2$ concentration from 0.5% to 2%. Minimum POD activity was observed in 2% $CaCl_2$-treated fruits after 120 days of storage (Fig. 2a), which is similar to the results of Shirzadeh *et al.* (2011). The study by EL-hallali *et al.* (2003) showed that Ca^2 reduces chilling injury and POD activity in fleshes and peels of fruits stored at low temperatures for prolonged periods. Ranadive and Haard (1977) identified a correlation between peroxidase activity and lignification in cell walls of fruits. Ca^{2+} appears to be necessary for POD activity because it induces the cross-linking of polygalacturonan chains into a structure that can be recognized by isoperoxidase (Ponel *et al.*, 1999).

The results also showed that the storage time and $CaCl_2$ had a significant effect on CAT activity of fruits (Fig. 2b). These findings indicate that maximum CAT was observed in 2% $CaCl_2$-treated fruits, whereas the minimum CAT activity was recorded in the control. It has been reported that calcium treatments maintained a higher CAT activity in loquat (Akhtar *et al.*, 2010) and apricot (Ali *et al.*, 2013b) during cold storage. Reduced electrolyte leakage through calcium treatment expands enzyme antioxidant activity, the cell wall integrity, and consistency (Mortazavi *et al.*, 2007). Enhancement of tissue browning in the controls could possibly be associated with the increase in CAT activity. This might be due to the reason that calcium lagged

higher respiration rates in the treated fruits as compared with the controls and lower $CaCl_2$ concentrations.

Calcium treatment is a safe and applicable method of increasing the shelf life and reducing the chilling injuries in persimmon during cold storage. Future studies are needed to explore persimmon postharvest physiology by dipping postharvest fruits in calcium chloride solution with more variable concentrations.

References

1. Akhtar, A., N.A. Abbasi, and A. Hussain. 2010. Effect of Calcium Chloride Treatments on Quality Characteristic of Loquat Fruit During Storage. Pak. J. Bot. 42(1):181-188.
2. Ali, S., T. Masud , K.S. Abbasi, T. Mahmood, S. Abbasi, and A. Ali. 2013a. Influence of $CaCl_2$ on Physico-Chemical, Sensory and Microbial Quality of Apricot cv. Habi at Ambient Storage. J. Chem. Bio. Phy. Sci. Sec. 3(4):2744-2758.
3. Ali, S., T. Masud, K.S. Abbasi, T. Mahmood and I. Hussain. 2013b. Influence of CaCl2 on Biochemical Composition, Antioxidant and Enzymatic Activity of Apricot at Ambient Storage". Pak. J. Nutr., 12 (5): 476-483.
4. Arnal, L and M.A. del Rio. 2004. Quality of Persimmon Fruit cv. Rojobrillante During Storage at Different Temperatures. Span. J. Agr. Res. 2(2):243-247.
5. Bailly, C., A. Benamar, F. Corbineau, and D. Côme. 1996. Changes in Malondialdehyde Content and in Superoxide Dismutase, Catalase and Glutathione Reductase Activities in Sunflower Seeds as Related to Deterioration during Accelerated Ageing. Physiol. Plant. 97(1):104–110.
6. Ben-Arie, R., I. Mignani., L.C. Greve., M. Huysamer. And J.M. Labavitch. 1995. Regulation of the ripening of tomato pericarpedaics by GA_3 and divalent cations. Physol. Plant. 93:99-107.
7. Chun, O.K., D.O. Kim, C.Y. Lee. 2003. Superoxide Radical Scavenging Activity of the Major Polyphenols in Fresh Plums. J. Agr. Food Chem. 51:8067–8072.
8. Conway, W.S., C.E. Sams, and K.D. Hickey. 2002. Pre- and Postharvest Calcium Treatment of Apple Fruit and its Effect on Quality. In: Tagliavini, M. *et al.* (eds). Proc. IS on Foliar Nutrition Acta Hort. 594:413-419.
9. Dhindsa, R. S. and W. Motowe. 1981. Drought Tolerance in Two Mosses: Correlation with Enzymatic Defense Against Lipid Peroxidation. J. Exp. Botany. 32:79-91.
10. de Souza, E.L., A.L.K. de Souza, A. Tiecher, C.L. Girardi, L. Nora, J.A. da Silva, L.C. Argenta, and C.V. Rombaldi. 2011. Changes in Enzymatic Activity, Accumulation of Proteins and Softening of Persimmon (*Diospyros kaki* Thunb.) Flesh as a Function of Pre-Cooling Acclimatization. Sci. Hort. 127:242–248.
11. Dey P.M., K. Brinson. 1984. Plant cell walls. Adv. Carbohy. Chem. Biochem. 42:265–382.
12. El- badawy, H.E.M. 2007. Trials to Improve Marketing Characteristics and Prolonging Storage Life of Persimmon and Mango Fruits. Ph.D thesis. Benha University. Egypt. 390 p.
13. El-hilali, F. A. Ait-Oubahou, A. Remah, and O. Akhayat. 2003. Chilling Injury and Peroxidase Activity Changes in "Fortune" Mandarin Fruit during Low Temperature Storage. Bulg. J. Plant Physiol. 29:44-54.
14. Food and Agriculture Organization (FAO), 2014. http://www.fao.org
15. Hussain, P. R., R.S. Meena, M.A.Dar, and A.M. Wani. 2012. Effect of Post-Harvest Calcium Chloride Dip Treatment and Gamma Irradiation on Storage Quality and Shelf-Life Extension of Red Delicious Apple. J. Food Sci. Technol. 94(4):415-426.
16. Garcia, J.M., S. Herrera, and A. Morilla. 1996. Effects of Postharvest Dips in Calcium Chloride on Strawberry. J. Agr. Food Chem. 44:30-33.
17. Goncalves, N.B.,V.D. de Carvalho, and J.R. de A. Goncalves. 2000. Effect of Calcium Chloride and Hot Water Treatment on Enzyme Activity and Content of Phenolic Compounds in Pinapples. Pesquisa Agropecuaria Brasileira. 35(10):2075-2081. (Portuguese/English Abestract).
18. Kader, A.A. and Rolle, R.S. 2004. The Role of Post-Harvest Management in Assuring the Quality and Safety of Horticultural Produce. Rome: FAO Agricultural Series Bulletin 152, 51 pp.
19. Kevers, C., M. Falkowski, J.O. TabartDefraigne, J. Dommes and J. Pincemail. 2007. Evolution of

Antioxidant Capacity During Storage of Selected Fruits and Vegetables. Plant Molecular Biology and Biotechnology, Plant Biology Institute. University of Liège, SartTilman, B-4000 Liege, Belgium.

20. Krammes, J.G., L.C. Argenta, and M.J. Vieira, M.J. 2006. Influence of 1-methylcycloprpene on Quality of Persimmon Fruit cv. 'Fuyu' after Cold Storage. In: Websrter, A.D. and Ramirez, H. (eds). X International Symposium on Plant Bioregulators in Fruit Production. Acta Hort. Saltillo, Mexico. PP:513-518.
21. Leong, L. P., and G. Shui. 2002. An Investigation of Antioxidant Capacity of Fruits in Singapore Markets. Food. Chem. 76:69–75.
22. Lester, G.E and M.A. Grusak. 1999. Postharvest Application of Calcium and Magnesium to ¡Honeydew' and ¡Netted' Muskmelons: Effects on Tissue Ion Concentrations, Quality and Senescence J. Amer. Soc. Hort. Sci., 124:545-552.
23. McCollum, T.G. and R.E. McDonald. 1991. Electrolyte Leakage, Respiration, and Ethylene Production as Indices of Chilling Injury in Grapefruit. HortScience 26:1191–1192.
24. Mahajan, B.V.C. and R.C. Sharma. 2000. Effects of Pre-Harvest Applications of Growth Regulators and Calcium Chloride on Physico-Chemical Characteristics and Storage Life of Peach (*Prunus persica* Batsch) cv. Shan-e-Punjab. Haryana. J. Hort. Sci. 29 (1&2):41-43.
25. Manganaris, G.A., M. Vasilakakis, I. Mignani, G. Diamantidis, and K. Tzavella-Klonari. 2005. The Effect of Preharvest Calcium Sprays on Quality Attributes, Physicochemical Aspects of Cell Wall Components and Susceptibility to Brown Rot of Peach Fruits (*Prunuspersica*L. cv.*Andross*). Sci. Hort. 107(1):43-50.
26. Mortazavi. N, R. Naderi , A. Khalighi , M. Babalar, and H. Allizadeh. 2007. The Effect of Cytokinin and Calcium on Cut Flower Quality in Rose (*Rosa hybrida* L.) cv. Illona. J. Food. Agr. Environ. 5:311-313.
27. Nakano, R., E. Ogura, Y. Kubo, and A. Inaba. 2003. Ethylene Biosynthesis in Detached Young Persimmon Fruit is Initiated in Calyx and Modulated by Water Loss from the Fruit. Plant Physiol. 131:276-286.
28. Picchioni, G. A., A. E. Watada, W. S. Conway, B. Whitaker, and C. E. Sams. 1998. "Postharvest Calcium Infiltration Delays Membrane Lipid Catabolism in Apple Fruit". J. Agr. Food Chem. 46:2452-2457.
29. Plewa, M. J., S.R. Smith, and E.D. Wanger. 1991. Diethyldithiocarbamate Suppresses the Plant Activation of Aromatic Amines into Mutagens by Inhibiting Tobacco Cell Peroxidase. Mutant Res. 247:57-64.
30. Penel, C., P. Cutsem, and H. Greppin. 1999. Interactions of a Plant Peroxidase with Oligogalacturonides in the Presence of Calcium Ions. Phytochemistry 51:193-198.
31. Ranadive, A.S. and N.F. Haard. 1972. Peroxidase Localization and Lignin Formation in Developing Pear Fruit. J. Food Sci., 37:381-383.
32. Rosen, J.C. and A.A. Kader. 1989. Postharvest Physiology and Quality Maintenance of Sliced Pear and Strawberry Fruits. J. Food. Sci. 54:656-659.
33. Safizadeh, M.R., M. Rahemi, and M. Aminlari. 2007. Effect of Postharvest Calcium and Hot-Water Dip Treatments on Catalase , Peroxidase and Superoxide Dismutase in Chilled Lisbon Lemon Fruit. Int. J. Agr. Res. 2(5):440-449.
34. Slinkard, K., and V.L. Singleton. 1977. Total Phenol Analyses: Automation and Comparison with Manual Methods. Amer. J. Enol. Viticult. 28:49-55.
35. Shirzadeh, E., V. Rabiei, and Y. Sharafi. 2011. Effect of Calcium Chloride (CaCl2) on Postharvest Quality of Apple Fruits. Afr. J. Agr. Res. 6(22):5139-5143.
36. Softner, R.A., W.S. Conway, and C.E. Sams. 1998. Effect of Postharvest Calcium Chloride Treatments on Tissue Water Relations, Cell Wall Calcium Levels and Postharvest Life of 'Golden Delicious' Apples. J. Amer. Soc. Hort. Sci. 123(5):893-897.
37. Su, X, Y. Jiang, X. Duan, H. Liu, Y. Li, W. Lin, and Y. Zheng. 2005. Effects of Oxygen on Skin Browning of Longan Fruit. Food Technol. Biotechnol. 43(4):359–365.
38. Subbiah, K. and R. Perumal. 1990. Effect of Calcium Sources, Concentrations, Stages and Number of Sprays on Physico-Chemical Properties of Tomato Fruits. South Indian Hort. 38(1):20-27.
39. Taira, S. 1996. Astringency in Persimmon. In: Linskens, H.-F. and J. F. Jackson . Modern Method of Plant Analysis, Fruit Analysis. (eds). Springer-Verlang, Berlin, 18:97-110.
40. Taira, S., M. Ono, and N. Matsumoto. 1997. Reduction of Persimmon Astringency by Complex Formation between Pectin and Tannin. Postharvest. Biol. Technol. 12:265-271.

41. Vicente, A.R., G.A. Martinez, A.R. Chaves, and P.M. Civello. 2006. Effect of Heat Treatment on Strawberry Fruit Damage and Oxidative Metabolism During Storage. Postharvest Biol. Technol. 40:116–122.

42. Voisine, R., L.P. Vezina, and C. Willemot. 1993. Modification of Phospholipids Catabolism in Microsomal Membranes of Irradiated Cauliflower (*Brassica oleracea* L.). Plant Physiol. 102:213-218.

43. Wada, L., and B. Ou, B. 2002. Antioxidant Activity and Phenolid Content of Oregon Caneberries. J. Agr. Food Chem. 50:3495–3500.

44. Wang, S.Y. and H.S. Lin. 2000.Antioxidant Activity in Fruits and Leaves of Blackberry, Raspberry, and Strawberry Varies with Cultivar and Developmental Stage. J. Agr. Food. Chem. 48(2):140-146.

45. White, P.J. and M.R. Brodley. 2003. Calcium in Plant. Ann. Bot. 92: 487-511.

46. Zeng, Q., A. Nakatsuka, T. Matsumoto and H. Itamura. 2006. Pre-Harvest Nickel Application to the Calyx of Saijo Persimmon Fruit Prolongs Postharvest Shelf Life. Postharvest. Biol. Technol. 42:98-103.