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EXTENDING POSTHARVEST QUALITY ATTRIBUTES OF GRAPES (V. vinifera L. cv. 'THOMPSON SEEDLESS') BY PREHARVEST CALCIUM PULVERIZATIONS

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ABSTRACT

Demand for fresh grape is increasing globally in accordance with the improvement in living standard since the grape berry contains large amounts of phytochemicals including anthocyanins, phenolics, flavonoids and resveratrol, which have been suggested to be responsible for human health benefits. However, table grapes easily undergo deterioration due to their soft texture and the high water content, which make it difficult to preserve without treatment. This study was thus conducted to evaluate the effect of preharvest calcium sprays on maintenance of postharvest quality of grapes (V. vinifera L. cv. 'Thompson Seedless'). Three preharvest calcium sprays were applied to leaves and developing green berries with or without leaf removal pruning (a traditional practice performed in commercial vineyards worldwide) during berry development stages. After harvest, grapes were cold stored (1°C, 90% R.H.) up to 3 months. Preharvest micronized calcium sprays, with or without leaf removal pruning, markedly extended the postharvest quality of grapes by delaying weight loss, reducing decay, maintaining rachis chlorophyll concentrations and preserving visual quality during the prolonged cold storage. Besides, in calcite-treated grapes, lower titratable acidity decrease courses with a subsequent lower maturity index during prolonged storage indicate that calcite sprays restricted postharvest physiological senescence of grapes. Overall findings indicated that preharvest calcite sprays may be an environmental-friendly, healthy and sustainable viticulture practice for extending postharvest quality of grapes.

Key words: table grape, organic sprays, micronized calcite, quality assurance, cold storage

INTRODUCTION

World population is expected to exceed 10 billion by 2050 [Béné et al. 2015], further adding to global food security concerns. Such increase translates into 33% more human mouths to feed, with the greatest demand growth in the poor communities of the world. According to Alexandratos and Bruinsma [2012], food supplies would need to increase by 60% in order to meet the food demand in 2050. Food availability and accessibility can be increased by

increasing production, improving distribution, and reducing the losses [Kader 2005]. Thus, reduction of postharvest food losses is a critical component of ensuring future global food security.

Table grapes, as non-climacteric fruits with a relatively low rate of physiological activity [Crisosto et al. 2001], are subject to severe postharvest losses during long-distance transport and storage [Li et al. 2015], which results in rachis (cluster stem) drying



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and browning, berry shatter, and even wilting and shriveling of berries [Sabir and Sabir 2013]. Inappropriate handling processes are the main reason that weaken the natural defenses of grapes and making the fresh grape more susceptible to decay and consequently deterioration. World losses of agricultural products during postharvest are 10-30%, and an estimated rate for grape is 27% [Romanazzi et al. 2007]. Gray mold infection (Botrytis cinerea) and stem (rachis) browning are the two main factors responsible for postharvest quality loss of table grapes [Crisosto et al. 2002, Jiang et al. 2015]. SO₂ fumigation is still the primary means for controlling berry decay due to its excellent effect on postharvest diseases [Droby et al. 2009, Lee et al. 2015]. However, SO₂ fumigation is becoming very restrictive because its residues are dangerous to people and may give a sulphurous flavour to the fruit. For table grapes, the problem related to the concentration of SO₂ in the storage room as well as in the shipping box are widespread global issues and several times lots of table grapes are blocked in the European Market due to high concentration of SO₂. Unfortunately, the concentration of SO₂ in the box is conditioned by the relative humidity (RH) inside the plastic bag and so difficult to control. Another problem associated with the use of SO₂ is the constant potential for injury to the berries and rachis [Crisosto et al. 2002] because the release of SO₂ depends on the RH inside the bag and so it is hard to obtain standard concentration in all the boxes. Rachis browning, the second most important storage problem [Lichter et al. 2006, 2011, Romanazzi et al. 2012], is considered to be the result of rachis desiccation [Li et al. 2015], which can occur partly during storage. It mainly affect the visual quality and today it is well established that consumer preference is a primary cause of food waste in surplus economies [IMECHE 2012]. Furthermore, rachis browning presents a major barrier to development of novel technologies for storage of table grapes [Li et al. 2015], since the green rachis in clusters of table grapes provide an important indication of the freshness of the produce after storage [Balic et al. 2012].

Recently, growing consumer awareness of the potential harm of chemical treatments for the control of insects, diseases and physiological disorders, has led to the development of non-damaging physical treatments for this purpose in horticultural produce. Therefore, more environmentally friendly and harmless compounds should be developed as alternative methods for postharvest diseases [Sabir et al. 2011].

Calcium, as a versatile signaling ion with special functions, serves as a major regulatory ion in horticultural crops [Aghdam et al. 2012]. It is considered to be an important mineral element that regulates fruit quality, specifically, maintenance of fruit firmness, a decrease in postharvest decay and incidence of physiological disorders such as water core, bitter pit and internal breakdown [Lurie 2009]. It is an important component of the plant cell wall, and binds together the strands of pectin helping to maintain fruit firmness. Calcium binding to cell wall components may also reduce the accessibility of cell wall degrading enzymes to their substrates [Vicente et al. 2009] and by this way storage and shelf life of fruits can be enhanced. Preharvest calcium sprays are one of the most important practices of the new strategies applied in the Integrated Fruit Production systems [Manganaris et al. 2005], improving fruit characteristics and minimizing fungicide sprays towards the end of the harvest period [Sabir et al. 2014], since they enhance fruit resistance to brown rot [Conway et al. 1994]. Sprays with calcium chloride based formulas are extensively used [Manganaris et al. 2005], whereas micronized calcium sources are promoted as alternative sources, characterized by a high absorption capacity [Sabir et al. 2012]. In spite of these facts, interestingly, our literature investigation yielded no satisfactory knowledge regarding quality response of grapes to preharvest calcium applications. Therefore, this study was conducted to evaluate the effects of preharvest calcium (as micronized calcite) spays on postharvest quality of table grape in comparison to the traditional pruning practice performed worldwide in commercial vineyards for quality improvement.

MATERIALS AND METHODS

Study design. A vineyard experiment in factorial format based on randomized complete block design with three replications, consisted of six ten-year-old

healthy vines of 'Thompson Seedless' (syn. 'Sultani Çekirdeksiz') grape cultivar (cv.) was conducted. Treatments were, (i) traditional summer pruning (removal of three leaves below clusters for each shoot), (ii) pulverization of micronized calcite [Ca-CO₃ (40%), SiO₂ (4%), MgO (1%) and Fe₂O₃ (1%)] at 0.5% concentration [Kara and Sabir 2010], and (iii) nano-size calcite plus pruning. Leaf removal, as traditional canopy management practice in commercial vineyards [Lee and Skinkis 2013] recommended to enhance berry quality and decrease disease incidence, improving the canopy microclimate of the fruiting zone [Risco et al. 2014] has been performed at berry set stage and considered as control treatment. Calcite sprays were performed to leaves and green berries three times during berry development stages, namely at berry set and two additional applications with 15 d intervals, using 1000 L solution per hectare [Dilek and Sabir 2016]. Table grape bunches, belonging to different preharvest applications, were harvested at commercial maturity stage (around 21.5 ±1.5°Brix) and transported to the laboratory for initial analyses and processing. Bunches were graded for uniformity of color and size, and grapes without visible damage on their skin or visible microbial infection were selected. For each group (pruning, nanosize calcite and pruning plus nanosize calcite), nine packages (three replications × three storage times) were prepared by placing about 500 g of table grapes inside 30 × 40 cm polyamide/polyethylene plastic bags. Finally, samples were stored for up to three months in a cold room at 1°C and 90% RH. Samples of fresh fruits were taken (three packages from each group) for monthly analyses.

Quality indexes. Individual samples were weighed initially and after each storage duration to obtain weight loss, expressed as %. Total soluble solids content (SSC) expressed as °Brix was measured with a portable refractometer (Atago, Tokyo, Japan) in grape juice obtained by whisking the berries from each replication in a blender (1 min, 14 000 rpm) and then filtering the juice. Titratable acidity (TA) was determined by titrating 10 mL of juice using NaOH 0.1 mol L⁻¹ to pH 8.1 [AOAC 1984]. Results were expressed as g tartaric acid per

100 g fresh weight (FW). Maturity index (MI) was calculated as SSC/TA ratio. The pH was measured using a pH meter (Crison, Barcelona, Spain). The colour parameters L^* (lightness), a^* (redness) and b^* (yellowness) were measured at three random points on the peel surface of ten berries from each replication with a colorimeter (CR-400, Konica Minolta, Osaka, Japan) in reflectance mode. Hue angle $(h^\circ = \tan^{-1} (b^*/a^*))$ and chroma $(C^* = (a^{*2} + b^{*2})^{1/2}))$ were also calculated.

Decay incidence and visual quality. Decay was quantified by counting the number of decayed berries in each cluster, multiplying the total number of decayed berries per replication by the average berry size, and calculating the percentage of decayed berries with respect to the weight of the entire replication. Visual quality was scored on a 9 to 1 scale, where 9 = excellent, fresh appearance, 7 = good, 5 = fair (limit of marketability), 3 = fair, 1 = unusable.

Rachis chlorophyll and total phenols. Total chlorophyll was determined spectrophotometrically as described by Agar et al. [1997] with slight modifications. One gram of blended grape tissue was homogenized with 15 mL chloroform–methanol (2 : 1, v/v) for 1 min. Extracts were filtered with filter paper and solutions supplemented with chloroform–methanol to 25 mL final volume. Total chlorophyll was determined by measuring the absorbance of solution in the spectrophotometer at 663 and 645 nm against chloroform–methanol blank. Results were calculated using the McKinney equation and expressed as mg kg⁻¹.

The following extraction procedure was used for total phenol determinations. A 5 g portion of berry tissues without seeds was homogenized in methanol for 1 min and then centrifuged at $4000 \times g$ for 30 min at 5°C. Total phenols were determined according to the method of Singleton et al. [1999]. A 100 μ L aliquot of each extract was mixed with 1.58 mL of water, 100 μ L of Folin–Ciocalteu's reagent and 300 μ L of sodium carbonate solution (200 g L⁻¹). The absorbance at 760 nm was read after 2 h. The content of total phenols was calculated on the basis of the calibration curve of gallic acid and was expressed as mg gallic acid 100 g⁻¹ FW.

Statistical analyses. Statistical analyses were performed in triplicate on three different batches. The mean values and standard deviation were calculated. The data shown in the figures are the average of all repetitions, where the error bars are the standard deviations. Experimental data were submitted to one-way analysis of variance and Student's t-test (P < 0.05) using the software SPSS 13.0 for windows.

RESULTS AND DISCUSSION

Weight loss is an easy and objective measure often used for valid evaluation of the response of horticultural commodities to treatments [Crisosto et al. 2011]. In the current study, both the application and storage duration significantly influenced weight loss of grapes. At the end of storage, the lowest weight loss was found in bunches of calcite spray treatments (4.3%), while the greatest weight loss (7.5%) occurred in pruning application (fig. 1). The calcite sprays markedly retarded the weight loss of clusters, most probably owing to its protective effects on cell wall. It is well-known that calcium, as a constituent of the cell wall, plays an essential role in forming cross-bridges which influence cell wall strength and is regarded as the last barrier before cell separation [Fry 2004]. Exogenously applied calcium stabilizes the plant cell wall [Aghdam et al. 2012] and protects it from cell wall degrading enzymes [White and Broadley 2003] such as polygalacturonase (PG), a prominent cell wall degrading enzyme synthesized during ripening [Hadfield and Bennett 1998]. It is already well-documented by MacDougall et al. [1995] that the PG activity is blocked by high calcium concentrations in plant cell. Such protective effects of preharvest calcium treatments have also been reported for several commodities, including peaches [Manganaris et al. 2005], nectarines [Crisosto et al. 2000] and apples [Chardonnet et al. 2003].

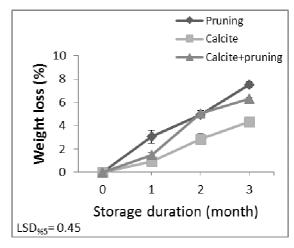


Fig. 1. Changes in weight loss (%) of grapes during the prolonged cold storage. Error bar stands for the standard deviation of the mean of triplicate determinations

Table 1. Changes in SSC (°Brix), TA and pH values of grapes during the prolonged cold storage. Means not connected by same letter are significantly different at 5% level by LSD (± standard deviation)

Quality features	Preharvest	Storage duration (month)				
	applications	0	1	2	3	
SSC	Pruning	23.0 ±0.00 ^{ab}	22.8 ±0.35 ^b	23.4 ±0.33 ^{ab}	23.5 ±0.38 ^a	
	Calcite	23.0 ± 0.00^{ab}	21.5 ± 0.81^{c}	21.3 ± 0.58^{c}	23.5 ± 0.35^{a}	
	Calcite + pruning	20.0 ± 0.00^{d}	21.4 ± 0.58^{c}	21.9 ± 0.15^{c}	23.6 ± 0.05^{a}	
TA	Pruning	0.75 ± 0.01^{ab}	0.68 ± 0.01^{e}	$0.62 \pm 0.02^{\rm f}$	0.52 ± 0.02^{g}	
	Calcite	0.78 ± 0.00^{a}	0.74 ± 0.04^{ab}	0.76 ± 0.04^{ab}	$0.72 \pm 0.03^{b-d}$	
	Calcite + pruning	$0.69 \pm 0.01^{c-e}$	0.73 ± 0.01^{bc}	0.68 ± 0.06^{de}	0.69 ± 0.02^{de}	
рН	Pruning	3.27 ± 0.00^{a}	3.12 ± 0.08^{e}	3.16 ± 0.02	$3.06 \pm 0.01^{\rm f}$	
	Calcite	$3.20 \pm 0.01^{b-d}$	3.04 ± 0.02^{f}	3.15 ± 0.07^{de}	3.14 ± 0.05^{de}	
	Calcite + pruning	$3.22 \pm 0.00^{a-c}$	$3.17 \pm 0.03^{c-e}$	3.15 ± 0.01^{de}	3.26 ± 0.02^{ab}	

LSD for SSC: 0.66; TA: 0.05; pH: 0.06

Table 1 shows the effect of the different treatments on different must (grape juice) chemical parameters of grapes during storage. There were statistically significant changes in SSC, TA and pH values of must at harvest as well as storage duration. The highest SSC value at harvest was obtained from pruning and calcite treatments (with the same value 23.0 °Brix) while the lowest SSC was obtained from calcite + pruning (20.0 °Brix). SSC underwent a slight increase through the storage and reached to a maximum 23.6 °Brix (calcite + pruning), though the differences were insignificant at the end of the storage. Several researchers reported general increases in SSC of grapes during cold storage [Sabir et al. 2011], due to gluconeogenesis pathway [Famiani et al. 2009] or water loss [Li et al. 2015]. About the calcium treatment, studies on various peach cvs, revealed that SSC was not markedly influenced by the preharvest calcium sprays [Crisosto et al. 2000, Serrano et al. 2004], most probably due to fruit tissue differences, such as hairiness, which may solely affect the entrance of calcium into the fruit. Berry TA content of non-treated pruning vine underwent a progressive decrease during storage. After a three-month storage, TA contents of both calcite and pruning + calcite treatments were significantly higher than nontreated vines. Differences in pH values among the treatments at harvest were statistically significant. The pH showed a general decrease along the storage time,

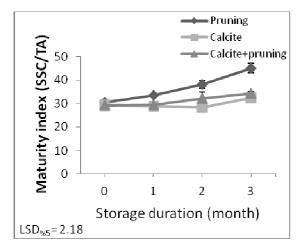


Fig. 2. Changes in maturity index (SSC/TA) of grapes during the prolonged cold storage. Error bar stands for the standard deviation of the mean of triplicate determinations

except for calcite + pruning where an increase was detected at the final analysis. Sabir and Sabir [2013] found a slight decrease in pH of 'Red Globe' cv., while the pH value of 'Müşküle' cv. was almost constant. Sanchez-Ballesta et al. [2006] also reported that pH value remains fairly constant during long-term cold storage. Hence, literature indicates that the must pH may show alteration among the cvs during the cold storage. MI values in response calcite sprays were significantly lower than that of non-sprayed grapes (fig. 2). Relatively lower TA decrease courses, with a subsequent lower MI in calcite-treated grapes indicate that preharvest calcite sprays obviously restricted postharvest physiological senescence of grape berries during the prolonged storage. Exogenous applied calcium has been reported to reduce decay, delay ripening and extend postharvest life of various fresh fruits such as peaches and nectarines [Serrano et al. 2004], apples [Siddiqui and Bangerth 1995] and kiwi fruit [Gerasopoulos et al. 1996]. Yang et al. [2010] studied the molecular mechanisms of calcium beneficial effects and isolated genes from tomato coding for calmodulin, a calcium specific protein and SR/CAMTA (Stress Responsive/CaM-binding Transcriptional Activator), a calmodulin-binding transcription factor. They suggested that calcium regulates fruit development and ripening by forming a calcium/calmodulin complex to activate SISRs (fruit specific calmodulin genes).

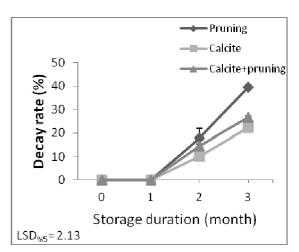


Fig. 3. Changes in decay rate (%) of grapes during the prolonged cold storage. Error bar stands for the standard deviation of the mean of triplicate determinations

During the first month of storage, there was no decayed berry across the treatments (fig. 3). However, the berries suffered from an accelerated decay rates during the prolonged storage. At the end of the storage, with a significant differences among the treatment, the highest and lowest decay rates were obtained from pruning (26.5%) and calcite (17.3%) treatments, respectively. It has already been well-established that calcium improves and maintains cell wall components [Vicente et al. 2009], by binding the carboxyl groups of the pectic homogalacturonan backbone, as postulated by the egg-box model [Grant et al. 1973], and may protect the pectic backbone from polygalacturonase-mediated depolymerisation [Wehr et al. 2004].

In extending postharvest quality of produces, consumer acceptability is a prime consideration. As illustrated in Figure 4, the grape belonging to the calcite treatment maintained its initial visual quality during the first month of storage. After the first month, the visual quality of overall grapes underwent a noticeable decline. At the end of the three-month storage, significant differences were revealed among the treatments. The highest value on the visual quality was obtained from calcite treatment (5.7), followed by calcite plus pruning (4.4) and pruning (4.0) treatments. Among the treatments, calcite alone was able to keep grape visual quality above marketability limit at the end of 3 month storage. Shriveling, resulting from severe water loss

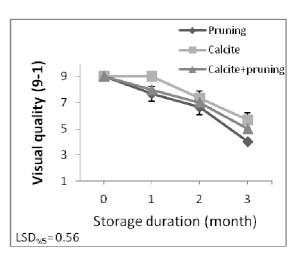


Fig. 4. Changes in visual quality (9–1 scale) of grapes during the prolonged cold storage. Error bar stands for the standard deviation of the mean of triplicate determinations

during postharvest handling [Sabir et al. 2011], is a common and very pronounced feature that reduces postharvest visual quality and limits the postharvest life of grapes [Crisosto et al. 2002]. Studies revealed that preharvest calcium treatment increases tissue firmness in perishable horticultural produces [Siddiqui and Bangerth 1995, Gerasopoulos et al. 1996]. In the present study, markedly lower weight loss and significantly higher visual quality in calcite-sprayed grapes during the storage verify the positive effect of calcite sprays on visual quality of grapes.

Table 2. Changes in L^* , C^* and h° values of grapes during the prolonged cold storage. Means not connected by same letter are significantly different at 5% level by LSD (\pm standard deviation)

Quality features	Preharvest applications	Storage duration (month)				
Quality leatures	r renarvest applications	0	1	2	3	
	Pruning	46.08 ±2.2 ^{a-d}	44.77 ±0.5 ^{b-e}	43.11 ±2.2 ^e	40.12 ±0.9 ^f	
L^*	Calcite	$45.15 \pm 1.4^{b-e}$	46.97 ± 1.3^{ab}	$43.93 \pm 1.2^{d-e}$	$44.38 \pm 0.6^{\text{c-e}}$	
	Calcite + pruning	$46.59 \pm 2.1^{a-c}$	48.16 ± 0.8^{a}	$45.40 \pm 0.7^{b-d}$	$46.04 \pm 0.6^{a-d}$	
	Pruning	19.95	21.91	24.29	24.85	
C^*	Calcite	18.52	20.13	23.48	22.28	
	Calcite + pruning	19.94	22.29	24.42	24.11	
	Pruning	106.44	105.85	100.73	97.65	
h°	Calcite	110.93	109.79	106.55	97.42	
	Calcite + pruning	106.14	103.92	99.49	94.91	

LSD for L: 2.27

Table 2 shows the development of different color coordinates for the treatments during the cold storage. L^* and h° decreased with storage time across the treatments in contrast to C^* which progressively increased. Treatments did not lead to any significant differences in C^* or Hue values, though L^* values among the treatments were significant, possible due to enzymatic browning which is a common problem seen in grape storage [Sabir et al. 2011]. At the end of storage, the highest L^* value was obtained from calcite + pruning treatment, followed by calcite. This indicate that calcite treatment slow down the browning process caused by non-enzymatic and enzymatic reactions as reported by different authors [González-Barrio et al. 2005, Pastor et al. 2011], studying on postharvest color response of various table grapes to various treatments.

Treatments significantly affected the pre- and poststorage total chlorophyll concentrations. At harvest total chlorophyll concentrations were 3.92, 3.54 and 3.26 mg kg⁻¹ for calcite, calcite plus pruning and pruning treatments respectively. Rachis chlorophyll concentration gradually decreased along with the prolonged storage (fig. 5). Loss in total chlorophyll concentration of rachis during the storage was also greatest in clusters of pruning treatment, whereas

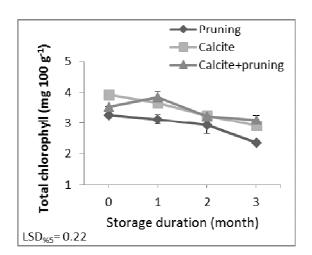


Fig. 5. Changes in total chlorophyll concentration (mg 100 g⁻¹) of grapes during the prolonged cold storage. Error bar stands for the standard deviation of the mean of triplicate determinations

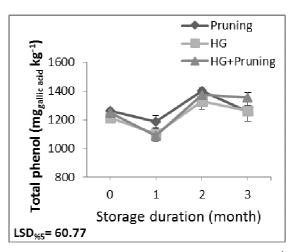


Fig. 6. Changes in total phenol content (mg_{gallic acid} kg⁻¹) of grapes during the prolonged cold storage. Error bar stands for the standard deviation of the mean of triplicate determinations

calcite treatment with or without pruning significantly maintained the total chlorophyll. However, rachis in the calcite treatments with or without pruning, still appeared to retain a green appearance up to the third month of cold storage. At the end of storage, with significant differences among the treatments, highest chlorophyll concentration was detected in calcite plus pruning treatment (3.1 mg 100 g⁻¹), followed by calcite (2.91 mg 100 g⁻¹), while the rachis of pruning treatment was as low as 2.36 mg 100 g⁻¹ rachis tissue. The green rachis in clusters of table grapes provide an important indication of the freshness of the produce after storage. Rachis browning is associated mainly with dehydration and ethylene biosynthesis [Li et al. 2015]. Maintaining grape clusters under high RH is a prerequisite to prevent rachis browning since, beyond a certain water-loss threshold, which is around 3%, water loss has been shown to cause extensive browning [Crisosto et al. 2001, Lichter et al. 2011]. This is in agreement with the current study in which rachis chlorophyll degradation was accompanied by reductions in weights, resulting from gradual water loss. However, high RH is a two-edged sword as it supplies water for pathogens and thereby results in loss of grapes due to decay [Li et al. 2015]. Studies revealed a tight relationship between weight loss and rachis chlorophyll degradation [Cefola et al. 2011,

Lichter et al. 2011]. Crisosto et al. [1994] reported a rachis browning of 'Thompson Seedless' table grape when cluster weight loss was 3.1 or 3.6%, while there was no rachis browning when cluster weight loss was 1.4 or 2.4%. Present investigations on rachis chlorophyll concentration, including weight loss due to dehydration during storage, indicate that preharvest micronized calcium spray is an effective and sustainable way for maintenance of rachis quality.

Among the large number of fruits, grapes are one of the most important sources of phenolic compounds, which have beneficial effects on human health by acting to neutralize free radicals [Orak 2007]. At harvest, differences of treatments for the total phenol content of grapes were insignificant, ranging from 1214.2 to 1264.2 mg gallic acid kg⁻¹ for calcite and pruning, respectively (fig. 6). Total phenol of all the grapes decreased during the first month and then increased significantly up the second month of storage, regardless of the treatments. After a slight decrease, total phenol significantly varied from 1257.2 to 1355.8 mg gallic acid kg⁻¹ for pruning and calcite plus pruning treatments, respectively. Cefola et al. [2011] revealed that neither preharvest soil factor nor postharvest modified atmosphere condition affected the total phenolic content of the grape cv. 'Victoria' during 15 day storage at 5°C. Previously, Cantwell [1995] also detected low variations in such attributes during the cold storage of various nonclimacteric horticultural commodities. Considering these literature examples, the current findings could most probably be attributed to the postharvest physiological characteristics of table grape, which is classified as a non-climacteric fruit, as already indicated by Kader [2002].

CONCLUSIONS

Preharvest micronized calcium sprays on leaves and clusters, with or without leaf removal pruning, markedly extended the postharvest quality of grapes by delaying weight loss, reducing decay, maintaining rachis chlorophyll concentrations and preserving visual quality during the cold storage at 1°C, 90% RH up to 3 months. External calcium sprays also

retained must acidity and corresponding maturity index during prolonged storage. Overall findings indicated that preharvest calcium treatments may be an environmental-friendly, healthy and sustainable treatment for extending postharvest quality of grapes, without adverse effect on postharvest physiology of produces, and thus may be considered as an effective alternative for common chemical treatments.

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