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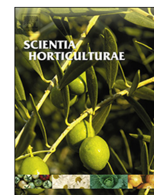

Comparison of nano-calcium and calcium chloride spray on postharvest quality and cell wall enzymes activity in apple cv. Red Delicious

Shiva Ranjbar, Majid Rahemi*, Asghar Ramezanian

Department of Horticultural Science, School of Agriculture, Shiraz University, Shiraz, Iran

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ABSTRACT

The aim of the experiment was to compare the effects of preharvest nano-calcium and calcium chloride spraying on postharvest quality and the cell wall enzyme activities of the apple fruit (*Malus domestica* L. cv. Red Delicious) at harvest and during storage (1, 2, 3, 4 month). Sprayings on apple trees were started from 70 d after full-bloom until one month before harvesting. They were sprayed 5 times, intervals of two weeks, with nano-calcium (0, 1.5, 2, and 2.5%), and calcium chloride (0, 1, 1.5, and 2%) solutions. After harvest, some fruit were transported to the laboratory to evaluate the parameters while other fruit were stored at 0 °C and at 90% RH for 4 months. Measurements were performed on fruit firmness, weight loss, titrable acidity (TA), total soluble solids (TSS), total phenolic content (TPC), browning, total antioxidant activity (TAA), and fiber content at 0, 1, 2, 3, and 4 months of storage period. Furthermore, assessments were made regarding enzymes in the cell wall such as polygalacturonase (PG), pectin methylesterase (PME), and β-galactosidase (β-Gal). Results showed that firmness, TA, TPC, TAA, and fiber content increased in fruit that treated with both nano-calcium and calcium chloride as compared to control fruit, however, these parameters decreased by prolonging the storage time. Meanwhile, weight loss, TSS, and internal browning increased during storage time, but, this increase in treated fruit was less than control fruit. Moreover, during storage, lower activities of PG, PME, and β-Gal enzymes were observed in fruit that sprayed with both calcium fertilizers. In addition, In short, it was observed that the quality of apple fruit treated with nano-calcium was better than those treated with calcium chloride in all parameters. So, it can be considered to use nano-calcium fertilizer instead of calcium chloride fertilizer for improving quality and storability of fruit such as apple.

1. Introduction

Apple fruit is one of the major fruit that grow in temperate zones. Nowadays, this fruit has received more attention because of its fibre as it has positive effect on human health, which causes to increase consumption, and phenolic compounds that are major antioxidants of diet (Scalbert et al., 2005). However, during storage, the loss of physical and chemical quality of apples could be a problem (Kov and Felf, 2003), and the softening could cause difficulties in postharvest handling (Paull et al., 1997). One of the factors that cause the increase in fruit softening is cell wall degrading enzymes. These include polygalacturonase (PG), pectin methylesterase (PME), and β-galactosidase (β-Gal) (Brummell and Harpster, 2001; Tavarini et al., 2008; Opazo et al., 2013). Such enzymes facilitate the solubilization of cell wall pectin. Therefore, in this case calcium as one of the essential elements plays a key role. Calcium binds with the carboxyl groups located at the backbone of pectin homogalacturonan, as hypothesized by the model of eggbox (Braccini and Pérez, 2001). This causes an increase in fruit firmness and also stabilizes the cell wall of plant, protecting it from the degradation of enzymes (White and Broadley, 2003). Therefore, the softening of fruit is delayed by calcium applications, and storage life is increased. In addition, the decrease in physiological disorders such as internal browning by calcium is another point in maintaining the quality of fruit during storage (Martín-Diana et al., 2007). Moreover, calcium leads to a reduced ripening and the delayed senescence of fruits (Lester and Grusak, 2004; Mahajan and Dhatt, 2004; Singh et al., 2007). Since calcium is an important element, as mentioned above, it is necessary to evaluate the efficacy of new forms of calcium fertilizers such as nano-calcium.

One of the new technologies recently used in agriculture is nanotechnology. Nanotechnology means technology in the case of atoms to achieve something beneficial through the manipulation (Tantawy et al., 2014). In fact, a new powerful technology causes most of the changes in agriculture such as developing food quality. Therefore, Nano- fertilizers can bring about many advantageous as compared to conventional fertilizers. Compounds in the nano scale result in an increase in the

* Corresponding author.
E-mail address: rahemi@shirazu.ac.ir (M. Rahemi).

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surface-to-volume ratio, which usually occurs by decreasing the size of the particle, thereby increasing the activity of the particles as compared to other compounds and increasing their effectiveness (Miller and Senjen, 2008). Therefore, they can be influenced on crops because of their ability for higher absorbance, more penetration into plants, and greater transportation within the plants cell quickly (Benzon et al., 2015) result in using efficiency of the nutrient. Moreover, they are required in fewer amounts than conventional chemical fertilizers (Rameshaiah et al., 2015) because only small fraction of fertilizer reaches to the plant by using conventional fertilizers, which is much less than the minimum required concentration of plant. So, frequent application of fertilizer is required to the target point. Furthermore, the cost can be reduced if we use nano- fertilizers instead of conventional fertilizers. The objective of this study was to compare the effects of preharvest nano-calcium (Nano-Ca) sprays with calcium chloride ($CaCl_2$) on the quality and storability of the ‘Red delicious’ apple fruit.

2. Materials and methods

2.1. Plant material

A population of 15-year old apple trees (*Malus domestica* L. cv. Red Delicious) were sprayed with 4 levels of calcium chloride ($CaCl_2$) (35% calcium, Merck, Germany) (0, 1, 1.5, and 2%) and 4 levels (0, 1.5, 2, and 2.5%) of nano-calcium (Nano-Ca) (7% calcium, Khazra, Sodour Ahrar Shargh Co., Iran). The control group was without calcium solution treatment. The treatments were applied 5 times through the whole experiment, and there was an interval of 2 weeks between each treatment. The treatments started 70 d after full-bloom (July 2015) and continued until one month before the harvesting of fruit. For maximum calcium absorption, Tween-20 (2 g L^{-1}) was added to solution sprays and sprays were performed until run off. Since starch test was used for commercial maturity of control fruit, apple fruit from all treatments was harvested at the commercial maturity of the control fruit and was transported to the laboratory. Then, fruit were categorized, some of them were selected to evaluate the parameters before storing (at harvest) while other fruit were stored at 0 °C and 90% RH for 1, 2, 3, and 4 months. Then, fruit quality was measured every month.

2.2. Weight loss (%)

Weight loss was measured by the formula:

$$[\text{Fruit weight loss } (\%) = (\text{Initial weight} - \text{final weight})/\text{Initial weight}] \times 100$$

2.3. Fruit firmness

Fruit firmness was measured on both sides of each fruit with Effegi penetrometer fitted with 11 mm diameter prob.

2.4. Titrable acidity (TA) and total soluble solids (TSS)

TSS (%) was determined with a digital refractometer (Atago, Japan) at room temperature. TA was determined by titration an aliquot (10 mL) of the juice to pH 8.2 with 0.1 N NaOH (normality of NaOH solution) and expressed as g malic acid L^{-1}.

2.5. Total phenolic content (TPC)

Folin-Ciocalteau colorimetric method was used for determining the TPC (Singleton et al., 1999), the absorbance was measured at 750 nm by UV/Vis spectrophotometer (T60 uv–vis). Gallic acid was used as a standard and the results were expressed as g kg^{-1} fresh weight.

2.6. Total antioxidant activity (TAA)

Antioxidant activity of the samples was evaluated through the free radical scavenging DPPH (2, 2-diphenyl-1-picrylhydrazyl, free radical) method of Meyers et al. (2003). 50 μL of the fruit extract was added to 950 μL of DPPH radical, then, the sample of each fruit was vortexed, and incubated at room temperature in darkness for 30 min. Absorption was measured with a spectrophotometer (T60 uv–vis) at 517 nm. The percentage of scavenged radicals was calculated using the following formula:

$$\text{Scavenged Percentage} = [(\text{A Control} - \text{A Sample})/\text{A Control}] \times 100$$

A Control = the absorbance of the control
A Sample = the absorbance of the sample

2.7. Fiber content

Fiber content of fruit was evaluated by the method of Maynard (1997). Accordingly, 2 g slice of each fruit was boiled with 200 mL of sulphuric acid for 30 min (alkaline digestion stage). Then, these were filtered through muslin and washed with boiling water until there was no acidic content. Then, they were boiled with 200 mL of sodium hydroxide solution for 30 min (acid digestion stage). Again, they were filter through muslin cloth and washed with 25 mL of boiling 1.25% sulphuric acid, three 50 mL portions of water and 25 mL alcohol. The residues of samples were removed, and then they were transferred to the ashing dish (w_1). The residues were dried for 2 h at 130 °C. After cooling, they were weighed in a desicator (w_2). Finally, they were heated again for 30 min at 600 °C, and were weighed again after cooling (w_3).

$$\text{Crude fiber content } (\%) = [(W2 - W1) - (W3 - W1)/\text{Weight of the sample}] \times 100$$

2.8. Browning

Browning of fruit was measured by method of Coseteng and Lee (1987). Fifty g of apple fruit was weighed and then was added to 100 mL distilled water in 250 mL beaker. They were transferred to the blender and homogenized for 1 min. The homogeneous mixture was placed at room temperature for 1 h. 10 mL of the supernatant was transferred to test tube, then, 15 mL 95% ethanol was added to them and was centrifuged at 800 × g for 15 min. The absorption of the supernatant was read at 440 nm by spectrophotometer (T60 uv–vis) and considered as indicative of the browning rate of the sample.

2.9. Enzyme activities assay

2.9.1. Polygalacturonase (PG)

The activity of polygalacturonase enzyme was determined by incubating 50 μL of extract in a 1 mL solution including polygalacturonic acid (25 g L^{-1}) and 40 mM sodium acetate (pH 5.0) for 1 h at 30 °C. Then, 1.5 mL of the sample tubes were boild for 10 min, and absorbance was measured at 410 nm after cooling at room temperature. The measurements were performed by UV/Vis spectrophotometer (Pharmacia LKB. U/Trospec III. Sweden). Freshly prepared P-hydroxybenzoic acid hydrazide (50 g L^{-1}) was added to the reaction (York et al., 1985). The galacturonic acid was used for the standard curve. Then, PG activity was calculated in unit on the weight of fresh fruit.

2.9.2. Pectin methylesterase (PME)

A volume of solution 500 μL including 0.0012 μL of bromothymol blue, 3 mM potasium phosphate buffer (KH_2PO_4, $_3H_2O$) pH 7.5, 0.5% Pectin (w/v, including 100 mM NaCl and 0.01% NaN_3) and 2.5 mM

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