

Impact of Postharvest Calcium Treatments on Storage Life, Biochemical Attributes and Chilling Injury of Apricot

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ABSTRACT

Fruits of two apricot (*Prunus armeniaca* L.) cultivars 'Bagheri' and 'Asgarabadi' were treated with 1 or 3% of Calcium Chloride (CaCl₂) or Calcium propionate (Ca pro) and stored at 1°C, 90% RH for 21 days (d). Fruits were sampled weekly and stored for 2 days at 20°C for shelf life study. Chilling Injury (CI), firmness, color, Titratable Acidity (TA), Soluble Solid Content (SSC), fruit calcium concentrations, Pectin MethylEsterase (PME) enzyme activity and pectin content were monitored during the storage period. CI was first detected in the control fruit after 7 days, and incidence and severity of CI in control fruit was higher than in calcium treated fruit after 14 days. Calcium treatments increased fruit calcium concentrations and delayed ethylene production, as well as softening and color changes. PME activity and water-insoluble pectin decreased while water-soluble pectin increased during fruit storage. Higher calcium concentrations may help to maintain fruit quality by alleviating CI incidence and inhibiting ripening in fruit stored at low temperature.

Keywords: Cell wall, Pectin Methyl Esterase (PME), Postharvest, Storage disorder.

INTRODUCTION

Calcium has an important role in plant cell physiology. It has been reported that Ca is an important intracellular messenger, mediating responses to hormones, biotic and abiotic stress signals and further a variety of developmental processes (Reddy and Reddy, 2004). Calcium plays a very important role in the structure of the cell wall in which cross-link free carboxyl groups on adjacent polygalacturonate chains present in the middle lamella of the plant cell wall contribute to cell to cell adhesion and cohesion, thus leading to the higher firmness of the fruit tissues (Burns and Pressey, 1987). Calcium ion differs from other nutritional elements by being imported into fleshy fruit only in small amounts, much less than into the leaves. Calcium uptake and

distribution in the plant is influenced by water movement to transpiring organs and relative rate of Ca use along the transport pathway (Saure, 2005). Although, Ca is sufficiently available in the soil of most orchards, but localized Ca deficiency may become a problem in several fruit crops, with the risk of large economic losses. Fruit accumulates most Ca during the first 15 to 30 days after fruit setting, after this time fruit Ca assimilation in the fruit is practically undetectable (Bemadac *et al.*, 1996). It has been reported that fruit with low Ca concentrations showed a higher susceptibility to Ca deficiency problems. The improvement of the fruit metabolizable Ca concentration is a very difficult process which drives the continuous search for new, more assimilable Ca compounds (Verlag *et al.*, 2003).

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The involvement of Ca in the regulation of fruit maturation and ripening process has been well established (Ferguson, 1984). Pre-harvest and postharvest treatments with calcium salts have been effective in controlling several physiological disorders, delaying ripening, improving fruit quality, maintaining fruit firmness and reducing the incidence of fungal pathogens (Manganaris *et al.*, 2005; Wójcik and Lewandowski, 2003).

Apricot is a highly perishable fruit, needing low temperature for storage and transportation system (Agar *et al.*, 2006; Crisosto, 2000). In addition, stone fruits such as apricots are very sensitive to low temperature and peaches and nectarines exhibit Chilling Injury (CI) after long periods of cold storage (Lurie and Crisosto, 2005). The main symptoms of peach and nectarine CI are Internal Browning (IB) and flesh mealiness (Brummell *et al.*, 2004). Plums and apricots can also be affected, although the symptoms are slightly different with a gel-like region forming near the stone (Taylor *et al.*, 1993). The onset of CI symptoms determines the postharvest storage potential and development of these chilling disorders reduces consumer acceptance and thus limits fruit storage life.

Different treatments are used to alleviate CI and prolong fruit storage period. Plant growth regulators such as jasmonic acid (Jin *et al.*, 2009), salicylic acid (Cao *et al.*, 2010) and polyamines (Koushesh Saba *et al.*, 2012) may reduce the impact of different stress conditions on horticultural crops (Shen *et al.*, 2000). Exogenous applications of calcium markedly increase the calcium concentration in the flesh and affect some of the changes associated with fruit ripening, disorders and senescence (Poovaiah, 1986). Postharvest calcium treatments include dipping, vacuum and pressure infiltration alone, or in combination with heat treatments or modified atmosphere, have been shown to improve peach (Manganaris *et al.*, 2007) and strawberry fruit shelf-life (García *et al.*, 1996). Apricot cultivars showed different responses to postharvest calcium treatments (Antunes *et al.*, 2003) and reports in other fruits are also inconsistent. Iran is native to apricot and is one

of the main apricot producers (Arzani *et al.*, 2005), therefore the enhancement or improvement of postharvest techniques for successful storage and handling of harvested fruit to the target market such as Europe is an important task for the Iranian apricot industry.

The objective of the current study was to investigate if postharvest CaCl₂ and Ca pro application to apricot before storage at 1°C would prolong the fruit storage period. In addition, effects of treatments on flesh browning, changes in fruit quality, ethylene production, cell wall changes and activities of PME enzymes in the fruit were evaluated.

MATERIALS AND METHODS

Plant Material and Treatment

Two commercial apricot (*Prunus armeniaca* L.) cultivars, 'Bagheri' and 'Asgarabadi', were harvested according to commercial harvest and fruit color change (Koushesh Saba *et al.*, 2012). Harvested fruit were immediately transported by a ventilated car to the Pomology lab of the Department of Horticultural Science at Tarbiat Modares University (TMU) in Tehran. Fruit were selected for uniformity in size, shape and color, without any mechanical damage, blemishes or any pest and diseases. Thirty apricots were sampled and analyzed immediately in order to monitor fruit characteristics at day zero (day 0). The other harvested fruit randomly distributed into 20 groups. Post-harvest calcium treatments were applied with four replications. Fruit were dipped at 20°C for 4 minutes in 1 or 3% Calcium Chloride (CaCl₂), 1 or 3% Calcium propionate (Ca pro), or distilled water (control). All solutions contained Tween-20 (2 g L⁻¹). Fruit were then placed on desiccant Kraft paper and allowed to dry before storage. All control and treated fruit were stored in the storage with 1°C and 90% RH and 1, 7, 14 and 21 days after storage; samples were transferred and stored at 20°C for another

two days prior to any measurements and analysis, to simulate the marketing process.

Fruit Quality Measurements

Fruit flesh color was determined on the opposite paped check of each fruit (15 fruits per replicate) using a Self-Contained Color Measurement Spectrophotometer (Hunter lab, Color Flex®, Reston, VA, USA). Values a^* and b^* were recorded and converted to hue angle using the formula $h^\circ = \tan^{-1}(b^*/a^*)$. Subsequently flesh firmness was measured using a hand-held Effegi penetrometer fitted with an 8 mm tip. Values are expressed as Newton. A wedge-shaped slice of flesh was taken from each fruit and slices were pooled and juiced. SSC was determined using an Atago (Tokyo, Japan) refractometer. TA was measured by titrating 10 mL juice with 0.1N NaOH to an endpoint of pH 8.2 and expressed as the percentage of malic acid.

Ethylene Production

Ethylene was measured as described previously by Koushesh Saba *et al.* (2012). Fruit were removed from storage and allowed to equilibrate with 20°C. Four replicates of three fruit per treatment were placed in a 1 L glass jar hermetically sealed for 1 hour. One mL of headspace gas was withdrawn with a gas syringe, and the ethylene was quantified using a GC (Agilent 6890N, USA) equipped with a Flame Ionization Detector (FID) and a 50-m stainless steel column (Agilent Technology Inc. model 19095P-S25 HP-Plot AL/S). The carrier gas (helium) flow rate was 30 mL min⁻¹. The column temperature was 70°C, and injector and detector temperatures were 180°C. Results were expressed as nL g⁻¹ h⁻¹.

Chilling Injury (CI) Evaluation in the Fruit Symptoms related to chilling injury including gel breakdown or flesh browning (Crisosto, 2000) were visually assessed. A severity index was determined as follows:

$[(\% \text{ of fruit with slight disorder} \times 1) + (\% \text{ of fruit with medium disorder} \times 2) + (\% \text{ of fruit with severe disorder} \times 4)]/4$. Slight, medium and severe CI disorder was calculated and considered when it was less than 25%, between 25 and 50% and more than 50% of the fruit flesh showed symptoms, respectively.

Calcium Determination

To determine fruit calcium content, a wet oxidation procedure was applied. Sampled apricot fruit was oven-dried at 70°C and then grounded to a powder. Grounded fruit samples (0.3 g) were taken and put into the digestion tube. Then, 2.5 ml of pre-mixed digestion solution including 3.5 g of selenium and 7.2 g of salicylic acid (C₇H₆O₃) in 1,000 ml of sulphuric acid (H₂SO₄) was added and allowed to digest for 24 hours. The samples (tubes) were then placed on the termolyne type hot plate at 130°C for 2 hours then cooled to room temperature. The contents of the digestion tube were then transferred to a 100 ml volumetric flask and 3 ml of hydrogen peroxide was added. Finally, the samples were heated at 330°C for 3 hours to decolorize the digested samples and diluted with 48 ml of distilled water. The amount of Ca in the samples was determined using atomic absorption spectrophotometer (Shimadzu, Japan). Results were expressed as mg Kg⁻¹ dry weight (mg kg⁻¹ DW).

Cell Wall

Alcohol-Insoluble Solids (AIS) were prepared according to the method described previously by Rose *et al.*, (1998). Water-Soluble Pectin (WSP) and Water-Insoluble Pectin (WIP) fractions were sequentially extracted from AIS (Manganaris *et al.*, 2006). Five mg of AIS was extracted twice in 5mL of distilled water for 30 min and centrifuged at 17,000×g for 30 minutes. The supernatants were collected, combined, and



designated as the WSP fraction. The pellet was dissolved in 2 mL of concentrated H₂SO₄, stirred for 5 min, and the volume was adjusted to 10 mL with distilled water, and designated as the WIP fraction. Aliquots of these fractions were used for uronic acid determination by the m-hydroxybiphenyl method (Blumenkrantz and Asboe-Hansen, 1973), using galacturonic acid as a standard.

Pectin Methyl Esterase (PME) Assay

The extraction of the PME enzyme was performed using the method described by Denès *et al.* (2000). Activity of PME was determined titrimetrically by measuring the release of carboxylic acid per unit of time, at 30°C as described by Terefe *et al.* (2009). Activity was quantified by adding crud extract enzyme to a 3.5 mg mL⁻¹ of apple pectin solution (70-75% degree of esterification, Fluka) containing 0.125M NaCl at pH 7.0 and 25°C. During the hydrolysis of pectin at 30°C, the pH was maintained at 7.5 by adding 0.01N NaOH solutions. The consumption of NaOH per unit of time was recorded for a 15 minutes reaction period. Note that, the activity of PME was proportional to the rate of NaOH consumption (dVNaOH/dt). The activity Unit (U) is defined as the amount of enzyme required to release 1 ml of carboxyl group per minute under the assay condition. All assays were done in triplicates.

Statistical Analysis

Data for the analytical determinations were subjected to Analysis Of Variance (ANOVA). Sources of variation were storage time periods and treatments. The mean values were calculated and reported as the mean±SE (n= 4). Least Significant Difference (LSD) test at *P*= 0.05 was used to compare means between treated fruit and control at each sampling time. All analyses were performed with MSTATC software.

RESULTS

Chilling Injury

CI was first detected after 7 days of storage for both 'Bagheri' and 'Asgarabadi', thereafter, CI incidence and severity were increased with longer storage life (Figure 1). In control fruit CI at 7 days was 3.7 and 4.3 in 'Bagheri' and 'Asgarabadi' respectively and increased to 65 and 69 after 21 days in cold storage. Calcium treatments decreased CI by the 14th day and the difference was evident with control, thereafter CI increased in treated fruit to almost the same level as control.

Ethylene Production

Fruit in both cultivars were at the early climacteric stage at harvest, ethylene production increased rapidly in control fruit until 14 days, reaching a maximum of 25.56 and 33.98 nL g⁻¹ h⁻¹ in 'Bagheri' and 'Asgarabadi' respectively. Calcium treatments suppressed ethylene production by the 7th day and delayed the onset of climacteric phase of ethylene production. Ethylene production in 1 and 3% of calcium propionate and 1% of calcium chloride increased after 7 days storage and reached to maximum by the 14th day (Figure 2). Although, there was no significant difference in ethylene production in calcium treated and untreated fruit after 14 days but at the same time that difference was evident in 3% calcium chloride in 'Asgarabadi' cultivar.

Fruit Quality

Fruit firmness decreased rapidly in both treated and control fruit. Three percent of Calcium chloride and propionate were more effective than 1% of both calcium sources and decreased softening rate, the difference was evident by the 14th day (Figure 3).

SSC increased during fruit storage, it was 17.9 and 15.3% at first and reached to 20.6

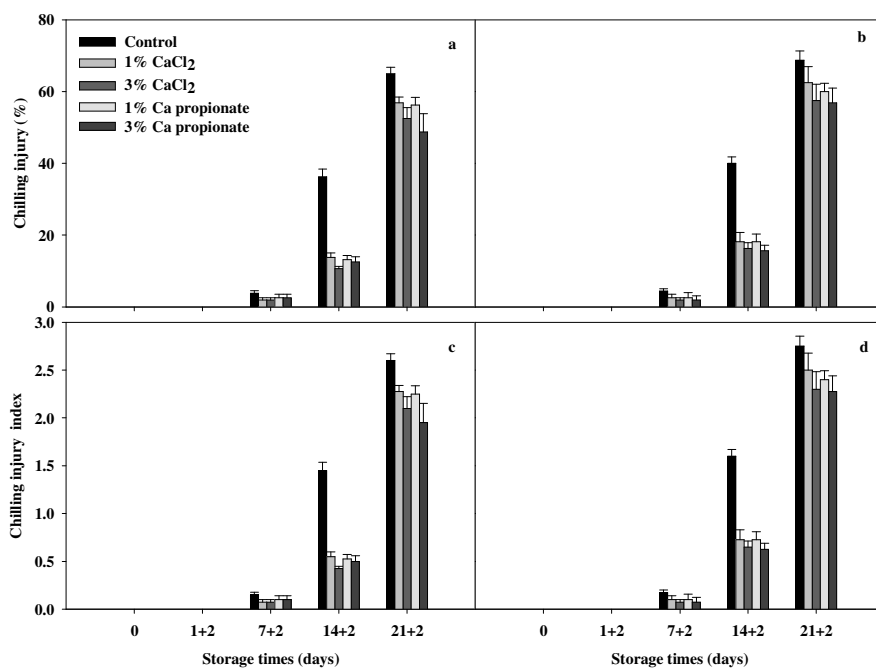


Figure 1. Chilling Injury (CI) incidence in (a) ‘Bagheri’ and (b) ‘Asgarabadi’ apricot cultivars; and CI index for (c) ‘Bagheri’, and (d) ‘Asgarabadi’ apricot cultivars after treatment with 1 and 3% CaCl₂ or Calcium propionate at harvest and storage at 1°C. Mean±Standard error (n= 4).

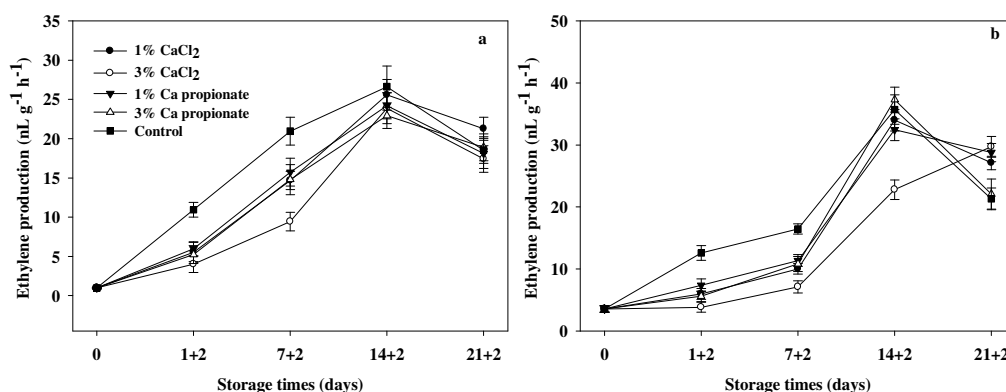


Figure 2. Ethylene production in (a) ‘Bagheri’, and (b) ‘Asgarabadi’ apricot cultivars after treatment with 1 and 3% CaCl₂ or Calcium propionate at harvest and storage at 1°C. Means±Standard error (n= 4).

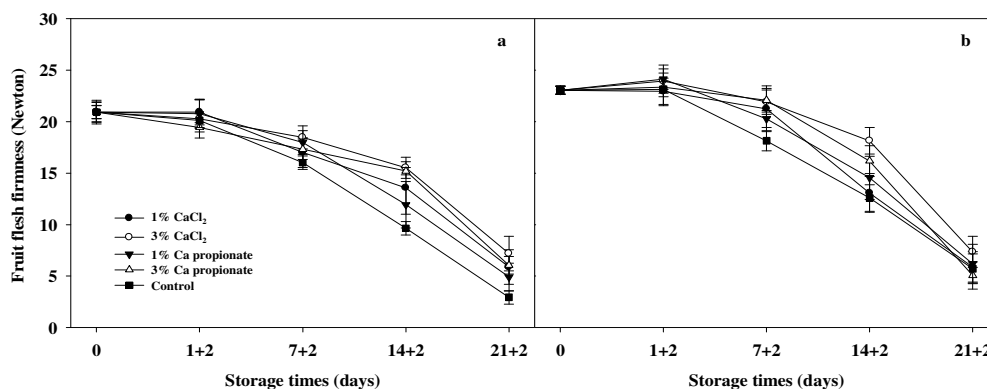


Figure 3. Fruit firmness in (a) ‘Bagheri’, and (b) ‘Asgarabadi’ apricot cultivars after treatment with 1 and 3% CaCl₂ or Calcium propionate at harvest and storage at 1°C. Means±Standard error (n= 4).



and 18.3% in ‘Bagheri’ and ‘Asgarabadi’ cultivars respectively. Although TA decreased, SSC and TA were not influenced by calcium treatments, but h° value decreased during fruit storage. In addition, fruit background color was stable in treated fruit after 7 days of storage, but decreased thereafter. Calcium treatments delayed the reduction in h° during storage and 3% calcium chloride treatment was more effective than the other treatments (Figure 4).

Calcium Content

Fruit calcium content was in the stable condition after fruit harvest and during the

storage period. Calcium treatment applications increased the amount of fruit calcium content in all applied treatments and the highest level was observed in 3% of both calcium chloride and propionate (Figure 5).

Cell Wall Analysis

There was no change in the Water-Soluble Pectin (WSP) content until the first day of storage in both studied cultivars and afterwards it increased. WSP in calcium treated fruit in ‘Bagheri’ was lower than control at the 7th day but no significant difference was observed thereafter except for 3% calcium chloride, which was still lower at the 14th day. No difference was observed in treated

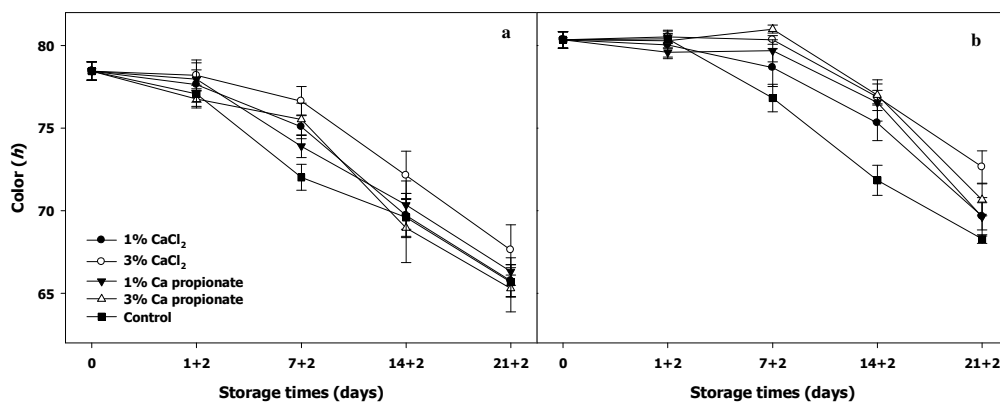


Figure 4. Color in (a) ‘Bagheri’, and (b) ‘Asgarabadi’ apricot cultivars after treatment with 1 and 3% of CaCl₂ or Calcium propionate at harvest and storage at 1°C. Means±Standard error (n= 4).

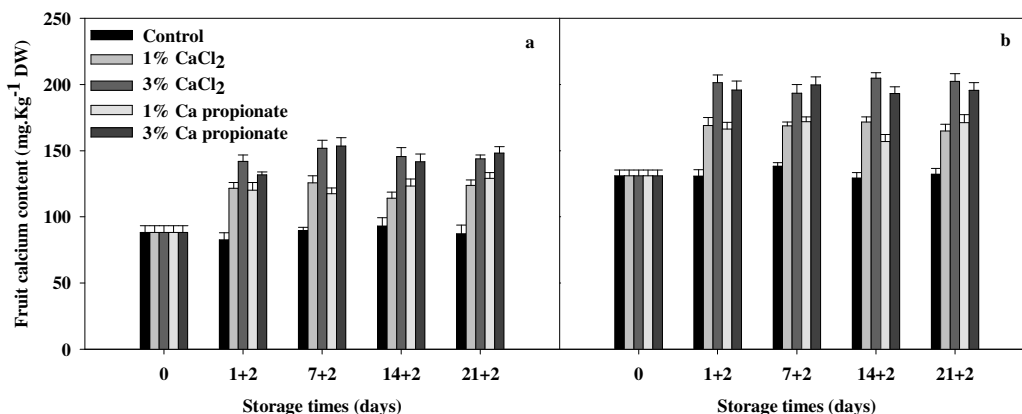


Figure 5. Fruit calcium concentration in (a) ‘Bagheri’, and (b) ‘Asgarabadi’ apricot cultivars after treatment with 1 and 3% of CaCl₂ or Calcium propionate at harvest and storage at 1°C. Means±Standard error (n= 4).

and untreated fruit in ‘Asgarabadi’ cultivar (Figures 6-a and -b). Water-Insoluble Pectin (WIP) significantly decreased, from 301 and 341 $\mu\text{g mg}^{-1}$ cell wall to 254 and 279 $\mu\text{g mg}^{-1}$ cell wall in ‘Bagheri’ and ‘Asgarabadi’ respectively, during fruit storage. WIP was significantly greater in 3% calcium chloride treatment than the other treatments in ‘Asgarabadi’ cultivar (Figures 6-c and -d).

PME Enzyme

PME activity decreased in ‘Bagheri’ during storage at low temperature and was not affected by calcium treatments. PME activity in ‘Asgarabadi’ cultivar was stable after the first day of storage and then decreased but the activity was still higher in control than calcium treated fruit at the 7th day of storage (Figure 7). Enzyme activity was stable in 1% of both calcium chloride and propionate between 7 and 14 days while it was decreased in other treatments and control in the same storage time. Changes in

PME activity were significant in the both studied cultivars during storage at low temperature.

DISCUSSION

It has been reported that stone fruit are susceptible to CI when stored at low temperature (Lurie and Crisosto, 2005). Our previously reported results (Koushesh Saba *et al.*, 2012) indicated that ‘Bagheri’ and ‘Asgarabadi’ apricot cultivars showed CI disorder during storage at low temperature. Both studied cultivars in the present research developed CI after 7 days at 1°C and the incidence increased thereafter. However, calcium treatments significantly reduced CI incidence in both cultivars by 14 days. Despite high level of calcium content in treated fruit, a similar incidence level was observed in either treated or control fruit after 21 days. A negative correlation between tissues calcium content and the

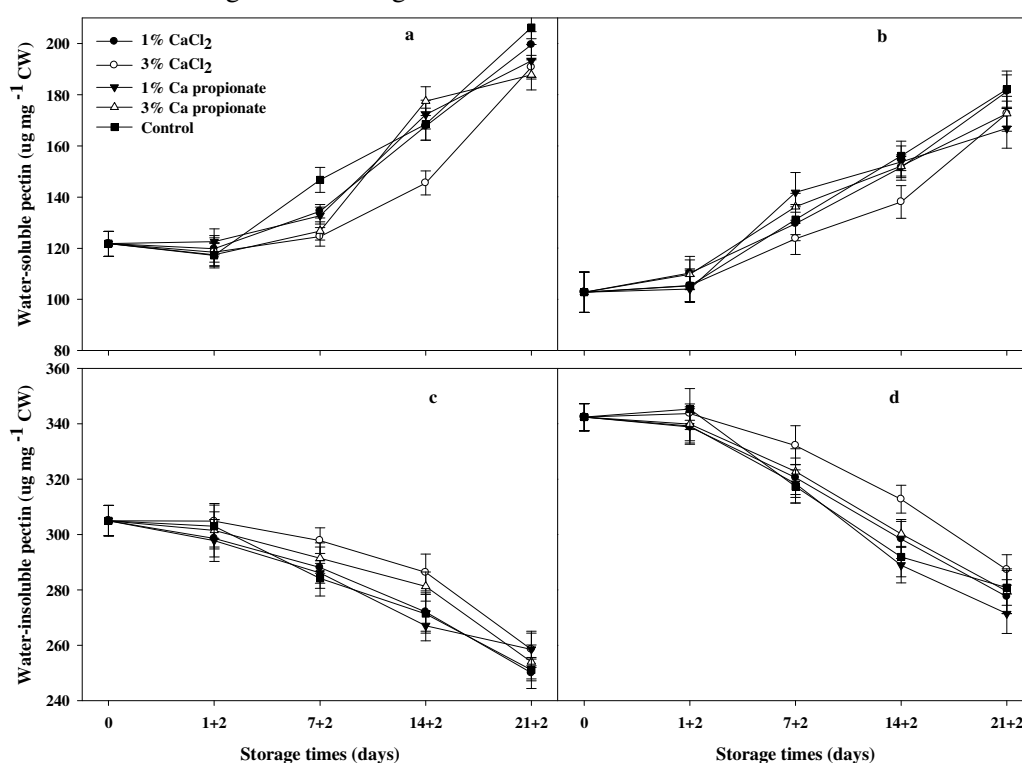


Figure 6. Water-soluble pectin in (a) ‘Bagheri’, and (b) ‘Asgarabadi’ apricot cultivars; and Water-insoluble pectin for (c) ‘Bagheri’, and (d) ‘Asgarabadi’ apricot cultivars after treatment with 1 and 3% of CaCl₂ or Calcium propionate at harvest and storage at 1°C. Mean±Standard error (n= 4).

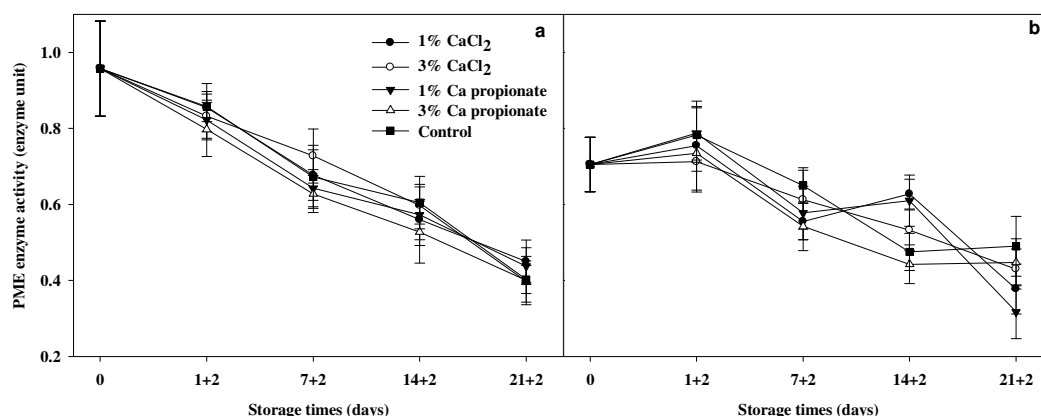


Figure 7. PME enzyme activity in (a) 'Bagheri', and (b) 'Asgarabadi' apricot cultivars after treatment with 1 and 3% of CaCl₂ or Calcium propionate at harvest and storage at 1°C. Means±Standard error (n= 4).

susceptibility of fruit or vegetables to chilling injury has been reported in some literatures. Wang (2010) found a negative correlation between fruit calcium content and CI incidence while Crisosto (2000) showed calcium spray had no effect on CI onset in peach fruit. Calcium ions bind cell walls and maintain the texture properties and semi-permeable properties of cell membranes, it is also known to serve as a secondary messenger in cells (Ferguson, 1984; Poovaiah, 1986). It has been reported that loss of cellular calcium compartmentalization is a consequence of chilling injury (Hewajulige *et al.*, 2003; Wang, 2010).

Fruit flesh texture is a critical quality attribute in the consumer acceptability. Calcium and boron interact with pectin compounds and form cross-linked polymer network which makes cell wall constituents firmer (Dong *et al.*, 2000). Calcium content of the fruit peel and flesh was significantly increased by at least 50% by calcium sprays, compared to unsprayed fruit (Elmer *et al.*, 2007; Manganaris *et al.*, 2005; Tzoutzoukou and Bouranis, 1997). In both studied 'Bagheri' and 'Asgarabadi' cultivars fruit calcium content increased by treatments applications. On the other hand some literature showed fruit flesh calcium content is not related to calcium spray or dipping unless minor changes happen in fruit peel. The uptake of exogenous calcium ions by

ripe strawberry fruit has been related to an increase in the proportion of ionically bound pectins (Lara *et al.*, 2004), thus contributing to the maintenance of cell-to-cell adhesion and the stability of the cell wall, both of which contribute to fruit firmness. Both calcium sources retained fruit firmness and high treatment concentrations were more efficient. It is generally believed that calcium is involved in improving fruit texture through its structural role in the cell walls as the element serves as the cementing material that cross-links to the pectin macromolecules and thus contributes to integrity of the cell wall and the mechanical performance of fruit tissues (Huang *et al.*, 2008; Richardson, 1998). The slower softening is probably associated with effects of calcium treatments on ethylene production. So that, in current study 3% of CaCl₂ and to a lesser extent other calcium treatments suppressed ethylene production of fruit by 14 days of storage. Figures 2-a and -b). These results are similar to those who found that calcium could delay ethylene production in climacteric fruit (Ben-Arie *et al.*, 1995; Chira *et al.*, 2006; Tzoutzoukou and Bouranis, 1997).

Calcium treatments could also affect other fruit quality attributes. SSC and TA are among widely used harvest index and quality attributes of fruit (Antunes *et al.*, 2003). In the present research, SSC increased during fruit storage while TA was

decreased. Similar results were reported during peach fruit ripening (Puerta-Gomez and Cisneros-Zevallos, 2011). Pre harvest calcium treatment has been reported to delay SSC and TA changes (Liu *et al.*, 2009; Serrano *et al.*, 2004). Contrarily, in the current study postharvest calcium treatment was not effective on SSC and TA changes. It has been reported that pre-harvest calcium treatment delayed color change of peach and nectarine during postharvest storage (Serrano *et al.*, 2004). Calcium treatments retain fruit initial color but, apricot cultivar responses were different so that 3% CaCl₂ retained 91 and 95% of initial fruit flesh color in 'Bagheri' and 'Asgarabadi' cultivars respectively after 14 days. These effects could be the result of calcium capacity to bind cell wall and delay the senescence process by affecting texture integrity.

Pre-harvest application of calcium induced low PME activity and decreased WSP in orange (Dong *et al.*, 2009; Manganaris *et al.*, 2007). It has also been observed that exogenous calcium significantly stimulated PME activity (Leiting and Wicker, 1997). The enzyme activity in two studied apricot cultivars showed some fluctuation and inconsistency, but the reduction in overall enzyme activity was observed during cold storage. The results showed that PME activity was not affected by calcium treatments. Our method of PME assay may not be sensitive enough to pick up the very small differences in the enzyme activity which may be influenced by applied treatments.

PME removes the methyl group of the galacturonic acid polymers, which then enables PG to depolymerize the de-esterified polygalacturonic chain, and reduces its molecular weight (Brummell and Harpster, 2001). Pre harvest calcium chloride application decreased the soluble polyuronide content in apricot fruit by 29% (Tzoutzoukou and Bouranis, 1997). On the other hand, Chardonnet *et al.* (2003) reported no direct relation between uronic acid and fruit calcium content. Manganaris *et al.* (2007) speculated that peach fruit treated

with calcium had higher WSP and to somewhat lower WIP. The current study showed that two studied apricot cultivars had a slight difference in response. Generally, the applied treatments specially 3% of calcium treatment induced lower WSP by 7 days in both studied cultivars and by 14 days in 'Bagheri' cultivar. Contrary to WSP, Water In-soluble Pectin (WIP) was decreased over the storage time period, so the direct relation was found between calcium treatments and WIP.

In conclusion our research indicated that 3% postharvest calcium treatment could maintain fruit quality and alleviate chilling injury of apricot fruit by suppression of ethylene production and improving fruit texture attributes.

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اثر تیمار پس از برداشت کلسیم بر عمر انباری، خصوصیات بیوشیمیایی و خسارت سرمازدگی زردآلو

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چکیده

میوه های دو رقم زردآلو (*Prunus armeniaca* L.) باقری و عسگرآبادی با کلرید کلسیم و پروپیونات کلسیم (غلظت ۱ و ۳ درصد) تیمار شده و سپس میوه ها به مدت 21 روز در سردخانه با دمای ۱ درجه سانتیگراد نگهداری شدند. نمونه برداری هر هفته یک بار صورت گرفته و میوه ها بعد از خارج شدن از سردخانه به مدت ۲ روز در دمای ۲۰ درجه سانتیگراد قرار گرفتند. صفاتی مانند خسارت سرمازدگی، سفتی بافت میوه، تولید اتیلن، رنگ، اسیدیته قابل تیتراسیون، محتوی مواد محلول کل، محتوی کلسیم میوه، فعالیت آنزیم پکتین متیل استراز و محتوی پکتین در مدت نگهداری مورد مطالعه قرار گرفت. اولین علائم سرمازدگی بعد از هفت روز مشاهده شد و علائم عارضه و شدت آن در میوه های شاهد بیشتر از میوه های تیمار شده در روز چهاردهم بود. تیمار کلسیم غلظت درونی کلسیم را افزایش داده و باعث تاخیر در تولید اتیلن و نیز نرم شدن و تغییر رنگ گردید. در طول مدت نگهداری زردآلو در سردخانه فعالیت آنزیم پکتین متیل استراز و محتوی پکتین نامحلول کاهش یافت در حالیکه محتوی پکتین محلول افزایش پیدا کرد. احتمالاً افزایش غلظت کلسیم درونی از طریق کاهش علائم سرمازدگی و جلوگیری از رسیدگی میوه در حفظ کیفیت میوه در سردخانه مفید است.