Sprays Mixing Resveratrol and Benzylaminopurine Previous Harvest Helps to Preserve Postharvest Quality in Cherimoya

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Abstract

Previously, separated sprays of 1.6 mM resveratrol (RVS) and 1.0 mM 6-benzylaminopurine (BAP) before harvest of cherimoya fruit (Annona cherimola Mill.) reduced the postharvest rate of pulp and peel softening, and the loss of peel color, respectively. Aim of present research was to reduce both, postharvest fruit softening and peel color loss in cherimoya 'Ruth' and 'Fino de Jete' by applying a mixing solution of 1.6 mM resveratrol and 1.0 mM benzylaminopurine at 8 or 15 days before harvest. After fifteen days stored at room temperature, the color parameter L, indicating the darkening of the fruit peel, was reduced up to 41% for 'Ruth' and 58% for 'Fino de Jete' by RVS and BAP sprays. RVS and BAP reduced fruit and peel softening in 'Ruth'; but higher effect, i.e. over 100 N and 60 N in relation to control at 7 days in storage, was observed in 'Fino de Jete' fruit and peel, respectively. The higher enzymatic activity of polygalacturonase (PG), possible inducing fruit and peel softening, was retarded for two days in RVS-BAP treated fruit in relation to control.*

Keywords: fruit softening, pectinmethylesterase, peel color, polygalacturonase, postharvest quality.

Introduction

The cherimoya (*Annona cherimola* Mill.) fruit has exceptional nutritional and sensory qualities (Vanhove and Van Damme, 2013). However, in part due to cherimoya fruit high perishability, most of the production of this commodity is usually sold only in local markets, near the production areas (Pinto *et al*., 2005). After harvest cherimoya fruit ripens quickly, becoming quite soft, the peel darkens, and it becomes quite difficult to handle without damaging the commodity. Thus, it does not preserve well for shipping and retail sale (Leal, 1990; Morales *et al*., 2014). Recommendations for handling and store cherimoya fruit include its cold storage at temperatures around 8 and 15°C (Pareek *et al*., 2011). Moreover, the use of control atmospheres reduce fruit softening, ethylene production, and keep the chlorophyll content of the peel (Del Cura *et al*., 1996); heat shock treatments with water vapor raising between 48 and 52°C for 60 min reduced fruit ripening (Alique *et al*., 2009). Recently, the addition of 1.6 mM of resveratrol (RVS), 15 or 8 days before harvest, reduced the rate of cherimoya fruit softening with or without simulation of transport (Morales *et al*., 2014; Morales *et al*., 2016). On the other hand, spraying 1.0 mM benzylaminopurine (BAP), 15 or 8 days before harvest, reduced the peel color decay in cherimoya fruit 'Fino de Jete' (Morales *et al*., 2015).

RVS is a stilbene related to grapevine resistance to fungal diseases ant it is biosynthesized in response to biotic and abiotic stresses (Adrian *et al*., 1997). Moreover, RVS exogenous application increased storage life in apple (*Malus communis* L.), avocado (*Persea americana* Mill.), tomato (*Solanum lycopersicum*), pepper (*Capsicum annuum*), strawberry (*Fragaria* × *ananasa*) and grapes (*Vitis vinifera*) (Jiménez *et al*., 2005); whereas in mandarin 'Satsuma' (*Citrus unshiu* Marc.) inhibited the discoloration of the peel; former effect was attributed to the antioxidant properties of the stilbene (Cherukuri *et al*., 2007). Van Buren (1986) suggested that RVS might promote cell-wall lignification and prevent hydrolysis of pectic polymers; Morales *et al*. (2014, 2016) hypothesized that this former property of RVS might be related with the beneficial results obtaining with this plant phenolic reducing cherimoya fruit postharvest softening. On the other hand, it was found that RVS improved photosynthetic efficiency; enhancing the green color of the leaves along with increased resistance to some plant pathogens in wheat plants (Pociecha *et al*., 2014).

The BAP has been used to extend the shelf life of some horticultural commodities. For example, in summer squash (*Cucurbita maxima*) kept the fruit quality during storage by slower dehydration and remained firmer than the control (Massolo *et al*., 2014). Although in summer squash color was not affected by 1 mmol l^{-1} BAP (Massolo *et al*., 2014), the application of 50 ppm BAP to broccoli (*Brassica oleracia italica*) florets maintained the hue angle for over 100 h, whereas in the control, the hue angle decreased after 70 h at 20°C, in darkness (Downs *et al*., 1997). Further research has determined that exogenous application of BAP in broccoli reduced the degradation of photosynthetic proteins (Costa *et al*., 2005) and the expression of chlorophyll degrading genes (Gómez-Lobato *et al*., 2012). Specifically in fruit crops, addition of 10^{-3} M of BAP to banana (*Musa acuminata*) retarded the loss of chlorophyll a and b, along with the accumulation of monoacylglycerol, triacylglycerol and free fatty acids in the fruit peel (Aghofack-Nguemezi and Manka'abiengwa, 2012). Similar results were reported in litchi fruits (*Litchi chinensis*) applying 100 mg l^{-1} BAP, the reduced loss of chlorophyll in the pericarp was related to lower activity of chlorophyllase (Wang *et al*., 2005). For cherimoya 'Fino de Jete' the addiction of 1 mM BAP resulted in a reduction of postharvest darkening of the fruit peel by 38% (Morales *et al*., 2015).

As in other regions of Latin America, one of the challenges in the cherimoya chain production is to improve the postharvest handling. Thus, in this work, the objective was to determine if the previous observed beneficial results of separated pre-harvest application of RVS and BAP in cherimoya fruit could resulted and even improved with a mixed RVS-BAP solution.

Materials and methods

Vegetative material

For present work, in 2012 we used the world widely cropped cherimoya fruit 'Fino de Jete' and a local selection, named 'Ruth'. Cherimoya fruit of 'Ruth' were produced from open-pollination in a commercial orchard located in Achichipico, Yecapixtla, Morelos, Mexico (18° 94' 62"N, 98° 82' 75" W and 1944 m elevation). Fruits of cherimoya 'Fino de Jete' were hand-pollinated in the same season in an experimental orchard at the Center for Scientific and Technological Research of the State of Mexico (CICTAMEX), Salvador Sánchez Colín Foundation, located in Coatepec de Harinas, Mexico (18° 46' 38" N, 99° 46'38"W and 2240 m elevation).

Application and handling

A distilled water solution containing 1.6 mM RVS and 1.0 mM BAP, both reagents from SIGMA, or a control solution (only distilled water) was applied by brush (Morales *et al*., 2014, 2015) to 81 randomly selected fruit growing in the middle and basal parts of 6 trees, in each orchard, at 15 or 8 days before harvest (DBH). Fruit selection was based on the day of fruit set and the color peel at the time of application (Morales *et al*., 2014, 2015). The amount of RVS-BAP solution was approximately 3 ml per fruit. Once the fruits were harvested, they were conventionally packaged and transported to the Laboratory of Horticulture, Autonomous University of the State of Mexico. The fruit was stored at room temperature (RT), i.e., between 14 and 18 ºC. Subsequently, they were analyzed every third day starting one day after harvest and lasting till 15 days after harvest (DAH).

Postharvest analysis

Changes in fresh weight, skin color with a photo-colorimeter, fruit and peel firmness with a texturometer, and concentrations of reducing sugars and ascorbic acid were recorded as described previously by Morales *et al*. (2014). The activity of pectinmethylesterase (PME) was determined in the peel of the fruit as described by Hagerman and Austin (1986).

Briefly, approximately 5 g of plant tissue was processed in an omnimixer with 15 ml of 1 M NaCl and 10 g l^{-1} PVPP. The suspension was stirred for 4 h and then filtered through Whatman No. 42 filter paper. The filtrate was adjusted to pH 7.5 with NaOH, and the extract was used to test the PME activity. All steps in the preparation of extracts were performed at -4 °C. The activity was assayed in a reaction mixture containing 600 µl of pectin 0.5% w/v, 150 µl of bromothymol blue 0.01% in phosphate buffer 0.003 M pH 7.5; 100 µl of water pH 7.5 and 100 µl of enzyme extract. The reduction of the optical density was determined at 620 nm at 37 °C. The results were expressed as the change in optical density (ΔOD) in three minutes per gram of peel. The activity of polygalacturonase (PG) in cherimoya peel was tested using the method of MacDonnell *et al*. (1945). The reaction mixture consisted of 2.5 ml of polygalacturonic acid 0.2% (w/v) and 0.75 ml of the enzyme extract. This solution was incubated at 30 °C for one hour, filtered with Whatman No. 1 filter paper, and the loss of viscosity was compared to the control. The activity was expressed as the percentage of decrement in viscosity after one hour at 30°C.

Statistical analysis

The results were analyzed using a completely randomized design; based in previous reports (Morales *et al*., 2014) and the results immediately after harvest, each cultivar was statistically analyzed individually with RVS-BAP doses as the fixed factor in the software SPSS19. When the F value was significant, a comparison of means with Tukey's test ($\alpha < 0.05$) was also performed.

Results and discussion

As expected, cherimoya fruit quality is so far related to genetic conditions and there were differences between the fruit quality of ʿFino de Jete' and 'Ruth' for all variables but the color factor b*, reducing sugar and ascorbic acid contents (data not shown).

Color L*, a*, b*

For luminosity (L*), factor a* and b*, in both cultivars there was significant difference between the application of RVS and BAP ($\alpha \leq 0.05$) and control fruits (Figure 1). For valor L*, in 'Ruth' the application at 8 DBH generated more brilliant color fruits than treatment at 15 DBH and control; i.e. after 15 days in storage control fruits presented 15 and 21 units less than cherimoya treated at 15 or 8 DBH. In 'Fino de Jete', the fruits treated at 15 or 8 DBH were 12 units more brilliant than control fruits at the end of the storage time. In both, 'Ruth' and 'Fino de Jete', for color a*and b* the control fruits were more red (a*) and less yellow becoming green-blue (b*), implying the start of tanning, than those cherimoyas treated with RVS-BAP. Fruits treated with RVS-BAP remained green and yellow (Figure 1).

Morales *et al*. (2014) reported that the application of RVS in cherimoya 'Fino de Jete' did not prevent the development of a tanned peel, but the application of 1.0 mM BAP reduced the degreening rate of the peel in cherimoya 'Fino de Jete' stored at room temperature (Morales *et al*., 2015). It is known that after harvest, the peel color becomes darker, a regular process due to enzymatic oxidation by polyphenol oxidase (PPO) and peroxidase (POD) (Prieto *et al*., 2007). However, in some fruits and vegetables, application of BAP maintained the green color of harvest during postharvest (Zhu *et al*., 2004; Zaicovski *et al*., 2008; Aghofack-Nguemezi and Manka'abiengwa, 2012), by reducing chlorophyll loss and the activity of chlorophyllase (Wang *et al*., 2005). Although in wheat RVS increased the photosynthetic rate, according to previous experiences (Morales *et al*., 2014, 2015) in this work the reduction in green peel color might be attributed to BAP.

Fruit weight loss and softening

The results showed that in both cultivars, there was significant difference ($\alpha \leq 0.05$) among treatments in the kinetics of fruit weight (Figure 2) and fruit and peel softening (Figure 3). The application of 1.6-1.0 mM RVS-BAP, on both dates, reduced the rate of weight loss by 5.5% for 'Ruth' and by 9.9% for 'Fino de Jete' at the end of the storage time, in relation to control. On the other hand, when RVS-BAP was applied at 8 DBH, the fruit softening rate was reduced by 6.5% and 7.2%, respectively, for 'Ruth' and 'Fino de Jete'; whereas in the fruit treated 15 DBH, the rate of fruit softening was reduced by 3.1% and 10.3%, respectively. For the peel, in 'Ruth', when RVS-BAP were applied at 15 DBH, the prevention of softening in almost all the stored period, was greater (5.2%) ($\alpha \leq 0.05$) than those results presented for 8 DBH application and control. For 'Fino de Jete', application at 8 and 15 DBH were clearly superior to control with an interesting efficiency at 7 DAH with over 60 N of difference in relation to control.

PME and PG activities increased in both 'Ruth' and 'Fino de Jete', in the first days after harvest. However, the maximum activity of PME was observed at 5 DAH in both, treated and non-treated fruit; but for PG activity, the higher value for non-treated fruit was observed almost two days earlier than in treated fruits (Figure 4).

Morales *et al*. (2014) decreased the rate of cherimoya fruit softening by 78% and 54% for 'Fino de Jete' and 'Bronceada', respectively, by applying 1.6 mM RVS at 8 or 15 DBH; but the addition of 1.0 mM BAP did not reduce fruit softening rate but reduced fruit weight loss (Morales *et al*., 2015). In this work, application of those both products, RVS and BAP, reduced fruit weight loss and softening, that means a double effect in relation of previous reports. Goñi *et al.* (1997) mentioned that water loss in harvested fruits is due to the intrinsic physiological processes of respiration, transpiration and osmotic adjustments; whereas Jiménez *et al*. (2005) suggested that fruit firmness is closely related to dehydration. Thus, reduction in water loss has been related to stabilization and strengthening of the cell walls (Conway and Sams, 1983). In other vegetables, the effect of BAP reducing weight loss has been related to a cell wall reinforcement (Massolo *et al*., 2014) and reduction in the respiration rate (An *et al*., 2006). On the other hand, Van Buren (1986) explained that because RVS is a polyphenol, it promotes cell-wall lignification, preventing hydrolysis of the pectic polymers. Present effect of BAP and RVS strengthening the firmness of the peel promotes cherimoya fruit resistance to handling and transportation (Morales *et al*., 2014).

Several authors have pointed out that postharvest cherimoya softening is related to the increase of the activities of PG, PME, endoglucanases, xyloglucan and genes related to endotransglycosylases and expansins (Escribano *et al*., 1997; Sánchez *et al*., 1998; Li *et al*., 2009). Results of present work agreed with those authors observing an increase in PG and PME activities along with the fruit softening process. Our observations support the idea of the effectiveness of RVS reducing the rate of softening in cherimoya (Morales *et al*., 2014) and show that this effect is not lost when RVS is applied in combination with BAP. For this work, PG activity seems to be closer related to softening of cherimoya fruit as its higher activity was retarded two days in fruit treated with BAP and RVS in relation to control (Figure 8). Similar results were obtained in custard apple fruit (*Annona squamosa*) after dipping fruit in hot water, which resulted in lower softening rate (Vyas *et al*., 2015).

Reducing sugars and ascorbic acid

The values for reducing sugar in cherimoya 'Ruth' presented differences ($\alpha \leq 0.05$) for some days, but they were inconsistent along the postharvest period. On the other hand, in 'Fino de Jete', from day 7 of storage, the control fruits always had lower values than those observed with the application of RVS-BAP. Previously, Morales *et al*. (2014) reported that 15 DAH the reducing sugar amount in cherimoya 'Fino de Jete' treated with 1.6 mM RVS at 8 DBH was lower than control fruit; they explained this effect suggesting that RVS reduced senescence, which may, in turn, reduce the rate of synthesis of reducing sugars. The contraire effect observed in this work might be related to the effect of BAP or a possible effect of the environment, as the year reported by Morales et al. (2014) is different to the year of this study. However, this report reaffirms that RVS and BAP has no adverse effects on cherimoya fruit sensory quality (Morales *et al*., 2014, 2015); in complement, for ascorbic acid, there were any significant differences among treatments ($\alpha \leq 0.05$). A decrease in ascorbic acid content (data not shown) was observed in both cultivars from harvest to ripening, as reported previously (Morales *et al*., 2014).

Conclusions

Preharvest application of a solution containing 1.6 mM resveratrol and 1.0 mM benzylaminopurine may improve the handling and transportation of cherimoya fruit by reducing the peel and flesh softening along with reduction in peel color loss. For color, application of these compounds 8 days before harvest was better for cherimoya 'Ruth' whereas for cherimoya 'Fino de Jete' both dates generated similar beneficial results. For fruit and peel softening, the application 15 days before harvest was better for both cultivars. Higher activity of polygalaturonase was retarded two days in treated fruit in relation to control. Any constant effect in reducing sugars and ascorbic acid contents was observed. Use of plant phenolics and cytokinins have potential for improving postharvest of cherimoya fruit.

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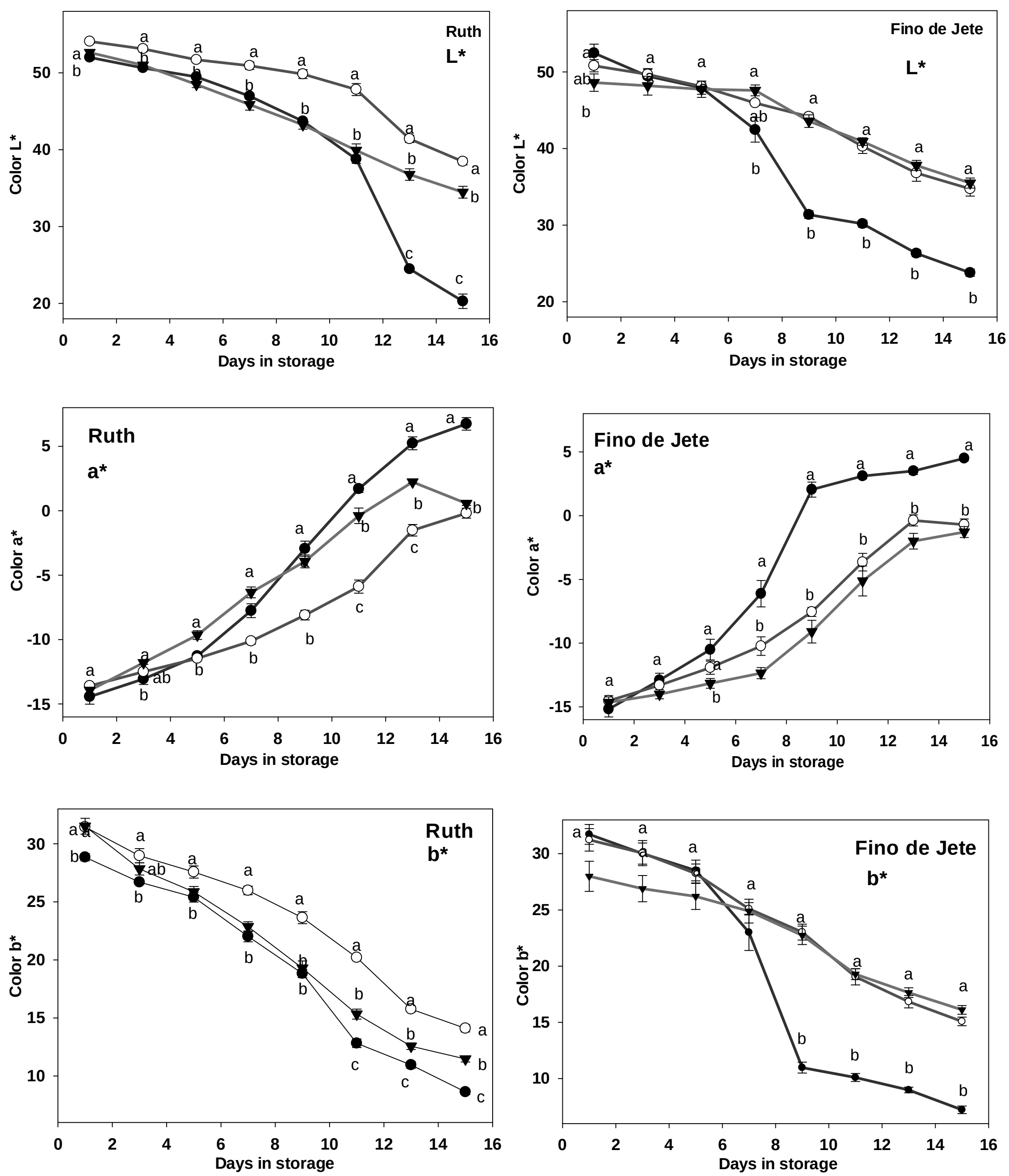

Figure 1. Color L*, a*and b*in ‘Ruth’ and ‘Fino de Jete’ cherimoya fruits stored at room temperature after preharvest treatment with 1.6 mM resveratrol - 1.0 mM 6-benzylaminopurine, at 8 (○) or 15 (▼) days before harvest and control (●). Different letters indicate significant difference in relation to days in storage. Data are the average of 9 replications, one fruit each ± EE.

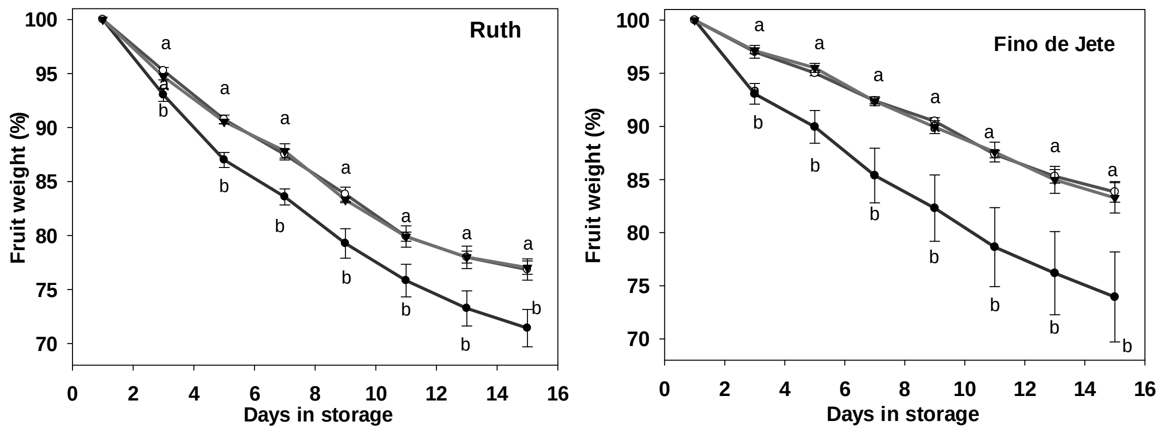

Figure 2. Weight loss in ‘Ruth’ and ‘Fino de Jete’ cherimoya fruits stored at room temperature and previously treated with 1.6 mM resveratrol - 1.0 mM 6-benzylaminopurine, at 8 (○) or 15 (▼) days before harvest and control (●). Different letters indicate significant difference in relation to the day in storage. Data are the average of 9 replications, one fruit each ± EE.

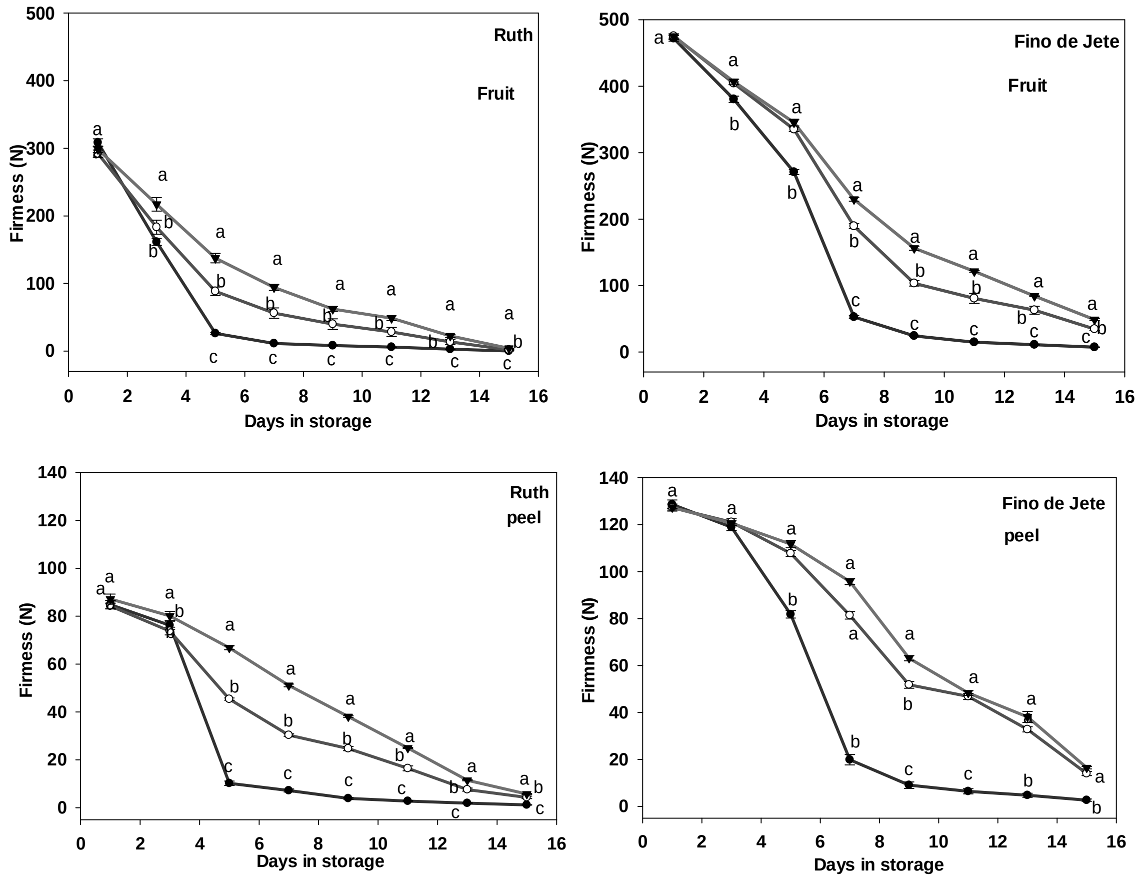

Figure 3. ‘Ruth’ and ‘Fino de Jete’ cherimoya fruit and peel firmness, following storage at room temperature and previous application of 1.6 mM resveratrol - 1.0 mM6-benzylaminopurine, at 8 (○) or 15 (▼) days before harvest and control (●). Different letters indicate significant difference in relation to the day in storage. Data are the average of 9 replications, one fruit each ± EE.

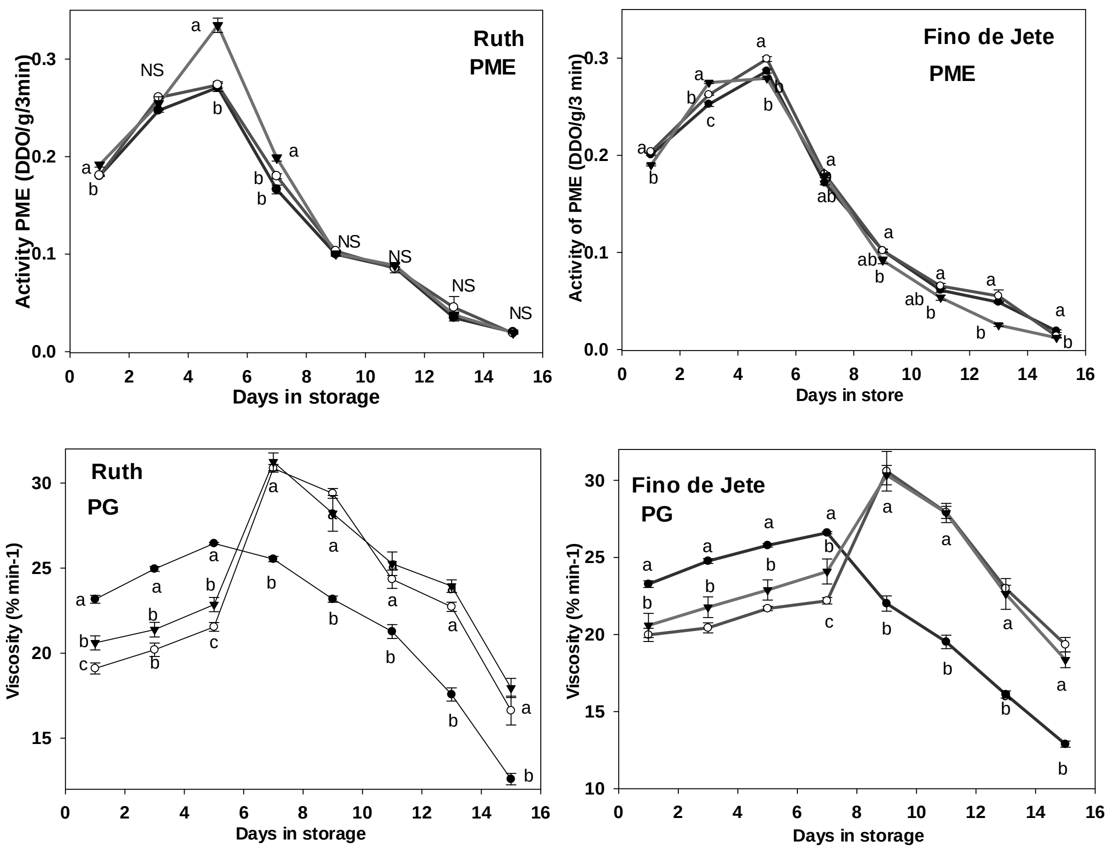

Figure 4. Activities of pectin methylesterase (PME) and polygalacturonase (PG) in ‘Ruth’ and ‘Fino de Jete’ cherimoya fruit stored at room temperature and treated previously with 1.6 mM resveratrol - 1.0 mM 6-benzylaminopurine, at 8 (○) or 15 (▼) days before harvest and control (●). Different letters indicate significant difference in relation to the day in storage. Data are the average of 9 replications, one fruit each ± EE.

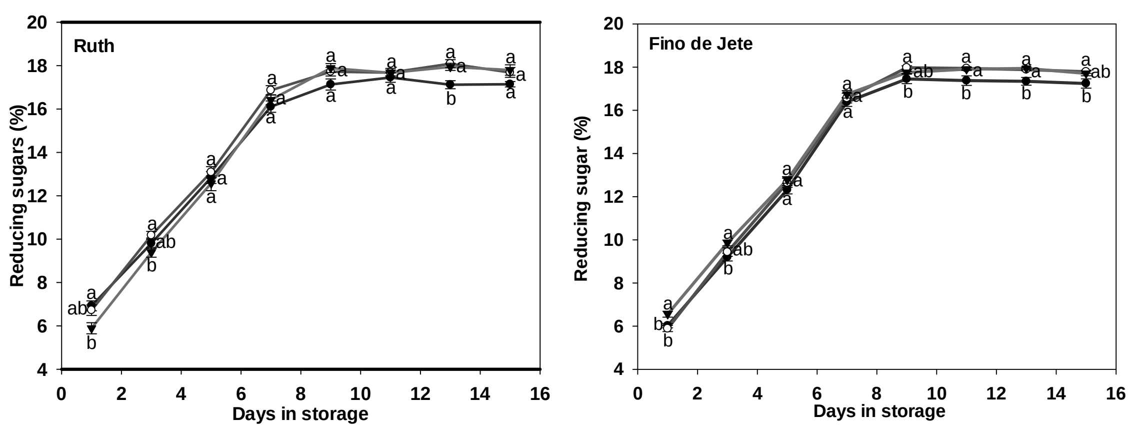

Figure 5. Reducing sugars in ‘Ruth’ and ‘Fino de Jete’ cherimoya fruit stored at room temperature after treatment with 1.6 mM resveratrol - 1.0 mM 6-benzylaminopurine, at 8 (○) or 15 (▼) days before harvest and control (●). Different letters indicate significant difference in relation to the day in storage. Data are the average of 9 replications, one fruit each ± EE.