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Effect of Malic Acid and Calcium Treatments on Quality Characteristics of Apple Fruits During Storage

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ABSTRACT

Apple texture can deteriorate during cold storage, resulting in softness and mealiness. The purpose of this work was to estimate shelf-life and to study the behavior of 'Jonagold' apples kept at 0-2°C in a normal atmosphere. The experiment was started in season 2010-2011 and fruit weight losses, fruit firmness, Total Soluble Solid (TSS), 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 th days of postharvest life. The fruits were immersed in distilled water, three malic acid concentrations (0, 100 and 150 mg^{-1}) or at three calcium concentrations (0, 0.35 and 0.7% w/v). Results showed that fruit weight loss significantly decreased in malic acid+calcium treatments in comparison to control. Also, results showed that malic acid+calcium treatments increase fruit firmness, Catalase activity (CAT), Titratable acidity (TA) and Perlim index while decreasing of pH, Total soluble solids/Titratable acidity ratio and Peroxidase activity (POD) during cold storage at 0-2°C for 5 month ($p \leq 0.05$). The results showed that malic acid+calcium treatments application was influenced on ethylene in comparison to control. In general, this experiment showed that post-harvest malic acid+calcium treatments prevented fruit softening and decreased weight losses.

Key words: Total soluble solids, Titratable acidity, malic acid, calcium, apple

INTRODUCTION

Apple fruits mostly stored after harvest. The fruits quality determination includes color, surface damage, acidity, sugars and soluble solids (Omaima and Haggag, 2007). 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). With present day technology it is possible to store apples in excellent physical condition well into the year following their harvest but fruit quality depends strongly on temperature and humidity during storage (Varela *et al*., 2005). Apple is a climacteric fruit with a long post-harvest life in cool storage. Losses in fruit quality are mostly due to its relatively high metabolic activity during storage (Fattahi *et al*., 2010). Cool storage is widely used to reduce respiration rate, ethylene production and extend the shelf-life of fruits. Calcium (Ca^{2+}) 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; Dzomeku *et al.*, 2008; Naeem *et al.*, 2009; Al-Hamzawi, 2010). Pre- and postharvest application of calcium may delay senescence in fruits with no detrimental effect on consumer acceptance (Lester and Grusak, 1999). Exogenously applied calcium stabilizes the plant cell wall and protects it from cell wall degrading enzymes (White and Broadley, 2003). Faust (1989) asserted that Calcium (Ca^{2+}) in the apoplast exerts a binding effect in the complex of polysaccharides and proteins comprising the cell wall and that cytoplasmic Calcium (Ca^{2+}) may regulate several enzyme activities. Postharvest calcium dips can increase calcium content considerably compared to preharvest 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). Malic acid is a well known organic acid that presumably can reduced the number of bacteria in the solution and with decrease ACC-oxidase activity cause delay the onset of hydrolysis of structural cell components, decrease ethylene production and sensitivity (Kazemi *et al.*, 2010). Previous study had revealed that MA (malic acid) sprays during growth period increased chlorophyll content of cut flowers while Ca Calcium spray caused extended post harvest vase life (Darandeh *et al.*, 2010). However, No evidence was found for the hypothesis Malic acid application on the quality and storage life of apple fruit during storage. In the present study, we test the hypothesis Malic acid, calcium or their combination 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, Total soluble solids/Titratable acidity, pH, Ethylene production, Thiault index, Perlim index, Peroxidase and Catalase enzyme activities were measured at 5 month of postharvest life. *Malus domestica* var. 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 four groups, each group containing 150 fruits in four replicates and immersed into solution of (0.35 and 0.7% (w/v) Ca, three malic acid concentrations (0, 100 and 150 mg^{-1}) and in distilled water as control for 10 min. Fruits were then dried for about 24 h 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.

Weight loss: Physiological weight loss was determined by using Tefera *et al.* (2007) method, by periodical weighing of apples 20, 40, 60, 80,100,120 and 150 days after storage. The differential weight losses were calculated for each interval and converted into percentage by dividing the change with the initial weight recorded on each sampling interval. The cumulative Physiological weight loss was expressed in percent with respect to different treatments. The difference between the initial weight and successive weights gave the rate of weight loss (as percentages).

Fruit firmness: Firmness was measured on two opposite peeled sides using a pressure meter (OSK, 10576) 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 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 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: The maturity index was evaluated as the TSS/TA ratio (i.e., ratio increasing with maturity) (Schirra *et al*., 2004).

pH: pH of the juice were measured using a pH meter (Jenway, 3020).

Peroxidase activity (POD): Peroxidase activity was measured based on the method of Chance and Maehly (1955).

Catalase activity (CAT): CAT activity was measured based on the method of Aebi (1984). The activity was measured in a reaction mixture (2.0 mL final volume) composed of 30% H_2O_2 in 50 mM NaKPi, pH 7.0 and 1.5 mL of enzyme extract. Samples without H_2O_2 were used as a blank. The decomposition of H_2O_2 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-ionisation detector at 120°C. The carrier gas was nitrogen at a flow rate of 20 mL min^{-1}.

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 and MA on weight losses of stored fruits are listed in Table 1. Results showed that Dipped fruits in Ca+MA solution at different concentration prevented weight loss in comparison with control ($p = 0.05$). Maximum weight loss occurred in control treatment while lowest loss was recorded in 150 mg^{-1} MA+ 0.7% (w/v) Ca (Table 1). Calcium applications have 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. The above mentioned results are agreed with those recorded by Mahajan and Dhatt (2004). They reported that pear fruit treated with Ca proved to be most effective in reducing weight loss compared to non treated fruit during a 75 days storage period. The above mentioned results are agreed 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 150 mg^{-1} MA+0.7% (w/v) Ca were effective in Firmness for 5 month more than the other treatments in during storage.

Table 1: Mean comparison of fruit weight loss, firmness, ethylene, TSS, TA, POD, CAT, TSS/TA, pH, thiault and perlim indexes in different concentration Ca+MA solution during 5 month storage at 0-2°C

Time storage (day)	Treatment		Weight loss (%)	Firmness (kg)	Ethylene[1] (μL kgh^{-1})	TSS (%)	TA (%)[2]	POD[3] (Ua. mg^{-1} prot)	CAT[4] (Ua .mg^{-1} prot)	TSS/TA	pH	Thiault index	perlim index
	MA (mg^{-1})	Ca (w/v)%											
20	0	0.00	0.049a	1.7b	2.10a	12.95a	41.03b	4.12a	5.08b	0.32a	3.87a	1577c	89.61b
	100	0.35	0.033b	1.8a	2.05a	12.20a	52.75a	1.73b	5.64b	0.23b	3.79a	1614.7b	94.17a
	150	0.70	0.022c	0.2a	1.22b	12.90a	46.88ab	1.27c	8.31a	0.24b	3.92a	1630.2a	93.20a
40	0	0.00	0.047a	1.52b	2.27a	13.67a	00.36a	3.19a	4.24b	0.38a	3.90a	1603.02c	88.89a
	100	0.35	0.043b	1.60a	2.15b	12.85b	36.15a	2.63b	4.00b	0.35a	3.87a	1527.06b	86.16a
	150	0.70	0.032c	1.63a	1.47c	12.90b	36.15a	1.12c	7.41a	0.35a	3.87a	1532.09a	86.41a
60	0	0.00	0.08a	1.44c	3.02a	13.77a	32.23b	4.00a	3.61b	0.43a	4.01a	1575c	86.63a
	100	0.35	0.07b	1.59b	2.35b	13.30a	36.05a	3.78b	3.21b	0.37b	3.94b	1615b	87.42a
	150	0.70	0.04c	1.63a	2.17c	13.60a	37.04a	1.47c	6.14a	0.34b	3.88b	1703a	85.27a
80	0	0.00	1.4a	1.44c	3.42a	13.80a	30.52b	17.58a	1.00c	0.45a	4.03a	1562b	85.62a
	100	0.35	1.2b	1.51b	2.55b	13.42a	30.50a	2.30b	2.75b	0.38a	3.96b	1571b	87.41b
	150	0.70	1.00c	1.79a	2.50c	13.50a	36.40a	2.33b	5.01a	0.37a	3.93b	1589a	88.33a
100	0	0.00	1.9a	1.39b	3.82a	13.85a	25.95b	3.24a	6.00b	0.53a	4.19a	1521b	82.67a
	100	0.35	1.6b	1.50a	2.05b	13.55a	32.22a	1.50b	6.00b	0.42b	4.08ab	1555b	85.78a
	150	0.70	1.4c	1.46a	2.02b	12.65a	35.67a	0.97c	7.81a	0.38b	3.93b	1597a	88.45a
120	0	0.00	0.2a	1.34b	3.88a	13.98a	25.10b	4.00a	4.48b	0.56a	4.23a	1525b	82.53b
	100	0.35	1.6b	1.47a	3.47a	13.60a	27.27ab	3.00b	4.5b	0.54a	4.15a	1508c	82.62b
	150	0.70	1.1c	1.46a	2.10c	13.77a	31.40a	1.75c	6.22a	0.42b	4.18a	1575a	86.57a
150	0	0.00	3.1a	1.29c	3.90a	14.04a	21.32b	0.71b	0.4c	0.66a	4.37a	1501b	80.47b
	100	0.35	3.1a	1.38b	3.50a	13.63a	27.06a	2.55a	2.8b	0.52b	4.22a	1499b	81.74b
	150	0.70	2.6b	1.48a	3.15b	13.82a	31.22a	0.76c	3.12a	0.44b	4.30a	1571a	86.05a
F-test probabilities													
	Ca (w/v)%		0.05	0.01	0.03	0.89	0.05	0.039	0.04	0.063	0.114	0.04	0.02
	MA (mg^{-1})		0.02	0.01	0.001	0.61	0.04	0.03	0.03	0.054	0.63	0.04	0.05

Means in each column followed by similar letters are not significantly different at 5% level (LSD test). [1]Total soluble solids, [2]Titratable acidity, [3]Peroxidase activity, [4]Catalase activity

The results indicate that maximum firmness was recorded in 150 mg^{-1} MA+0.7% (w/v) Ca as compared to control, while minimum firmness was recorded in control during 5 month ($p = 0.05$). 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 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 postharvest application of apple by Ca decreased softening and kept firmness during storage also, Casero *et al.* (2004) reported 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. Also, the results indicate that maximum TA was observed in 150 mg^{-1} MA+0.7% (w/v) 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. This result was in agreement with the report of Manganaris *et al.* (2005) that suggested postharvest application of apple by Ca dips did not effect of TA % in peaches during storage. The TSS/TA ratio increased with increasing the storage duration. But dipped fruits in Ca+MA solution at different concentration prevented increasing of TSS/TA ratio in comparison with control ($p \leq 0.05$).

POD and CAT enzyme activities: The results indicate that maximum POD activity was recorded in control as compared to 0.35 and 0.7 % (w/v) Ca and other treatment (Table 1). 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. 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 150 mg^{-1} MA+0.7% (w/v) while the lowest Catalase activity (CAT) was recorded in control. High Ca concentrations result in decreased flesh browning symptoms which are directly associated with calcium content in fruits (Hewajulige *et al.*, 2003).

Ethylene: The results in Table 1 show that the storage period has a significant effect on ethylene of fruits ($p = 0.05$). The results indicate that maximum ethylene was observed in control treatment, while, the lowest ethylene was recorded in 150 mg^{-1} MA+0.7% (w/v). 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). This result was in agreement with the report of Mortazavi *et al.* (2007) that suggested postharvest application of apple by Ca decreased electrolyte leakage and increased the cell wall integrity and stability. Also, This result was in agreement with the report of Kazemi *et al.* (2010) that showed the treatment MA treatment improved flower quality with reduced ACC-oxidase activity (ACO), ethylene production and microbial population in vase solution of carnation cut flowers. 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

The effect of MA on senescence indices, which is reported here for the first time, could be promising. MA and Calcium dips retarded metabolism as indicated by the lower respiration rates of MA+calcium treated samples. MA+calcium dips improved the firmness of apple. MA+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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