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Physical and metabolic changes induced by mechanical damage in 'dwarf-prata' banana fruits kept under cold storage

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Abstract

The purpose of this work was to demonstrate the physical and metabolic alterations induced by mechanical damage on 'Dwarf-Prata' banana fruits kept under low temperature (15 ºC and 89 % of RH). A split plots in time assay within a randomized complete block design with three replications was used, constituted of control and four mechanical injuries: cut, abrasion, impact and compression, with samplings along time. Evaluated characteristics were: percentage of accumulated weight loss, daily rate of weight loss, electrolyte leakage of the damaged peel region, soluble sugars and starch, respiration, ethylene production and enzymatic activity of polyphenol oxidase (PPO) and peroxidase (POD). The percentage of accumulated weight loss, the daily rate of weight loss, respiration and ethylene production were determined daily on duplicate samples, consisting of three fruits. Electrolyte leakage, soluble sugar levels and starch and the PPO and POD enzymes activities were determined at 1, 3, 5, 8 and 11 days after the mechanical injuries treatments. The results show that all fruits subjected to mechanical injury increase weight loss (%), electrolyte leakage (%) and PPO average activity, also showing accelerated peel color evolution rate and anticipated climacteric peak, compared to control. The damage caused by abrasion caused higher accumulated weight loss (%). The starch conversion to soluble sugars in the pulp was affected by impact damage. The impact and compression damages anticipated climacteric ethylene peak and, consequently, fruit ripening. The impact damage greatly increased PPO and POD activities.

Keywords: polyphenol oxidase, peroxidase, peel color index, darkening, post-harvest.
Abbreviations: PPO_polyphenol oxidase, POD_peroxidase.

Introduction

Brazilian production of bananas is predominantly focused on the domestic market, usually being harvested, handled and transported in a defective and inadequate way, contributing to substantial losses in the post-harvest phase (Alves, 1997), which are very important from the economic and nutritional standpoint (Dadzie and Orchard, 1997). Mechanical damage is a major cause of post-harvest losses in banana fruits (Dadzie and Orchard, 1997; Ferris et al., 1995; Lladó and Dominguez, 1998), resulting in reduced shelf-life and non-acceptance of the fruit by the consumer. Mechanical damage can occur at any point in the supply chain of the 'Dwarf-Prata' banana – *Musa* ssp. (Maia et al., 2007), due to the technology used in harvest and post-harvest (Li and Thomas, 2014). Banana fruits show physical and physiological responses to mechanical damages that are mainly linked to the appearance, with advanced symptoms of necrosis in the damaged region (Lladó and Dominguez, 1998; Li and Thomas, 2014). The fruit that undergoes mechanical injury ripens faster due to increased respiration and ethylene production, greater mass loss (water loss), higher invasion of microorganisms (Dadzie and Orchard, 1997; Lladó and Dominguez, 1998; Zeebroeck et al, 2007; Fischer et al, 2009; Pedreschi et al., 2013; Li and Thomas, 2014; Bugaud et al., 2014) and higher enzymatic activity in the damaged area (Lladó and Dominguez, 1998; Li and Thomas, 2014; Bugaud et al., 2014). The increased respiration rate is considered responsible for the production of compounds that offer resistance to microbiological attack. However, according to Zhu et al. (2012), ethylene production by *Botritis cinerea* contributes to ethylene production in the *B. cinerea*–grape system, and ethylene may be involved in modulating both hyphal growth and pathogenesis. Ethylene evolution on the wound may trigger the ripening of injured and healthy fruits nearby, stored in the same box, chamber or deposit (Chitarra and Chitarra, 1990; Kuang et al., 2013). The result of these changes is a poor quality product (banana fruits) with high rates of post-harvest losses (Dadzie and Orchard, 1997; Li and Thomas, 2014). The injury also promotes disruption of epidermal cells, triggering enzymatic reactions and, consequently, the appearance of darkened areas in the affected region due to the leakage of cell liquid and exposure to enzymatic action, which promotes the oxidation of phenolic compounds into quinones (Radiet al., 1997). In response to certain types of stress, there is also an increased activity of enzymes responsible for defensive mechanisms of plant tissues, such as peroxidase (Lagrimini, 1987), which is connected to the synthesis of lignin (Lagrimini, 1987) and polyphenol oxidase, which is involved in the darkening of the damaged region (Mayer and Harel, 1979; Bower and Cutting, 1988), as a result of the oxidation of phenols (Enzimelab, 2004). There are numerous studies in the literature reporting the effects of low temperatures in the preservation of fruits by reducing the metabolic activities directly or indirectly related

to the ripening process (Marriott, 1980; Kays, 1991; Rocha, 2005).
However, there are few reports on the effects of cold on fruits subjected to mechanical injuries. The objective of this study was to determine the physical and metabolic changes induced by mechanical stress in 'Dwarf-Prata' bananas kept in a cold chamber.

Results

Fresh mass loss

The fresh mass loss (%) of 'Dwarf-Prata' banana fruits showed a linear increase over time in all treatments. However, the control fruits presented, after 11 days of evaluation, 1.14% fresh mass loss. Meanwhile, the values of fresh mass loss of fruits subjected to damage by cutting, abrasion, impact and compression after 11 days of evaluation were 2.42%, 3.68%, 1.84% and 1.54%, respectively (Fig. 1). At the end of the evaluations, the fruits damaged by cutting and abrasion had lost 11.2% and 11.8% of fresh mass, while the fruits damaged by impact, compression and control had lost 9.7%, 8.1% and 8 4%, respectively, nine days after treatment. There was no significant effect of the damage x time interaction regarding fruits daily fresh mass loss (%). However, the damage by abrasion caused average loss in daily fresh mass (%) significantly superior to the other treatments. Furthermore, the damage by cutting caused average daily fresh mass loss (%) of the fruits superior to the control, while the other treatments did not differ from it (Table 1). Although the daily fresh mass loss tends to be higher in fruits damaged by cutting and abrasion than in fruits from other treatments, the effect of the type of mechanical damage in the daily fresh mass loss was not significant. The loss of fresh mass weight was of 1,1, 1,4, 1,4, 1,2, 1,1% for control, cutting, impact, abrasion and compression, under room temperature, respectively (Maia et al., 2011).

Electrolyte leakage

Electrolyte leakage (%) is related to the integrity of the plasma membrane, being inversely proportional to it (Fergunson and Watkins, 1981). Because of that, it was observed, in all treatments, increases in electrolyte leakage (%) during time (Fig. 2), indicating, as expected, the integrity loss of the plasma membranes of the fruit cells, as the ripening and senescence occur processes (Kays, 1991).
All fruits that suffered mechanical damage showed electrolyte leakage values (%), starting from the 5^{th} evaluation day, higher than the control values. Fruits subjected to abrasion injury showed, at the end of the evaluations, higher electrolyte leakage than the other treatments (Fig. 2). That indicates that abrasion severely affects, in the spot it was applied, the integrity of the plasma membranes, although it does not promote fruit ripening before the other studied injuries (Fig. 5), which could explain the higher electrolyte leakage (%) values. Total electrolyte leakage of the fruits in room temperature was of 64,1% in the last evaluation day, while the fruits damaged by cutting, impact, compression and control achieved values of 62,9%, 47,2%, 44,5% and 36,2% (Maia et al., 2011).

Sugars and starch content

The soluble sugars content in the pulp of 'Dwarf-Prata' bananas increased during time in all treatments (Fig. 3). Compression and abrasion damages caused, at the end of evaluations, the highest total sugar contents in the pulp, 17.1 and 16.5%, respectively. Fruits subjected to damages by cutting and impact, on the other hand, showed at the end of the evaluations the lowest sugars content values, 15.0% and 15.1%. Fruits subjected to impact damage showed at the end of the evaluations the lowest values of soluble sugars contents and highest starch contents in the pulp, compared to the other treatments (Figs. 3 and 4). It was observed, in the fruits of all treatments, a reduction in starch content in their pulps during the evaluation period. According to the coefficients of the adjusted models, the conversion speed of starch into sugars increased in fruits subjected to damage by abrasion and compression (Fig. 4). The soluble sugars content in the pulp increased in the fruits of all treatments during the evaluation period of mechanical damage at room temperature, while starch levels decreased (Maia et al., 2011). The soluble sugars content in fruits injured by cutting, impact, abrasion and compression reached 23.9%, 21.5%, 18.2% and 23.0%, respectively, at the end of the evaluation period, while the control reached 20.1% at room temperature (Maia et al., 2011). In the last evaluation day (sixth day), the starch content in the pulp of the fruits damaged by impact kept at room temperature was 7.2%, whereas the starch content in the pulp of the fruits damaged by cutting, abrasion, compression and control were 3.8%, 2.1%, 2.9% and 6.7%, respectively (Maia et al., 2011).

Peel color index

All treatments showed increases on peel color index over time (Fig. 5), which characterizes fruit ripening. The mechanical damage treatments showed higher speed of peel color index evolution compared to control, and the fruits subjected to damage by impact and compression showed the highest values. Fruits subjected to these two types of mechanical damage were the only ones to reach values close to the peel color index 7, while the damages by cutting and abrasion indexes reached the peel color index above and close to 6 and the control a peel color index below 6 (fig. 5).

CO_2 and ethylene production

All mechanical injury treatments caused anticipation of the climacteric peak of CO_2 production of 'Dwarf-Prata' bananas compared to the control. Fruits damaged by cutting, abrasion, impact and compression reached the climacteric peak in the eighth (66.81 mg kg^{-1} h^{-1} of CO_2), seventh (67.32 mg kg^{-1} h^{-1} of CO_2), sixth (74.79 mg kg^{-1} h^{-1} of CO_2) and fourth (73.56 mg kg^{-1} h^{-1} of CO_2) day after treatments (Fig. 6), when these fruits showed peel color index between 4 and 5. Compared to ethylene production in 'Dwarf-Prata' bananas, only the fruits subjected to damage by impact and compression showed anticipation of the peak, at four and six days after treatment, respectively. The control and the fruits subjected to damage by abrasion and cut showed maximal ethylene production seven days after treatment (Fig. 7).

Polyphenol oxidase and peroxidase

With the exception of fruits subjected to damage by impact, there was no peak of polyphenol oxidase (PPO) activity during the evaluation period (Fig. 8). Treatments of damage by cutting, abrasion, impact and compression showed overall average PPO activity of 424, 490, 551 and 464 units g^{-1}, respectively. The control, in turn, showed a mean of 420 units g^{-1}. These results indicate that mainly the damages by abrasion and impact cause increased activity of this oxidative enzyme. The maximum peroxidase (POD) activity in the peel of not subjected to mechanical damage 'Dwarf-Prata'

Table 1. Daily fresh mass loss (%) of 'Dwarf-Prata' bananas in function of types of mechanical damage.

Control	Cutting	Abrasion	Impact	Compression
0.13 c	0.25 b	0.37 a	0.20 bc	0.7 bc

Means followed by the same letter do not differ between themselves by the Tukey's test at a 5% level of significance ($p<0.05$).

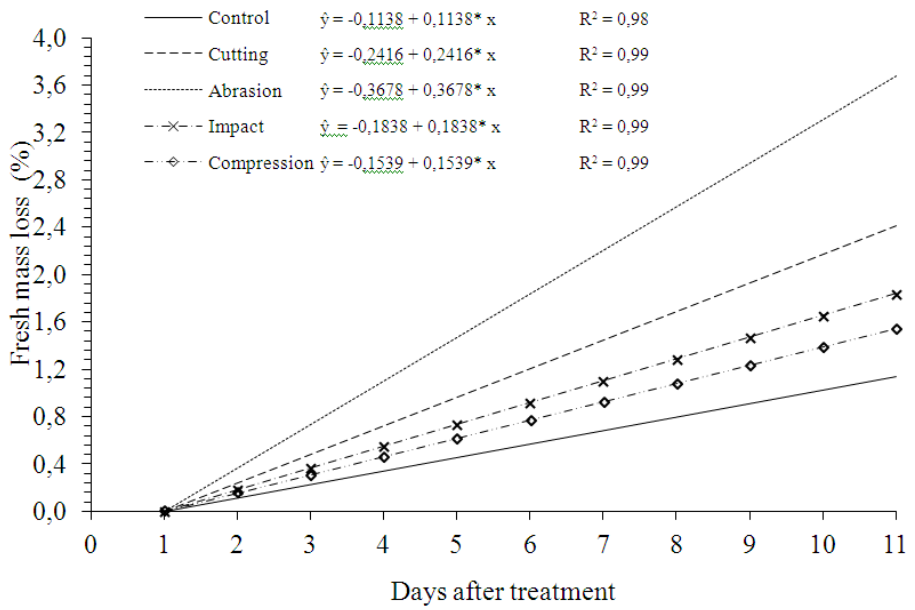

Fig 1. Fresh mass loss (%) of 'Dwarf-Prata' bananas during storage time at 15 °C and 89% RH, according to the type of mechanical damage. *Significant at 0.1% significance by t test.

bananas and in those subjected to compression and impact damages occurred on the fifth and eighth day after the treatment, respectively. In fruits subjected to damage by cutting and abrasion, POD activity increased up to 11 days after treatment (Fig. 9). The highest values observed were 319, 1793, 2094, 3793 and 120 units g^{-1} for the control and the damages by cutting, abrasion, impact and compression, respectively. As to damage by cutting, no POD activity peak was observed, but an increase of enzyme activity occurred until the last day of evaluation (4,860.7 units g^{-1}). At the peaks of POD activity, the values increased until 310.4%, 956.6% and 618.4% for fruits damaged by cutting, abrasion and impact, respectively, compared to the control.

Discussion

The results confirm that the damage by cutting and abrasion cause remarkable increase in fresh mass loss of fruits compared to control, even when they are kept at low temperature (15 °C) and high relative humidity (89%). According to Maia et al. (2011), an increased fresh mass loss of fruits was observed during the assessments in treatments at room temperature. According to Kays (1991), the maximum fresh mass loss allowed in many fruits without causing negative consequences to the appearance of the fruit is, on average, of 9.7%. However, as bananas are sold by weight along the market chain, damaged fruits, especially by cutting and abrasion, will bring greater financial loss. There was also severe dehydration in the affected region of the fruits damaged by abrasion and cutting over time. Injuries by cutting and abrasion make 'Prata' bananas more susceptible to fresh mass loss at room temperature (Maia et al., 2011). Abrasion severely affects, in the spot it was applied, the integrity of the plasma membranes, although it does not promote fruit ripening before the other studied injuries. The reason is that damages by abrasion cause breakdown of great part of the epidermal cells. Furthermore, the integrity of cell membranes is affected by all types of mechanical damages, since the electrolyte leakage can be used as a reference of the integrity of the membranes or modifications in selective permeability (Fergunson and Watkins, 1981). In addition, during ripening the membranes gradually lose their selective permeability and breakdown (Kays, 1991). Zhou et al. (2007) also observed that mechanical damages affect the plasma membrane in pears. Sugars content increased during time in all treatments, demonstrating fruit ripening, which is also followed by this phenomenon (Salomão, 1995; Wills et al., 1998). Such content increases with ripening due to hydrolysis of accumulated starch (Marriott, 1980; Kays, 1991), during fruit growing (Robinson, 1996). By the low values of soluble sugar contents (%) observed in the pulps of the fruits (Fig. 3), as well as by the high values of starch content (%) (Fig. 4), it may be presupposed that these ripening processes were not totally complete due to the low storage temperature. Beyond that, the lowest values of soluble sugars contents and highest starch contents in the pulp of the fruits subjected to impact, compared to the other treatments, suggest that this kind of injury compromises the conversion of starch into sugar in the affected region, and agree with the observations done by Maia (2001). Probably, the cells of the pulp affected by the impact injury collapse, compromising the activity of the enzymes that break the starch. Fruits of all treatments showed a sigmoid pattern for the soluble sugars contents (%) in the pulp. This behavior occurs in three different phases: the first phase is characterized by a slow increase, the second by an exponential increase and the third by stabilization. The reduction in the starch content in the fruits pulps results confirms the conversion of starch in soluble sugars while the fruit ripens. Furthermore, with the exception of the fruits subjected to damage by impact, the fruits damaged by

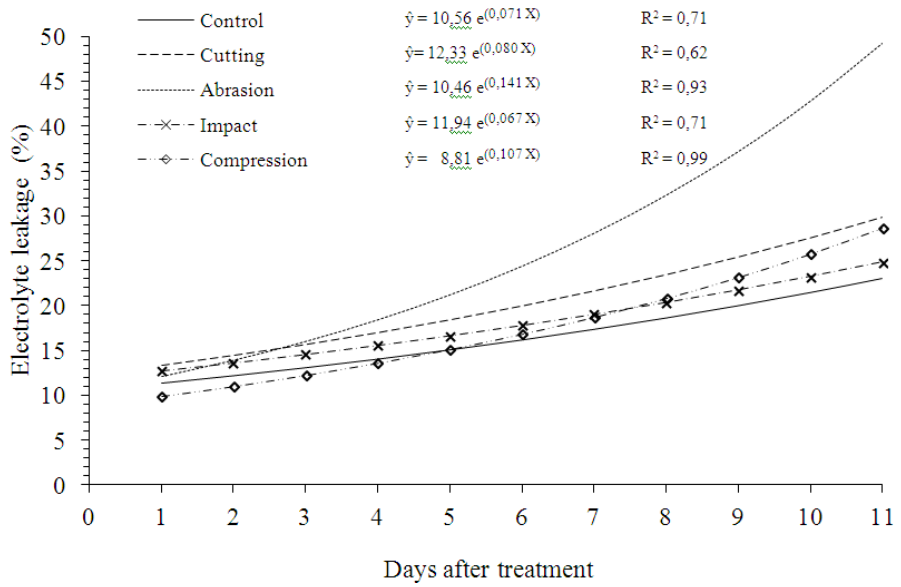

Fig 2. Electrolyte leakage (%) of 'Dwarf-Prata' banana peels during storage time at 15 ºC and 89% RH, in function of the type of mechanical damage.

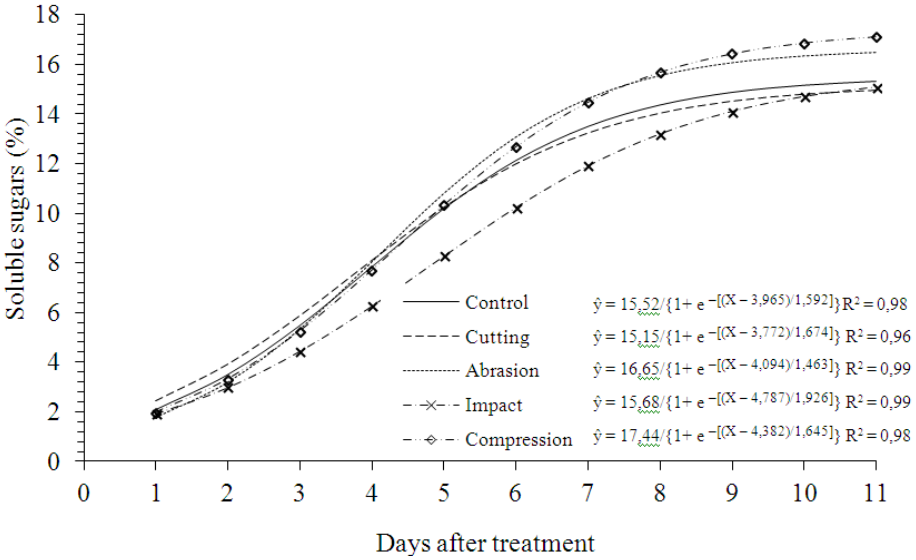

Fig 3. Soluble sugars (%) in the pulp 'Dwarf-Prata' bananas during storage time at 15 °C and 89% RH, in function of the type of mechanical damage.

cutting, abrasion and compression showed, at the end of the evaluations, inferior starch contents in the pulp than the control. These data agree with the ones observed on the evaluations of peel color stage, in which the fruits of these treatments appeared to be always in more advanced stages than the control. In general, the amounts of total sugars in the pulps of damaged fruits were higher than those in control fruits during the evaluation period. This indicates that mechanically damaged fruits have a faster conversion of starch into sugar, i.e., the pulp ripens faster. Mechanical damage stimulates the respiratory process and the biosynthesis of ethylene. The consequence is the increased activity of enzymes of starch degradation by α-amylase, β-amylase and glycosidases. Increased action of these enzymes accelerates degradation and available amount of sucrose and glucose, which are the main respiratory substrates to increase the respiration of the mechanically damaged fruit (Paliyathet al, 2008; Taiz and Zeiger, 2010). The evolution of the peel color index, described by Dadzie and Orchard (1997), results in degradation of chlorophyll, which allows visualization of carotenoids (ROCHA, 1984). This phenomenon occurs as the fruit ripens (Marriott, 1980, Wills et al., 1998). As the fruits that are in the peel color index 6 generally are in final stages of ripening and beginning senescence, the mechanical injury reduces the shelf life of 'Dwarf-Prata' bananas, contributing to the increase in post-harvest losses. Although all fruits submitted to mechanical injury have exceeded the peel color index 6 at the end of the evaluations, the levels of soluble sugars of these fruits were lower than expected for the 'Dwarf-Prata' bananas, the same way as the starch contents were higher than expected at this stage of ripening. This shows that, in this condition, of 15 °C and 90% relative humidity, the conversion of starch into sugars is slower than degradation of chlorophyll during the process of fruit ripening, i.e., there is a loss of synchronization between the two phenomena. This suggests that this temperature is not ideal for fruit ripening (Marriott, 1980). The anticipation of the climacteric peak of CO_2 production of 'Dwarf-Prata'

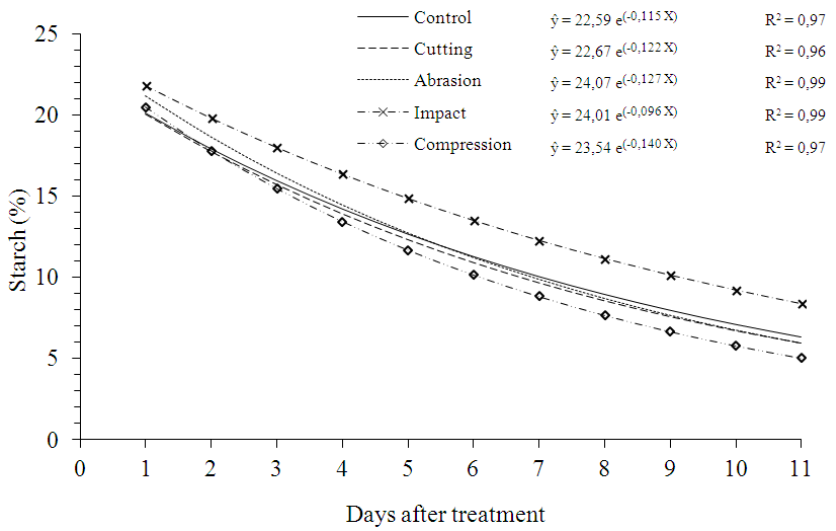

Fig 4. Starch (%) in the pulp of 'Dwarf-Prata' bananas during storage time at 15 ºC and 89% RH in function of type of mechanical damage.

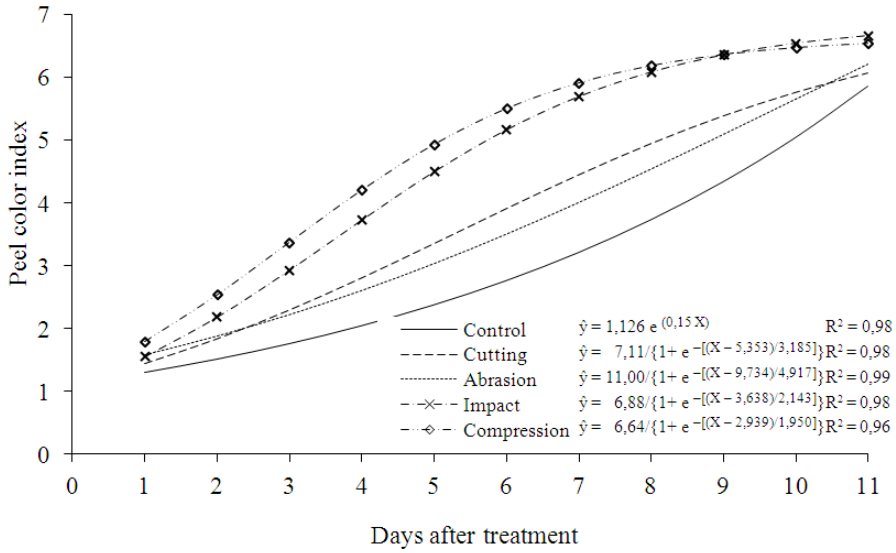

Fig 5. Evolution of peel color index of 'Dwarf-Prata' bananas during storage time at 15 °C and 89% RH in function of type of mechanical damage.

bananas compared to the control are consistent with the assertions of Dadzie and Orchard (1997) and Lladó and Dominguez (1998), who mention the anticipation of the climacteric peak of the banana in response to mechanical injury. This anticipation is related to the stress response of the fruit and, therefore, to the triggered defense processes (Taiz and Zeiger, 2010). The consequence of this is a faster ripening of this fruit and a reduced shelf life. As only the fruits subjected to impact and compression damage anticipated the peak of ethylene production furthermore to reducing shelf life of fruits subjected to damage by impact and compression by accelerating ripening, the evolution of ethylene from the wounded part may trigger the ripening of injured and healthy fruits nearby, stored in the same box, chamber or tank (Chitarra and Chitarra, 1990), resulting in higher losses. According to Maia et al. 2011, the PPO in the peel of 'Dwarf-Prata' bananas, under environmental conditions, reached the activity peak on the seventh day after treatment, when the fruits were damaged by impact (1,888.9 units g^{-1}), abrasion (1083.3 units g^{-1}) and cutting (718.3 units g^{-1}). Activity peaks of PPO were also reached in fruits damaged by compression (579.4 units g^{-1}) and in control fruits (571.4 units g^{-1}), showing the response of fruits to these lesions. These results indicate an increase of up to 25.7%, 89.6% and 230.6% in the enzymatic activity of fruits damaged by cutting, abrasion and impact, compared to the control. The PPO, when 'in vivo', is found attached to cell membranes and is activated only when released from these. When the cell membrane suffers some damage, the enzyme is released and activated, oxidizing phenolic compounds that are converted into quinines (Underhill and Critchley, 1995). Therefore, as seen for the results of electrolyte leakage, mechanical injury promotes destruction of the integrity of cell membranes and consequent mixing between substrate and oxidative enzymes, resulting in the darkening observed in the region affected by the cutting, abrasion and impact damages. The presence of these phenolic compounds in the region of injury is confirmed by the evaluated anatomical cuts. This confirms that the increase in PPO activity is

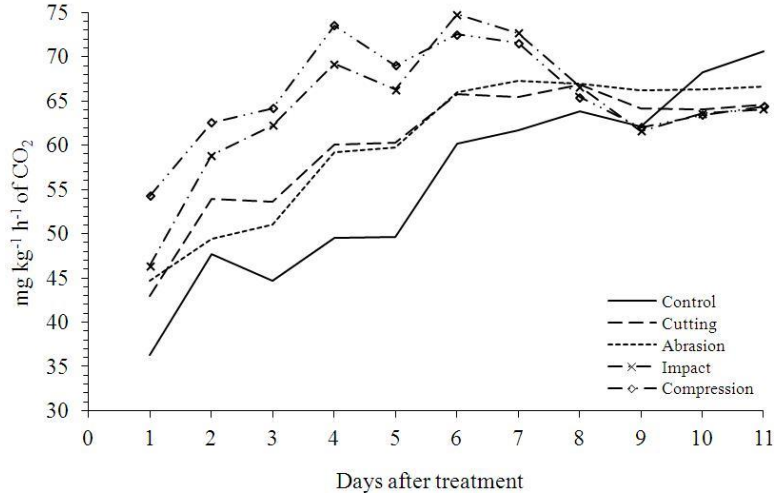

Fig 6. Respiratory rate of 'Dwarf-Prata' bananas during storage time at 15 ºC and 89% RH in function of type of mechanical damage.

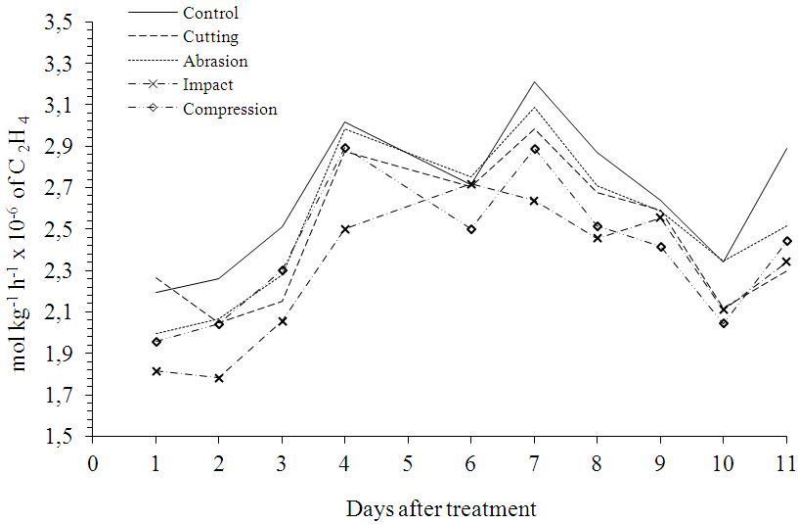

Fig 7. Ethylene production in 'Dwarf-Prata' bananas during storage time at 15 ºC and 89% RH in function of the type of mechanical damage.

directly related to cell disruption. It can be noted that the POD activity was greatly increased by the damages by cutting, abrasion and impact. Since this enzyme, besides being linked to the processes of oxidation of phenols and has activity related to stress (Mizobutsi, 2002; Enzimelab, 2004, International Working Group on Plant Peroxidases, 2004; Taiz and Zeiger, 2010), it becomes evident, along with an increase on the average PPO activity, the response of defense of fruits to mechanical injury. In mechanically damaged fruits kept at room temperature, according to Maia et al. (2011), POD activity reached a peak in fruits damaged by abrasion (12.515.3 units g^{-1}), impact (8,509.5 units g^{-1}) and compression (1,726.3 units g^{-1}) on the seventh day after treatment and in the control fruits (1,184.5 units g^{-1}) on the fifth day after treatment, although in this case the peak was less evident. The increase in the activity of these enzymes promote oxidative darkening of the injured area due to oxidation of phenols (Mizobutsi, 2002; Enzimelab, 2004, International Working Group on Plant Peroxidases, 2004; Taiz and Zeiger, 2010), resulting in unpleasant aspect of the fruit to the consumer, although, in most cases, it is suitable for consumption. By comparing the processes of daily and accumulated fresh mass loss (%), starch degradation and consequent formation of soluble sugars in the pulp, peel color evolution rate, production and peak of CO_2, PPO and POD activities in the peel of the fruits of the same treatment, stored in room conditions and in cold chamber, it was observed that those stored under room conditions have a higher intensity and speed of the phenomena. Therefore, maintaining the fruits at 15 ºC and 90% relative humidity may reduce the deleterious effects of mechanical damage, with exceptions to the content of soluble sugars, which was sub-optimal because of slow hydrolysis of starch, even with the fruits reaching peel color index close to 7.

Materials and Methods

Study site

The experiment was conducted in the Laboratories of Post-Harvest and Fruit Analysis from the Fruit Production Sector of the Department of Plant Sciences at the Universidade Federal de Viçosa. The 'Dwarf-Prata' (AAB) banana fruits (*Musa* spp.), with 36 ± 2 mm diameter, were obtained in a commercial orchard of the Cachoeira do Salto Farm, owned by Agropecuária Veloso Maia, located in the city of Verdelândia, Minas Gerais State (15º 24' S, 43º 43' W, 480

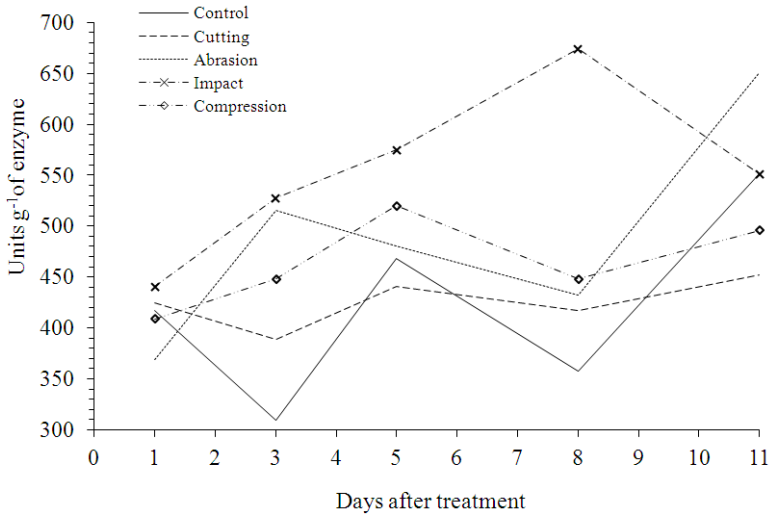

Fig 8. Polyphenol oxidase activity in the peel of 'Dwarf-Prata' bananas during storage time at 15 °C and 89% RH in function of type of mechanical damage.

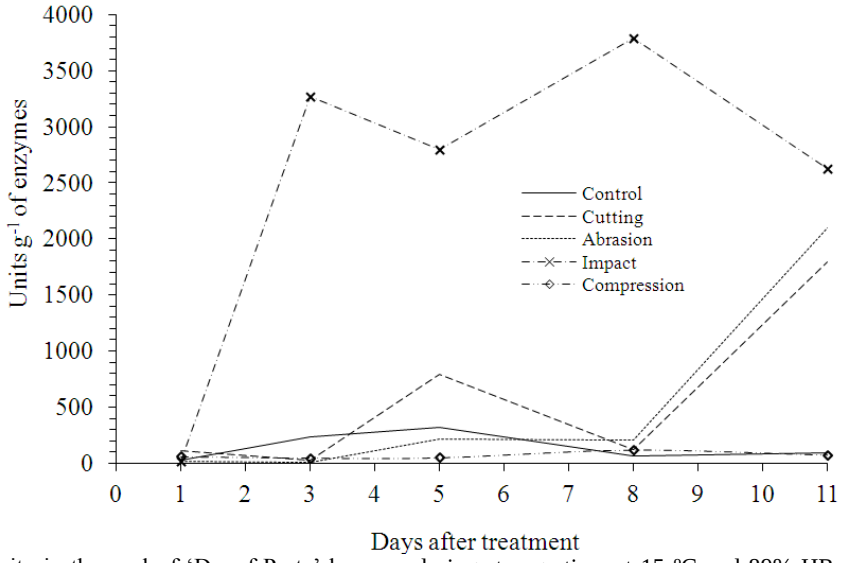

Fig 9. Peroxidase activity in the peel of 'Dwarf-Prata' bananas during storage time at 15 ºC and 89% HR in function of types of mechanical damage.

m altitude, climate type Aw, according to Köppen's classification).

Fruit harvest

The bunches were harvested in May 2004, when the central fruit of the second hand reached the diameter of 36 ± 2 mm. Once harvested, the bunches were washed with water in the packing house at the property, being then dehanded. The second, third and fourth hands of each bunch were identified, wrapped in bubble wrap and packed in corrugated cardboard boxes. Those were then transported to the Postharvest Laboratory at the Universidade Federal de Viçosa, where the fruits were individualized by a cut close to the floral cushion and washed in detergent solution at 0.2% for five minutes. After this process, the fruits were packed in plastic boxes lined with shredded paper and kept in a cold chamber at 15 ± 1 °C and 89 ± 2% of relative humidity up to and after the moment of treatment application.

Treatments

It was used a split plot design, with five treatments consisting of a control or absence of mechanical damage (T1) and four sources of mechanical damage: cut (T2), abrasion (T3) impact (T4) and pressure or compression (T5), with samples over time for a period of 11 days in a completely randomized design with three replicates and three fruits per plot. The fruits (peel color index: 1) (Dadzie and Orchard, 1997) were damaged by cutting, abrasion, impact or compression and pressure. The fruits were damaged, one by one, in the central region, on both sides, in two areas of 10 cm^2 each (2 cm by 5 cm), totaling 20 cm^2. The damage was done by cutting with a knife in three cuts with 5 cm, spaced 1 cm apart, to a depth of 2 mm. Some fruits were damaged by rubbing number 80 sandpaper on the area delimited above. Fruits damaged by impact, according to the methodology described by Dadzie and Orchard (1997), were obtained by leaving a steel ball (66 g) fall on the fruits of a height of 1.5 m. The impact energy was 0.9712 J.

The fruits damaged by compression were obtained by maintaining a mass of 3 kg for 5 minutes over them.

Laboratory analyses

The following characteristics were evaluated: percentage of accumulated fresh mass loss, daily rate of fresh mass loss, i.e., the percentage of daily loss of fresh mass, electrolyte leakage of the damaged region of the peel, contents of soluble sugars and starch, respiration, ethylene production and enzymatic activity of polyphenoloxidase and peroxidase. The percentage of accumulated mass loss, the daily rate of fresh mass loss, respiration and ethylene production were determined daily on duplicate samples, consisting of three fruits. The electrolyte leakage, the contents of soluble sugars and starch and the enzymatic activity of polyphenol oxidase (PPO) and peroxidase (POD) were determined at 1, 3, 5, 8 and 11 days after the treatments. For this, the fruits were randomly sampled during the ripening process. They were weighed together to determine the daily rate of fresh mass loss measured as described by Dadzie and Orchard, 1997. The electrolyte leakage was determined using the methodology described by Whilton et al. (1992), whereby a piece of the damaged peel of 1 cm diameter per fruit was removed. This section was washed in distilled water and the surface dry on absorbent paper and then incubated for 2 hours in a capped test tube containing 18 mL of distilled water and kept in ambient temperature. After this period, the electric conductivity was measured with a conductivity meter (model CG 853, Schott). Subsequently, the test tubes containing the peel samples were autoclaved at 121 °C and 0.15 Mpa for 30 min and the reading of electric conductivity was repeated. The results were expressed as the ratio between the values of the first and the second measurement multiplied by 100.

Subsamples were taken from the pulp region and ground, approximately 500 mg for the determination of sugar and starch contents, and 1,000 mg of peel to determine the activity of PPO and POD. These samples were frozen in liquid nitrogen (-196 °C) and stored at -20 °C until the evaluations. To determine sugar and starch contents, the samples were homogenized in a Polytron with 80% ethanol and centrifuged at 2,000 x g for 10 min. The precipitate was extracted and centrifuged again for another three times and the supernatants were combined and the volume completed to 25 mL. Then, 10 mL of the supernatant were collected and completely evaporated in a rotary vacuum evaporator at about 45 °C, and the residue was resuspended in 5 mL of distilled water and stored at -20°C until analysis. From this extract, aliquots were used to determine the soluble sugars by anthrone reaction (Hodge and Hofreiter, 1962). The residue of the alcoholic extractions was treated with water:perchloric acid 52% in 1:1.3 before centrifugation and centrifuged three times at 2000 x g for 10 min, after reaction in periods of 20, 30 and 20 minutes for the three extractions , respectively. The resulting supernatants were combined and completed with distilled water in a volume of 50 mL and used for quantification of starch by the method of McCready et al. (1950) modified by Patel (1970). PPO and POD activities were determined by the method described by Silva (1981) and Flurkey and Jen (1978), with modifications. To obtain the enzymatic extract, samples were placed in a test tube with 5 mL of 0.2 mol L^{-1} phosphate buffer (pH 6.0), cooled and homogenized in a Polytron at 20,500 rpm for 40 s. The suspension was centrifuged at 10,000 x g for 21 min at 4 °C. After centrifugation, the extract of supernatant or enzyme was placed into a test tube in an ice bath. To determine the PPO activity, 1.3 mL of 0.2 mol L^{-1} phosphate buffer (pH 6.0) and 1.5 mL of 0.2 mol L^{-1} catechol were filled in a test tube. This tube was placed in a thermostatic bath at 25 °C to stabilize the temperature. Subsequently, 30 µL of the enzyme extract were added, followed by homogenization and then seven absorbance readings were made every 30 s in a spectrophotometer, at a wavelength of 425 nm. Results were expressed as units of enzyme per gram of sample, calculated from the amount of extract that caused an increase in absorbance of 0.001 units per minute. To determine POD activity, 2.0 mL of frozen 0.2 molL^{-1}phosphate buffer (pH 6.0) with 0.5% guaiacol, were and placed in a test tube. This tube was placed in a thermostatic bath at 30 °C to stabilize the temperature. The tube was then added 250µL of the enzyme extract and 250 µL of hydrogen peroxide at 0.08%, followed by homogenization. Immediately, seven absorbance readings were taken every 30 s in a spectrophotometer at 470 nm. Results were expressed in units of enzyme per gram of sample, based on the amount of extract that induced an increase in absorbance of 0.001 units per minuto. All reagents were stored in a refrigerator. The catechol, guaiacol and hydrogen peroxide solutions were prepared and stored in the dark. To determine ethylene production, which was expressed in µmol of ethylene produced per 1 kg of fruit for 1 hour by gas chromatography, three fruits were placed in a container with a capacity of 3.3 L, hermetically sealed, for 55 minutes. Evolution rates of ethylene were determined by gas chromatography. It was used a gas chromatographer Model 37-D from Scientific Instruments C.G. Ltd., equipped with a flame ionization detector and a stainless steel column filled with PORAPAK R, with the following working conditions: column temperature of 80 °C, vaporizer temperature of 100 °C, temperature of the detector of 140 °C, N_2 flow of 30 mL min^{-1}, H_2 flow of 30 mL min^{-1}, synthetic air flow (20% O_2 and 80% N_2) of 210 mL min^{-1}. A sample was injected with a volume of 3 mL and a standard (White Martins) containing 99.5% of ethylene was used.

Statistical analysis

Data were subjected to analysis of variance and regression. The linear and nonlinear models were selected in accordance to the potential to explain the biological phenomenon in question, to the coefficient of determination and to the significance of the regression coefficients, using the t test up to the level of 10% of significance. For the data on respiration, ethylene production and activity of enzymes PPO and POD, descriptive analysis was applied.

Conclusions

Considering the 'Dwarf-Prata' bananas stored in cold chamber, it is concluded that: Mechanical injury on banana fruits increases fresh mass loss (%), electrolyte leakage (%) and average polyphenol oxidase activity, and accelerates the evolution of peel color index and anticipates the climacteric peak, compared to control.Damages caused by abrasion cause higher losses of daily and accumulated fresh mass (%). Damage caused by impact hinders the conversion of starch to soluble sugars in the pulp. Damages caused by impact and compression anticipate the ethylene production peak and fruit ripening. Damage caused by impact greatly increases the activity of polyphenol oxidase and peroxidase.

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