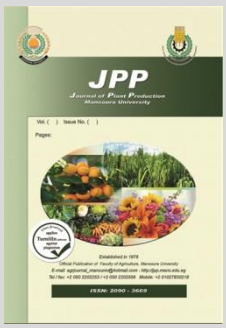
Journal of Plant Production

Journal homepage & Available online at: www.jpp.journals.ekb.eg

Effect of Calcium Nanoparticles Coupled with Oxalic Acid in Minimizing Rachis Browning Incidence and Its Relation to Phenolic Compounds in 'Superior Seedless' Grapes During Shelf Life

Asmaa S. M. Omar[1*] and M. S. Aboryia[2]

[1]Pomology Department, Faculty of Agriculture, Mansoura University, Mansoura, Egypt
[2]Pomology Department, Faculty of Agriculture, Damietta University, Egypt



ABSTRACT

The rachis browning phenomenon is an urgent problem in the grape trade where it is not acceptable to customers. The research was conducted in two successive growing seasons (2020-2021) to assess the influence of calcium nanoparticles (CaNPs) coupled with oxalic acid (OXA) in various concentrations on decreasing the browning phenomenon of rachis and maintaining the quality of 'Superior Seedless' grape bunches during shelf-life period. Bunche samples were taken from a private vineyard when SSC was about 16%. The grapes were stored for three days at laboratory temperature (at 27 ± 1 ° C and 46 ± 2% relative humidity). Result showed that the CaNPs-20 mM OXA significantly maintained the freshness of clusters rachis by reducing the rate of water loss, repressing the development of brown pigments and preserving the hue angle of rachis. Also, it maintained the soluble solids content and SSC:TA-ration, while it delayed the decreases of total acidity and ascorbic acid content, which showed the better maintenance of chemical content in the treated bunches. Besides, a notable decrease in the enzyme activities of cell walls such as polygalacturonase, xylanase, cellulase, and pectinase was observed. Also, the contents of total phenol and flavonoid have increased, while polyphenol oxidase and phenylalanine ammonia-lyase have decreased. Also, the CaNPs-20mM OXA reduced malondialdehyde accumulation, lipoxygenase activity and the rachis electrolyte leakage percentage throughout the shelf-life duration. summary, CaNPs-20 mM OXA proved to be an effective treatment for maintaining the quality of 'Superior Seedless' bunches during the shelf-life duration.

Keywords: Superior Seedless; Shelf-life; Browning; Nano-calcium particles; Cell wall enzymes.

INTRODUCTION

Grapes in Egypt in recent years have been classified second position after citrus fruits according to the cultivated area and the amount of the crop produced (Ministry of Agriculture, 2015). The harvest area is 78,853 hectares and produces 1,759,472 tons (FAOSTAT, 2018). Currently, one of the internationally known grape cultivars is 'Superior Seedless' that achieves high income (Menora *et al*., 2015). It is preferred by purchasers for its high nutritional value, high taste, multi-purpose uses, and greater economic return (Koyama *et al*., 2020). The 'Superior Seedless' grape is one of the vines that appeared at the end of June on the Egyptian market for one to three weeks before the countries of East Asia. This is considered an advantage in export, which makes its price higher than any other source country. It also spread to different regions and grown in different climates. It has grown very well in Egyptian conditions and is harvested in green for European customers (Artés-Hernández *et al*., 2006). Generally, grape has a low physiological activity possesses and a higher respiration rate, as it is considered a non-climacteric fruit. Thus, its life is relatively short, which enhances the rachis browning (RB).The rachis browning (RB) is one of the most detrimental post-storage disorder for grape as it makes storage so difficult for longer periods, and is related to the looseness both of quality and acceptance during the series of marketing (Artés-Hernández *et al*., 2006; Lichter *et al*., 2011). RB is related to water loss through rachis, which begins after harvest (Champa *et al*., 2015), and then through the enzymes that break down the cell wall (Huang *et al*., 2013). Then more rapid activity in polyphenol oxidase (PPO) and loss of the content of rachis phenolic compounds during the shelf-life (Yoruk and Marshall, 2003).

Physiologically, RB occurs through the oxidation of phenolic compounds such as flavonoids, scientifically ortho-diphenols (O-diphenols, 1,2-diphenols) to semiquinones and quinones. Polyphenol oxidase (PPO) and peroxidase (POD) enzymes are involved (Pourcel *et al*., 2007). Besides, phenylalanine ammonia lase (PAL) is also involved (Ranjbaran *et al*., 2011). Browning occurs in many different fruits during shelf-life, in many studies, it was examined using different materials such as organic acids like ascorbic acid, citric acid and oxalic acid (Moon *et al*., 2020) to determine the frequency of browning and reduce it. Oxalic acid (OXA) is an organic acid that is mainly used as an anti-stress, programmed cell death, and redox hemostasis in plant cells. Also, it controlled fruit ripeness after harvest by reducing ethylene production (Wu *et al*., 2011). Also, a new technique, the application of nanoparticles, has been applied on fruits.

Therefore, this study aims to evaluate the effect of mixing calcium nanoparticles (CaNPs) with oxalic acid (OXA) in various concentrations on reducing the browning incidence in rachis and keeping fruit quality of 'Superior Seedless' grape bunches during the shelf life.

MATERIALS AND METHODS

Experimental layout

The experiment was carried out on 'Superior Seedless' grapes in a private vineyard for two seasons (2020-

* **Corresponding author.**
E-mail address: asmaa2007@mans.edu.eg
DOI: 10.21608/jpp.2022.229367

2021) to investigate the influence of calcium nanoparticles (CaNPs) coupled with oxalic acid (OXA) at different concentrations (CaNPs-0 mM OXA, CaNPs- 10 mM OXA and CaNPs-20 mM OXA) as the first factor while the second factor was the shelf-life period (one, two and three days after the initial time) on browning appearances in rachis of grape bunches as a factorial experimental design. The grape bunches (288 bunches) were collected while SSC was about 16% at harvest time and divided to two groups. The first one (144 bunches) was used to evaluate the water loss %, (RB index) rachis browning index, and the rachis hue angle during the 3 days of the experiment. Whereas, the second samples (144 bunches) was subjected to monitor chemical traits involved the activities of cell wall degradation enzymes, contents of phenolic compounds, and browning enzyme activities.The bunches were divided into 4 treatments, each has 36 bundles distributed on three replicates (12 bundles/replicate). All tested grape bunches were stored for 3 days at a laboratory temperature of 27 ± 1 ° C and 46 ± 2% air relative humidity.

Formation of (CaNPs) including (OXA)

Calcium nanoparticles (CaNPs) were prepared with a small modification based on the protocol according to Yugandhar and Savithramma (2013). It was systematized by adding oxalic acid (OXA) at 10 and 20 mM. All concentrations of OXA were mixed in a solution of $CaCl_2$ at 50 mM using distilled water. The product mix was kept on a controller at 5000 rpm for 1 hour and then brought to laboratory temperature for 2 to 3 days.

Nanoparticles proprieties were tested by UV-vis spectroscopy, Zeta potential, and transmission electron microscope (TEM)

The size of original Ca^{++} particles and the screening of the synthesis calcium nanoparticles were examined by using theUV-vis spectroscopic analysis from ATI Unicom. Identifying the UV-Vis spectroscopy of the blended mineral nanoparticles was reported at 240-440 nm. The investigation was carried out at 25 ° C using quartz cuvettes (1 cm optical path, Figure 1). A zeta potential analysis was carried out to describe the situation on the nanoparticle surface (NPs) and to check the stability of the nanoparticle mixture in the long term. The CaNPs associated with the surface charge of oxalic acid (OXA) was determined by using Malvern Instruments Ltd Zeta Potential Ver. 2.3 in the central laboratory of the electron microscope unit of the Faculty of Agriculture, Mansoura Univ., Egypt. CaNPs-OXA has an outside charge that draws a light film of ions of various charges onto the surface of CaNPs-OXA. It has a bilayer distribution of ions when it spreads in suspension. The electrical potential at the end of the bilayer is identified because the particles zeta potential have rates between +100 mV and -100 mV (Figure 2). The Calcium nanoparticles (CaNPs) with oxalic acid (OXA) are integrated and the zeta potential data was -4.74 mV (more establishment).

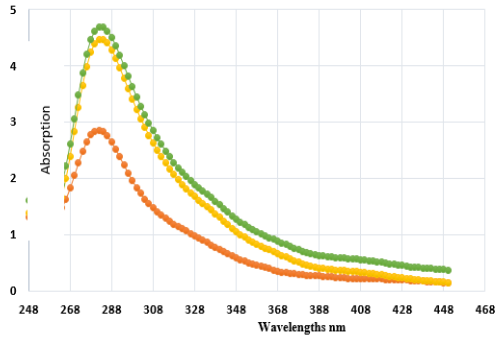


Figure 1. UV-visible absorption spectra of synthesis calcium nanoparticles (CaNPs) blended with different concentrations of oxalic acid (0, 10, and 20 mM) depicting a peak at 270 nm.

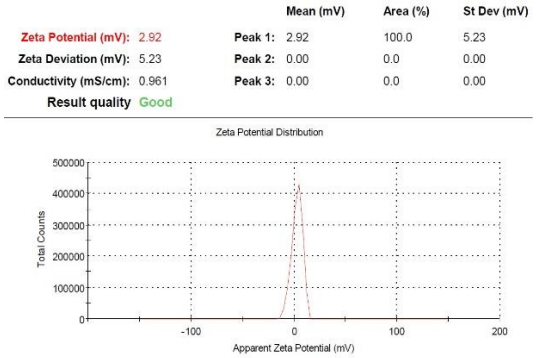


Figure. 2. Zeta potential distribution for calcium nanoparticles with oxalic synthesized

Nanoparticles with zeta potential data between +25 mV and below -25 mV usually show high equilibrium (Soheyla and Foruhe, 2013). The size of the CaNPs-OXA formation and the morphological results of the acquired nanoparticles (NPS) was established utilizing transition electron microscopy (JEOL TEM-2100) linked to a Charge-Coupled-Device camera at an accelerating charge of 200 kV. Any sample of the combined mineral CaNPs-OXA is fixed by adding a mixture of samples to copper-covered carbon networks, which are then soluble and can slowly disappear before the TEM image is taken (Figure 3). TEM estimates were shown in the central laboratory of the electron microscope unit.

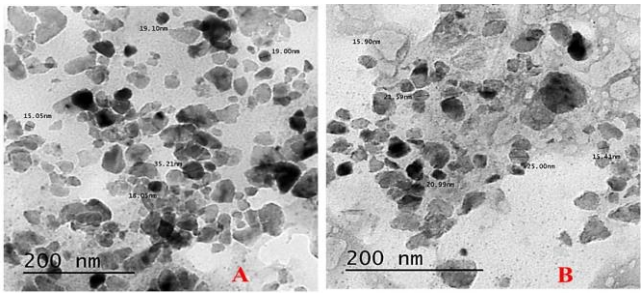


Figure. 3 The transmission electron microscopy (TEM) was performed for the synthesized nanoparticles at 200 nm. The size of the particles for CaNPs (A) is between 15.05 and 35.21 nm, while the size of CaNPs with oxalic acid is around 15.90 to 25.00 nm. The shape of the particles is spherical, and the small numbers are tetragonal. The particles in the case of CaNPs are more aggregated than CaNPs with oxalic acid particles (B).

CaNPs-OXA treatment protocol

When arriving at the laboratory, concurrently grape bunches of 'Superior Seedless' were divided to 4 groups and each soaked for 3 minutes at laboratory temperature in one treatment as follows: T1: control (without treatment), T2: CaNPs-0 mM OXA (only calcium nanoparticles without OXA), T3: CaNPs-10 mM OXA and T4: CaNPs-20 mM OXA.The treated bunches were stored at temperature of 27 ± 1 ° C and 46 ± 2% relative humidity for three days. A representative sample of untreated bunches were kept for 0 day of analysis.

Physical and chemical quality analysis of bunches

The water loss % from the grapes was determined over 3 days of shelf life based on the initial weight at the begining of study. The method (Crisosto *et al*., 2001) was used for the rachis browning index (RB-index). The color of the rachies was monitored by photography throughout the shelf-life. Images were evaluated by using ImageJ Ver software Ver., 1.43 USA to receive RGB signals to calculate the hue angle of rachis based on the method (Khojastehnazhand *et al*., 2010). Berry samples were selected from different treated bunches to estimate soluble solids content (SSC%) using a Carlzeiss handheld refractometer. The total acidity (TA) was calculated by titration with 0.1 N NaOH (AOAC, 1995), and the SSC: TA ratio was calculated as maturity index. For ascorbic acid (AsA), the content measurement was estimated by a titrimetric method using 2,6-dichlorophenol-indophenol and 6% oxalic acid according to (AOAC, 1995).

Cell wall degradation enzymes activities

Rachis sample (1 gm) grind with Tris-HCl (20 mM) at pH 7, and confused at 15,000 rpm for 20 min below cooling (4 °C). The clear sample extracted was stored at −20 °C for measurements enzymes: (polygalacturonase; PG), (xylanase; XLN), and (cellulase; CEL). The determination of the reduction end of galacturonic acid, xylose, and carboxymethyl cellulose as sources, gives an indication of enzymes activities (Collmer *et al*., 1988). The blend of reaction (1000 µL) contained; 200 µL of 200 mM of derivation buffer sodium acetic acid ($C_2H_3NaO_2$) at pH 5.0, 100 µl of 200 mM sodium chloride (NaCl), and 300 µl poly-galacturonic acid (PGA), all the blend at a sensible volume in a total volume (1 ml). The increase in the substrate was an indication of the occurrence of the response. The reaction mixture was incubated in a water bath at 37 °C for one hour. From this point, 500 µl of dinitro ($C_7H_4N_2O_7$) salicylic acid as reagent was combined and solved in a water bath for ten minutes, then the mixtures had been cooled until came to room temperature. These mixtures were evaluated on a spectrophotometer; at 560 nm for both polygalacturonase (PG) and xylanase (XYL) and 540 nm for cellulase (CEL). The amount of enzyme producing 1 µM of reducing sugar (RS) was the way to express the 1 unit of enzyme activity, measuring RS released per min at 37 °C. As for determination of pectinase (PT) activity, it was determined by mixing 500 µl (0.36% w / v) polygalacturonase with 50 mM Tris-HCl carrier at 8.5 pH, 300 µl 4 mM calcium chloride, 600 µl proteins and 600 µl water analyzed and then the reaction mixture kept at 37 °C for 3 hours (Collmer *et al*., 1988). Pectinase (PT) was evaluated on a spectrophotometer at 232 nm (Payasi and Sanwal, 2003). The mg $^{-1}$ units of protein were used to express the specific activity of enzymes, hence the method according to Bradford (1976) to determine the total content of soluble proteins in the enzyme extract.

Phenolic compounds and browning enzyme activities

The measurements of phenolic compounds such as total phenol (TP) and flavonoid (FL) were accomplished as follow: TP in treated grapes was assessed by applying Folin-Ciocalteu reagent with gallic acid as the reactor and measured at a wavelength of 750 nm.The data were addressed as mg gallic acid equivalents (GAE) mg 100 g^{-1} FW (Hoff and Singleton, 1977). The total substance of flavonoids (FL) was analyzed utilizing the method of (Zhishen *et al*., 1999). Polyphenol Oxidase(PPO) was extracted by using 1.0 g of rachis sample that homogenized with 20 mM (Tris- Hydrochloride) buffer, pH 7.0. At 16,000 rpm for 6 min under cooling at 4 °C, the mix was centrifuged and the pure extraction was kept below -20 °C toward determination of PPO. The catechol substrate was used to measure PPO activity. The extract (200 µl) was directly added to 3 ml of 20 mM catechol dissolved in 100 mM Na PO_4^- buffer, PH 7.0 (Jiang *et al*., 2004). The increased activity was reported at 400 nm on a spectrophotometer for 3 minutes. The activity was presented with a 0.1 min^{-1} difference in absorbance. Phenylalanine ammonia-lyase (PAL) was determined according to the protocol of (Ke and Saltveit, 1986), and the PAL activity was estimated under laboratory conditions. The activity recorded at 290 nm is based on the production of cinnamic acid.

Lipid peroxidation, lipoxygenase activity (LOX), and Electrolyte leakage percentage (EL%)

Malondialdehyde (MDA) is an important marker for damage caused by lipid peroxidation processes. Rachis sample (2.5 g) was used and the 1,1,3,3-tetraethoxypropane (Sigma, USA) in the range of 0-2 mM (TBARS) as a standard curve, which was relative to 0-1 mM of MDA (Iturbe-Ormaetxe *et al*.,1998). Lipoxygenase (LOX), 1g of rachis sample for monitoring the LOX activity which is presented as 0.01 unit min^{-1} mg $^{-1}$ protein (Pinto *et al*., 2007). Rachis electrolyte leakage (EL%) was calculated using the following formula: EL%) = (Initial EL after 3-hours/Final EL after boiling sample) × 100 (Xua *et al*., 2012).

Statistical analysis

A factorial experiment was laid in a completely randomized block design. The Tukey's-HSD test was performed using the JMP Pro 16 program (SAS Institute, Cary, NC), with a significance level of $P<0.05$ to compare the data means. Data in each table represents the interaction effects between calcium nanoparticles coupled with oxalic acid and the shelf-life duration as factors. Pearson's correlation matrix and principal component analysis (PCA) were used to determine the relationships between the analyzed parameters.

RESULTS AND DISCUSSION

Effect of CaNPs-OXA on the physical characteristic of bunches

Table 1 shows the effect of CaNPs-OXA treatments at $P<0.05$ on the physical characteristic of the grape bunch through the shelf-life duration in days. Firstly, water loss%

of bunches through 3 days of shelf-life is viewed as stress by which restricts the storage ability of bunches. Observably, water loss increased during the experimental time (days). Clearly, the control treatment (untreated bunches) showed more rapid water loss throughout shelf-life duration, while, CaNPs-OXA treatments resulted in less water loss up to the end of study. The results showed that treated fruit with CaNPs-20 mM OXA had the lowest water loss at the end of shelf-life duration compared to the control so this treatment is being considered the best treatment in terms of reduced in water loss. Regard fully, for the rachis browning index (RB-index), which is an indicator of evaluation rachis browning symptoms during shelf-life. It appeared from the 2nd day of the experiment then it increased and differentiated independently up to the 3rd day of the shelf-life period while, it appeared in the 3rd day only in case of bunches treated with CaNPs-20 mM OXA. In fact, with increasing OXA concentrations, RB-index decreased. Fruit treated with CaNPs-OXA 20 mM had lowest RB-index at 3rd day of shelf-life compared to the control as shown in (Figure 5). Furthermore, another indicator to evaluate the variation of the effect of CaNPs-OXA treatments on rachis browning of Superior Seedless is the rachis color hue angle. The green color of the rachis is an important indicator of the freshness of bunches of table grapes during the storage process. Clearly, the hue of rachis color declined gradually and slowly according to CaNPs-OXA treatments during shelf-life, while control displayed more rapid decreases in it. The CaNPs-OXA 20 mM showed significant preservation in the rachis color and maintained the freshness in clusters rachis significantly by suppressing the development of brown pigments during shelf-life. In control clusters, the rachis senescence have been accelerated by water loss and through degradation of photosynthetic pigments. These results could be explained due to two ways. Firstly, the existence of calcium in the soaking solution could support the cell wall, which decreases the cell wall dysfunction during shelf-life through formation of calcium pectate by reaction of calcium with pectic acid, resulting in more hardness and preservation the structure of cell wall (Lee *et al*., 2003; Ranjbar *et al*., 2018). White and Broadley (2003) provied that Ca accumulates in the cell walls causing more cross linking of the pectic polymers, which is responsible for increasing the cohesion and strength of cell, also it maintains integrity and functionality of the membrane fruit, this results in low water loss from the fruit (Mahajan and Dhatt, 2004) and therefore reducing the water loss%. Also, Ranjbar *et al*. (2018) showed that nano-calcium treated fruits were firmer than CaCl 2 treated fruits and this could be due to more permeation of nanoparticles and more reliable absorption by fruit tissues. Secondly, OXA plays an important role on the maintenance and stabilization of cell integrity and on the reduction of the hydrolysis of cell wall components in the mesocarp (Valero and Serrano 2010). Recent studies reported that OXA postharvest treatments in asparagus preserved quality by reducing the rate of respiration during cold storage (Barberis *et al*., 2019). Also, OXA plays an important role in the defense mechanisms of various fruits against various stress conditions (Jin *et al*., 2014). There is accumulating evidence to suggest that the effectiveness of OXA treatment for mitigating damage from cold storage originates from the suppression of oxidative damage (Ding *et al*., 2007). These results are consistent with the previous reports on various fruits, i.e. apple, banana, litchi, and peach (Huang *et al*., 2013).

Table 1. The effect of nano-calcium coupled with oxalic acid at different concentration (0, 10, and 20 mM) on water loss percentage %, rachis browning index and rachis color hue angle of 'Superior Seedless' grapes during three days of the shelf-life.

Treatments	Shelf life duration in days			
	D0	D1	D2	D3
		Water loss %		
Control	0.00± 0.00 ^{i}	19.91 ± 0.043 ^{e}	24.76 ± 0.063 ^{b}	30.29 ± 0.003^{a}
CaNPs-$_{0\ mM}$ OXA	0.00± 0.00 ^{i}	15.92 ± 0.077 ^{g}	21.39 ± 0.047 ^{cd}	25.39 ± 0.047^{b}
CaNPs-$_{10\ mM}$ OXA	0.00± 0.00 ^{i}	14.12 ± 0.006 ^{h}	19.54 ± 0.071 ^{e}	22.16 ± 0.047^{c}
CaNPs-$_{20\ mM}$ OXA	0.00± 0.00 ^{i}	13.87 ± 0.081 ^{h}	17.58 ± 0.057 ^{f}	20.11 ± 0.017^{e}
		Rachis browning index(RB-index)		
Control	1.00 ± 0.00 ^{e}	1.07 ± 0.005 ^{e}	2.56 ± 0.577^{c}	4.81 ± 0.047 ^{a}
CaNPs-$_{0\ mM}$ OXA	1.00 ± 0.00 ^{e}	1.00 ± 0.000 ^{e}	1.59 ± 0.031^{de}	3.43 ± 0.071 ^{b}
CaNPs-$_{10\ mM}$ OXA	1.00 ± 0.00 ^{e}	1.00 ± 0.000 ^{e}	1.02 ± 0.005^{e}	2.33 ± 0.079 ^{cd}
CaNPs-$_{20\ mM}$ OXA	1.00 ± 0.00 ^{e}	1.00 ± 0.000 ^{e}	1.00 ± 0.001^{e}	1.02 ± 0.005 ^{e}
		Rachis color hue angle (h^{o})		
Control	118.36 ± 0.00 ^{a}	98.13 ± 0.037 ^{g}	87.29 ± 0.091^{j}	77.66 ± 0.017 ^{l}
CaNPs-$_{0\ mM}$ OXA	118.36 ± 0.00 ^{a}	109.35 ± 0.052 ^{e}	91.78 ± 0.101^{h}	85.09 ± 0.072 ^{k}
CaNPs-$_{10\ mM}$ OXA	118.36 ± 0.00 ^{a}	113.51 ± 0.039 ^{c}	107.41 ± 0.074^{f}	90.86 ± 0.054 ^{i}
CaNPs-$_{20\ mM}$ OXA	118.36 ± 0.00 ^{a}	116.22 ± 0.077 ^{b}	111.63 ± 0.035^{d}	98.62 ± 0.045 ^{g}

The data main of both seasons was analyzed using two way (Factorial design). Each value represents mean and ± SE (n=3) replicates. The superscript letters different (P<0.05) and represent the significantly between treatments mains using Tukey's-HSD Test.

Effect of CaNPs-OXA on chemical quality attributes of bunches

Table 2 depicts the change in the chemical analysis of bunches (berry quality) such as soluble solids contents (SSC%), total acidity (TA%), SSC: TA-ratio, and ascorbic acid content (AsA) affected with CaNPs-OXA treatments and the shelf-life duration in days. Clearly, the chemical quality elements show a significant interaction at P<0.05, when the shelf-life period and CaNPs-OXA applications were analyzed. The change in both SSC% and SSC:TA-ration increased significantly and rapidly with control treatment. On the other hand, the CaNPs-20 mM OXA treatment maintained both SSC% and SSC:TA-ration all over the shelf-life duration. Regards to the TA% and AsA content, they decreased throughout the shelf-life period. However, CaMPs-OXA treatments have maintained the

contents of TA and AsA compared with the control. In addition, the CaNPs-20 mM OXA treatment had higher TA and AsA content than fruit treated with other treatments. As the decreases were delayed by this treatment, which showed the better maintenance of acidity and ascorbic acid content. Contrariwise, the control bunches exhibited the minimum level of TA and AsA content on the 3rd day. The CaNPs-20 mM OXA significantly (P<0.05) maintained the chemical analysis of bunches (berry quality). Calcium causes a slowdown hydrolysis of polysaccharides to monosaccharides, besides slows down in the metabolism and respiration, it causes a delay in ripening and reduces the SSC% of fruits during storage (Ranjbar *et al*., 2018). Also, Ali *et al.* (2013) showed that Ca treatments delay the syndrome of postharvest senescence by reducing the change in compounds such as acids and sugars. In a similar context, Bhat *et al.* (2012) and Torres *et al.* (2009) showed that calcium applications on pear and atemoya fruits, respectively can delay changes in SSC. Also, preharvest nanocalcium treated sweet pepper had less SSC than control fruit through storage period (Amini *et al*., 2016). The decrease in TA and AsA content during shelf-life may be related to its consumption in the respiration process (Ghafir *et al*., 2009). Ranjbar *et al*. (2018) illustrated that nano-calcium treated fruits had the highest TA content compared to the untreated fruits where, Ca plays an important role in; delaying the ripening, reducing the respiratory rate, and reducing the production of ethylene. Calcium treated fruits exhibited more preservation of acids content during storage due to decreased softening rate, which affects on the glycolytic enzyme system (Torres *et al*., 2009). Similarly, Manganaris *et al*. (2005) and Ishaq *et al*. (2009) on peach and apricot, respectively, reported that the $CaCl_2$ application maintained acid contents during storage period. Besides, nano-calcium treatment on sweet pepper increased total acid content (Amini *et al*., 2016). The application of oxalic acid led to a significant increase in ascorbic acid after 84 days of cold storage by reducing the ascorbic acid oxidation (Kayashima and Katayama, 2002). OXA treatments showed a reduction in production of ethylene and it may be argued occur because of the inhibition of the activities of ethylene biosynthesis enzymes (Wang *et al*., 2009) which delay the ripening process (Valero *et al*., 2011) and therefore maintain the sugar and acid contents in the treated fruits.

Table 2. The effect of nano-calcium coupled with oxalic acid at different concentration (0, 10, and 20 mM) on the chemical quality attribute: soluble solids content (SSC%), total acidity (TA%), SSC: TA-ratio, and ascorbic acid content (AsA) of 'Superior Seedless' grapes during three days of the shelf-life.

Treatments	Shelf life duration in days			
	D0	D1	D2	D3
	Soluble solids contents (SSC%)			
Control	16.39 ± 0.022 ^{a}	16.54 ± 0.020 ^{d}	16.68 ± 0.012 ^{b}	16.89 ± 0.008 ^{a}
CaNPs-$_{0\ mM}$ OXA	16.39 ± 0.022 ^{a}	16.41 ± 0.005 ^{e}	16.53 ± 0.008 ^{d}	16.67 ± 0.015 ^{bc}
CaNPs-$_{10\ mM}$ OXA	16.39 ± 0.022 ^{a}	16.35 ± 0.012 ^{e}	16.41 ± 0.012 ^{e}	16.57 ± 0.012 ^{cd}
CaNPs-$_{20\ mM}$ OXA	16.39 ± 0.022 ^{a}	16.21 ± 0.106 ^{f}	16.34 ± 0.005 ^{e}	16.39 ± 0.008 ^{e}
	Total acidity (TA%)			
Control	0.784 ± 0.011 ^{a}	0.702 ± 0.054 ^{h}	0.662 ± 0.024 ^{i}	0.588 ± 0.030 ^{j}
CaNPs-$_{0\ mM}$ OXA	0.784 ± 0.011 ^{a}	0.763 ± 0.041 ^{d}	0.724 ± 0.016 ^{f}	0.704 ± 0.042 ^{h}
CaNPs-$_{10\ mM}$ OXA	0.784 ± 0.011 ^{a}	0.777 ± 0.060 ^{b}	0.730 ± 0.028 ^{e}	0.715 ± 0.026 ^{g}
CaNPs-$_{20\ mM}$ OXA	0.784 ± 0.011 ^{a}	0.782 ± 0.031 ^{a}	0.778 ± 0.051 ^{b}	0.767 ± 0.018 ^{c}
	SSC: TA-ratio			
Control	20.89 ± 0.013 ^{h}	23.55 ± 0.079 ^{c}	25.17 ± 0.082 ^{b}	28.70 ± 0.058 ^{a}
CaNPs-$_{0\ mM}$ OXA	20.89 ± 0.013 ^{h}	21.49 ± 0.061 ^{g}	22.82 ± 0.050 ^{e}	23.66 ± 0.102 ^{c}
CaNPs-$_{10\ mM}$ OXA	20.89 ± 0.013 ^{h}	21.04 ± 0.046 ^{h}	22.49 ± 0.032 ^{f}	23.18 ± 0.092 ^{d}
CaNPs-$_{20\ mM}$ OXA	20.89 ± 0.013 ^{h}	20.72 ± 0.175 ^{i}	20.99 ± 0.038 ^{h}	21.35 ± 0.034 ^{g}
	Ascorbic acid content (mg 100 g^{-1} FW) (AsA)			
Control	3.91 ± 0.037 ^{a}	2.63 ± 0.013 ^{e}	2.03 ± 0.011 ^{g}	1.79 ± 0.008 ^{h}
CaNPs-$_{0\ mM}$ OXA	3.91 ± 0.037 ^{a}	2.86 ± 0.085 ^{d}	2.52 ± 0.015 ^{e}	2.16 ± 0.021 ^{g}
CaNPs-$_{10\ mM}$ OXA	3.91 ± 0.037 ^{a}	3.44 ± 0.223 ^{b}	2.59 ± 0.008 ^{e}	2.35 ± 0.015 ^{f}
CaNPs-$_{20\ mM}$ OXA	3.91 ± 0.037 ^{a}	3.84 ± 0.020 ^{a}	3.72 ± 0.021 ^{a}	3.15 ± 0.029 ^{d}

The data main of both seasons was analyzed using two ways (Factorial design). Each value represents mean and ± SE (n=3) replicates. The superscript letters different (P<0.05) and represent the significantly between treatments mains using Tukey's-HSD Test.

Effect of CaNPs-OXA on cell wall degeneration enzymes activities

Table 3 shows the alteration in the activity of the enzymes of cell wall degradation as polygalacturonase (PG), xylanase (XYL), cellulase (CEL), and pectinase (PT) activities (U mg^{-1} protein), during the shelf-life duration in days for 'Superior Seedless' bunches treated with CaNPs-OXA treatments. The cell membrane degradation enzyme activities present a significant values at P<0.05 when the shelf-life period and CaNPs-OXA treatments were considered. Observably, PG activity increased during the experimental time up to 2nd day and then decreased slightly in the 3rd day. While, XYL and CEL activety increased sharply up to the end of duration. PT activety increased slightly throghout the shelf-life period. Clearly, the control treatment (untreated bunches) showed more rapid and sharp activety in all cell wall degradation enzyme as the shelf-life time was prolonged. Meanwhile, CaNPs-OXA treatments resulted in less activity in cell wall degradation enzymes up to the end of study. With further observation, the higher the concentration of OXA treatments, the lower the activity of these enzymes. Also, the results showed that treated bunches with CaNPs-OXA 20 mM had the lowest activety of cell wall degradation enzymes at the end of shelf-life duration. So, CaNPs-OXA 20 mM being considered the best treatment in terms of reduced the activity of cell wall degradation enzymes during the shelf-life period. The cell wall degradation enzymes play an important role in the hydrolysis of cell walls then the fruit softening (Prasanna *et al*., 2007). Tapre and Jain (2014)

showed that the breakdown of the important compound in many fruits, rhamnogalacturonan pectin, occurs by the polygalacturonase (PG) enzyme which, catalyzes the hydrolytic cleavage of α (1, 4) galacturonan bonds. Therefore, inhibition of the activity of the PG enzyme is important to maintain the strength of the cell wall and not to degrade it (Agusti *et al*., 2004). Zhi *et al* (2017) explened that the reduction in the activety of PG, CEL and other enzymes responsible for modifying cell wall structure can occur by doing the Ca treatment which delays the breakdown the polysaccharides of cell wall and maintaining its strength. Li *et al*. (2011) on Jujube fruits and Madani *et al*. (2014) on papaya fruits, showed that calcium treatment reduced PG enzyme activity due to higher calcium pectate in treated fruits. Another role of calcium, it binds with the negative charges on the cell wall that are created by the activity of the cell wall degradation enzymes thus decreases the expansion and the torn of cell, this way reduces the fruit softening process. Actually, Madani *et al*. (2014) showed that nano-calcium solution generates carboxyl groups by the reaction of pectin with Ca^{2+} ions, which form salt-bridge cross-links. There is a lot of evidence about the role of calcium in increasing the cell wall pectin characteristics and in maintaining integrity of cell wall by reducing the solubilization of uronic acids in the pectin and reducing the disassembly of cellulose-hemicellulose network (Tsantili *et al*. 2002; Zhi *et al*. 2017). Cellulase inhibition at later stage may be due to the influx of internal calcium and thus inhibiting the enzyme (Jawandh *et al*. 2009). Calcium ions would be maintaining pectins ionically-bound structure and middle lamella through an up-regulation of pectin methylesterase activity and its related gene expression, allowing a higher number of Ca_2^+ ⁻pectin bridges to be formed and, simultaneously down-regulating the activity and gene expression of well-known pectin degrading enzymes (Langer *et al*., 2019). In addition, the presence of OXA with CaNP mixture could play an important role in the systemic protective stress response, which is programmed in plant cells through cell death and redox homeostasis, as well as in the antisenescence impact, especially in fruits after harvesting (Wu *et al*., 2011).

Table 3. The effect of nano-calcium coupled with oxalic acid at different concentration (0, 10, and 20 mM) on the cell wall degradation enzymes activities: Polygalacturonase (PG), Xylanase (XYL), Cellulase (CEL), and Pectinase (PT) of 'Superior Seedless' grapes during three days of the shelf-life.

Treatments	Shelf life duration in days			
	D0	D1	D2	D3
	Polygalacturonase (PG unit mg^{-1} protein)			
Control	0.63 ± 0.005 ^{k}	0.75 ± 0.009 ^{e}	0.87 ± 0.003 ^{a}	0.79 ± 0.065 ^{d}
CaNPs-$_{0\ mM}$ OXA	0.63 ± 0.005 ^{k}	0.73 ± 0.013 ^{f}	0.83 ± 0.008 ^{b}	0.71 ± 0.023 ^{g}
CaNPs-$_{10\ mM}$ OXA	0.63 ± 0.005 ^{k}	0.71 ± 0.025 ^{g}	0.81 ± 0.005 ^{c}	0.68 ± 0.034 ^{h}
CaNPs-$_{20\ mM}$ OXA	0.63 ± 0.005 ^{k}	0.65 ± 0.016 ^{j}	0.67 ± 0.011 ^{i}	0.64 ± 0.008 ^{j}
	Xylanase (XYL; unit mg^{-1} protein)			
Control	4.19 ± 0.017 ^{h}	13.02 ± 0.337 ^{c}	16.91 ± 0.870 ^{b}	20.26 ± 0.197 ^{a}
CaNPs-$_{0\ mM}$ OXA	4.19 ± 0.017 ^{h}	10.44 ± 0.583 ^{d}	13.26 ± 0.560 ^{c}	16.54 ± 0.583 ^{b}
CaNPs-$_{10\ mM}$ OXA	4.19 ± 0.017 ^{h}	7.55 ± 0.565 ^{f}	8.75 ± 0.588 ^{e}	10.64 ± 0.268 ^{d}
CaNPs-$_{20\ mM}$ OXA	4.19 ± 0.017 ^{h}	4.93 ± 0.332 ^{h}	6.09 ± 0.315 ^{d}	6.91 ± 0.055 ^{fg}
	Cellulase (CEL; unit mg^{-1} protein)			
Control	3.98 ± 0.032 ^{i}	11.23 ± 0.028 ^{f}	16.93 ± 0.583 ^{c}	24.85 ± 0.594 ^{a}
CaNPs-$_{0\ mM}$ OXA	3.98 ± 0.032 ^{i}	10.71 ± 0.243 ^{f}	14.46 ± 0.521 ^{d}	20.43 ± 0.560 ^{b}
CaNPs-$_{10\ mM}$ OXA	3.98 ± 0.032 ^{i}	9.24 ± 0.265 ^{g}	12.56 ± 0.481 ^{e}	17. 58 ± 0.557 ^{c}
CaNPs-$_{20\ mM}$ OXA	3.98 ± 0.032 ^{i}	6.65 ± 0.588 ^{h}	9. 57 ± 0.583 ^{g}	11. 07 ± 0.586 ^{f}
	Pectinase (PT; unit mg^{-1} protein)			
Control	0.50 ± 0.005 ^{h}	0.72 ± 0.003 ^{e}	0.98 ± 0.066 ^{b}	1.14 ± 0.020 ^{a}
CaNPs-$_{0\ mM}$ OXA	0.50 ± 0.005 ^{h}	0.70 ± 0.006 ^{e}	0.90 ± 0.015 ^{c}	0.96 ± 0.018 ^{b}
CaNPs-$_{10\ mM}$ OXA	0.50 ± 0.005 ^{h}	0.65 ± 0.025 ^{f}	0.87 ± 0.032 ^{c}	0.90 ± 0.009 ^{c}
CaNPs-$_{20\ mM}$ OXA	0.50 ± 0.005 ^{h}	0.54 ± 0.041 ^{g}	0.70 ± 0.017 ^{e}	0.78 ± 0.005 ^{d}

The data main of both seasons was analyzed using two ways (Factorial design). Each value represents mean and ± SE (n=3) replicates. The superscript letters different (P<0.05) and represent the significantly between treatments mains using Tukey's-HSD Test.

Effect of CaNPs-OXA treatments on phenolic compounds and browning enzymes activities

Table 4 displays the variations in phenolic compounds (TP and FL) and browning enzymes activities (PAL and PPO) affected with CaNPs-OXA treatments and the shelf-life duration in days. Clearly, all parameters show a significant interaction at P<0.05, when the shelf-life period and CaNPs-OXA applications were analyzed. It was expected that the longer of the shelf-life period causes in rapidly decrease of TP and FL contents. With more observation, it was found that CaNPs-OXA treatments caused the highest TP and FL contents while, control had the lowest contents at the end of shelf-life time. On the other hand, the CaNPs-OXA 20 mM treatment maintained both elements all over the shelf-life duration. Concomitant with the decrease in TP and FL, an increment in PAL and PPO activities were observed. Where, CaNPs-OXA treatments had lower PAL and PPO activities compared to the control. In addition, the CaNPs-OXA 20 mM treatment had the lowest PAL and PPO activities than bunches treated with other treatments. CaNPs-OXA 20 mM treatment maintained the apperance of rachis and decreased the browning by lower losses of the phenolic compounds and decrese the activity of the browning enzymes. Where, calcium prevents the phenolic compounds oxidation by increasing the permeability of the membrane and contents of polysaccharide in the cell wall of fruit (Dunn and Able, 2006). It is also known that calcium reduces the rate of aging, which leads to less utilize of phenol (Lester and Grusak, 1999). Torres *et al*. (2009) proved that Ca reduces the activity of the PPO indirectly way. On the other side, the accumulation of TPC

have been promoted during storage by OXA treatments due to the activation of PAL, a key enzyme of the phenylpropanoid pathway. PPO activity inhibits by OXA treatments due to chelate copper from the active site of the enzyme since oxalic acid has a high affinity to form metal complexes with copper ion (Pourcel *et al*., 2007). These results were consistent with previous studies in pomegranate, mango and plum (Sayyari *et al*., 2010, Zheng *et al*., 2012 and Martínez-esplá *et al*., 2019). These responses suggested that the rachis browning occurrence is usually related to the consumption of the phenolic compounds as a substrate to PPO and PAL enzymes. Therefore, the decreases in the activity of PAL and PPO activities after bunches soaked in CaNPs-OXA 20 mM might indicate the limiting of losses of TP and FL during the storage period then less rachis browning.

Table 4. The effect of nano-calcium coupled with oxalic acid at different concentration (0, 10, and 20 mM) on phenolic compounds (total phenol; TP, and flavonoids; Fl) and browning enzymes activities (Polyphenol Oxidase; PPO, and Phenylalanine ammonia-lyase; PAL) of 'Superior Seedless' grapes during three days of the shelf-life.

Treatments	Shelf life duration in days			
	D0	D1	D2	D3
	Total phenol content (TP mg 100 g^{-1} FW)			
Control	38.96 ± 0.323 ^{a}	30.00 ± 0.361 ^{cd}	17.37 ± 6.876 ^{e}	18.76 ± 0.592 ^{e}
CaNPs-$_{0\ mM}$ OXA	38.96 ± 0.323 ^{a}	31.74 ± 0.364 ^{bcd}	27.34 ± 0.846 ^{d}	26.58 ± 0.571 ^{d}
CaNPs-$_{10\ mM}$ OXA	38.96 ± 0.323 ^{a}	34.16 ± 0.606 ^{dbc}	30.28 ± 0.311 ^{cd}	29.35 ± 0.600 ^{cd}
CaNPs-$_{20\ mM}$ OXA	38.96 ± 0.323 ^{a}	38.10 ± 0.984 ^{a}	36.85 ± 0.583 ^{ab}	35.94 ± 0.568 ^{ab}
	Flavonoid content (Fl mg 100 g^{-1} FW)			
Control	18.10 ± 0.302 ^{a}	15.66 ± 0.623 ^{cd}	11.74 ± 0.594 ^{f}	9.85 ± 0.568 ^{g}
CaNPs-$_{0\ mM}$ OXA	18.10 ± 0.302 ^{a}	16.70 ± 0.334 ^{bc}	15.60 ± 0.548 ^{d}	12.95 ± 0.588 ^{e}
CaNPs-$_{10\ mM}$ OXA	18.10 ± 0.302 ^{a}	17.84 ± 0.020 ^{a}	17.27 ± 0.290 ^{ab}	15.75 ± 0.603 ^{cd}
CaNPs-$_{20\ mM}$ OXA	18.10 ± 0.302 ^{a}	18.03 ± 0.035 ^{a}	17.90 ± 0.058 ^{a}	17.67 ± 0.024 ^{ab}
	Polyphenol Oxidase (PPO; unit mg^{-1}protein)			
Control	0.22 ± 0.005 ^{i}	0.27 ± 0.013 ^{e}	0.37 ± 0.008 ^{b}	0.45 ± 0.008 ^{a}
CaNPs-$_{0\ mM}$ OXA	0.22 ± 0.005 ^{i}	0.26 ± 0.009 ^{f}	0.34 ± 0.006 ^{c}	0.38 ± 0.025 ^{b}
CaNPs-$_{10\ mM}$ OXA	0.22 ± 0.005 ^{i}	0.24 ± 0.042 ^{gh}	0.31 ± 0.005 ^{d}	0.34 ± 0.036 ^{c}
CaNPs-$_{20\ mM}$ OXA	0.22 ± 0.005 ^{i}	0.22 ± 0.003 ^{i}	0.24 ± 0.011 ^{h}	0.25 ± 0.071 ^{g}
	Phenylalanine ammonia-lyase (PAL; unit mg^{-1} protein)			
Control	8.76 ± 0.137 ^{h}	16.37± 0.040 ^{ef}	24.45± 0.589 ^{c}	38.75± 0.594 ^{a}
CaNPs-$_{0\ mM}$ OXA	8.76 ± 0.137 ^{h}	16.03± 0.031 ^{ef}	20.99± 0.318 ^{d}	30.88± 0.589 ^{b}
CaNPs-$_{10\ mM}$ OXA	8.76 ± 0.137 ^{h}	14.44± 0.361 ^{f}	17.21± 1.955 ^{e}	24.28± 0.595 ^{c}
CaNPs-$_{20\ mM}$ OXA	8.76 ± 0.137 ^{h}	11.15± 0.603 ^{g}	11.16± 0.755 ^{g}	12.58± 0.855 ^{g}

The data main of both seasons was analyzed using two ways (Factorial design). Each value represents mean and ± SE (n=3) replicates. The superscript letters different (P<0.05) and represent the significantly between treatments mains using Tukey's-HSD Test.

Effect of CaNPs-OXA treatments on lipid peroxidation (malondialdehyde; MDA), lipoxygenase (LOX), and rachis electrolyte leakage percentage (EL%)

Table 5 illustrates the change accumulation of lipid peroxidation (malondialdehyde; MDA), lipoxygenase (LOX) activity, and rachis electrolyte leakage percentage (EL%) during the experimental duration in days. Clearly, the CaNPs-OXA treated bunches exhibited a significantly (P<0.005) lower level of MDA, LOX and EL% by the end of storage compared to the control bunches.

Table 5. The effect of nano-calcium coupled with oxalic acid at different concentration (0, 10, and 20 mM) on lipid peroxidation accumulation (malondialdehyde; MDA), lipoxygenase enzyme activity (LOX), and rachis electrolyte leakage percentage of 'Superior Seedless' grapes during three days of the shelf-life.

Treatments	Shelf life duration in days			
	D0	D1	D2	D3
	Lipid peroxidation (malondialdehyde; MDA mM g^{-1} FW)			
Control	0.13 ± 0.005 ^{k}	0.26 ± 0.010 ^{f}	0.34 ± 0.005 ^{c}	0.45 ± 0.060 ^{a}
CaNPs-$_{0\ mM}$ OXA	0.13 ± 0.005 ^{k}	0.24 ± 0.008 ^{g}	0.29 ± 0.022 ^{e}	0.38 ± 0.011 ^{b}
CaNPs-$_{10\ mM}$ OXA	0.13 ± 0.005 ^{k}	0.20 ± 0.021 ^{h}	0.24 ± 0.071 ^{g}	0.30 ± 0.056 ^{d}
CaNPs-$_{20\ mM}$ OXA	0.13 ± 0.005 ^{k}	0.16 ± 0.041 ^{j}	0.19 ± 0.021 ^{i}	0.20 ± 0.015 ^{h}
	Lipoxygenase activity (LOX, Unit mg^{-1} protein)			
Control	0.50 ± 0.005 ^{f}	0.62 ± 0.005 ^{bc}	0.63 ± 0.018 ^{ab}	0.65 ± 0.045 ^{a}
CaNPs-$_{0\ mM}$ OXA	0.50 ± 0.005 ^{f}	0.59 ± 0.008 ^{d}	0.60 ± 0.035 ^{cd}	0.61 ± 0.023 ^{cd}
CaNPs-$_{10\ mM}$ OXA	0.50 ± 0.005 ^{f}	0.55 ± 0.012 ^{e}	0.56 ± 0.042 ^{e}	0.59 ± 0.021 ^{d}
CaNPs-$_{20\ mM}$ OXA	0.50 ± 0.005 ^{f}	0.51 ± 0.005 ^{f}	0.54 ± 0.011 ^{e}	0.54 ± 0.062 ^{e}
	Rachis electrolyte leakage %			
Control	7.14 ± 0.020 ^{l}	20.79 ± 0.294 ^{g}	29.96 ± 0.324 ^{d}	37.67 ± 0.288 ^{a}
CaNPs-$_{0\ mM}$ OXA	7.14 ± 0.020 ^{l}	19.52 ± 0.357 ^{h}	28.24 ± 0.413 ^{e}	36.06 ± 0.320 ^{b}
CaNPs-$_{10\ mM}$ OXA	7.14 ± 0.020 ^{l}	16.64 ± 0.571 ^{i}	21.54 ± 0.583 ^{f}	30.92 ± 0.571 ^{c}
CaNPs-$_{20\ mM}$ OXA	7.14 ± 0.020 ^{l}	10.74 ± 0.591 ^{k}	14.65 ± 0.521 ^{j}	19.54 ± 0.568 ^{h}

The data main of both seasons was analyzed using two ways (Factorial design). Each value represents mean and ± SE (n=3) replicates. The superscript letters different (P<0.05) and represent the significantly between treatments mains using Tukey's-HSD Test.

More observably, the behavior of soaked bunches in CaMPs-OXA 20mM treatment was more stable. Which means that this treatment reduces the degree of cell breakdown, maintains the quality and prolongs the storage

period, giving more protection to Superior Seedless bunches during shelf-life duration. Tian *et al*. (2013) showed that reactive oxygen species increase during the process of fruits ripening due to increase oxidative metabolism, this can increase the aging rate and cause cell membranes damages. In fact, Ca regulates the activity of target enzymes by inducing the active conformation, such as phosphodiesterase GAD, Ca^{2+}-ATPase and PDE (Ranty *et al*., 2006), reduces the ROS, decreases membrane lipid peroxidation damage by regulating its own concentration and stabilizes the basic structure of the lipid layer, which plays a vital role in maintaining the integrity of the cell membrane (Snedden and Fromm, 2010). Likewise, Mirdehghan and Ghotbi (2014) observed an increase in the antioxidant activities of pomegranate fruits by Ca treatments. Also, calcium applications on persimmon fruit could preserve the rate of total antioxidant activity at the highest level during cold storage (Bagheri *et al*., 2015). On other side, the differences in MDA accumulation and LOX activity could be described by the fact that OXA minimizes the lipid peroxidation process and improves the antioxidant activity during the shelf-life duration (Zhang and Tian, 2006 and Huang *et al*., 2013).

Multivariate analysis of 'Superior Seedless' parameters

A PCA for all parameters data collected from bunches during shelf life was carried out to test different CaNPs-OXA treatments (CaNPs-OXA 0 mM, CaNPs-OXA 10 mM, and CaNPs-OXA 20 mM) applied to 'Superior Seedless' grapes. The PCA separated the effects of CaNPs coupled with OXA at different concentrations during shelf life. PC1 explained 79.3 % of the variability in the data, while PC2 explained 9.77 % of the variability (Fig. 4A).

Figure 4B shows the positive correlation between RB-index with all the variables except hue angle, TA%, AsA content %, and phenolic compounds (TP and FL).

The enzymatic browning (PPO and PAL), and cellular metabolism enzyme activities (PG, CEL, PT, and XYL) were positively correlated with the RB-Index. Also, three valuables (MDA, IL%, and LOX) had a positive correlation with the RB-Index. Pearson's correlation coefficient among the studied parameters shows the correlation and indicates these results (Table 6).

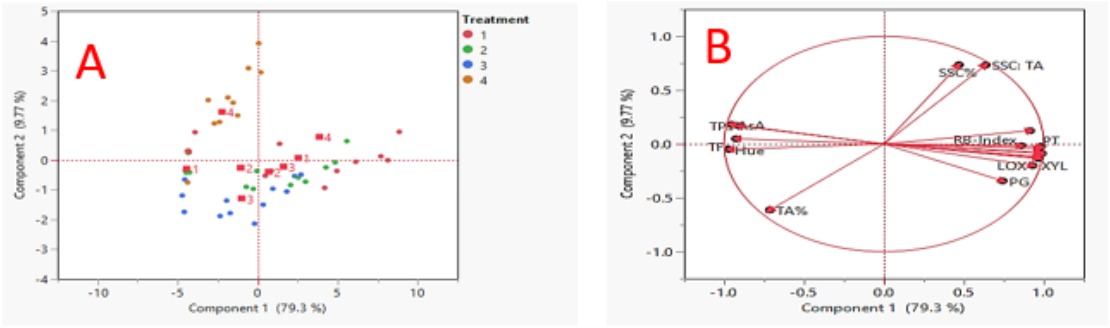


Figure 4. Principal component analysis (PCA) representing shelf-life duration in days and four treatment for Superior Seedless grapes, plotted with the contribution of each parameter on the four PCA axes (A) and all of the physiological and biochemical parameters measured in bunches during the shelf-life period (B). Principal component analysis (PCA) variable correlation.

Table 6. Pearson's correlation coefficient among the studied parameters of 'Superior Seedless' grape treated with CaNPs-OXA during shelf life.

	WL %	RB-Index	hº	SSC %	TA %	SSC: TA	AsA	TP	FL	PPO	PAL	PG	CEL	PT	XYL	MDA	EL %	LOX
WL%	*1.0000																	
RB-Index	0.6695	1.0000																
hº	-0.8708	-0.8349	1.0000															
SSC%	0.4273	0.4035	-0.4825	1.0000														
TA%	-0.8044	-0.5126	0.7127	-0.6706	1.0000													
SSC: TAttS SC:TA	0.6905	0.5118	-0.6525	0.7641	-0.9362	1.0000												
AsA	-0.8702	-0.7581	0.9334	-0.3208	0.5808	-0.4711	1.0000											
TP	-0.7767	-0.7453	0.8485	-0.3481	0.5421	-0.4746	0.8838	1.0000										
FL	-0.7286	-0.9092	0.8870	-0.4648	0.5725	-0.5466	0.8492	0.8971	1.0000									
PPO	0.8337	0.9057	-0.9378	0.3709	-0.5769	0.5070	-0.9313	-0.8919	-0.9228	1.0000								
PAL	0.8324	0.9351	-0.9211	0.3681	-0.5681	0.5033	-0.9095	-0.8498	-0.9184	0.9742	1.0000							
PG	0.6918	0.4266	-0.6585	0.1594	-0.3892	0.2491	-0.7928	-0.8126	-0.6631	0.7272	0.6316	1.0000						
CEL	0.9238	0.8708	-0.9529	0.4063	-0.6821	0.6030	-0.9397	-0.8547	-0.8810	0.9623	0.9723	0.6614	1.0000					
PT	0.9288	0.8006	-0.9367	0.3827	-0.6837	0.5999	-0.9508	-0.8609	-0.8333	0.9478	0.9207	0.7477	0.9699	1.0000				
XYL	0.8270	0.8328	-0.9287	0.4016	-0.6027	0.5126	-0.9399	-0.8859	-0.9405	0.9352	0.9356	0.7757	0.9265	0.9025	1.0000			
MDA	0.8850	0.8864	-0.9530	0.3912	-0.6453	0.5557	-0.9522	-0.8845	-0.9227	0.9736	0.9806	0.7125	0.9836	0.9520	0.9719	1.0000		
EL%	0.9214	0.8171	-0.9611	0.3646	-0.6569	0.5531	-0.9631	-0.8498	-0.8480	0.9480	0.9493	0.6999	0.9847	0.9678	0.9303	0.9744	1.0000	
LOX	0.8528	0.7059	-0.8859	0.2965	-0.5757	0.4554	-0.9497	-0.8931	-0.8483	0.8786	0.8707	0.8275	0.8952	0.8887	0.9464	0.9318	0.9083	1.0000

Values express average values per shelf-life duration in days (three days) of Superior Seedless grape. WL%—water loss percentage; RB index—rachis browning index; hº—berry color hue angle; SSC%—total soluble solids; TA%—total acidity ; SSC:TA ratio between SSC and TA; AsA—ascorbic acid content; TP—total phenol content; FL—flavonoid content; PPO—polyphenol oxidase; PAL—phenylalanine ammonia-lyase; PG—polygalacturonase; CEL—cellulase; PT—pectinase; XYL—xylanase; MDA—malondialdehyde accumulation; EL%—electrolyte leakage percentage;LOX—lipooxegenase .

CONCLUSION

By observing the behavior of 'Superior Seedless' grapes, treated with solutions of nano-calcium coupled with different concentrations of oxalic acid during shelf-life, it is noted that the nano-form of calcium chloride mixed with oxalic acid at a concentration of 20 mM gives the best result for reducing the symptoms of rachis browning during the shelf-life. Where, it minimizes cell wall degradation enzymes activities. Also, it reduces lipid peroxidation process (a lack of accumulation of MDA, LOX inactivity and less rachis electrolyte leakage percentage). Besides, less oxidation of the phenolic compounds by less PPO and PAL activity means less rachis browning (Figure 5), which reflects on rachis and berry color quality.

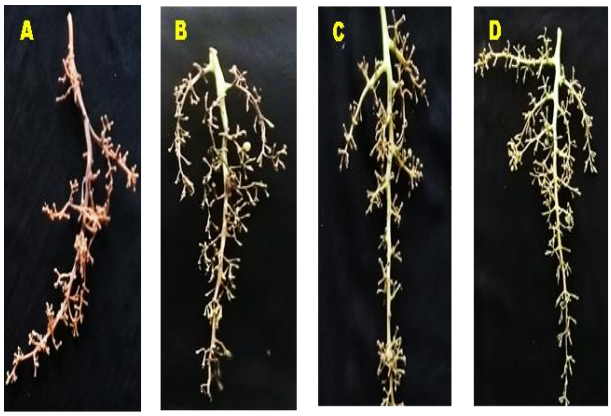


Figure 5. Effect of CaNPs-OXA treatments and control on browning incidence on the 3rd day of the shelf life. The control treatment (A); CaNPs-OXA 0 mM (B); CaNPs-OXA 10 mM (C); and CaNPs-OXA 20 mM (D)

REFERENCES

Agusti, M.; Juan, M.; Martinez-fuentes, A.; Mesejo, C.; Almela, V. (2004). Calcium nitrate delays climacteric of persimmon fruit. J. Appl. Biol. Res., 144: 65–69.

Ali, S.; Masud, T.; Abbasi, K.S.; Mahmood, T.; Abbasi, S.; Ali, A. (2013). Influence of $CaCl_2$ on physico-chemical, sensory and microbial quality of Apricot cv. Habi at ambient storage. J. chem. biol. phys. Sci., 3: 2744–2758.

Amini, F.; Bayat, L.; Hosseinkhani, S. (2016). Influence of preharvest nano- calcium applications on postharvest of sweet pepper (*Capsicum annum*). Nusantara Bioscie.,8: 215–220.

AOAC. (1995). Association of Official of Analytical Chemist (15th ed.), AOAC, Washington, USA.

Artés-hernández, F.; Tomás-barberán, F.A.; Artés, F. (2006). Modified atmosphere packaging preserves quality of SO_2-free 'Superior Seedless' table grapes. Postharvest Biol. Technol., 39: 146-154.

Bagheri, M.; Esna-ashari, M.; Ershadi, A. (2015). Effect of postharvest calcium chloride treatment on the storage life and quality of persimmon fruit (*Diospyros kaki Thunb. cv.* Karaj). Int J Hortic Sci Technol, 2: 15–26.

Barberis, A.; Cefola, M.; Pace, B.; Azara, E.; Spissu, Y.; Serra, P. A.; Logrieco, A. F.; D'hallewin, G.; Fadda, A. (2019). Postharvest application of oxalic acid to preserve overall appearance and nutritional quality of fresh-cut green and purple asparagus during cold storage: A combined electrochemical and mass-spectrometry analysis approach. Postharvest Biol. Technol., 148: 158–167.

Bhat, M. Y.; Ahsan, H.; Banday, F. A.; Dar, M. A.; Wani, A. I.; Hassan, G. I. (2012). Effect of harvest dates, pre harvest calcium sprays and storage period on physico-chemical characteristics of pear CV. Bartlett. J. Agric. Res. Develop., 2: 101–106.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 248–254.

Champa, W. A. H.; Gill, M. I. S.; Mahajan, B. V. C.; Arora, N. K. (2015). Preharvest salicylic acid treatments to improve quality and postharvest life of table grapes (*Vitis vinifera* L.) cv. Flame Seedless. J. Food Sci. Technol., 52: 3607–3616.

Collmer, A.; Ried, J. L.; Mount, M. S. (1988). Assay methods for pectic enzymes. Methods Enzymol., 161: 329–335.

Crisosto, C. H.; Corzo, P.; Palou, L.; Mitchel, F. G.(2001). Table grape packaging Influences 'Flame Seedless' and 'Red Globe' storage quality. Center Vally Postharvest Newsletter, 1–10.

Ding, Z.; Tian, S.; Zheng, X.; Zhou, Z. W.; Xu, Y. (2007). Responses of reactive oxygen metabolism and quality in mango fruit to exogenous oxalic acid or salicylic acid under chilling temperature stress. Physiol. Plant., 130: 112–121.

Dunn, A. and Able, J. (2006). Preharvest calcium effects on sensory quality and calcium mobility in strawberry fruit. Acta Hortic., 708: 307–312.

FAOSTAT. (2018). Source FAOSTAT .

Ghafir, S. A. M.; Gadalla, S. O.; Murajei, B. N.; El-Nady, M. F. (2009). Physiological and anatomical comparison between four different apple cultivars under storage conditions. Afr. J. Plant Sci., 3: 133–138.

Hoff, J. E. and Singleton, K. I. (1977). A method for determination of tannin in foods by means of immobilized enzymes. J. Food Sci., 42: 1566–1569.

Huang, H.; Zhu, Q.; Zhang, Z.; Yang, B.; Duan, X.; Jiang, Y. (2013). Effect of oxalic acid on antibrowning of banana (*Musa spp*., AAA group, cv. 'Brazil') fruit during storage. Sci. Hortic., 160: 208–212.

Iturbe-ormaetxe, I.; Escuredo, P. R.; Arrese-igor, C.; Becana, M. (1998). Oxidative damage in pea plants exposed to water deficit or paraquat. Plant Physiol., 116: 173–181.

Ishaq, S.; Rathore, H. A.; Masud, T.; Ali, S. (2009). Influence of postharvest calcium chloride application, ethylene absorbent and modified atmosphere on quality characteristics and shelf life of apricot (*Prunus armeniaca* L.) fruit during storage. Pak. J. Nutr., 8: 861–865.

Jawandh, S. K.; Mahajan B. V. C.; Gill, P. S. (2009). Effect of pre-harvest treatments on cellulase activity and quality of pear fruits under cold storage conditions. Notulae Sci. Biol., 1:88–91.

Jiang, Y.; Joyce, D. C.; Jiang, W.; Lu, W. (2004). Effects of chilling temperatures on ethylene binding by banana fruit. Plant Growth Regul., 43: 109–115.

Jin, P.; Zhu, H.; Wang, L.; Shan, T.; Zheng, Y. (2014). Oxalic acid alleviates chilling injury in peach fruit by regulating energy metabolism and fatty acid contents. Food Chem., 161: 87–93.

Kayashima, T. and Katayama, T. (2002). Oxalic acid is available as a natural antioxidantin some systems. Biochim. Biophys. Acta, 1573: 1–3.

Ke, D. and Saltveit, M. E. (1986). Effects of calcium and auxin on russet spotting and phenylalanine ammonia-lyase activity in iceberg lettuce. HortScience, 21: 1169–1171.

Koyama, R.; Silva borges, W. F.; Colombo, R. C.; Hussain, I.; De souza, R. T.; Roberto, S. R. (2020). Phenology and yield of the hybrid seedless grape 'BRS Melodia' grown in an annual double cropping system in a subtropical area. *Horticulturae, 6*, 3.

Khojastehnazhand, M.; Omid, M.; Tabatabaeefar, A. (2010). Development of a lemon sorting system based on color and size. Afric. J. Plant Sci., 4: 122-127.

Langer, S. E.; Marina, M.; Burgos, J .L.; Martínez, G. A.; Civello, P. M.; Villarreala, N. M. (2019). Calcium chloride treatment modifies cell wall metabolism and activates defense responses in strawberry fruit (*Fragaria* × *ananassa, Duch*). J. Sci. Food Agric., 99: 4003-4010.

Lee, R. C.; Hrmova, M.; Burton, R. A.; Lahnstein, J.; Fincher, G.B. (2003). Bifunctional family 3 glycoside hydrolases from barley with a-L-arabinofuranosidase and b-D-xylosidase activity. J. Biol. Chem., 278: 5377–5387.

Lester, G.E. and Grusak, M.A. (1999). Postharvest application of calcium and magnesium to honeydew and netted muskmelons: effects on tissue ion concentration, quality, and senescence. J. Am. Soc. Hortic. Sci., 124: 545–552.

Li, L.; Ban, Z.; Li, X.; Xue, T. (2011). Effect of 1-methylcyclopropene and calcium chloride treatments on quality maintenance of '*Lingwu Long*' Jujube fruit. J. Food Sci. Technol., 1–7.

Lichter, A.; Kaplunov, T.; Zutahy, Y.; Daus, A.; Alchanatis, V.; Ostrovsky, V. (2011). Physical and visual properties of grape rachis as affected by water vapor pressure deficit. Postharvest Biol. Technol., 59: 25–33.

Madani, B.; Mohamed, M. T. M.; Watkins, C. B.; Kadir, J.; Awang, Y.; Shojaei, T. R. (2014). Preharvest calcium chloride sprays affect ripening of 'Eksotika' papaya fruit during cold storage. Sci. Hortic., 171: 6–13.

Mahajan, B. V. C. and Dhatt, A. S. (2004). Studies on postharvest calcium chloride application on storage behaviour and quality of Asian pear during cold storage. Int. J. Agric. Environ. Food Sci., 2: 157–159.

Manganaris, G.A.; Vasilakis, M.; Migani, I.; Diamantidis, G.; Tzavella-klonari, K. (2005). The effect of preharvest calcium sprays on quality attributes, physicochimical aspects of cell wall components and suseptibility to brown rot of peach fruit (*Prunus persica* L. cv. Andross). Sci. Hortic., 107: 43–50.

Martínez-esplá, A.; Serrano, M.; Martínez-romero, D.; Valero, D.; Zapata, P. J. (2019). Oxalic acid preharvest treatment increases antioxidant systems and improves plum quality at harvest and during postharvest storage. J. Sci. Food Agric., 99: 235–243.

Menora, N. D.; Veena, J.; Vinod, K..; Vijaya, D.; Debnath, M. K.; Santosh, P.; Padmavathamma, A. S.; Variath, M. T.; Somshekhar, B.; Santosh, K. (2015). Influence of rootstock on bud break, period of anthesis, fruit set, fruit ripening, heat unit requirement and berry yield of commercial grape varieties. Int. J. Plant Breed. Genet., 9: 126–135.

Ministry of Agriculyure (2015). Agriculture Directorates of Governorates. 1-450.

Mirdehghan, S.H. and Ghotbi, F. (2014). Effects of salicylic acid, jasmonic acid, and calcium chloride, on reducing chilling injury of pomegranate (*Punica granatum* L.) Fruit. J. Agric. Sci. Technol., 16 (1): 163–173.

Moon, K. M.; Kwon, E. B.; Lee, B.; Kim,C. Y. (2020). Recent trends in controlling the enzymatic browning of fruit and vegetable products. Molecules, 25: 2754.

Payasi, A. and Sanwal, G. G. (2003). Pectate lyase activity during ripening of banana fruit. Phytochemistry, 63: 243-248.

Pinto, M. D. C.; Tejeda, A.; Duque, A. L.; Mac´ıas, P. (2007). Determination of lipoxygenase activity in plant extracts using a modified ferrous oxidation−xylenol orange assay. J. Agric. Food Chem., 55: 5956-5959.

Pourcel, L.; Routaboul, J. M.; Cheynier, V.; Lepiniec, L.; Debeaujon, I. (2007). Flavonoid oxidation in plants: from biochemical properties to physiological functions. Trends Plant Sci., 12: 29–36.

Prasanna, V.; Prabha, T. N.; Tharanathan, R.N. (2007). Fruit ripening phenomena: an overview. Crit. Rev. Food Sci. Nutr., 47: 1–19.

Ranjbaran, E.; Sarikhani, H.; Bakhshi, D.; Pouya, M. (2011). Investigation of salicylic acid application to reduce postharvest losses in stored 'Bidaneh Ghermez' table grapes. Int. J. Fruit Sci., 11: 430–439.

Ranjbar, S.; Rahemi, M.; Ramezanian A. (2018). Comparison of nano-calcium and calcium chloride spray on postharvest quality and cell wall enzymes activity in apple cv. Red Delicious. Sci. Hortic., 240, 57-64.

Ranty, B.; Aldon, D.; Galaud, J. P. (2006). Plant calmodulins and calmodulin-related proteins: multifaceted relays to decode calcium signals. Plant Signal. Behav., 1 (3): 96–104.

Sayyari, M.; Valero, D.; Babalar, M.; Kalantari, S.; Zapata, P. J.; Serrano, M. (2010). Prestorage oxalic acid treatment maintained visual quality, bioactive compounds, and antioxidant potential of pomegranate after long-term storage at 2 °C. J. Agric. Food Chem., 58: 6804–6808.

Snedden, W.A. and Fromm, H. (2010). Calmodulin as a versatile calcium signal transducer in plants. New Phytol., 151 (1): 35–66.

Soheyla, H. and Foruhe, Z. (2013). Effect of zeta potential on the properties of nano-drug delivery systems - a review (Part 2). Trop. J. Pharm. Res., 12: 265–273.

Tapre, A. R. and Jain, R.K. (2014). Pectinases: enzymes for fruit processing industry. Int. Food Res. J., 21(2): 447–453

Tian, S.; Quin, G.; Li, B. (2013). Reactive oxygen species involved in regulating fruit senescence and fungal pathogenicity. Plant Mol. Biol.,82 (6): 593–602.

Torres, L. M. A. R.; Silva, M. A.; Guaglianoni, D. G.; Neves, V. A. (2009). Effects of heat treatment and calcium on postharvest storage of atemoya fruits. Alim. Nutr., Araraquara, 20: 359–368.

Tsantili, E.; Konstantinidis, K.; Athanasopoulos, P. E.; Pontikis, C. (2002). Effects of post-harvest calcium treatments on respiration and quality attributes in lemon fruit during storage. J. Hortic. Sci. Biotechnol., 77: 479–484.

Valero, D. and Serrano, M. (2010). Postharvest Biology and Technology for Preserving Fruit Quality, 1st ed.; CRC Press: Boca Raton, FL, USA.

Valero, D.; Diaz-mula, H.M.; Zapata, P. J.; Casyillo, S.; Guillen, F.; Martinez, R. D.; Serrano, M. (2011).. 'Postharvest treatments with salicylic acid, acetyl salicylic acid or oxalic acid delayed ripening and enhanced bioactive compounds and antioxidant capacity in sweet cherry'. J. Agric. Food Chem., 59 (10): 5483–5489.

Wang, Q.; Lai, T.; Qin, G.; Tian, S. (2009). Response of Jujube fruits to exogenous oxalic acid treatment based on proteomic analysis. Plant Cell Physiol., 50 (2): 230–242.

White, P.J. and Broadley, M.R. (2003). Calcium in plants. Ann. Bot., 92: 487–511.

Wu, F.; Zhang, D.; Zhang, H.; Jiang, G.; Su, X.; Qu, H.; Jiang, Y.; Duan, X. (2011). Physiological and biochemical response of harvested plum fruit to oxalic acid during ripening or shelf-life. Food Res. Int., 44: 1299–1305.

Xua, M.; Dongb, J.; Zhanga, M.; Xua, X.; Sunb, L. (2012). Cold-induced endogenous nitric oxide generation plays a role in chilling tolerance of loquat fruit during postharvest storage. Postharvest Biol. Technol., 65: 5–12.

Yoruk, R. and Marshall, M.R. (2003). A Survey on the potential mode of inhibition for Oxalic acid on polyphenol oxidase. J. Food Sci., 68: 2479-2485.

Yugandhar, P. and Savithramma, N. (2013). Green synthesis of calcium carbonate nanoparticles and their effects on seed germination and seedling growth of *Vigna mungo* (L.). Hepper. Int. j. adv. res., 1(8): 89–103.

Zheng, X. L.; Tian, S. P.; Gidley, M. J.; Yue, H.; Li, B. Q. (2012). Effects of exogenous oxalic acid on ripening and decay incidence in mango fruit during storage at room temperature. Postharvest Biol. Technol., 45: 281–284.

Zhang, X. and Tian, S. (2006). Effect of oxalic acid on control of postharvest browning of litchi fruit. Food Chem., 96: 519–523.

Zhi, H. H.; Liu, Q. Q.; Dong, Y.; Liu, M. P.; Zong, W. (2017). Effect of calcium dissolved in slightly acidic electrolyzed water on antioxidant system, calcium distribution, and cell wall metabolism of peach in relation to fruit browning. J. Hortic. Sci. Biotechnol. 92(6): 621–629.

Zhishen, J.; Mengcheng, T.; Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem., 64: 555-559.

تأثير جزيئات النانوكالسيوم الممزوجة بحمض الأكساليك في تقليل حدوث التلون البنى على عناقيد العنب السبريوروعلاقته بالمركبات الفينولية خلال فترة التداول

أسماء سعيد مصطفى عمر[1] و محمد سعد أحمد أبورية [2]

[1]قسم الفاكهة ـ كلية الزراعة- جامعة المنصورة

[2]قسم الفاكهة ـ كلية الزراعة- جامعة دمياط

أجريت هذه التجربة خلال موسمين متتاليين (2020 و 2021) على عناقيد عنب السبريور وكان الغرض من هذة الدراسة هو تقليل حدوث ظاهرة التلون البنى بعناقيد العنب لهذا الصنف وذلك بإستخدام خليط من جزيئات النانوكالسيوم الممزوجة بحمض الاوكساليك بتركيزات مختلفة: CaNPs-OX $_{0\ mM}$ ، CaNPs-OX $_{10\ mM}$، CaNPs-OX $_{20\ mM}$ وذلك خلال ثلاثة ايام اثناء فترة التداول على درجة حرارة 27±1C° ورطوبة نسبية 2% ± 46. تم اخذ العينات بصورة يومية لدراسة محددات الجودة الفيزيائية والكيميائية. أظهرت النتائج أن التركيز CaNPs-OXA 20 ملي مولار حافظ على نضارة العناقيد من خلال تقليل معدل فقد الماء ، وتقليل ظهور التلون البنى والحفاظ على زاوية الصبغة. كما أظهرت النتائج ان نفس المعاملة حافظت على محتوى المواد الصلبة الذائبة و نسبة المواد الصلبة الذائبة/ الحموضة، بينما أخرت إنخفاض محتوى الحموضة الكلية و محتوى حمض الأسكوربيك. إلى جانب ذلك، لوحظ انخفاض ملحوظ في أنشطة إنزيمات تدهور الجدر الخلوية مثل polygalacturonase (PG) ، و cellulase (CEL) ، و xylanase (XYL) ، و.pectinase (PT) . أيضًا زاد محتوي الفينول الكلي (TP) والفلافونويد (FL) وانخفض نشاط كل من إنزيمات اللون البني ، polyphenol oxidase (PPO)، phenylalanine ammonia-lyase (PAL). أيضًا قللت االمعاملة CaNPs-OXA 20 تراكم malondialdehyde (MDA) ونشاط lipoxygenase (LOX) ونسبة التسرب الكهربائى٪ . باختصار، أثبتت المعاملة CaNPs-OXA 20 أنها علاج فعال للحفاظ على جودة عناقيد العنب السبريور خلال فترة التداول.