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MECHANICAL INJURIES AND THEIR EFFECTS ON THE PHYSIOLOGY OF 'GOLDEN' PAPAYA FRUIT

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Key words: Carica papaya L., postharvest, impact, abrasion, cut.

ABSTRACT

Papaya fruit is highly perishable, resulting in one of the highest loss indexes of any fruit. In addition, postharvest handling is injurious, resulting in mechanical injuries that induce ripening and reduce the shelf life of the fruit. The aim of the present work was to evaluate the effects of mechanical injuries on the postharvest physiology of 'Golden' papaya fruit. For this purpose, 'Golden' papaya fruit at the early ripening stage was subjected to three types of controlled mechanical injuries: abrasions, cuts and bruises. Abrasions were made with 200 mesh sandpaper over a peel surface area of 6 cm2. Cuts were made by creating incisions 30 mm long and 5 mm deep. Bruises were produced by the impact due to dropping the fruits from a height of 2 m onto a hard surface. Each fruit was injured twice on opposite sides at the largest diameter and received only one type of injury. Intact fruits were used as controls. The injured areas were marked, and the fruits were stored at 22ºC and 85% RH for 8 days. Impact injury accelerated respiratory activity and ethylene production. The papaya fruits subjected to impact injury showed higher 1-aminocyclopropane-1-carboxylate (ACC) oxidase activity than those uninjured. Electrolyte leakage was higher in fruits injured during the first days of storage, mainly by impact, which had immediate effects on electrolyte leakage. In conclusion, the postharvest physiology of 'Golden' papaya fruits was affected by mechanical injury, primarily injury caused by impact.

DAÑOS MECÁNICOS Y SUS EFECTOS SOBRE LA FISIOLOGÍA DE FRUTAS DE PAPAYAS 'GOLDEN'

Palabras clave: Carica papaya L., poscosecha, impacto, abrasión, corte.

RESUMEN

La papaya es una de las frutas que registra los más altos niveles de pérdidas, por ser altamente perecible, además el manejo poscosecha adoptado es agresivo y resulta en muchos daños mecánicos, que acelerón la maduración y reduce la vida útil de frutas. El objetivo de este estudio fue evaluar los efectos de las lesiones mecánicas sobre la fisiología poscosecha de papayas 'Golden'. Los daños mecánicos de abrasión, corte y amasado fueron reproducidos en los frutos de madurez 1. La abrasión se hizo con lija de grano 200 en un área de 6 cm2. Para el corte se realizaron incisiones de 30 mm de longitud y 5 mm de profundidad. La lesión amasado fue causada por el impacto y los frutos se dejaron caer desde una altura de 2 m. Cada fruta ha sufrido dos daños en lados opuestos en la región de mayor diámetro y recibió solamente un tipo de lesión. Frutos intactos se utilizaron como controle. Las áreas dañadas fueron demarcadas y los frutos almacenados a 22ºC y 85% de humedad relativa durante 8 días. La lesión por impacto aceleró la tasa de respiración y la producción de etileno. La actividad de la ACC oxidasa en las papayas sometidas a la abrasión y el impacto fue mayor en comparación con los frutos sometidos al corte y los del control. La fuga de electrolitos fue mayor en frutos con heridas en los primeros días de almacenamiento, en particular en los sometidos al impacto, que mostraron un efecto inmediato sobre la fuga de electrolitos. La fisiología poscosecha de papayas 'Golden' fue afectada en función de las lesiones mecánicas, principalmente por el impacto.

INTRODUCTION

The papaya has a climacteric respiration pattern, a short postharvest life and reaches the full ripening stage over the course of a few days. The changes resulting from ripening occur soon after the harvesting of physiologically mature fruit, triggered by ethylene production and an increased respiratory rate. These changes characterize the papaya as a highly perishable fruit postharvest (Kader, 2002). The ripening of climacteric fruits is characterized by an increased respiratory rate followed by autocatalytic ethylene production (Prasanna et al., 2007).

The amino acid methionine is the biological precursor of ethylene in higher plants and is converted into ethylene by a biosynthetic pathway that consists of two enzymatic reactions. In the first, S-adenosyl-methionine (SAM) is converted into aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACCS), and the second reaction is catalyzed by the enzyme ACC oxidase (ACCO), which is responsible for the conversion of ACC to ethylene (Dorling and McManus, 2012). Ethylene production is strongly stimulated by exogenous factors such as mechanical injuries in addition to autocatalytic response. Ethylene is involved in physiological events during the growth and development of plants as both a cause, as in ripening, and an effect, as in a stress response (Bleecker and Kende, 2000; Druege, 2006).

Among the causes of papaya postharvest losses, the majority are the result of mechanical injuries, which are defined as plastic deformation, surface disruption and plant tissue destruction caused by external forces that lead to physical or physiological, chemical and biochemical changes (Chen et al., 2007; Sivakumar and Wall, 2013). In general, mechanical injuries stress the plant, resulting in increased ethylene production and disorders related to cellular compartmentalization (De Martino et al., 2006). An increased respiratory rate, deterioration and a reduction in the postharvest lifetime of fruits subjected to mechanical injury have been reported (Zhou et al., 2007; Maia et al., 2011; Montero et al., 2012). The aim of this study was to evaluate the effects of mechanical injuries on the postharvest physiology of ‘Golden’ papaya fruit.

MATERIALS AND METHODS

Fruit selection

Golden papaya fruits obtained from a commercial orchard in Espírito Santo, Brazil were used for the experiments. First, fruits at ripening stage 1 (defined by a yellow peel color over up to 15% of the surface) were packed in a cardboard box and transported in a refrigerated truck at 10°C from the producer to the Supply Center in Campinas, Brazil. The fruits were then transported to the Postharvest Physiology and Technology Laboratory at ESALQ/USP in Piracicaba, Brazil. Papayas were selected for uniform color and the absence of defects in the peel and subjected to abrasion, cutting and bruising via controlled mechanical injury methods.

Mechanical injury methods

Abrasion was performed with 200 mesh sandpaper. An adhesive label with a hole having an area of 6 cm2 was affixed to the fruit in contact with the peel surface. The sandpaper was rubbed against the peel once by hand to cause the injury to the area bounded by the hole in the label. The cut injury was caused by cutting the fruit to a depth of 5 mm along an incision 30 mm long using a blade fixed on a wooden foundation that provided a force of 7.5 kg, to which the fruits were subjected for 15 minutes. Bruises were produced in papayas by impact. One papaya was placed in a fine mesh bag 15 cm long and 10 cm wide to prevent the rotation of the fruit during the fall; the bag also had a hole approximately 3 cm in diameter. The fruit was dropped from 2 m into a wooden box having a hard bottom, which was painted with red ink. The interior sides of the wooden box were padded with polystyrene foam 3 cm thick to minimize the impact between the fruit and the sides of the box after the fall of the fruit. At the moment of impact, the damaged area of the fruit was marked by the red ink on the box surface through the hole in the bag. Each fruit was injured twice on opposite sides at the widest diameter and received only one type of injury. The damaged areas of the fruits were demarcated, and they were stored at 22ºC and 85% RH for 10 days. Intact fruits were held under the same conditions as controls. Evaluations of respiratory activity and ethylene production were performed daily, and ACC oxidase activity and electrolyte leakage were evaluated every two days.

Measurement of respiratory activity and ethylene production

To measure respiratory activity and ethylene production, the fruits were placed in 1.7 L airtight glass jars with lids containing silicone septa; the jars had been previously equilibrated to the temperature and relative humidity of the experimental storage conditions. After 1 hour, gas samples were withdrawn from each jar through the silicone septum with a Hamilton Gastight syringe. The samples were analyzed for CO_2 and ethylene using a Thermo Electron Trace 2000GC gas chromatograph equipped with two flame ionization detectors (FID) set at 250ºC, two injectors both set at 100ºC, two Porapack N columns (CO_2 column, 4 m; C_2H_4 column, 1.8 m) set at 100ºC and a methanator adjusted to 350ºC for CO_2 analysis. The respiratory activity and ethylene production were calculated from the jar volume, the fruit mass and the time that the jars were closed (60 minutes). The results were expressed as CO_2 (mL kg^{-1} h^{-1}) and C_2H_4 (µL kg^{-1} h^{-1}) for respiratory activity and ethylene production, respectively. Eight replicates were performed for each assay, and each replicate consisted of the measurement of one fruit.

Measurement of ACC oxidase activity

ACC oxidase was extracted and assayed as described by Karakurt and Huber (2003) with several modifications. Two grams of papaya pulp from the injured region, excluding the peel, were homogenized in 6 mL of extraction solution containing 0.1 M Tris (pH 7.4), 10% glycerol and 30 mM sodium ascorbate. The slurry was filtered through 4 layers of cheesecloth and centrifuged at 14000 rpm for 30 minutes. Enzyme activity was assayed at 25°C in 1 mL of a reaction mixture containing 0.4 mL of the enzyme extract, 30 mM sodium ascorbate, 0.1 mM $FeSO_4$, 1 mM ACC and extraction buffer in 20 mL vials stoppered with rubber septa. The reaction mixture was incubated at 25°C for 2 hours, and 1 mL of gas was withdrawn from the headspace and analyzed for ethylene using a Thermo Electron 2000GC Trace gas chromatograph. ACC oxidase activity was expressed as C_2H_4 (µL kg^{-1} h^{-1}). For the ACC oxidase activity assays, 4 replicates consisting of 1 fruit each were assayed, and each replicate was performed in duplicate.

Measurement of electrolyte leakage

The level of damage in the injured tissue was assessed by electrolyte leakage according to the methodology described by Serek et al. (1995). A cylinder of tissue (1 cm in diameter and 1 cm long) was collected from the injured region of each fruit without removing the peel using a metal sampler. The cylinders were washed with deionized water, dried lightly with paper towels and then incubated for 2 hours in 30 mL of deionized water at room temperature. After this period, the electrical conductivity of this solution was measured with a conductimeter (MS Tecnopon - MCA-150). After incubation, the samples were autoclaved at 120°C and 1.5 atm for 30 minutes to destroy the selective permeability of the membrane and allow for complete electrolyte leakage. After autoclaving, the electrical conductivity of the surrounding water was measured again, and the results were expressed as the ratio between the first and second readings multiplied by 100. For the electrolyte leakage assay, 4 replicates consisting of one fruit each were assayed, and each replicate consisted of 4 cylinders.

Statistical analyses

The experimental design for physicochemical analysis, was completely randomized with factorial scheme 4 x 4 (type of injury x day of storage after the injury), with four replications for physicochemical analysis and eight replications for physiological analysis, in a total of 64 and 128 samples, respectively, being each sample represented by one papaya The results were evaluated via analysis of variance (ANOVA) and the Tukey's test ($P \leq 0.05$).

RESULTS

Respiratory activity and ethylene production

The bruise injury caused by impact interfered in the respiratory activity (Figure 1A). The fruit displayed high CO_2 production during storage ($P \leq 0.05$). For the cut injury, the respiration rate was 1.3 times higher than in the fruits with abrasion injury or fruits without injury. Among all the injuries, fruits damaged by abrasion showed the lowest respiratory activity, which did not differ significantly ($P \geq 0.05$) from fruits without injury.

The impact mechanical injury resulted in higher ethylene production compared with fruits that received other types of injuries and control fruits (Figure 1B). Twenty-four hours after injury, papayas subjected to the impact injury showed a significant increase in ethylene production ($P \leq 0.05$). In contrast, fruits that received cuts or abrasions and fruits without mechanical injury showed an increase in ethylene production starting at the 3rd day of storage. The ethylene production by the fruit subjected to impact was 1.32 mL kg^{-1} h^{-1}. This production was 2.6 times greater than that of fruits that received abrasion injuries and 1.7 times greater than that of fruits that received cutting injuries and control fruits. There were no differences in ethylene production among the fruit subjected to cutting or abrasion and fruits without mechanical injury ($P \geq 0.05$).

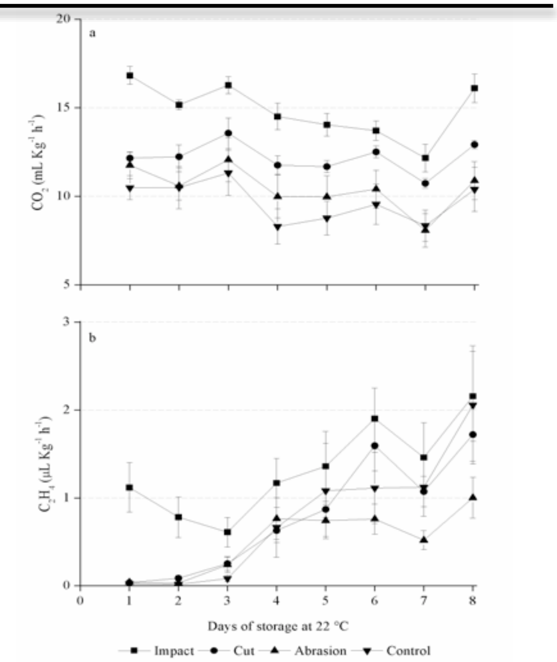

Figure 1. Respiratory activity (a) and ethylene production (b) in Golden papaya fruit subjected to mechanical injury and stored at 22±1°C and 85±5% RH. Vertical bars represent the mean error (n=8).

ACC oxidase activity

The ACC oxidase activity in papayas subjected to abrasion and impact injuries was greater than in fruits subjected to cut injuries and control fruits ($P \leq 0.05$) (Table 1). Papayas subjected to abrasion showed high ACC oxidase activity two days after the injury. This level of activity was maintained until the 6th day of storage.

Comparing the ACC oxidase activity (Table 1) with the ethylene production of fruits subjected to abrasion (Figure 1B), increased levels of ACC oxidase did not result in increased production of ethylene. Fruits subjected to abrasion showed high levels of ACC oxidase; however, they showed lower ethylene production among all treatments.

Papayas subjected to impact and cut injuries also showed high ACC oxidase activity in the first days of storage. For fruits without injury, the enzyme activity was constant and did not differ during storage from that in fruits subjected to cut injuries (Table 1). For these treatments, differences in ACC oxidase activity did not result in differences in ethylene production (Figure 1B). The control fruits showed lower expression of enzyme activity but did not differ from fruits that received a cutting injury. The reduction of ACC oxidase activity was coincident for all treatments at the end of storage.

Table 1 - ACC oxidase activity (C_2H_4 mL kg^{-1} h^{-1}) in Golden papaya fruit subjected to mechanical injury and stored at 22±1°C and 85±5% RH. Enzyme activity is expressed as ethylene production.

	Mechanical Injury			
	Impact	Cutting	Abrasion	Control
Mean	0.041 a	0.034 b	0.045 a	0.032 b
F Test	0,0012*			
Mechanical Injuries	Days of storage at 22±1 ºC 2	4	6	8
Impact	0.046 ABab	0.055Aa	0.038ABbc	0.026Ac
Cutting	0.038BCab	0.050ABa	0,022Cc	0.025Abc
Abrasion	0.051Aa	0.040Bab	0.050Aab	0.038Ab
Control	0.025Cb	0.039Ba	0.035BCab	0.030Aab
F Test	0.0006*			
DMS	0.0067			
CV (%)	26.62			

**Significant at 5%. Means followed by same capital letter in the column, and tiny line, do not differ by Tukey's test (P≤0.05).*

Electrolyte leakage

The injury caused by impact had an immediate effect on electrolyte leakage. Two days after the application of mechanical injury, these fruits showed 67% more electrolyte leakage than the control fruits. Impact injury also caused a greater release of electrolytes than cut or abrasion injuries (P≥0.05) (Figure 2), indicating the severity of the injury to the tissue. These results are correlated with the respiration and ethylene production results (Figure 1), in which it was observed that the fruits subjected to impact injury matured faster. Fruits damaged by abrasion displayed greater electrolyte leakage on the 4th day after mechanical injury (P≤0.05). In fruits subjected to cutting injury, the same behavior was also observed on the same date. Fruits subjected to mechanical injury had 50% more electrolytes extravasated from their cells than the control fruits, even on the 4th day after the injury (Figure 2). However, decreases in electrolyte leakage in fruit subjected to impact and abrasion injuries were observed on the 4th and 6th days of storage, respectively, in other words, after the maximum ion release of the fruits after these treatments. The leakage of the fruits that were not subjected to mechanical injury was the highest on the 6th day of storage (P≤0.05) (Figure 2).

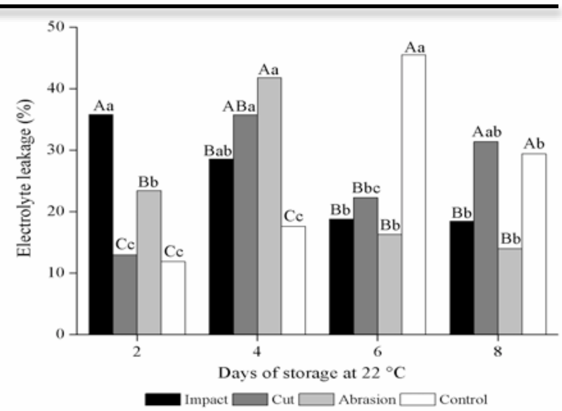

Figure 2. Electrolyte leakage (%) in 'Golden' papayas fruit subjected to mechanical injury and stored at 22±1°C and 85±5% RH for 8 days. Means followed by the same capital letter within each storage day, and over the shortest storage period for each injury, do not differ by Tukey's test (P≤0.05).

DISCUSSION

The diffusion of gases in tissues is facilitated by mechanical injury and results in increased respiration due to CO_2 loss, which accumulates in the intercellular spaces (Kader, 2002). Increased respiration may also be attributed to the decarboxylation of malic acid, which flows out of damaged cells after mechanical injury (Mattiuz and Durigan, 2001). In the present study, we observed high CO_2 production due to mechanical injury, where the highest levels were caused by impact-based injury. This type of injury has also been reported to be responsible for increases in the respiratory activity of citrus fruit (Scherrer-Montero et al., 2011), melon (Perea et al., 2006), tomatoes (Paiva et al., 2012) and potato tubers (Rady and Soliman, 2013).

In addition to cut injury also influenced the postharvest physiology of papaya. According to De Martino et al. (2006), this type of injury causes cell disruption, which leads to acids in vacuoles being transported to the respiratory system in an attempt to repair the damage (Mattiuz and Durigan, 2001). The abrasion injury induced less CO_2 and ethylene production than those impact-based injuries, whose behavior it also reported by Quintana and Paull (1993) in papayas submitted to the similar damages and stored at 25ºC.

The differences between the metabolic activities of papayas submitted to different types of injury are important because the respiratory intensity and storage duration are inversely related; i.e., a higher respiration rate in a particular fruit leads to a shorter shelf life (Kader, 2002). Furthermore the ethylene accelerates the deterioration and senescence of plant tissues and promotes the ripening of climacteric fruits, resulting in differences in the physiological ages of intact and injured tissues (Giovannoni, 2007).

We investigated the relationship between ACC oxidase activity and ethylene production in fruits subjected to mechanical injuries. After abrasion injuries, we found that the increased levels of ACC oxidase observed did not result in increased ethylene production, possibly because the enzyme activity was measured only in the exact place where the mechanical injury occurred although the ethylene production was evaluated in the fruit as a whole. Zheng et al. (2005) verified that the ACC oxidase activity is stimulated in the region of damaged tissue in persimmon fruit, and the synthesis of ethylene also appears to be concentrated in this region, which is similar to that observed in this work.

Bender et al. (2003) studied the effect of heat treatment on the activity of ACC oxidase in mango and found that there was an increase in enzyme activity as a result of stress caused by heat treatment. However, the authors found no increase in ethylene production, even with the ACC oxidase activity at higher levels. McCollum et al. (1995) observed the same difference in enzyme activity and ethylene production in cucumbers subjected to hydrothermal stress and said that there may be a second pathway that influences the regulation of enzymes related to ethylene biosynthesis. However, the authors did not suggest a likely pathway or indicate what mechanisms may be involved in this regulation.

Our experiments revealed a reduction in ACC oxidase activity at the end of storage in fruits submitted to different types of mechanical injury. According to Concellón et al. (2005), the reduction of ACC oxidase activity likely occurs by due to cellular decompartmentalization, and the conversion of ACC (aminocyclopropane-1-carboxylic acid) by ACC oxidase to ethylene requires membrane integrity. Antunes and Sfakiotakis (2000) reported that injured plant tissue senesces more quickly, which may reduce ACC oxidase activity.

Plant tissues often respond to mechanical stress, chilling injury or high temperatures with an increase in electrolyte leakage (Mirdehghan et al., 2007; Zhou et al., 2007; Chen et al., 2012). Electrolytes are ions that are normally contained within cell membranes, which are sensitive to many types of stress that are associated with increased permeability and loss of membrane integrity (Saltveit, 2002; Rinaldo et al., 2010). According to Lewis et al. (2008), the first responses of a fruit to mechanical injury are rapid changes in membrane permeability and bioelectric potential.

It was observed that the impact injury promoted immediate increase in electrolyte leakage was followed by a decrease, while the damage type abrasion and cut promoted a two-day delay in this raise. The reason is that damage by abrasion causes the disruption of a large quantity of epidermis cells. Moreover, the integrity of cell membranes is affected by all types of mechanical damage, since the electrolyte leakage serves as a reference of the membrane integrity or alteration of selective permeability and was closely related to the shelf-life of papaya fruits (Huang et al., 2005). Furthermore, during the normal ripening process, the membranes gradually lose the selective permeability and break, as found in papayas not injured on the 6th day. The different behavior of this variable emphasizes the negative effect of mechanical injuries on papayas ripening, due the loss of permeability has occurred in these fruits earlier, indicating faster ripen. The reduction of values suggests an attempt to restoration of membranes in damaged tissues, while undefined tendency of cut injury may be indicative that the tissue failed to repair the damage. Zhou et al. (2007) also observed that mechanical damage affected plasma membrane of pear.

In conclusion, impact injury in 'Golden' papaya caused an increase in respiratory activity, ethylene production, increased the activity of ACC oxidase and showed an immediate effect on electrolyte leakage.

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