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Calcium salts as an alternative to preserve minimally processed table grape quality

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Abstract

The effects of calcium lactate, propionate, chloride, ascorbate, and propionate (5 g L^{-1}) combined with heat treatment (60 °C, 2 min), on the quality parameter of minimally processed table grapes, stored for 21 days at 5 °C, were investigated. Berries sanitised with NaOCl (100 mg L^{-1}) were used as control. The respiration rate of Ca-treated berries remained below that of the control up to 17 days. No differences were found on the atmosphere composition. Firmness was maintained on berries treated with Ca until 14 days of storage, especially on the propionate and ascorbate treatments. The bound, free, and total Ca measured values were higher on Ca-treated berries. The functional quality did not show differences among the treatments. The Ca treatment combined with heat treatment, especially with the addition of ascorbate and propionate, maintained the firmness of the berries but did not show major effects on the other quality parameters evaluated.

Keywords: heat treatment, firmness, bound Ca, functional properties, *Vitis vinifera*

Introduction

Grapes (*Vitis vinifera* L.) are extensively cultivated worldwide, and its annual output has reached 74 million tons (FAO, 2019). Grapes are a non-climacteric fruit with a relatively low metabolic activity that can be associated with long storage periods. However, during this period, physiological disorders can occur, limiting the postharvest storage capacity. Rachis browning and subsequent berry shatter is one of the main postharvest problems, which consequently cause significant economic losses, mainly in susceptible varieties (Mirdehghan & Rahimi, 2016; Zhang et al., 2019a). Thus, the development of minimally processed products using grapes as raw materials can be an alternative to reduce losses and waste, especially considering that processing involves rachis elimination.

One of the processes that limit the useful life of whole and minimally processed fruits is the loss of firmness or softening. It occurs as a consequence of the maturation progress and is associated with the dismantling and degradation of the cell wall as a result of enzymatic and non-enzymatic transformations that mainly affect the pectin (Liu et al., 2009; Silveira et al., 2011; Hocking et al., 2016). In minimally processed products, the unit operations carried out during the processing generates a stress situation that accelerates maturation. As a result, events, such as loss of firmness, can increase (Oms-Oliu et al., 2010).

In vegetable tissues, calcium (Ca^{2+}) exists in two forms: Ca linked to negatively charged carboxyl groups of polygalacturonic acids from the mid-lamella pectin and Ca linked to negatively charged head groups of plasma membrane phospholipids and proteins, as free Ca that remain unbound in the solution (Ngamcheuachit et al., 2014).

Firmness loss is associated with the levels of Ca present in the tissues, because it is responsible for

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the formation of bridges with the antiparallel chains of homogalactans of the pectin with negatively charged carboxyl groups, forming the structures called 'eggboxes'. In this way, cell integrity and cell cohesion are maintained (Lionetti et al., 2010; Liu et al., 2017).

Calcium applications have been used to stabilise cell membranes through the formation of Ca pectates, thus increasing the rigidity of the cells and making them less accessible to enzymatic attack (Lui et al., 2017).

Furthermore, free Ca present in the cytosol directly influences the regulation of the expression and synthesis of enzymes and proteins involved in the maintenance of membrane integrity and the reduction of active oxygen species production, thus reducing the consumption of intracellular antioxidants and maintaining their quantities (Wang, 2014). In this sense, Zhi et al. (2017) reported that a $Ca(NO_3)_2$ treatment reduces the production of active oxygen species and malondialdehyde production, considered a marker for measuring membrane degradation, and increases the levels of enzymatic and non-enzymatic antioxidants in peach.

The combination of heat with Ca salts has had a greater effect than that obtained by a single Ca application. The use of temperatures between 40 °C and 60 °C will determine a greater activity of the enzyme pectin methyl esterase that demethylates the pectin forming anionic carboxyl groups, allowing Ca ions to form salt bridge cross-links (Silveira et al., 2011; Ando et al., 2017).

This study aimed to evaluate the effects of heat treatments with Ca salts on the firmness, metabolic activity, Ca content, and functional properties of minimally processed table grapes.

Materials and Methods

Minimally processed fresh grapes

The work was performed on the Centro de Estudios Postcosecha (CEPOC), Facultad de Ciencias Agronómicas, Universidad de Chile (Santiago, Chile) with 'Black Seedless' table grapes acquired from Agrofruta Ltda. (Copiapó, Chile). Before the processing, the grapes were stored at 0 °C and 90-95% relative humidity.

The minimal process was performed in a conditioned handling room at 5 °C, and clusters of grape were shattered manually. Then, the berries were submerged on cold water (5 °C) to diminish the contact of the peduncle insertion zone damaged with O_2. Grapes in the control treatment were sanitised with sodium hypochlorite (Clorox Chile SA, Santiago, Chile) at 100 mg L^{-1} for 1 min at 5 °C and rinsed in cold water for 1 min.

Berries in the Ca treatments were submerged in a solution of Ca lactate (Merck, Darmstadt, Germany), Ca carbonate, Ca propionate or Ca ascorbate at 60 °C for 2 min. The Ca concentration of each salt was 5 g L^{-1}. All of them were provided by Sigma-Aldrich (St. Louis, MO, USA).

Then, the control and Ca treatment berries were placed in a stainless-steel mesh to drain water excess. An approximate of 120 g of berries were packaged in heat-sealed 10 x 15 cm low-density polyethylene bags (40 µm thickness, 6,000 mL O_2 m^{-2} d^{-1}) (FR400, Plastic Film Sealer, China), that were maintained in refrigeration at 5±1 °C for 21 days simulating the shelf-life period. Three randomly bags were analysed after 1, 7, 14 and 21 days storage.

Respiration rate

The respiration rate was determined in 120 g of berries placed in 500 mL hermetic jars in a humidified atmosphere. At the corresponding analysis days, the jars were closed for 2 h, and gaseous samples were taken from the headspace through a silicone septum using a plastic syringe and analysed by gas chromatograph (Hewlett Packard 5890 Series II, USA) with a thermal conductivity detector (TCD) and a column GFT Porapak Q 80/100. The temperature of TCD was 200 °C, and the injector and oven worked at 50 °C. He was used as the gas carrier at 45 psi. The calibration standards of CO_2, O_2, and N_2 with concentrations of 0.9%, 18.2% and 81.5%, respectively, were used. Gases were provided by Indura (Santiago, Chile). Determinations were made in triplicate, and the respiration rate was expressed as mg CO_2 kg^{-1} h^{-1}.

Atmosphere composition

The O_2 and CO_2 concentrations inside the bags were measured by a portable gas analyser (Checkpoint, PBI Dansensor, Ringsted, Denmark). For instrument calibration, atmospheric air was used (0.03% CO_2 and 21% O_2). Bags atmosphere determination was made through a silicon septum affixed outside the bags. Values were expressed as percentage (%).

Firmness

Firmness was measured by a texture analyser (Brookfield Engineering, CT3, Middleboro, Massachusetts, USA) in the equatorial zone of the berries through a puncture test. For this, a 4.5 mm-diameter flat-head stainless-steel cylindrical probe descended at a speed of 50 mm s^{-1} exerted a pressure to deform the berries by 10 mm. At each sampling day (0, 7, 14, and 21 days), the firmness of 18 berries from each treatment was monitored. The results were expressed in N mm^{-1}.

Ca determination

Bound Ca was determined by a flame atomic absorption spectrophotometer (Beijing Beifen-Ruili Analytical Instrument (Group) Co., WFX-110B/120B/130B, Beijing, China) at 422.7 nm. For the determination, 2 g of berries peels were placed in Petri dishes and dried at 65 °C in a forced circulation drying oven (Lab Tech, LDO-50F, Santiago, Chile), until the weight remained constant. Immediately, dry peels were subjected to digestion (Environmental Express, HotBlock®SC154, South Carolina, USA) with 6 mL of nitric acid (65% purity, Merck, Darmstadt, Germany) and 12 mL of perchloric acid (60% purity, Merck, Darmstadt, Germany). The digestion was performed for 15 h. From 0-12 h temperature was 50 °C, and then the temperature increased by 50 °C per h until it reached 200 °C. After the digestion, a supernatant was made up to 25 mL by adding ultrapure water.

For a free Ca determination, 2 g of grape peels were homogenised in an Ultra-Turrax® (Ika Laboratory Equipment, T18, Staufen, Germany) with 15 mL of ultrapure water for 5 min at 14,000 rpm to break down cells and release calcium from the cytoplasm and mitochondria. The samples were made up to 25 mL with ultrapure water and centrifuged at 3,000 rpm for 3 h. Ca was determined in the supernatant in the same spectrophotometer than bound Ca.

The total Ca content was expressed by the sum of the free and bound Ca. Determinations were made in triplicate, and the results were expressed as mg Ca 100 g^{-1} FW.

Total polyphenol content and total antioxidant capacity

For the extraction, 1 g of berries peels were weighed and homogenised (Ika Laboratory Equipment, T18, Staufen, Germany) for 1 min at 14,000 rpm with 9 mL of methanol. The samples were refrigerated in the dark for 24 h at 5 °C and centrifuged (Hermle Z 326 K, Hermle Labortechnik, Wehingen, Germany) at 3,000 rpm for 20 min at 4 °C.

Total polyphenol content (TPC) determination was performed by combining 19.2 µL of the extract with 29 µL of the Folin-Ciocalteu reagent (Merck) 1N and 192 µL of a solution of 0.4% NaOH and 2% Na_2OH (Merck) in ELISA plates (JET Biofil, Shanghai, China). After 1 h of incubation at room temperature, absorbance was measured at 750 nm in a microplate reader (Asys UVM 340, Biochrom, Cambridge, UK). Three repetitions were performed. The results were expressed as g of gallic acid equivalents (GAE) per kg of fresh weight (g kg^{-1}).

Total antioxidant capacity (TAC) was measured by the ferric reducing antioxidant power (FRAP) assay proposed by Benzie and Strain (1999) and the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) antioxidant assay developed by Brand-Williams et al. (1995). For FRAP determination was made following the methodology described by Silveira et al. (2018).

For DPPH determination, a 21 µL aliquot of the extract was added to a mixture of 194 µL of a DPPH methanolic solution. This solution had its absorbance adjusted to 1.1 at 515 nm.

Both determinations were made in the microplate reader (Asys UVM 340, Biochrom, Cambridge, UK) used for TPC determination.

Results of three repetitions, were expressed as g Trolox equivalent (TE) per kg of fresh weight (g kg^{-1}) in both determinations.

Statistical analysis

A completely randomised design was used where the experimental unit was a bag containing 120 g of berries. For the respiration rate measurements, the experimental unit corresponded to a glass container. Two-way ANOVA ($P \leq 0.05$) was performed, with treatments and storage time as factors. Mean ± standard error of each analysed parameter, was reported. When statistically significant differences were identified, Tukey's test ($P \leq 0.05$) was used to means separate. All statistical analyses were run in Infostat version 2018 (Universidad Nacional de Córdoba, Argentina).

Results and Discussion

Respiration rate

At the beginning, the berries treated with Ca salt showed a lower respiration rate compared with the control, and those treated with Ca propionate showed a lower value (Table 1). In the subsequent analyses moments and until the 17th day, the Ca treatments continued to show lower respiratory activities although no differences were found between them. Control respiration decreased during the storage period, whereas the respiration of the Ca treatments increased. After 21 days, no differences were observed between the treated and control groups with values between 5.41 and 5.53 mg CO_2·kg h^{-1}, respectively.

Generally, the Ca treatment can effectively reduce metabolic activities. Wang et al. (2014) reported that a Ca chloride treatment was able to reduce the CO_2 production of cherries by 30% compared with those not treated. Furthermore, Silveira et al. (2011) showed a decrease in the CO_2 production of minimally processed Galia melon immersed in propionate, lactate, ascorbate, and Ca chloride compared with the control but only after 10 days of storage at 5 °C.

Table 1. Respiration rate of minimally processed 'Black Seedless' table grape, treated with Ca salts and packaged in modified atmosphere at 5 °C.

Treatment	Day 1	Day 5	Day 9	Day 13	Day 17	Day 21
Control	7.09 ± 0.06 Aa	6.59 ± 0.13 Aab	6.02 ± 0.21 Abc	5.69 ± 0.16 Ac	5.64 ± 0.09 Ac	5.54 ± 0.09 Ac
Ca lactate	3.82 ± 0.49 Bbc	3.58 ± 0.06 Bc	4.28 ± 0.24 Bbc	4.47 ± 0.15 Bb	4.59 ± 0.11Bab	5.43 ± 0.05 Aa
Ca chloride	3.81 ± 0.56 Bc	3.93 ± 0.08 Bbc	4.15 ± 0.21Bbc	4.75 ± 0.06 Bab	4.78 ± 0.16 Bab	5.42 ± 0.12 Aa
Ca propionate	2.85 ± 0.11 Cb	3.59 ± 0.21Ba	4.76 ± 0.03 Ba	4.91 ± 0.08 ABa	5.06 ± 0.11ABa	5.48 ± 0.08 Aa
Ca ascorbate	3.99 ± 0.44 Bd	4.02 ± 0.25 Bcd	4.53 ± 0.21Bbcd	4.88 ± 0.03 ABabc	4.89 ± 0.07 ABab	5.49 ± 0.11 Aa

Values are means ± standard error (n= 3)

Means followed by different letters, uppercase for treatment and lowercase for time, are statistically different according to Tukey's test at $p \leq 0.05$

Atmosphere composition

Atmosphere composition did not show differences between the treatments on any of the evaluation days for CO_2 and O_2 values. Oxygen concentrations were between 16.3% and 16.8% on day 1 and reached values between 8.8% and 9.8% after 21 days of storage. Then, CO_2 values varied between 2.8% and 3.2% on day 1, attaining values between 4.2% and 5.1% on day 21 (data not shown).

Firmness

The calcium treatments allowed the berries to be firmer until 14 days of storage, as shown in Figure 1. On day 1, no differences were found between the Ca salts used, with all the firmness values higher than those of the control. However, after 7 and 14 days of storage, the highest firmness corresponded to the berries treated with Ca propionate and ascorbate with values between 22%–23% and 18%–19% higher than the control, respectively.

The firmness of the control remained practically constant throughout the storage period, and, in general, the firmness of the Ca treatments was maintained until 14 days and decreased after 21 days.

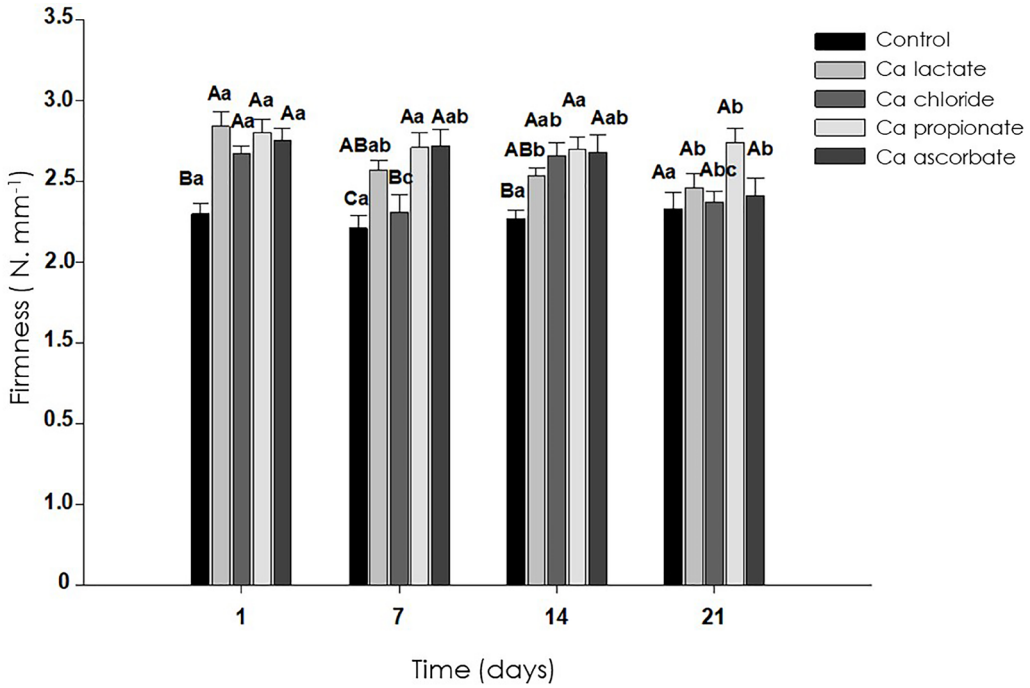

Figure 1. Flesh firmness of minimally processed 'Black Seedless' table grape treated with Ca salts and packaged in modified atmosphere at 5 °C. Vertical bars indicate the standard error of the means (n = 3).

In a work where the softening process in two varieties of table grapes was studied, the variety with the highest content of Ca showed higher uronic acids in the cell wall material and remained firmer, confirming the roles of the Ca bridge formation and cell wall integrity as related to a firmer grape berry (Balic et al., 2014).

In addition, the synergistic effect of Ca and heat treatments on firmness maintenance was previously reported in different minimally processed products (Silveira et al., 2011; Chong et al., 2015; Zhang et al., 2019b). This would be linked to the increase in pectin methylesterase (PME) activity promoted by the heat treatment, which, in the presence of exogenous Ca, promotes its combination with non-esterified C-6 from galacturonic acid residues. In this way, an increase in pectin stability occurs due to the improvement of the integrity and mechanical properties

of the cell wall (Ando et al., 2017; Zhang et al., 2019b).

In a study carried out on asparagus, the synergistic effect between temperature, Ca and PME in firmness maintenance was demonstrated. Here, the asparagus was dipped in solutions of 1% Ca lactate and 1% Ca lactate + 0.1% PME at temperatures between 50 °C and 90 °C for up to 100 min. The highest firmness was obtained when Ca lactate + PME solution was used at a temperature of 60 °C for 20 min. According to the authors, this result can be explained by the fact that infusing vegetables with Ca or Ca and PME at moderate temperatures can stimulate PME activities and result in a firmer texture (Peng et al., 2019).

Calcium content

Free Ca content is showed in Figure 2, where the control presented, at all the analysed moments, the lowest Ca amount. Initially, the treated berries presented between 57% and 70% more content than the control. After 21 days, the greatest differences were observed between the control and Ca lactate and propionate that presented about 100% and 70% more free Ca, respectively.

Ca levels of different treatments remained almost constant during the 21 days of refrigerated storage.

On the first day, the berries treated with the different Ca salts presented between 12% and 22% more bound Ca than the control (Figure 3). This difference was maintained in the following evaluation moments. From day 7, differences between the Ca treatments were found where Ca propionate and ascorbate presented higher values than those measured in berries treated with Ca chloride and lactate.

Moreover, in this case, bound Ca measured in the different treatments remained unchanged during the storage period.

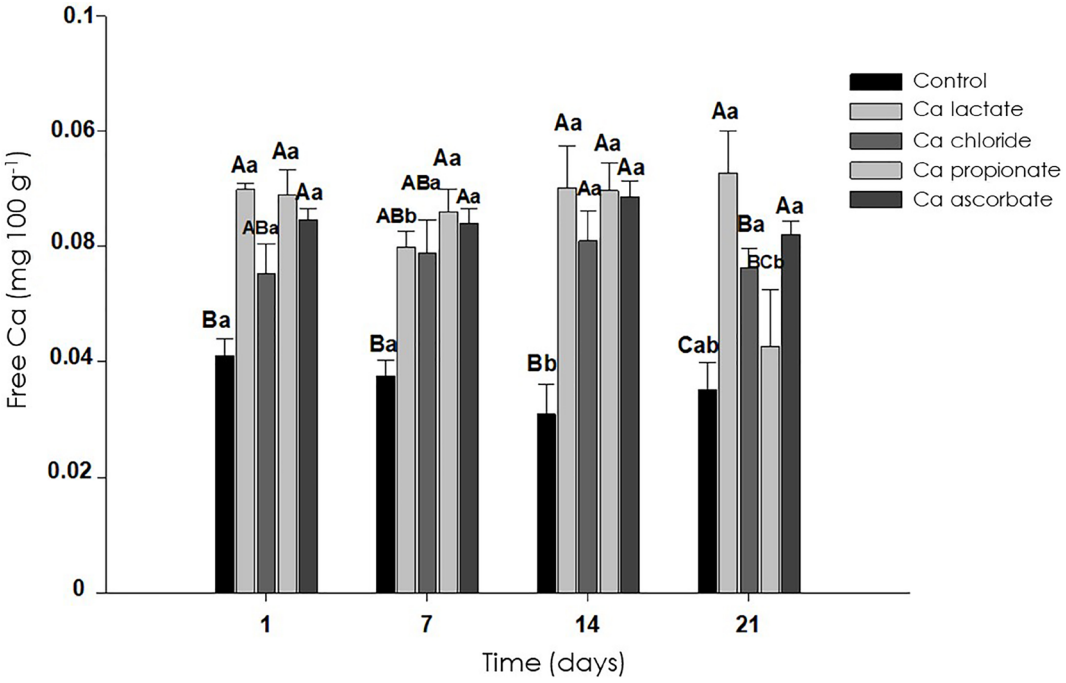

Figure 2. Free calcium (mg Ca 100 g^{-1}) of minimally processed 'Black Seedless' table grape treated with Ca salts and packaged in modified atmosphere at 5 °C. Vertical bars indicate the standard error of the means (n = 3). Means followed by different letters, uppercase for treatments and lowercase for time, are statistically different according to Tukey test at $P \leq 0.05$.

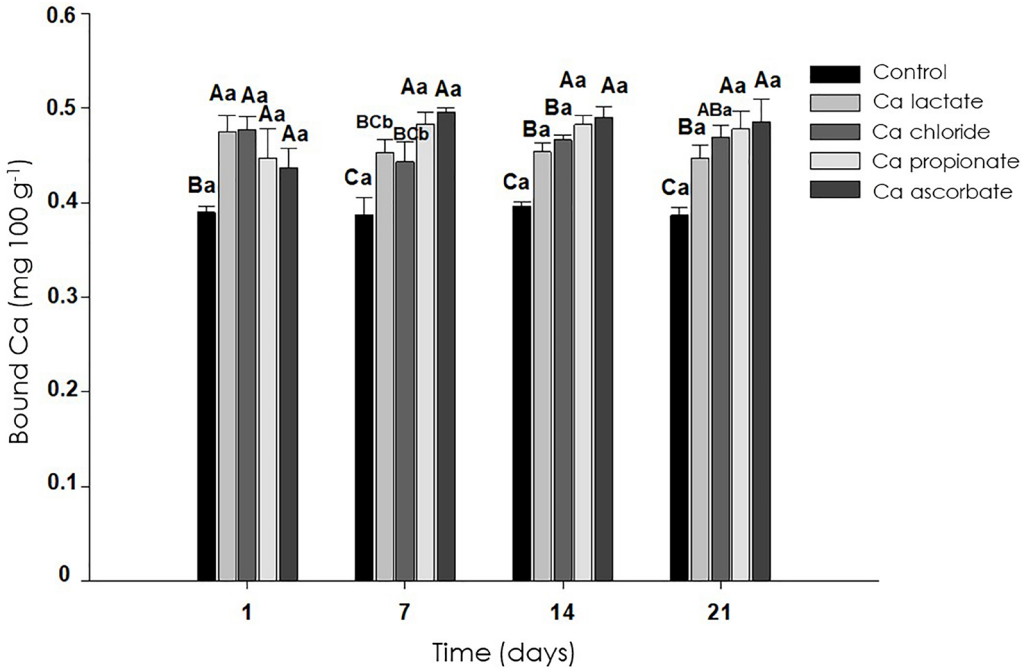

Figure 3. Bound calcium (mg Ca 100 g^{-1}) of minimally processed 'Black Seedless' table grape treated with Ca salts and packaged in modified atmosphere at 5 °C. Vertical bars indicate the standard error of the means (n = 3). Means followed by different letters, uppercase for treatments and lowercase for time, are statistically different according to Tukey test at $P \leq 0.05$.

The total Ca content is shown in Figure 4. This fraction is composed of bound and free Ca, which, as shown in the figure, represents 10% of the total Ca. The total Ca showed the same trend observed in the free and bound Ca fractions. In all the analysed moments, the levels measured in the treated berries were between 20% and 30% higher than those of the control. The highest values measured corresponded to the propionate and ascorbate treatments. The total Ca level was kept almost constant during the 21 days of the trial.

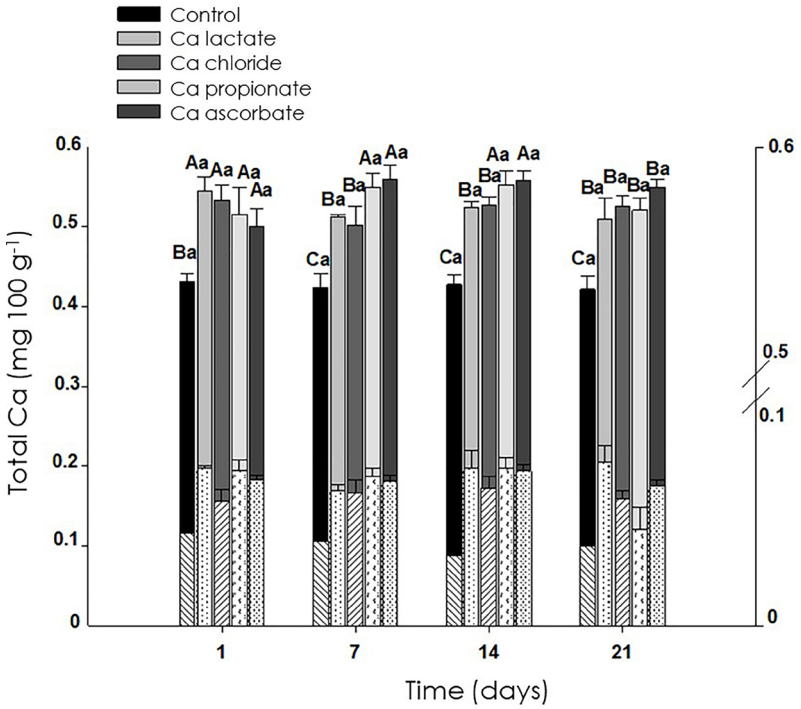

Figure 4. Total calcium (mg Ca 100 g^{-1}) of minimally processed 'Black Seedless' table grape treated with Ca salts and packaged in modified atmosphere at 5 °C. Vertical bars indicate the standard error of the means (n = 3). Means followed by different letters, uppercase for treatments and lowercase for time, are statistically different according to Tukey test at $P \leq 0.05$.

The Ca increase because of the Ca treatment, combined or not with the heat treatment, was also reported as in whole as in minimally processed products. Although, few of them have reported the behaviour of different Ca fractions, the total Ca is usually presented.

In sweet cherry, the application of $CaCl_2$ at concentrations between 0.2% and 2% in hydro-cooling (0 °C) for 5 min determined increases in the total Ca of tissues of up to 85% in the Sweetheart and 188% in the 'Lapins' cultivars (Wang et al., 2014). In the same way, Naser et al. (2018) mentioned that the combination of Ca lactate and heat treatment was more effective in increasing endogenous Ca levels in the whole persimmon.

In a previous work carried out on minimally processed Galia melon, the levels of free and bound Ca of melon dipped in different Ca salts (60 °C for 1 min) showed an increase related to the control treatment. The increase in Ca, mainly in the bound fraction, is responsible for maintaining the flesh firmness (Silveira et al., 2011). More recently, Peng et al. (2019) also found an increase in the free and bound Ca fractions in asparagus after immersion in Ca lactate. However, a significant correlation ($p < 0.05$) was only observed between the texture and bound Ca content in the tissue when its cell integrity was maintained. Therefore, it is important to quantify the effect of the applied Ca source on the bound Ca fraction to guarantee the effectiveness of the Ca treatment.

Total polyphenol and total antioxidant capacity

TPC remained constant throughout the storage period and without differences between the treatments (Table 2).

Table 2. Total polyphenol content (GAE g kg^{-1}) of minimally processed 'Black Seedless' table grape, treated with Ca salts and packaged in modified atmosphere at 5 °C.

Treatment	Day 1	Day 7	Day 14	Day 21
Control	24,558 ± 677 Ab	25,297 ± 506 Ab	25,341 ± 741 Ab	28,289 ± 1,485 Aa
Ca lactate	25,812 ± 2,188Aa	25,047 ± 772 Aa	26,562 ± 1,607Aa	27,213 ± 254 Aa
Ca chloride	26,067 ± 370 Aa	25,351 ± 1,793Aa	26,451 ± 1,258Aa	27,444 ± 1,246 Aa
Ca propionate	25,947 ± 64 Aa	26,469 ± 834 Aa	27,110 ± 259 Aa	28,336 ± 505 Aa
Ca ascorbate	26,461 ± 537 Ab	27,215 ± 235 Ab	27,878 ± 610 Ab	29,744 ± 285 Aa

Values are means ± standard error (n= 3)

Means followed by different letters, uppercase for treatment and lowercase for time, are statistically different according to Tukey's test at $p \leq 0.05$

TAC measured by DPPH showed no difference between the treatments (Table 3). Moreover, gradual differences were observed on the TAC measured by FRAP on days 7 and 14 and between the control and berries treated with Ca ascorbate (Table 3). In both methods, TAC increased during the 21 days of storage.

Table 3. Total antioxidant capacity (TE g kg^{-1}) of minimally processed 'Black Seedless' table grape, treated with Ca salts and packaged in modified atmosphere at 5 °C.

Treatment			FRAP	
	Day 1	Day 7	Day 14	Day 21
Control	12,137 ± 919 Ac	10,045 ± 211 Cbc	11,461 ± 1840 Bb	14,133 ± 972 Aa
Ca lactate	12,391 ± 413 Aa	11,702 ± 147 BCa	12,487 ± 533 ABa	13,197 ± 486 Aa
Ca chloride	12,066 ± 904 Ab	12,750 ± 502 ABab	13,430 ± 360 ABab	13,751 ± 1014 Aa
Ca propionate	12,936 ± 1133Ab	13,141 ± 989 BCab	13,261 ± 591 ABab	14,273 ± 1008 Aa
Ca ascorbate	13,155 ± 797 Aa	14,241 ± 598 Aa	13,834 ± 624 Aa	14,123 ± 449 Aa
			DPPH	
Control	44,641 ± 471 Ac	47,166 ± 525 Ac	47,925 ± 851 Ab	51,112 ± 1123 Aa
Ca lactate	45,625 ± 1161Ab	48,847 ± 226 Ab	51,867 ± 2538 Aa	51,283 ± 2142 Aa
Ca chloride	46,876 ± 797 Ab	47,779 ± 826 Ab	53,668 ± 683 Aa	51,042 ± 502 Aa
Ca propionate	44,880 ± 256 Ab	48,826 ± 224 Aa	51,025 ± 1666 Aa	51,057 ± 2412 Aa
Ca ascorbate	47,850 ± 1185Ab	49,143 ± 297 Ab	54,141 ± 1727 Aa	53,835 ± 2069 Aa

Values are means ± standard error (n= 3)

Means followed by different letters, uppercase for treatment and lowercase for time, are statistically different according to Tukey's test at $p \leq 0.05$.

Unlike the findings in this study, other authors mention that Ca treatments positively influence the TP and TAC of vegetable products. Naser et al. (2018) reported that although the TAC of the whole persimmon decreased over time, the Ca lactate treatment contributed to its maintenance.

According to Aguayo et al. (2015), the TAC measured by DPPH and FRAP in fresh-cut apples

decreased throughout the storage but was always higher in slices dipped in water at 55 °C with 6% Ca ascorbate for 2 min, as compared with those dipped in water at 4 °C for the same time. In the same work, TPC was not significantly affected as the reductions and increases were observed depending on the type of phenol considered. The differences with this work may be due to the Ca salt concentration used and the characteristics of the vegetable matrix, as in the case of the grape, where the berries remained practically unchanged after processing.

The relationship between Ca applications and antioxidant compounds can be attributed to the increase in cytosolic Ca, which in turn participated in regulating the expression and synthesis of enzymes and proteins linked to the membranes' integrity maintenance. Cellular membranes keeping stable, as a consequence of a reduction of the lipid peroxidation and free radicals that preserved the antioxidant level of the cell (Spinardi et al., 2005; Wang et al., 2014; Zhi et al., 2017).

Conclusions

Ca salts, especially ascorbate and propionate, combined with heat treatments, allowed maintaining the firmness and increasing the levels of bound and free Ca of berries. Nevertheless, they did not have a great impact on the quality and shelf life of minimally processed 'Black Seedless' table grapes.

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