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Influence of preharvest nano calcium applications on postharvest of sweet pepper (*Capsicum annum*)

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Abstract. *Amini F, Bayat L, Hosseinkhani S. 2016. Influence of preharvest nano calcium applications on postharvest of sweet pepper (*Capsicum annum*). Nusantara Bioscience 8: 215-220.* The effect of preharvest nano calcium treatment on the quality, physicochemical parameters and antioxidative enzyme activities of sweet pepper fruits were evaluated. For this purpose, sweet pepper plants treated with three concentrations (0, 4 and 8 g/L) of nano calcium in five steps (50, 60, 70, 80 and 90 days after pepper planting). 100 days after pepper planting, fruits were harvested and packaged in plastic bags and stored at 20±3 ^{0}C, then total soluble solids, pH, weight loss, carotenoids, electrolyte leakage, lipid peroxidation, titrable acidity, ascorbic acid, chlorophyll, calcium, protein and the activities of total antioxidant enzymes, catalase, and guaiacol peroxidase were evaluated at 0, 7, 14 and 21 days of storage period. Nano calcium treated fruits had lower levels of total soluble solids, pH, weight loss, carotenoids, electrolyte leakage, lipid peroxidation and higher levels of titrable acidity, ascorbic acid, chlorophyll, calcium and protein compared to the control plants. The increase percentage of titrable acidity, ascorbic acid, chlorophyll *a*, chlorophyll *b*, total chlorophylls, calcium and protein contents in plants treated with 4 g/L nano calcium and 21 days storage period were calculated 68.86%, 41.89%, 81.17%, 78.57%, 80.81%, 21.46% and 83.30% respectively. The maximum activities of total antioxidant enzymes, catalase and guailcol peroxidase were observed in 21 days control plants that application of nano calcium decreased these antioxidant activities. In most parameters 4 g/L concentration was more effective. Therefore, commercial application of preharvest nano calcium (4 g/L) can be considered for the maintenance of quality of sweet peppers during storage and marketing.

Keywords: Antioxidant enzymes, *Capsicum annum*, nano calcium, preharvest

INTRODUCTION

Sweet pepper (*Capsicum annum* L.) the second-most used vegetable worldwide (Mateos et al. 2013), grown in the tropical and sub-tropical zones of the world. Sweet pepper is characterized by its high levels of vitamin C (ascorbic acid), pro-vitamin A (carotene), calcium, antioxidant properties and vitamin E (Ramana-Rao et al. 2011). The consumption of 50-100 g fresh pepper fruits could provide 100% and about 60% of the advised daily amounts of vitamin C and A, respectively. The quality and texture of fruits are the important features for consumers. Preharvest calcium treatment is the safest and most effective method to improve the quality and extend the storage-life of fresh fruit (Tsantili et al. 2007).

Calcium is a secondary messenger that plays various tasks in adjusting physiological functions in fruits and vegetables during postharvest life (Soleimani-Aghdam et al. 2012). For vegetable producers, calcium's most important task during the crop fruiting stage is its role in cell wall/cell membrane stableness (Mayfeld and Kelley 2012). Preharvest treatment with calcium prevents fruit ripening and softening, delays senescence and a decrease in postharvest decay and incidence of physiological disorders such as water core (Tsantili et al. 2007). It has been reported frequently that calcium is one of the crucial nutritional components associated with enhancement the storage life and reducing the postharvest disorders of stored fruit (Holb et al. 2012). Calcium is important component of the plant cell wall, and binds together the strands of pectin helping to maintain fruit stiffness. It is also engaged in retaining membrane integrity. Calcium enhances fruit stiffness cross-linking the pectic polysaccharide chains by based on its divalent ionic character. Calcium binding to cell wall components may also diminish the availability of cell wall degrading enzymes to their substrates (Soleimani-Aghdam et al. 2012). Nanoparticles as a new generation of materials (Kmita et al. 2012) and the researchers of the world are seriously completing nanotechnology of various cancers treatment. The development of smart nanocarriers and nano anti-cancer drugs are the most important innovations in this direction (Ali et al. 2016). These drugs have many valuable properties such as targeted drug delivery and gene therapy modalities with lowest side effects (Ali 2011). The nano drugs are aimed selective and specific towards tumors (Ali et al. 2011). Nanodrugs are the great bullets, designed to be selective for tumor tissue and to act only on cancer cells and they are aimed selective and specific towards tumors (Saleem et al. 2013). Nanoparticles are atomic or molecular aggregates characterized by size less than 100 nm (Sabir et al. 2014). Nano compounds absorbed by plants rapidly and completely and supply nutrients deficiency and needs of plants (Harsini et al. 2014). In the present work, we evaluated the effect of preharvest nano calcium

applications on the quality and maintaining of sweet peppers during shelf life.

MATERIALS AND METHODS

Plant material and treatments

After sterilization of sweet pepper (*Capsicum annum* L.) seeds, they were cultured in cocopeat and peat moss with equal proportions. When the sweet pepper seedlings were at the 4 leaf stages, they were transferred to the common soil and were maintained under 25±3°C temperatures with the 16/8-hour light/dark photoperiod and 70% air relative humidity in a greenhouse at the research laboratory of Arak University in Arak city, Iran in 2015. 4 and 8 g/L concentrations of nano calcium were added to the soil in five steps. We started with, 50 and continued with 60, 70, 80 and 90 days after seeds planting. 100 days after plants planting, pepper fruits were harvested and packaged in plastic bags and stored at room temperature (20 °C) then the following parameters were measured at 0, 7, 14 and 21 days of storage period.

Total soluble solids (TSS), titrable acidity (TA) and pH

For measurement of TSS content of fruit, small cuts were made on the inner side of it, in order to ease the juice of the pepper sample to be squeezed. TSS measurements were taken by a digital refractometer. The TSS was expressed in Brix % (AOAC 1994). The titrable acidity (expressed as citric acid %) was determined by titrating 5-ml of juice with 0.1N sodium hydroxide, using phenolphthalein as an indicator (Mazumdar and Majumder 2003). The pH of the pepper samples was determined by the AOAC (AOAC 1994) method.

Ascorbic acid content

Determination of ascorbic acid (ASA) content was carried out based on 2,6 dichlorophenolindophenol (DCIP) method (Mazumdar and Majumder 2003). 2 g fine powder of pepper tissue was homogenized in 10 mL (3%) metaphosphoric acid for 10 min. Then vitamin C was determined by titration of 10 ml filtered sample by DCIP (0.86 mM) containing bicarbonate sodium (2.5 mM) and expressed as mg ascorbic acid /100gr FW.

Weight loss

Weight loss was recorded initially and weekly during storage. Weight loss was calculated as: weight loss= $(W_i - W_f)/W_i \times 100$, W_i being the initial sample weight and W_f the final sample weight. Results are reported as percentage weight loss.

Chlorophyll and carotenoids contents

Measurement of chlorophyll (Chl) *a*, Chl *b*, total Chl and total carotenoid can be determined in a whole-pigment extract of fruit tissue by UV-VIS spectroscopy and measurement absorbance of the extract at 470.0 nm, 648.6 nm and 664.2 nm (Lichtenthaler 1987).

Electrolyte leakage and lipid peroxidation

Electrolyte leakage (EL) was used to assess membrane permeability and it was also measured with an electrical conductivity meter. The procedure used was based on Vanstone and Stobbe (1977) method. Lipid peroxidation was measured as of the amount of malondealdehyde (MDA) determined by the thiobarbituric acid (TBA) reaction (Heath and Packe 1969).

Protein

Total protein content was measured by using of Bradford method (Bradford 1976). Bovine Serum Albumin was used as the standard.

Antioxidant activity

Leaf fresh materials (0.1 g) was powdered by liquid nitrogen and homogenized in 1 ml of 50 mM phosphate buffer (pH=7) which was containing 1 mM ethylene diamine tetra acetic acid (EDTA) by a homogenizer into microtubes. Insoluble materials were removed by Beckman refrigerated centrifuge at 13000 g for 20min at 4°C, and the supernatant was used as the source of enzyme extraction. The catalase (CAT) activity was assayed as described by Cakmak & Marschner (1992) and the activity was determined that it would decrease in the absorbance at 240 nm following the decomposition of H_2O_2. The guaiacol peroxidase (GPOX) activity was measured according to Polle et al. (1994) and by the monitoring of guaicol oxidation at 470 nm.

Total antioxidant activity was evaluated as the scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. For determination of this radical activity was used of Abe et al. (1998) method. Leaf fresh materials (100 mg) was powdered by liquid nitrogen, homogenized in 1ml of 90% ethanol and then maintained at 4°C for 24 h. Insoluble materials removed by centrifuge at 3500 g for 5 min. 20 µl of extracting solution was mixed with 800 µl of DPPH (0.5mM in ethanol). The absorbance of the resulting solution was measured at 517 nm after 30 min in darkness. The ability to scavenge the DPPH radical was expressed by inhibition percentage (I%).

Calcium content

0.25 g samples of pepper fruits were incinerated to produce ash in a furnace at 550 °C for 3 h. Calcium was dissolved in HCl: water (1:1, v/v), filtered and the filtrate was taken to 50 mL with water. Aliquots of the samples (25mL) were used for calcium analysis which was measured titrimetrically at pH 12 with a 0.024M EDTA by using calcon carboxylic acid as indicator. Results are expressed in mg of calcium per gram.

Statistical analysis

All data were analyzed by variance analysis using SPSS 16. Experiments were tested using completely randomized design in factorial form in three replicates. The data was represented as the means ± SE.

RESULTS AND DISUSSION

Total soluble solids (TSS), titrable acidity (TA) and pH

TSS and pH values of fruits increased gradually during the storage period (7, 14 and 21 days). Also results showed that nano calcium treatment reduced the TSS and pH values as compared to the control fruits (Table 1). The lowest amounts of TSS at all days of storage period were observed in nano calcium treatments and it wasn't different between 4 and 8 g/L concentrations of nano calcium. This reduction of TSS was due to the decrease of respiration and metabolic activity and as a result delaying the ripening process. Ramana-Rao et al. (2011) achieved similar results about TSS in sweet pepper. The highest level of pH was observed in the control fruits and pH value was highest in nano calcium treatments and 4 g/L concentration was more effective. In this study, TA levels decreased significantly with increasing of storage time. Also it observed that nano calcium application improved the TA levels in pepper fruits as compared to the controls (Table 1). This improvement in 4 g/L of nano calcium was better than 8 g/L. The maintenance of acidity in calcium treatment can be due to the decrement in metabolic changes of organic acid into carbon dioxide and water. Ibrahim (2005) and Ramana-Rao et al. (2011) reported the similar results.

Ascorbic acid (ASA) content

Analyze of results showed that ascorbic acid content of samples decreased slightly with storage time. Although treated fruits with both concentrations of nano calcium showed higher ascorbic acid content than the control fruits (Table 1). Stable form of vitamin C that is usually in the form of L-ascorbate has a key role in plant cells as an oxidizing agent (Macclean et al 2003). Ascorbic acid is capable to clean the superoxide and hydroxyl radicals, as well as regenerate a-tocopherol (Davey 2000). In Aguayo et al. (2010) study, calcium ascorbate treatments maintained vitamin C content at a higher level compared to the controls.

Weight loss

Our results showed that weight loss increased significantly with the increase storage time at both concentrations of nano calcium. Postharvest weight loss in vegetables is generally due to the loss of water through transpiration (Znidarcic et al. 2010). Also results demonstrated that nano calcium treatments resulted in reduced weight loss and 4 g/L concentration of nano calcium was more effective than 8 g/L concentration (Table 1). Calcium ions increase the stability of cell walls by binding non-sterified pectins and reduced ion leakage which could be responsible for the lower weight loss (Ramana-Rao et al. 2011). On the 21 days of storage period the samples treated with 4 g/L nano calcium had significantly lower weight loss (30.24%) than the controls (63.42%). Similar results observed in Angeletti et al. (2010) study that they showed preharvest calcium application improved weigh loss in two blueberry varieties.

Chlorophyll and carotenoids contents

Chl *a*, Chl *b* and total Chl of pepper fruits declined significantly during the storage period. Our study also exhibited that 4 and 8 g/L of nano calcium caused a significant increase in chlorophyll content of pepper (Table 2). Highest amounts of Chl *a* (2.35 mg/g), Chl *b* (1.42 mg/g) and total Chl (3.88 mg/g) were observed in control fruits. In this study, carotenoid content of pepper enhanced with the increase of storage time. Although application of nano calcium treatment caused the reduction in carotenoids content (Table 2). A property of fruit ripening is the change in color as a result of chlorophyll evanescence, when the yellowish coloration due to carotenoids becomes detectable. Chromoplast differentiation in fruit ripening is very often followed by carotenogenesis, a *de novo* carotenoid biosynthesis that enhances and even changes the strength and characteristics of the color in the ripe fruit (Hornero-Mendez and Minguez-Mosquera 2002). Chlorophyll and carotenoids contents of *Momordica charantia* decreased and increased respectively during ripening of fruit. Application 70 mM concentration of calcium pectate improved the pigment contents (Anbarasan and Tamilmani 2013).

Electrolyte leakage (EL) and lipid peroxidation (MDA content)

According to our results, electrolyte leakage (EL) and lipid peroxidation (MDA content) enhanced during storage period (7, 14 and 21 days). Treatment with 4 g/L and 8 g/L concentrations of nano calcium reduced significantly EL and MDA contents in pepper fruits (Figure 1A, B). About MDA, 4 g/L concentration was effective than 8 g/L, but about EL, effects of both concentrations were equal. Amounts of El and MDA in 4 g/L nano calcium on 21 days storage period were obtained 42% and 4.07 µmol/g, respectively. EL and MDA have been recognized as a proper indicator of membrane integrity. Increased EL and MDA contents by stored fruit over time are due to the loss of membrane integrity in relation to the breaking of membrane structural components such as phospholipids. Calcium is considered to be effective in holding membrane integrity by decreasing ion leakage and phospholipids losses in the cellular network (Bagheri et al. 2015). These results are in agreement with the results of Bagheri et al. (2015), who studied the effect of calcium chloride treatment on the EL and MDA contents of persimmon fruits.

Protein

During the storage times, total soluble protein content of pepper fruits decreased significantly as compared to the controls. Treatment with both concentrations of nano calcium improved protein content. The lowest amount of protein was obtained in control fruits (Figure 1C). The observed decrease in protein content as the period of storage increased might be due to increased utilization of the nutrients and reduced of protein production (Olusegun et al. 2012). Ca^{2+} could perform as signal molecule and by that adjust the expression of proteins. Ca^{2+} was necessary for accumulation of pathogenesis-related protein such as chitinase (Soleimani-Aghdam et al. 2012).

Table 1. Effect of preharvest nano calcium treatment on total soluble solids (TSS) %, titrable acidity (TA) %, pH, ascorbic acid (ASA, mg /100 g FW) and weight loss % of sweet pepper fruits during different storage periods. Values within a row with a letter in common are not significantly different according to Duncan's test'

Calcium (g/L)	Time (day)	Parameters				
		TSS	TA	pH	ASA	Weight loss
0	0	$1.6^{h} \pm 0.15$	$5.34^{a} \pm 0.08$	$5.92^{f} \pm 0.01$	$76.66^{a} \pm 1.4$	-
	7	$2.93^{e} \pm 0.08$	$4.14^{c} \pm 0.08$	$6.32^{e} \pm 0.01$	$58.4^{c} \pm 0.33$	$23.83^{e} \pm 0.84$
	14	$4.7^{c} \pm 0.15$	$2.32^{f} \pm 0.16$	$6.84^{b} \pm 0.02$	$42^{d} \pm 1.15$	$36.14^{c} \pm 0.66$
	21	$6.2^{a} \pm 0.06$	$1.06^{h} \pm 0.07$	$6.97^{a} \pm 0.01$	$20.67^{f} \pm 1.2$	$63.52^{a} \pm 0.57$
4	0	$1.6^{h} \pm 0.15$	$5.34^{a} \pm 0.08$	$5.92^{f} \pm 0.01$	$76.66^{a} \pm 1.4$	-
	7	$2.13^{g} \pm 0.08$	$5.11^{a} \pm 0.06$	$6.25^{e} \pm 0.02$	$64.33^{b} \pm 0.88$	$6.95^{i} \pm 0.22$
	14	$3.77^{d} \pm 0.03$	$3.69^{d} \pm 0.08$	$6.61^{d} \pm 0.06$	$55.66^{c} \pm 2.7$	$16.67^{g} \pm 0.82$
	21	$5.53^{b} \pm 0.03$	$1.79^{g} \pm 0.05$	$6.89^{ab} \pm 0.01$	$29.33^{e} \pm 2.03$	$30.24^{d} \pm 0.48$
8	0	$1.6^{h} \pm 0.15$	$5.34^{a} \pm 0.08$	$5.92^{f} \pm 0.01$	$76.66^{a} \pm 1.4$	-
	7	$2.53^{f} \pm 0.03$	$4.54^{b} \pm 0.07$	$6.28^{e} \pm 0.01$	$56.33^{c} \pm 1.2$	$12.41^{h} \pm 0.87$
	14	$4.03^{d} \pm 0.03$	$3.09^{e} \pm 0.06$	$6.73^{c} \pm 0.07$	$45.66^{d} \pm 1.76$	$20.02^{f} \pm 0.2$
	21	$5.77^{b} \pm 0.05$	$1.56^{g} \pm 0.06$	$6.92^{ab} \pm 0.01$	$22.40^{f} \pm 1.25$	$43.76^{b} \pm 1.36$

Table 2. Effect of prehrvest nano calcium treatment on Chl *a*, Chl *b*, total Chl and carotenoids of sweet pepper fruits during different storage periods. Values within a row with a letter in common are not significantly different according to Duncan's test'

Calcium (g/L)	Time (day)	Parameters			
		Chl *a*	Chl *b*	Total Chl	Carotenoids
	0	$2.35^{a} \pm 0.1$	$1.42^{a} \pm 0.11$	$3.88^{a} \pm 0.15$	$1.01^{j} \pm 0.06$
0	7	$1.38^{ef} \pm 0.02$	$0.99^{b} \pm 0.04$	$2.37^{bc} \pm 0.05$	$1.87^{g} \pm 0.03$
	14	$1.15^{g} \pm 0.02$	$0.47^{ef} \pm 0.01$	$1.62^{d} \pm 0.03$	$2.90^{d} \pm 0.03$
	21	$0.85^{h} \pm 0.02$	$0.14^{h} \pm 0.02$	$0.99^{e} \pm 0.05$	$4.09^{a} \pm 0.04$
	0	$2.35^{a} \pm 0.1$	$1.42^{a} \pm 0.11$	$3.88^{a} \pm 0.15$	$1.01^{j} \pm 0.06$
4	7	$1.88^{b} \pm 0.02$	$0.73^{cd} \pm 0.02$	$2.61^{b} \pm 0.03$	$1.34^{i} \pm 0.02$
	14	$1.77^{bc} \pm 0.03$	$0.55^{e} \pm 0.01$	$2.33^{c} \pm 0.05$	$2.22^{f} \pm 0.05$
	21	$1.54^{de} \pm 0.06$	$0.25^{gh} \pm 0.01$	$1.79^{d} \pm 0.06$	$3.44^{c} \pm 0.03$
	0	$2.35^{a} \pm 0.1$	$1.42^{a} \pm 0.11$	$3.88^{a} \pm 0.15$	$1.01^{j} \pm 0.06$
8	7	$1.60^{cd} \pm 0.03$	$0.84^{bc} \pm 0.03$	$2.43^{bc} \pm 0.04$	$1.60^{h} \pm 0.04$
	14	$1.55^{de} \pm 0.02$	$0.63^{de} \pm 0.04$	$2.18^{c} \pm 0.04$	$2.48^{e} \pm 0.03$
	21	$1.30^{fg} \pm 0.04$	$0.32^{fg} \pm 0.02$	$1.61^{d} \pm 0.02$	$3.75^{b} \pm 0.02$

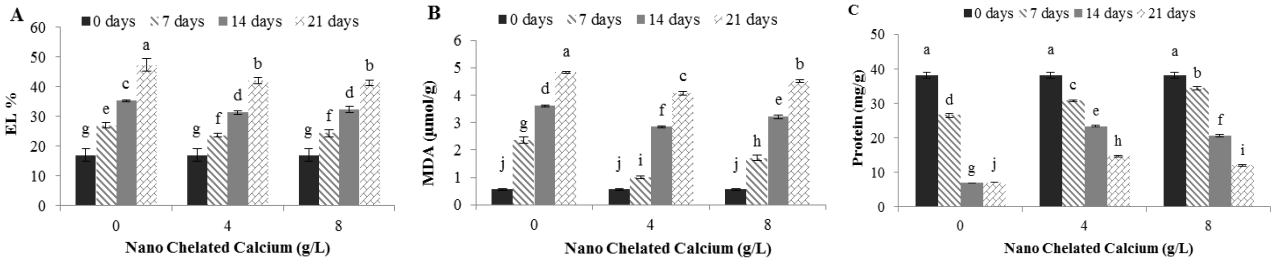

Figure 1. Effect of prehrvest nano calcium treatment on El (A), MDA (B) and protein (c) of sweet pepper fruits during different storage periods. Means followed by different letters are significantly different as determined by Duncan's test.

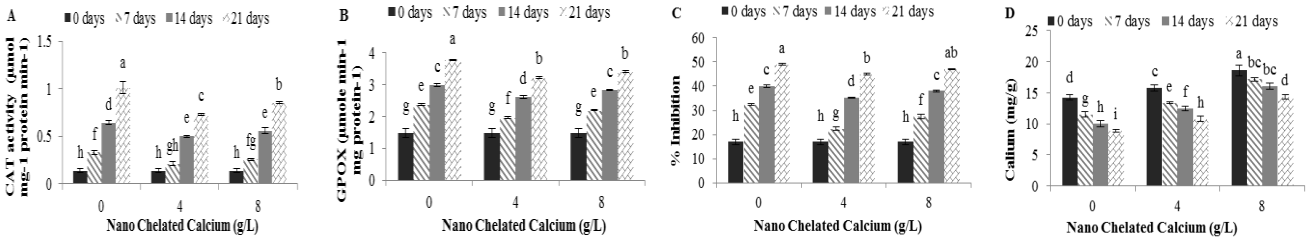

Figure 2. Effect of prehrvest nano calcium treatment on CAT (A), GPOX (B), I% (C) and calcium content (D) of sweet pepper fruits during different storage periods. Means followed by different letters are significantly different as determined by Duncan's test.

Antioxidants activity

Significant increases in CAT and GPOX activities were observed during the all of storage times (7, 14 and 21 days). Highest levels of CAT and GPOX were obtained in 21 days. Nano calcium treated pepper fruits showed significantly lower CAT and GPOX activities compared with the controls during the all of storage periods (Figure 2). 4 g/L of nano calcium had a better effect than 8 g/L in reduction of CAT and GPOX activities. I% enhanced significantly during the storage periods (Figure 2). The Increase of I% means more antioxidants have been produced. These results are in agreement with previous studies that showed total antioxidant activity was increased during storage (Awad and de-Jager 2003; Lata 2008; Moosavi-Dolatabadi et al. 2015). Treatment with nano calcium decreased I% levels in treated fruits. 4 g/L concentration was more effective (Figure 2). Different stress conditions lead to the over-production of reactive oxygen species (ROS) in plants which are highly active and harmful. ROS cause injury to proteins, lipids, carbohydrates, and DNA which finally result in oxidative stress. Plants have the potential to deal with ROS by synthesizing the antioxidant enzymes like CAT and GPOX (Seckin et al. 2010) as well as some non-enzymatic antioxidant. In response to stress, plants commonly enhance the activity of these enzymes (Xing et al. 2011) and decline in enzymatic capacity may be associated with a reduction in the capacity to prevent damage. Ramana-Rao et al. (2011) suggested that calcium treatments activate the defense-related enzymes and then improved protection of the fruits.

Calcium content

During the storage times, a reduction in calcium content of fruits was detected. Nano calcium treatment increased significantly the calcium content of pepper fruits. Highest amounts of calcium were observed in 8 g/L treatments. The 31% increase was calculated in pepper fruits with 8 g/L nano calcium treatment and in 0 day as compared to the non-treated plants. Angeletti et al. (2010) reported 10% increase in calcium content in two blueberries (*Vaccinium corymbosum*) varieties with preharvest calcium applications.

The results showed that early preharvest treatments with nano calcium were effective on pepper fruits quality. Both concentrations of nano calcium improved some parameters of pepper fruits such as total soluble solids, titrable acidity, pH, ascorbic acid, weight loss, chlorophyll, carotenoids, electrolyte leakage, lipid peroxidation and protein of sweet pepper. In the most parameters, 4 g/L of nano calcium was more effective than 8 g/L concentration. Application of nano calcium also maintained lower total antioxidant activities (I%), CAT and GPOX activities. With achievement these results, it can recommend preharvest nano calcium (especially 4 g/L concentration) application to maintain the quality of sweet peppers during postharvest storage.

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