

Full Length Research Paper

Effect of calcium chloride (CaCl₂) on postharvest quality of apple fruits

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The purpose of this research was to estimate the shelf-life and to study the behavior of '*Malus domestica* cv. 'Jonagold' apple kept at 0 to 2°C in a normal atmosphere. The effects of postharvest CaCl₂ applications on shelf-life and quality attributes of apple after harvest or cold storage up to 5 month were determined. The experiment was carried out in 2010 and 2011 and fruit weight losses, fruit firmness, total soluble solids (TSS), pH, titratable acidity (TA), TSS/TA ratio, ethylene production, peroxidase (POD) and catalase (CAT) enzyme activities were measured at 20, 40, 60, 80, 100, 120, and 150 days of postharvest life. The fruits were immersed in distilled water with calcium concentrations (0, 2 and 4% (W/V)). Results showed that fruit weight loss significantly decreased in calcium treatments in comparison to control. Also, results showed that calcium treatments increase fruit firmness, catalase activity, TA and Perlum index, while decreasing of pH, TSS/TA ratio and peroxidase activity during cold storage at 0 to 2°C for 5 month ($P \leq 0.05$). The results showed that calcium treatments application was influenced on ethylene in comparison to control. In general, this experiment showed that post-harvest Ca treatments prevented fruit softening and decreased weight losses.

Key words: Postharvest life, weight loss, firmness, calcium chloride.

INTRODUCTION

Apple (*Malus domestica* Borkh.) fruits are commonly stored for long periods at low temperatures under controlled atmosphere. Fruit quality and nutritional value decreases during storage. The shelf-life of apples is affected by a number of factors, such as growing, harvesting operations or storage conditions (Soliva-Fortuny et al., 2002). Losses in fruit quality are mostly due to its relatively high metabolic activity during storage (Fattahi et al., 2010). Calcium (Ca²⁺) has been extensively reviewed as both an essential element and its potential role in maintaining postharvest quality of fruit

and vegetable crops. The role of calcium in stabilizing cellular membranes and delaying senescence in horticultural and agronomy crops is well known (Poovaiah et al., 1988; Pervaiz et al., 2002; Hossain et al., 2005; Abdi et al., 2006; Misra and Gupta, 2006; Singh et al., 2006; Hosseini and Thengane, 2007; Naeem et al., 2009). Pre- and postharvest application of calcium may delay senescence in fruits with no detrimental effect on consumer acceptance (Lester and Grusak, 1999). Postharvest calcium dips can increase calcium content considerably compared to pre-harvest sprays, without causing fruit injury, depending on salt type and calcium concentration. Postharvest calcium application maintains cell turgor, membrane integrity, tissue firmness and delays membrane lipid catabolism, extending storage life of fresh fruits (Picchioni et al., 1998). Exogenously applied calcium stabilizes the plant cell wall and protects it from cell wall degrading enzymes (White and Broadley 2003).

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Abbreviations: TSS, Total soluble solids; TA, titratable acidity; POD, peroxidase activity; CAT, catalase activity; Ca, calcium.

The objective of this study was to determine the effect of calcium chloride application on the quality and storage life of apple fruit during storage.

MATERIALS AND METHODS

The experiment was started in season 2010/2011 and fruit weight losses, fruit firmness, total soluble solids, titratable acidity, TSS/TA, pH, ethylene production, peroxidase and catalase enzyme activities were measured at 5 month of postharvest life. *M. domestica* cv. 'Jonagold' was harvested at commercial maturity stage from an experiment orchard at the apple Research Institute of Iran (Zanjan, Iran). Fruits were subsequently transferred to laboratory and sorted based on size and the absence of physical injuries or infections. Fruits were randomly divided into three groups, each group containing 150 fruits in four replicates and immersed into solution of (2 and 4% (w/v) Ca and in distilled water as control for 10 min. Fruits were then dried for about 24 h and then stored at 0 to 2°C and 85 to 90 % relative humidity for 5 months. After 20, 40, 60, 80, 100, 120 and 150 days storage, 20 fruits per treatment were taken from cool storage for fruit quality assessment.

Fruit quality evaluation

Weight loss was determined by using Tefera et al. (2007) method. Fruit firmness was measured on two opposite peeled sides using a pressure meter (OSK 10576 CO., Japan) fitted with an 8 mm diameter flat tip. The firmness considered as an average peak force of 10 fruits and expressed as kg. Total soluble solid in the juice was determined with a hand- refractometer (NC-1, Atago Co., Japan) at room temperature and expressed as a percentage.

Titratable acidity was determined by titration an aliquot (20 ml) of the juice to pH 8.2 with 0.1N NaOH and the result was expressed as a percentage of malic acid. TSS/TA ratio was evaluated as the TSS/TA ratio (that is, ratio increasing with maturity) (Schirra et al., 2004). PH of the juice was measured using a pH meter (Jenway, 3020).

Peroxidase activity (POD)

A 1 g of the tissue located up to 1 cm beneath the peel was homogenized in a mortar with ice-cold 200 mM potassium phosphate buffer (pH 7.0) containing 5 mM Na₂EDTA, 10 mM Na₂S₂O₅, and 1% polyvinylpyrrolidone. After centrifugation (15,000×g, 15 min) the supernatant was used to determine POD (EC 1.11.1.7) activities. POD activity was measured after Chance and Maehly. The reaction mixture (2.0 ml final volume) consisted of 4.51 µl of 10 mM guaiacol, 2.9 ml of 50 mM NaPi, pH 7.0 and 1.5 ml of enzyme extract solution. The reaction was initiated via the addition of 3.35 µl of 30% H₂O₂. The activity of the mixture was determined spectrophotometrically at 470 nm after 10min at 20°C. Total protein concentration was measured by dye binding (Bradford, 1976). Enzyme activity was expressed in units of activity (U) mg⁻¹ protein.

Catalase activity (CAT)

A 1 g of the tissue located up to 1 cm beneath the peel was homogenized in a mortar with ice-cold 100 mM cold NaKPi, pH 7.0, 0.1% (w/v) PVPP, prepared and stored at 4°C, centrifuged at 4°C and 15,000×g for 15 min. The supernatant was recovered and used for the enzyme activity assay. CAT activity (EC 1.11.1.6) was

assayed after Aebi. The activity was measured in a reaction mixture (2.0 ml final volume) composed of 30% H₂O₂ in 50mM NaKPi, pH 7.0, and 1.5 ml of enzyme extract. Samples without H₂O₂ were used as a blank. The decomposition of H₂O₂ was followed spectrophotometrically by the decrease in A240. Enzyme activity was expressed in units of activity (U) mg⁻¹ protein.

Ethylene determination

Three fruits were enclosed in 3 L airtight jars for 1 h at 20°C. Ethylene measurements were performed by withdrawing 1 ml headspace gas sample from the jars with a syringe, and injecting it into a Varian 3300 gas chromatograph, equipped with a stainless steel column filled with Porapak, length 100 cm, diameter 0.32 cm, at 50°C and a flame-ionization detector at 120°C. The carrier gas was nitrogen at a flow rate of 20 ml/min.

Experimental design and statistical analysis

All data were analyzed for significant differences using analysis of variance (ANOVA) using the SAS (Statistical Analysis System) statistical package (SAS Institute, Cary, NC, USA). Data were then subjected to mean separation by the least significant difference test (LSD) at P<0.05.

RESULTS AND DISCUSSION

Weight loss

Effect of Ca on weight losses of stored fruits are listed in Table 1. Results showed that dipped fruits in Ca solution at different concentration prevented weight loss in comparison with control ($p \leq 0.05$). Maximum weight loss occurred in control treatment while lowest loss was recorded in 2% (w/v) Ca (Table 1). Calcium applications have been known to be effective in terms of membrane functionality and integrity maintenance which may be the reason for the lower weight loss found in calcium treated fruits. Mahajan and Dhatt (2004) reported that pear fruit treated with CaCl₂ proved to be most effective in reducing weight loss compared to non treated fruit during a 75 days storage period. The afore-mentioned results are in accordance with those recorded by Ashour (2000). He found that dipped fruits in Ca solution at different concentration reduce apple weight losses percentages.

Firmness

It is clear from the obtained data in Table 1 that dipping apple in 2 and 4% (w/v) Ca were effective in firmness for 5 month more than the other treatments in during storage. The results indicate that maximum firmness was recorded in 2% (w/v) Ca as compared to control, while minimum firmness was recorded in control during 5 month ($p \leq 0.05$). The retention of firmness in calcium treated fruits might be due its accumulation in the cell walls leading to facilitation in the cross linking of the

Table 1. Mean comparison of fruit Weight loss, Firmness, Ethylene, TSS, TA, POD, CAT, TSS/TA, pH in different concentration Ca solution during 5 month storage at 0 to 2 °C

Time of storage (day)	Treatment	Weight loss (%)	Firmness (kg)	Ethylene ($\mu\text{l kgh}^{-1}$)	TSS (%)	TA (%)	POD (Ua.mg-1 prot)	CAT (Ua.mg-1 prot)	TSS/TA	pH
	Ca (w/v)%									
20	0	0.035 ^a	1.65 ^b	2.53 ^a	11.25 ^a	48.15 ^b	3.49 ^a	5.32 ^b	0.24 ^a	3.99 ^a
	2	0.033 ^a	1.85 ^a	1.28 ^c	10.90 ^a	52.11 ^a	0.38 ^c	6.78 ^a	0.21 ^a	3.85 ^a
	4	0.032 ^a	1.84 ^a	1.56 ^b	10.89 ^a	55.25 ^a	1.92 ^b	4.65 ^b	0.19 ^a	3.80 ^a
40	0	0.045 ^a	1.56 ^b	2.64 ^a	12.75 ^a	36.40 ^c	10.54 ^a	4.10 ^c	0.35 ^a	3.95 ^a
	2	0.043 ^a	1.70 ^a	2.08 ^b	12.36 ^a	48.25 ^b	4.37 ^c	6.65 ^a	0.26 ^b	3.83 ^{ab}
	4	0.043 ^a	1.65 ^a	2.02 ^b	12.25 ^a	53.24 ^a	6.85 ^b	4.00 ^b	0.23 ^b	3.72 ^b
60	0	0.08 ^a	1.45 ^b	3.10 ^a	13.03 ^a	31.40 ^b	17.31 ^a	4.00 ^b	0.41 ^a	3.99 ^a
	2	0.05 ^b	1.69 ^a	2.26 ^b	12.50 ^a	43.92 ^a	7.23 ^b	6.02 ^a	0.28 ^b	3.85 ^b
	4	0.045 ^b	1.62 ^a	2.10 ^b	12.80 ^a	48.94 ^a	9.42 ^b	4.07 ^a	0.26 ^b	3.80 ^b
80	0	1.4 ^a	1.40 ^b	3.52 ^a	13.35 ^a	25.90 ^c	15.39 ^a	3.66 ^c	0.52 ^a	4.10 ^a
	2	1.3 ^a	1.62 ^a	2.38 ^b	12.72 ^a	35.97 ^b	5.17 ^b	12.75 ^a	0.35 ^b	3.96 ^b
	4	1.01 ^b	1.55 ^a	2.40 ^b	12.53 ^a	35.97 ^b	3.37 ^c	10.18 ^b	0.35 ^b	3.94 ^b
100	0	1.7 ^a	1.35 ^b	3.60 ^a	13.54 ^a	24.65 ^c	10.45 ^a	3.00 ^c	0.55 ^a	4.15 ^a
	2	1.5 ^b	1.53 ^a	2.43 ^b	13.08 ^a	35.97 ^b	5.00 ^b	8.47 ^a	0.36 ^b	3.98 ^a
	4	1.5 ^b	1.50 ^a	2.48 ^b	13.00 ^a	40.60 ^a	3.40 ^c	5.63 ^b	0.32 ^b	4.00 ^a
120	0	1.9 ^a	1.33 ^b	3.70 ^a	13.68 ^a	24.45 ^a	7.15 ^a	1.23 ^c	0.56 ^a	4.15 ^a
	2	1.7 ^{ab}	1.50 ^a	3.52 ^b	13.15 ^a	33.07 ^a	4.18 ^b	6.24 ^a	0.40 ^b	4.00 ^a
	4	1.6 ^b	1.48 ^a	3.00 ^b	13.08 ^a	33.07 ^a	3.28 ^b	3.12 ^b	0.40 ^b	4.08 ^a
150	0	3.02 ^a	1.30 ^b	4.25 ^a	13.00 ^a	21.72 ^b	3.00 ^a	0.82 ^c	0.60 ^a	4.18 ^a
	2	2.05 ^b	1.48 ^a	3.75 ^b	12.90 ^a	24.45 ^a	3.19 ^a	1.65 ^b	0.53 ^b	4.12 ^a
	4	2.45 ^b	1.45 ^a	3.42 ^c	13.02 ^a	24.45 ^a	2.36 ^b	2.28 ^a	0.53 ^b	4.15 ^a
<i>LSD- value</i>	Ca(w/v)%	0.89	0.65	0.98	0.63	6.23	2.78	3.15	0.63	1.05

Means in each column followed by similar letters are not significantly different at 5% level (LSD test).

pectic polymers which increases wall strength and cell cohesion (White and Broadley, 2003). This result was in agreement with the report of

Benavides et al. (2002) that suggested post-harvest application of apple by Ca decreased softening and kept firmness during storage. These

results are in agreement with those obtained by Casero et al. (2004). They reported that that dipped fruits in Ca solution at different

concentration increased apple firmness percentages.

Total soluble solids, pH, titratable acidity and TSS/TA ratio

TSS and pH were not influenced by the postharvest calcium dips, and slight differences existed. Results showed that postharvest calcium chloride dips did an effect of TA in apple in during storage. Also, the results indicate that maximum TA was observed in 4% Ca and lowest TA was recorded in control. Titratable acidity is directly related to the concentration of organic acids present in the fruit, which are an important parameter in maintaining the quality of fruits. Manganaris et al. (2005) has reported that postharvest calcium chloride dips did not affect TA in peaches during storage. The TSS/TA ratio increased with increasing the storage duration. But dipped fruits in Ca solution at different concentration prevented increasing of TSS/TA ratio in comparison with control ($p \leq 0.05$). The calcium chloride (2 and 4%) fruit had TSS/TA values lower than control.

POD and CAT enzyme activities

The results indicate that maximum POD activity was recorded in control as compared to 4% (w/v) Ca and other treatment. Minimum POD activity was recorded in 4 % (w/v) during 5 month (Table 1). All treatments had significant effect on the values of POD activity except control ($p \leq 0.05$). Previous studies (Lamikanra and Watson, 2001) indicated the ascorbate dependency of peroxidase (POD) enzymes in a number of commonly fresh-cut processed fruits whose activities appear to be related to the level of oxidative stress in cut fruit. The results of study El-hilali et al. (2003) on the "Fortune" mandarin fruit indicated the enhancement of POD activity in the peel of fruit stored at low temperature for prolonged periods and that Ca^{2+} and K^+ have an effect on chilling injury and enzyme activity. Ranadive and Haard (1972) identified a correlation between peroxidase activity and lignification in cell walls of pear fruit, and demonstrated differences in peroxidase activity at different Ca concentrations. Ca^{2+} appears to be necessary because it induces the cross-linking of polygalacturonan chains into a structure that can be recognized by its isoperoxidase (Penel et al., 1999). The results in Table 1 show that the storage period has a significant effect on catalase activity (CAT) of fruits ($p \leq 0.05$). The results indicate that maximum catalase activity (CAT) was observed in 2% (w/v) Ca, while the lowest catalase activity (CAT) was recorded in control. High calcium concentrations result in decreased flesh browning symptoms which are directly associated with calcium content in fruits (Hewajulige et al., 2003). Decreased electrolyte leakage by calcium application increases enzyme antioxidant activity, the cell wall integrity and stability (Mortazavi et al., 2007).

Ethylene

The results in Table 1 show that the storage period has a significant effect on ethylene of fruits ($p \leq 0.05$). The results indicate that maximum ethylene was observed in control treatment, while, the lowest ethylene was recorded in 4% (w/v) Ca. Oxidative membrane injury allows the mixing of the normally separated enzyme (PPO) and oxidizable substrates (polyphenols), which lead to browning (Hodges, 2003). High calcium concentrations result in decreased ethylene production, electrolyte leakage and flesh browning symptoms which are directly associated with calcium content in fruits (Hewajulige et al., 2003). Decreased electrolyte leakage by calcium application increases the cell wall integrity and stability (Mortazavi et al., 2007). Ethylene possesses an important role in integrating developmental signals and responses to abiotic stresses, like cold storage, and it has been suggested that calcium delays the onset of the ethylene climacteric period and climacteric peak (Ben-Arie et al., 1995).

Conclusion

Finally it was concluded that calcium dips retarded metabolism as indicated by the lower respiration rates of calcium treated samples. Calcium chloride dips improved the firmness of apple. Calcium concentration of treated samples was significantly greater ($p \leq 0.05$) than the control. Further studies are necessary to determine the sensory profile and the microbiological stability.

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REFERENCES

- Abdi GH, Khosh-Khm M, Eshghi S (2006). Effects of natural Zeolite on growth and flowering of strawberry. *Int. J. Agric. Res.*, 1(4): 384-389.
- Aebi H (1984). Catalase *in vitro*. *Methods Enzymol.*, 105: 121-126.
- Ashour NN (2000). Effect of environmental factors, calcium and potassium fertilization on yield and quality of apple. Ph.D. Thesis, Fac. Agric. Mansoura Univ, Egypt.
- Ben-Arie R, Mignani I, Greve LC, Huysamer m, Labavitch JM (1995). Regulation of the ripening of tomato pericarp discs by GA3 and divalent cations. *Physiol. Plant.*, 93: 99-107.
- Benavides A, Recasens I, Casero T, Soria Y, Puy J (2002). Multivariate analysis of quality and mineral parameters on Golden Smoothie apples treated before harvest with calcium and stored in controlled atmosphere. *Food Sci. Technol. Int.*, 1(8): 139-145.
- Casero T, Benavides A, Puy J, Recasens I (2004). Relationships between leaf and fruit nutrients and fruit quality attributes in Golden Smoothie apples using multivariate regression techniques. *J. Plant Nutr.*, 27: 313-324.
- Chance B, Maehly AC (1955). Assay of catalases and peroxidases. *Methods Enzymol.*, 2: 764-775.

- El-hilali F, Ait-Oubahou A, Remah A, Akhayat O (2003). Chilling injury and Peroxidase activity changes in "Fortune" mandarin fruit during low temperature storage. *Bulg. J. Plant Physiol.*, 29: 44-54.
- Fattahi J, Fifall R, Babri M (2010). Postharvest quality of kiwifruit (*Actinidia deliciosa* cv. Hayward) Affected by pre-storage application of salicylic acid. *South Western J. Hortic. Biol. Environ.*, 1: 175-186.
- Harker FR, Marsh KB, Young H, Murray SH, Gunson FA, Walker SB (2002). Sensory interpretation of instrumental measurements. 2. Sweet and acid taste of apple fruit. *Postharvest Biol. Technol.*, 24: 241-250.
- Hewajulige IGN, Wilson Wijeratnam RS, Wijesundera RLC, Abeysekere M (2003). Fruit calcium concentration and chilling injury during low temperature storage of pineapple. *J. Sci. Food Agric.*, 83: 1451-1454.
- Hodges DM (2003). *Postharvest oxidative stress in horticultural crops*. Foods Products Press/The Haworth Press, Binghamton, New York.
- Hossain AKM, Koyama H, Hara T (2005). Sugar compositions and molecular mass distributions of hemicellulosic polysaccharides in wheat plants under aluminum stress at higher level of calcium supply. *Asian J. Plant Sci.*, 4(1): 11-16.
- Hosseini GHT, Thengane R (2007). Salinity tolerance in cotton (*Gossypium hirsutum* L.) genotypes. *Inter. J. Bot.*, 3(1): 48-55.
- Lamikanra O, Watson MA (2001). Effects of ascorbic acid on peroxidase and polyphenoloxidase activities in fresh-cut cantaloupe melon. *J. Food Sci.*, 66: 1283-1286.
- Lester GE, Grusak MA (2004). Postharvest application of calcium and magnesium to honeydew and netted muskmelons: Effects on tissue ion concentrations, quality and senescence. *J. Am. Soc. Hort. Sci.*, 124: 545-552.
- Mahajan BVC, Dhatt AS (2004). Studies on postharvest calcium chloride application on storage behavior and quality of Asian pear during cold storage. *Int. J. Food Agric. Environ.*, 2: 157-159.
- Manganaris GA, Vasilakakis M, Mignani I, Diamantidis G, Tzavella-Klonari K (2005). The effect of preharvest calcium sprays on quality attributes, physicochemical aspects of cell wall components and susceptibility to brown rot of peach fruits (*Prunus persica* L. cv. *Andross*). *Sci. Hortic.*, 107(1): 43-50.
- Misra N, Gupta A (2006). Interactive effects of sodium and calcium on proline metabolism in salt tolerant green gram cultivar. *Ame. J. Plant Physiol.*, 1(1): 1-12.
- Mortazavi N, Naderi R, Khalighi A, Babalar M, Allizadeh H (2007). The effect of cytokinin and calcium on cut flower quality in rose (*Rosa hybrida* L.) cv. Illona. *J. Food Agric. Environ.*, 5: 311-313.
- Naeem M, Masroor M, Khan A, Morris JB (2009). Agro botanical attributes nitrogen-fixation, enzyme activities and nutraceuticals of Hyacinth bean (*Lablab purpureus* L.): A Bio-Functional Medicinal Legume. *Am. J. Plant Physiol.*, 4(2): 58-69.
- Penel C, Cutsem P, Greppin H (1999). Interactions of a plant peroxidase with oligogalacturonides in the presence of calcium ions. *Phytochemistry*, 51: 193-198.
- Pervaiz Z, Afzal M, Xi S, Xiaoe Y, Ancheng L (2002). Physiological Parameters of Salt Tolerance in Wheat. *Asian J. Plant Sci.*, 4: 478-481.
- Picchioni GA, Watada AE, Conway WS, Whitaker BD, Sams CE (1998). Postharvest calcium infiltration delays membrane lipid catabolism in apple fruit. *J. Agric. Food Chem.*, 46: 2452-2457.
- Poovaiah BW, Glenn GM, Reddy ASN (1988). Calcium and fruit softening: Physiology and biochemistry. *Hort. Rev.*, 10: 107-152.
- Ranadive AS, Haard NF (1972). Peroxidase localization and lignin formation in developing pear fruit. *J. Food Sci.*, 37: 381-383.
- Schirra M, Mulas M, Fadda A, Cauli E (2004). Cold quarantine responses of blood oranges to postharvest hot water and hot air treatments. *Postharvest Biol. Technol.*, 32: 191-200.
- Singh V, Singh D, Pathak SM (2006). Relationship of leaf and fruit transpiration rates to the incidence of softening of tissue in mango (*Mangifera indica* L.) Cultivars. *Ame. J. Plant Physiol.*, 1(1): 28-33.
- Soliva-Fortuny R, Oms-Oliu G, Martin-Belloso O (2002). Effects of ripeness stages on the storage atmosphere, color and textural properties of minimally processed apple slices. *J. Food Sci.*, 67: 1958-1962.
- Tefera A, Seyoum T, Woldetsadik K (2007). Effect of disinfection, packaging and storage environment on the shelf life of mango. *Biosyst. Eng.*, 97: 201-212.
- White PJ, Broadley MR (2003). Calcium in plants. *Ann. Bot.*, 92: 487-511.