Full Length Research Paper

Effects of postharvest applications of calcium nitrate and acetate on quality and shelf-life improvement of "Jonagold" apple fruit

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The effects of postharvest application of calcium were investigated on shelf-life and quality of apple fruits (*Malus domestica* cv. 'Jonagold') after harvest or cold storage up to 150 days. The fruits were immersed in deionised water or in different calcium sources (calcium acetate at two calcium concentrations 0.35 and $0.7\%_{w/v}$ and calcium nitrate at two calcium concentrations 0.5 and $1\%_{w/v}$. The experiment was carried out in 2010 to 2011 and fruit weight losses, fruit firmness, total soluble solids, pH, titratable acidity, total soluble solids /titratable acidity ratio, thiault index, perlim index, ethylene production, peroxidase and catalase enzyme activities were measured at 20, 40, 60, 80, 100, 120, and 150 days after harvest life. Results showed that all immersing conditions leaded to the best results of fruit quality assessments when compared with control. The calcium treatments increase fruit firmness, titratable acidity and catalase enzyme activity, while decreasing total soluble solids, weight losses percentages, ethylene production, TSS/TA ratio and Peroxidase enzyme activity during cold storage at 0-2°C for 150 days.

Key words: Shelf- life, "Jonagold" apple, calcium nitrate, calcium acetate.

INTRODUCTION

Apple is a climacteric fruit with a long post-harvest life in cool storage and the shelf-life of apple fruits is affected by many factors, such as growing condition, 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; Saure, 2005). Cool storage is widely used to reduce respiration rate, ethylene production and extend the shelf-life of fruits (Fattahi et al., 2010). Pre-harvest and postharvest calcium solution applications have been used to extend post harvest shelf life of fruits and vegetables (Poovaiah et al., 1988). Post harvest calcium dips can increase calcium content considerably compared to pre-harvest sprays, without causing fruit injury; depending on salt type and calcium concentration. Post harvest calcium application maintains cell turgor, membrane integrity, tissue firmness and delays membrane lipid catabolism, extending storage life of fruits (Garcia et al., 1996; Picchioni et al., 1998; Saure, 2005). Moreover: post harvest Ca treatments used to increase Ca content of the cell wall were effective in delaying senescence, resulting in firmer, higher quality fruit (Sams et al., 1993) that were less susceptible to disease during storage (Conway et al., 1991). Exogenously applied calcium stabilizes the plant cell wall and protects from cell wall degrading enzymes (White and Broadley, 2003; Saure, 2005). Faust (1989) asserted that Calcium (Ca²⁺) in the apoplast exerts a binding effect in the complex of polysaccharides and proteins comprising the cell wall, and that cytoplasmic Calcium may regulate several enzyme activities. The objectives of this study were to determine the effect of post harvest fruit immersion in different Calcium sources and the effect of Calcium concentrations on the quality and storage life of "Jonagold" apple fruit during cold storage.

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Abbreviations: TSS, Total soluble solid; TA, titratable acidity; POD, peroxidase activity; CAT, catalase activity; SAS, statistical analysis system; Ca, calcium.

MATERIALS AND METHODS

The experiment was carried out in 2010 to 2011 and fruit weight losses, fruit firmness, total soluble solids, titratable acidity, TSS/TA, pH, ethylene production, thiault index, perlim index, Peroxidase and Catalase enzyme activities were measured 5 month after harvest. "Jonagold"cultivar fruits were harvested in commercial maturity stage from an experiment orchard in the apple Research Institute of Iran (Zanjan, Iran). Fruits were subsequently transferred to the 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 replications and immersed into solution of (0.5 and 1.0% w/v CaN2O6. 4H2O and 0.35 and 0.7% w/v C4H6CaO4. XH2O) and in distilled water as control for 10 min. Fruits were dried for about 24hr and then stored at 0-2°C and 85-90% relative humidity for 5 months. After 20, 40, 60, 80, 100, 120 and 150th days storage, 20 fruits per treatment were taken from cool storage for fruit quality assessment. Studied characteristics and methods were included

Weight loss

Weight loss was determined by using as Tefera et al. (2007) method.

Fruit firmness

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 (TSS)

TSS in the juice was determined with a hand- refractometer (NC-1, Atago Co., Japan) at room temperature and expressed as a percentage.

Titratable acidity (TA)

TA 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 malice acid.

TSS/TA ratio

The maturity index was evaluated as the TSS/TA ratio (that is, ratio increasing with maturity as shown with Schirra et al. (2004).

PH

pH of the juice was measured using a pH meter according to Jenway, 3020).

Thiault index

Thiault index was calculated as follows: index = $[10 \times acidity (g/L) + sugar content (g/L)]$ based on Harker et al. (2002).

Perlim index

Perlim index was evaluated as follows: PI = [Kg/cm²×0.5+ Brix×

6.7+ malic acid (g/L) × 0.67] according to Lafer (1999).

Peroxidase activity (POD)

Peroxidase activity (POD) one gram 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% Polvinylpyrrolidone. After centrifugation (15,000× g, 15 min) the supernatant was used to determine POD (EC 1.11.1.7) activities according to Chance and Maehly (1955). The reaction mixture (2.0 ml final volume) consisted of 4.51µl 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 using addition of 3.35 µl of 30% H₂O₂. The activity of the mixture was determined spectrophotometrically at 470 nm after 10 min at 20°C. Total protein concentration was measured by dye binding as shown with Bradford (1976). Enzyme activity was expressed in units of activity (U) mg–1 protein.

Catalase activity (CAT)

Catalase activity (CAT) one gram 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 based on Aebi (1984). The activity was measured in a reaction mixture (2.0 ml final volume) composed of 30% H₂O₂ in 50 mM 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

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

Analysis of variance (ANOVA) was carried out using the SAS Software (Statistical Analysis System) statistical package (SAS Institute, Cary, NC, USA). Comparisons of means carried out by the least significant difference test (LSD) at P < 0.05.

RESULTS AND DISCUSSION

Effects on fruit firmness (kg)

Fruit firmness (kg) was affected by the Calcium source treatments during 150 days storage (Table 1). Also; it can be stated that all tested treatments have the significant highest effects on firmness during storage in compared with the control. A treatment of Calcium nitrate $(0.5\%_{w/v})$ and Calcium acetate $(0.35\%^{w/v})$ was significantly

Time storage (day)	Treatment CaN ₂ O ₆ .4H ₂ O w/v%	Weight loss (%)	Firmness (kg)	Ethylene (µl kgh⁻¹)	TSS (%)	TA (%)	POD (Ua.mg ⁻¹ prot)	CAT (Ua.mg ⁻¹ prot)	TSS/TA	рН	Thiault index	Perlim index
0.5	0.03b	1.99ª	1.80ª	11.90ª	67.75ª	3.30 ^b	8.86ª	0.18 ^b	3.65 ^b	173.2ª	103.28ª	
1	0.04ª	1.77 ^b	1.75ª	12.30ª	53.55 ^b	4.01ª	8.85ª	0.23ª	3.7a ^b	163.3 ^b	95.07⁰	
	0	0.04ª	1.47 ^b	2.47ª	13.92ª	41.85 ^b	10.5ª	4.11°	0.33ª	3.94ª	168.8ª	93.82ª
40	0.5	0.03 ^b	1.81ª	2.02 ^b	12.35 ^b	53.57ª	9.36 ^b	8.01ª	0.23 ^b	3.89ª	163.9a	95.39ª
	1	0.045ª	1.56 ^b	2. ^b	12.75 ^b	46.32ª	5.01°	6.45 ^b	0.38ª	3.89ª	160.8 ^b	91.77 ^b
	0	0.08ª	1.45°	3.00ª	13.80ª	33.05 ^b	17.31ª	3.36 ^b	0.42ª	4.06ª	156.1ª	87.64 ^b
60	0.5	0.07b	1.69ª	2.20 ^b	12.67 ^b	49.38ª	8.80 ^b	6.44ª	0.26 ^b	3.81 ^b	163.0ª	93.75ª
	1	0.07 ^b	1.55 ^b	2.12 ^b	12.70 ^b	44.88ª	5.60°	4.56 ^b	0.28 ^b	3.90 ^b	158.9ª	90.55 ^{ab}
	0	1.6ª	1.40 ^b	3.20ª	13.85ª	32.38a	17.58ª	2.18¢	0.43ª	4.07ª	158.2ª	86.80ª
80	0.5	1.5 ^b	1.52ª	2.42 ^b	12.85ª	36.82ª	7.62 ^b	6.36ª	0.35ª	3.95ª	152.4ª	85.76ª
	1	1.3 ^b	1.50ª	2.35 ^b	12.32ª	36.32ª	4.45°	3.12 ^b	0.34ª	4.01ª	146.3ª	83.00ª
	0	1.6ª	1.37 ^b	3.57ª	13.87ª	30.95°	10.12ª	1.57°	0.45ª	4.14ª	157.3ª	86.07ª
100	0.5	1.5 ^b	1.50ª	2.45°	13.05ª	36.80 ^b	6.50 ^b	6.00ª	0.35 ^b	3.98	154.5 ^{ab}	86.60ª
	1	1.3⁰	1.43 ^b	2.75 ^b	12.57ª	36.18ª	3.12°	2.82 ^b	0.35 ^b	4.03ª	148.8 ^b	83.87 ^{ab}
	0	2.4ª	1.54ª	3.85ª	13.90ª	25.10 ^b	3.00ª	1.42°	0.47ª	4.16ª	156.0ª	85.08ª
120	0.5	2 ^b	1.37 ^b	2.62°	13.12ª	29.27ª	3.19ª	5.3ª	0.41 ^b	4.10ª	150.7ª	83.78ª
	1	1.5°	1.4 ^{6a}	3.20 ^b	12.85ª	32.10ª	2.36 ^b	2.34 ^b	0.40 ^b	4.07ª	147.7ª	82.33ª
	0	2.8ª	1.34ª	3.87ª	14.81ª	22.57°	3.04ª	0.61°	0.62ª	4.41ª	158.9ª	84.76ª
150	0.5	2.4 ^b	1.40ª	3.17ª	13.62ª	30.57ª	2.03 ^b	1.35ª	0.45 ^b	4.29ª	154.3ª	78.74ª
	1	2.4 ^b	1.34ª	3.50ª	12.7 ^{5a}	27.62 ^b	1.73°	0.91 ^b	0.50 ^b	4.36ª	142.1ª	80.47 ^b
LSD test at <i>P</i> = 0.05	Ca _{w/v} %	0. 56	0.24	0.68	0.098	5.32	2.01	1.2	0.063	0.17	6.15	6.21

Table 1. Mean comparison of fruit Weight loss, Firmness, Ethylene, TSS, TA, POD, CAT, TSS/TA, pH, Thiault and Perlim indexes in different Ca concentrations during 5 month storage at 0-2^oC.

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

increased the fruit firmness in compared with the control fruits and gave the highest fruit firmness among other treatments. Calcium accumulation in the cell wall facilitates cross-linking of pectic polymers leading to a cell wall network that increases wall strength and cell cohesion (White and Broadley, 2003) with unbound Calcium ions to have little or no direct effect on tissue strength (Saftner et al., 1998).

This is in agreement with the report of Benavides et al. (2002) who suggested post harvest application of apple by Ca decreased softening and kept firmness during storage.

Also, Casero et al. (2004) reported that that dipped fruits in Ca solution at different concentration increased apple shelf life and storability. Similar results were obtained in 'Golden Smoothee' apple by Casero et al. (2004) who; indicated that fruit firmness shows a positive correlation with fruit Ca content and bitter pit incidence correlates negatively with this nutrient concentration.

Furthermore; Soure (2005) reported that Ca is known to stabilize cell membranes in 'fleshes fruit' and it may prevent physiological disorders attributed to Ca deficiency.

Effect on weight loss percentage

Effect of Calcium nitrate and Calcium acetate on weight losses of stored fruits are listed in Tables 1 and 2. It is clear that the percentage of weight losses of apple fruits was increased gradually with the progress of storage period in during storage. This loss was comparatively slow during the 20 days of storage, and then the rate of weight loss considerably increased in the end period of storage. The highest values of weight losses (%) were noticed by the untreated treatment. The lowest significant values of weight losses percentages were recorded by the treatment of Calcium nitrate 0.5% w/v and calcium acetate $0.35\%_{w/v}$ in 2.4 and 260% in storage, respectively. These results were in agreement with those recorded by Ashour (2000) who found that spraving "Anna" apple fruits with 0.5% Calcium chloride reduced fruit weight losses percentages. 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.

Effect on TSS and pH

TSS and pH were not influenced by the post harvest calcium dips, and slight differences existed (Tables 1 and 2).

Effect on TA and TSS/TA ratio

Results in Tables 1 and 2 indicated that TA percentage of "Jonagold"apple fruits showed a gradual statistically decrease as storage period advanced for treated and untreated fruits. The highest statistical values were recorded by calcium nitrate $0.5\%_{w/v}$ and calcium acetate $0.7\%_{w/v}$ treatments in during storage. These results were against with those reported by Ashour (2000) and Mohsen (1999) who reported the acidity decrease in "Anna" apple fruit; during storage which was treated with Calcium chloride spray or spray plus dipping. 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. TSS/TA ratio was significantly decreased in calcium treated "Jonagold"fruits, compared to the control fruits, whereas no differences were observed among the different calcium sources.

Effect on ethylene

"Jonagold" fruits dipped in Calcium nitrate and calcium acetate produced significantly lower levels of ethylene in compared with the control treatments. 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., 1995b).

Effect on proxidase and catalase enzyme activities

POD activity was lower in Calcium treated "Jonagold" fruits in compared with control fruits during 150 days in cold storage (Tables 1 and 2). The increased POD activity in control and treated fruits was connected with increasing storage period for 100 days, but after 100 days decreased POD enzyme activity in all treatments. The results showed that the storage period has a significant effect on CAT of fruits ($p \le 0.05$). However; results indicated that maximum CAT was observed in Calcium nitrate 0.5% w/v and Calcium acetate 0.35% w/v, while the lowest CAT was recorded in control.

El-hilali et al. (2003) reported that in the "Fortune" mandarin fruit; indicated the enhancement of POD activity in the peel of fruit stored at low temperature for prolonged periods and that Ca²⁺ and K⁺ have an effect on chilling injury and enzyme activity. Lee et al. (2007) founded that a reduction of peroxidase activity in fruit flesh has been observed following the application of 0.5% Calcium chloride. This tendency can be observed in the peroxidase activity of the cell wall and cytoplasm of fruit sprayed with Calcium chloride. Peroxidase activity in these organs may be associated with the Ca. Penel and Greppin (1994) showed that isoperoxidases exhibited an affinity for pectin in their Ca²⁺ induced conformations, via a variety of in vivo binding trials. Ca2+ 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). 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). The role of CAT activity may be of paramount importance in fruit ripening and senescence by removing, from the tissues, the excess of H₂O₂ that is produced mainly by sod activity

Time Storage (day)	Treatment C4H6CaO4. XH2O w/v%	Weight loss (%)	Firmness (kg)	Ethylene (µl kgh⁻¹)	TSS (%)	TA (%)	POD (Ua.mg ⁻¹ prot)	CAT (Ua.mg ⁻¹ prot)	TSS/TA	рН	Thiault index	Perlim index
0.35	0.041ª	1.90ª	1.25°	10.85ª	52.22ª	0.38°	6.78ª	0.21ª	3.84ª	146.6ª	87.62ª	
0.7	0.038ª	1.88ª	1.65 ^b	10.60ª	55.20ª	1.92 ^b	4.65 ^b	0.19ª	3.75ª	146.9ª	88.65ª	
	0	0.046ª	1.46 ^b	2.72ª	12.80ª	36.40°	9.00ª	4.11 ^b	0.35ª	3.98ª	151.5°	85.12°
40	0.35	0.044ª	1.72ª	2.07 ^b	12.40ª	46.45 ^b	2.36°	5.29ª	0.27 ^b	3.86 ^b	157.3 ^b	90.6 ^b
	0.7	0.042ª	1.64ª	2.00 ^b	12.30ª	55.25ª	3.00 ^b	4.15 ^b	0.22 ^b	3.79b	165.0ª	95.93ª
	0	0.07ª	1.42 ^b	3.07ª	13.35ª	31.40 ^b	12.22ª	4.00 ^b	0.43ª	4.00ª	152.3ª	84.15°
60	0.35	0.05 ^b	1.71ª	2.15 ^b	12.30b	44.48ª	4.45 ^b	4.56ª	0.28 ^b	3.88 ^b	154.2ª	88.85 ^b
	0.7	0.06 ^{ab}	1.63ª	2.07 ^b	12.40 ^b	51.90ª	3.08 ^b	3.98 ^b	0.24 ^b	3.86 ^b	162.7ª	94.05ª
	0	1.3ª	1.40 ^b	3.50ª	13.30ª	25.90°	15.39ª	3.66 ^b	0.51ª	4.15ª	146.2ª	80.19ª
80	0.35	1.2ª	1.64ª	2.40 ^b	12.7ª	35.97 ^b	5.17 ^b	3.80ª	0.35 ^b	3.96 ^b	149.9ª	84.7 ^{ab}
	0.7	1.00 ^b	1.56ª	2.42 ^b	12.50ª	35.97 ^b	3.37℃	3.48 ^b	0.26¢	3.95 ^b	159.6ª	91.58ª
	0	1.6ª	1.37 ^b	3.55ª	13.60ª	24.6°	16.96ª	2.85 ^b	0.55ª	4.19ª	148.2ª	80.64 ^b
100	0.35	1.4 ^b	1.56ª	2.40 ^b	13.32ª	34.70 ^b	5.20 ^b	3.14ª	0.38 ^b	3.99ª	155.2ª	86.54ª
	0.7	1.5 ^{ab}	1.54ª	2.52 ^b	12.65ª	40.60ª	4.15 ^b	3.01ª	0.31 ^b	4.08ª	154.09ª	87.44ª
120	0	1.8ª	1.36ª	3.55ª	13.67ª	24.45ª	10.53ª	1.07 ^b	0.56ª	4.18ª	148.7ª	80.79ª
	0.35	1.8ª	1.56ª	3.57ª	13.00ª	30.97ª	3.86 ^b	2.53ª	0.42 ^b	4.07ª	148.1ª	82.60ª
	0.7	1.6 ^b	1.54ª	2.90 ^b	13.05ª	33.07ª	3.23 ^b	2.42ª	0.39 ^b	4.11ª	150.8ª	84.08ª
150	0	3ª	1.31Þ	4.07ª	12.92ª	21.72 ^b	3.00ª	0.82°	0.59ª	4.31ª	138.07ª	75.49Þ
	0.35	2 ^b	1.54ª	3.60 ^b	12.80ª	25.60ª	3.19ª	1.65 ^b	0.50 ^b	4.22ª	140.6ª	78.06ª
	0.7	2.5 ^b	1.40 ^b	3.02°	13.05ª	26.37ª	2.36 ^b	2.28ª	0.49 ^b	4.35ª	144.1ª	79.3 ^{ab}
LSD test at <i>P</i> = 0.05	Ca _{w/v} %	0. 54	0.307	0.813	0.089	6.41	2.394	2.504	0.063	0.214	4.76	7.2

Table 2. Mean comparison of fruit weight loss, firmness, ethylene, TSS, TA, POD, CAT, TSS/TA, pH, thiault and perlim indexes in different Ca concentration during 5 month storage at 0-2°C.

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

(Monk et al., 1989; Foyer et al., 1997). H_2O_2 , capable of rapid diffusion across cell membranes, many act as a second messenger in selective induction of defense genes (Foyer et al., 1997).

Effect on Perlim and Thiault indexes

The Thiault and Perlim index increased with increasing Calcium concentrations in the storage

duration. Calcium acetate $(0.7\%_{w/v})$ and Calcium nitrate (0.5%) had a significantly influence in increasing the Perlim index in fruits in compared with control in the storage duration ($p \le 0.05$). The

results indicate that maximum Thiault and Perlim indexes were recorded in Calcium treatments as compared to other treatment. Growers use the Thiault index as an indicator of optimum ripeness for harvesting and consumption. Thiault values over 170 are considered acceptable for some apple varieties (Porro et al., 2002).

Conclusion

It was concluded that post harvest application of Calcium nitrate and acetate increased the storage life and some other post harvest characteristics in "Jonagold" apple fruits and these results can be applied by growers and traders which are interfered with apple production and tradition.

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