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RESEARCH ARTICLE

Cell wall biochemistry of sapodilla (*Manilkara zapota*) submitted to 1-methylcyclopropene

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Sapodilla (*Manilkara zapota*) is a climacteric fruit that ripens shortly after harvest. Studies on its conservation during storage have been mainly restricted to using low temperatures and modified atmospheres. In this study we investigated the influence of 1-methylcyclopropene (1-MCP) on the physiological and biochemical changes that sapodilla cell wall undergoes during ripening and evaluated its potential to preserve sapodilla fruits at postharvest. Fruits were treated with ethylene antagonist 1-MCP at 300 nL L^{-1} for 12 h and then stored under a modified atmosphere at 25ºC for 23 d. 1-MCP significantly delayed softening of sapodilla for 11 d as a consequence of inhibition of cell wall degrading enzyme activities, and thus 1-MCP-treated fruit exhibited a less extensive solubilization of polyuronides, hemicellulose and of free neutral sugar when compared to control fruit. Results suggest that delayed softening of sapodilla is largely dependent on ethylene production and perception.
Key words: cell wall hydrolases, ethylene, hemicellulose, neutral sugar, fruit ripening, uronides

Bioquímica da parede celular de sapoti (*Manilkara zapota*) submetido ao 1-metilciclopropeno: Sapoti (*Manilkara zapota*) é um fruto climatérico que amadurece rapidamente após a colheita e os estudos acerca da extensão de sua vida útil pós-colheita são principalmente restritos ao uso de baixa temperatura e atmosfera modificada. Este trabalho avaliou a influência do 1-metilciclopropeno (1-MCP) sobre o amolecimento e bioquímica da parede celular durante o amadurecimento de sapoti, assim como o seu potencial de conservação pós-colheita. Sapotis foram tratados com 1-MCP, um antagonista do etileno, a 300 nL L^{-1} por 12 h e, em seguida, armazenados sob atmosfera modificada a 25ºC por 23 d. O 1-MCP retardou significativamente o amadurecimento de sapoti por 11 d, via diminuição da atividade das enzimas hidrolíticas da parede celular e, portanto, os frutos tratados com 1-MCP exibiram menor solubilização dos poliuronídeos, hemicelulose e açúcares neutros livres quando comparados com os frutos-controle. O 1-MCP retardou o amaciamento do sapoti, indicando que esse processo é dependente da produção e percepção de etileno.
Palavras-chave: açúcares neutros, amadurecimento de frutos, etileno, hemicelulose, hidrolases da parede celular, uronídeos

INTRODUCTION

Ripening is the irreversible first step of senescence. The most characteristic alteration that fruits undergo during ripening is softening or loss of firmness of skin and flesh, which is a consequence of cell wall and middle lamellae degradation involving several hydrolytic enzymes (Dong et al., 2001; Mathooko et al., 2001; Jeong et al., 2002).

Ethylene is the hormone responsible for triggering and coordinating ripening events in climacteric fruits. Studies on gene expression have demonstrated that ripening is a programmed event that involves the controlled expression of specific genes, some of which are ethylene-dependent

(Giovannoni, 2001). Thus, if technologies are to be developed to maintain quality and enhance postharvest conservation of commodities, they will need to be based on previous knowledge of the physiological processes that control ripening and senescence.

The use of ethylene antagonists has been an important tool for clarifying the physiological role of ethylene in the process of fruit ripening and, also, as a postharvest treatment to broaden the conservation potential of fruits. 1-Methylcyclopropene (1-MCP) is the best-known and most studied of the ethylene inhibitors and has been shown to delay ripening of climacteric fruit (Jeong et al., 2002). 1-MCP postharvest treatment has been shown to reduce the activity of cell wall enzymes and delay the ripening of avocados for 4 d, while pears and plums treated with 1-MCP showed a reduction in ethylene production and respiratory rate (Dong et al., 2001; Mathooko et al., 2001; Jeong et al., 2002).

The sapodilla tree is a native species to Southern Mexico that is widespread throughout Brazil, although it is best acclimated to the tropical climate of the Northeast region (Bandeira et al., 2003). In Brazil, this species is mainly cultivated for its fruit and in spite of its great market potential, there are very few postharvest technologies developed for sapodilla fruit. Sapodilla is a climacteric fruit that when harvested at physiological maturity ripens within 8 d at 26ºC and while softening is the most obvious symptom of ripening there are no evident changes in skin color (Araujo-Neto et al., 2001; Morais et al., 2006). The studies on conservation during storage had been mainly restricted to use of low temperatures and modified atmosphere (Miranda et al., 2001) until postharvest treatment with 1-MCP showed that 200 and 400 nL L^{-1} concentrations delayed firmness loss and changes in pulp color of sapodilla (Morais et al., 2006). In this study we investigated the influence of 1-MCP at 300 nL L^{-1} on the physiological and biochemical changes that the sapodilla cell wall undergoes during ripening and evaluated its potential to preserve these fruit at postharvest.

MATERIAL AND METHODS

Plant material: Sapodilla [*Manilkara zapota* (L.) Von Royen 'Itapirema-31') fruits were harvested at physiological maturity when fully grown. The harvested fruits had a mean length of 6.2 and a diameter of 6.5 cm. The fruits were obtained from a commercial grower at Paraipaba, Ceará State, northeastern Brazil, and transported to Postharvest Physiology and Technology Laboratory in Fortaleza within 10 h after harvest. After selection for uniformity of size and developmental stage the fruits were soaked in 1000 mg L^{-1} of the fungicide Benomil® for 5 min, and then rinsed and dried. Fruits were sorted into two groups, one was treated with 300 nL L^{-1} 1-MCP for 12 h and the other was the control, without 1-MCP treatment.

1-MCP treatment: Fruits were exposed to 300 nL L^{-1} of 1-MCP in hermetically closed 186 L mini-chambers at 25 ± 2ºC for 12 h. The concentration used was achieved by releasing the gas from a commercial powdered formulation (Smartfresh® from Rohm and Haas, Philadelphia, USA) containing 0.14% of the active ingredient. Control fruits were maintained under identical conditions. Immediately after the chambers were opened, fruits were placed on polystyrene trays, four fruits per tray and covered with 12 μm PVC film and stored at 25 ± 2ºC and 70 ± 5% RH for 23 d. The PVC-modified atmosphere was used to reduce weight loss during storage (Miranda et al., 2002). Fruit samples were evaluated on harvest day and after 4, 8, 11, 14, 17, 20 and 23 d for firmness, cell wall structural polysaccharides and for cell wall enzymes. After firmness was measured, fruits were peeled and the mesocarpic tissue from each fruit was stored at -70ºC for posterior analysis.

Fruit firmness: Fruit firmness was measured on a TA.XT2i Stable Micro Systems® (Surrey, UK) automatic texture analyzer with a 6 mm plunger at a shearing speed of 1 mm s^{-1} to a depth of 15 mm. Measurements were carried out at two equidistant points on the equatorial region of whole, unpeeled fruits and results expressed in Newtons (N).

Cell wall material preparation and polysaccharide quantification: The cell wall material was extracted from the mesocarp (60 g) after homogenization in 70 mL of 80% ethanol at ambient temperature for 5 min using a Polytron followed by centrifugation at 6000 *g* for 12 min (Magalhães et al., 1996). The pellet was washed in 100 mL distilled water and centrifuged as above. The final pellet was suspended in 80 mL 50 mM KH_2PO_4 and 50 mM NaH_2PO_4 buffer, pH 4.5, and starch hydrolysed with amyloglucosidase (EC 3.2.1.3 – Merck, Germany, 28 U mL^{-1}) under agitation for 24 h in a water bath at 55ºC. The reaction was terminated by boiling for 5 min followed by cooling in an ice-cold water bath. To

obtain a starch-free sample, the mixture was centrifuged and the pellets were washed with 50 mL chloroform:methanol (1:1 v/v) and this procedure was repeated twice. Finally, the extracted cell wall material was washed with 50 mL acetone and dried at room temperature.

Hemicelluloses were extracted from 50 mg of cell wall material by hydrolysis in 3 mL trifluoroacetic acid (TFA) for 2 h at 120°C. The volume was adjusted to 50 mL with distilled water and then filtered through Whatman No. 1 paper. The filtrate represented the hemicellulosic fraction which was quantified by the anthrone method and expressed as a percentage of the isolated cell wall material (Yemm and Willis, 1994). Uronic acids were extracted from 50 mg of cell wall material by incubation with 3 mL 72% H_2SO_4 for 2 h. The volume was adjusted to 50 mL with distilled water and filtered through Whatman No. 1 paper. Uronic acids were quantified by the carbazole method, and expressed as a percentage of the isolated cell wall material (Bitter and Muir, 1962).

Cell wall sugar composition: Neutral sugars were determined from pectic and hemicellulosic cell wall fractions, but not from the cellulosic fraction, and derivatised as recommended previously (Albersheim et al., 1967). Cell wall material (5 mg) was hydrolyzed with 500 µL of 2 N TFA plus 200 µg inositol for 1 h at 121°C. The TFA was evaporated in a water bath (45°C) and 0.5 mL methanol added. Polysaccharides were solubilized with 150 µL of 1 N NH_4OH and then acetylated with 150 µL of 1 N acetic anhydride.

The composition of neutral sugars was determined on a gas chromatograph (VARIAN 3800, Palo Alto, USA). The acetylated derivatives were dissolved in 200 µL acetone and 2 µL of this mixture applied to the gas chromatograph fitted with an OV-DB 225 capillary column. The injector, column and detector temperatures were 250°C, 225°C and 300°C, respectively. The column flow rate was set at 1 mL min^{-1} and detection was by flame ionization. Carrier and auxiliary gases were instrument-grade He and O_2-free N_2, respectively. A mixture of sugars was used as standard: rhamnose, fucose, arabinose, xylose, mannose, galactose, glucose and inositol, at 1 mg mL^{-1} concentrations (DeVetten and Huber, 1990) and data expressed as percentage of neutral sugars in the isolated cell wall material.

Cell wall hydrolase assays: Pectinmethylesterase (PME, E.C. 3.1.1.11) was extracted and its activity determined (Jen and Robinson, 1984). Mesocarp tissue (5 g) was homogenized with 25 mL of ice-cold 0.2 N NaCl in a Polytron. The homogenate was filtered through Whatman No. 1 paper and the filtrate was collected as the enzyme crude extract. These former procedures were carried out at 4°C. For PME activity, the reaction mixture contained 5 mL of enzyme crude extract and 30 mL of pectin solution (1% v/w citrus pectin in 0.1 M NaCl) and the rate of pectin demethylation was monitored through titration with NaOH 0.025 M at pH 7.0 for 10 min. One unit of PME was defined as the amount of enzyme capable of demethylating pectin corresponding to the consumption of 1 nmol NaOH and results were expressed as U min^{-1} g^{-1} FW.

Polygalacturonase (PG, E.C. 3.2.1.15) was extracted and its activity determined (Pressey and Avants, 1973; Buescher and Furmanski, 1978). Mesocarp tissue (5 g) was homogenized with 50 mL of ice-cold water. The homogenate was filtered through Whatman No. 1 paper. The residue retained on the filter was washed in 20 mL of ice-cold water, resuspended in 20 mL of 1 N NaCl and stirred for 1 min. It was then adjusted to pH 6.0 and left for 1 h. The volume was completed to 30 mL with 1 N NaCl and filtered through Whatman No. 1 paper and the filtrate collected as the enzyme crude extract. All previous steps were conducted at 4°C. For the enzyme activity assay, the reaction mixture consisted of 200 µL enzyme crude extract plus 50 µL 0.25% polygalacturonic acid in 37.5 mM sodium acetate buffer, pH 5.0. The reaction mixture was incubated for 3 h at 30°C followed by immersion in a boiling water bath to stop the reaction. The reducing groups liberated were determined according to Somogyi technique (Nelson, 1944). One unit of PG activity corresponds to an increase in absorbance of 0.01 and results were expressed as U min^{-1} g^{-1} FW.

β-galactosidase (β-GAL, E.C. 3.2.1.23) was extracted and its activity determined (Dey and Pridham, 1969; Kitagawa et al., 1995). Mesocarp tissue (10 g) was homogenized with 20 mL of 0.1 M sodium acetate-phosphate buffer, pH 5.0, with 1% PVP and centrifuged at 10000 *g* for 15 min. The pellet was resuspended in 0.1 M acetate phosphate buffer, pH 5.0, plus 0.005 M 2-mercaptoethanol and then centrifuged as above. The pellet was resuspended in 0.02 M sodium acetate buffer, pH 5.0 plus 3 M NaCl, stirred for 12 h and then centrifuged again (14000 *g* for 20 min). The supernatant was dialyzed for 24 h against water and then collected as the enzyme crude extract. All previous steps were conducted at 4°C.

β-galactosidase was assayed by hydrolysis of *p*-nitrophenyl-β-galactopyranoside. The reaction mixture consisted of crude enzyme extract and 0.003 M substrate in McIlwaine buffer, pH 4.0. After 15 min at 37°C, the reaction was terminated by 0.1 M sodium carbonate and the *p*-nitrophenol released was measured spectrophotometrically at 400 nm. One unit of β-GAL activity corresponds to an increase in absorbance of 0.01 and results were expressed as U min^{-1} g^{-1} FW.

Statistical analysis: The experiment was conducted in a 2 x 8 factorial design with two treatment levels defined as the 1-MCP dosage (0 and 300 nL L^{-1}) and eight time-points of evaluation (0, 4, 8, 11, 14, 17, 20 and 23 d). The experimental plots were made up of three replicates with four fruits each and results were expressed as the mean ± SD.

RESULTS

Fruit softening: Sapodilla treated at postharvest with 1-MCP at 300 nL L^{-1} softened much slower than control fruits (Figure 1). After 8 d of storage at 25ºC and 70% RH, firmness of control fruits had decreased from 79.7 N on harvest to 7.7 N. Meanwhile, softening of 1-MCP-treated fruits was significantly delayed reaching 15.37 N after 11 d of storage. After storage for 23 d, no significant difference in firmness was found between treated and control fruits. The sharp decrease in firmness of control fruits observed up to day 8 could be correlated to climacteric ethylene synthesis (Morais et al., 2006).

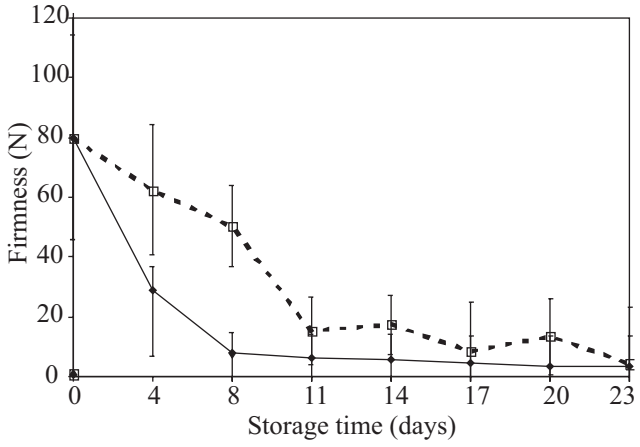

Figure 1. Changes in firmness of sapodilla treated with 300 nL L^{-1} 1-MCP (□) and (♦) control stored at 25 ± 2°C and 70 ± 5% RH. *n* = 3 ± SD.

Cell wall material: There was a large reduction in hemicellulose concentration during storage of sapodilla (Figure 2), although 1-MCP-treated fruits maintained higher levels than control fruits. During the 23 d of storage, the hemicellulose concentration nearly halved in treated fruits and declined more than 70% in their control counterparts.

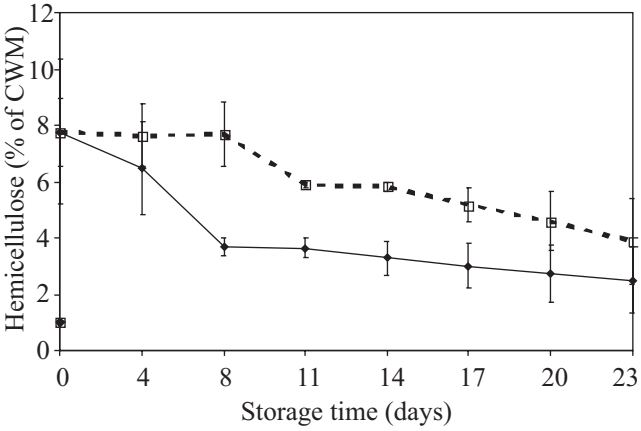

Figure 2. Changes in hemicellulose concentration from isolated cell wall material (CWM) during postharvest storage of sapodilla treated with 300 nL L^{-1} 1-MCP (□) and (♦) control, at 25 ± 2°C and 70 ± 5% RH. *n* = 3 ± SD.

The uronic acid concentration declined greatly during ripening up to 8 d of storage and then remained nearly constant for both control and treated fruits. However, the uronic acid levels were lower in the control (ca. 5%) than in 1-MCP-treated sapodilla (ca. 10%) (Figure 3).

Rhamnose, galactose, arabinose are monosaccharides characteristic of pectins, and in sapodilla their concentrations decreased sharply during storage (Figure 4A-C), with galactose and arabinose showing the highest levels. Mannose, glucose and xylose (Figure 4D-F) are hemicellulose-typical sugars and their decrease is consistent with the decline in hemicellulose concentration (Figure 2) during storage of sapodilla. Xylose is the most abundant among the neutral sugars quantified in sapodilla. The decline in xylose and glucose concentration was smaller than for the other monosaccharides, probably due to the fact these sugars are part of the hemicellulosic fraction which had a smaller decline than the pectic fraction of the sapodilla cell wall (Figure 3).

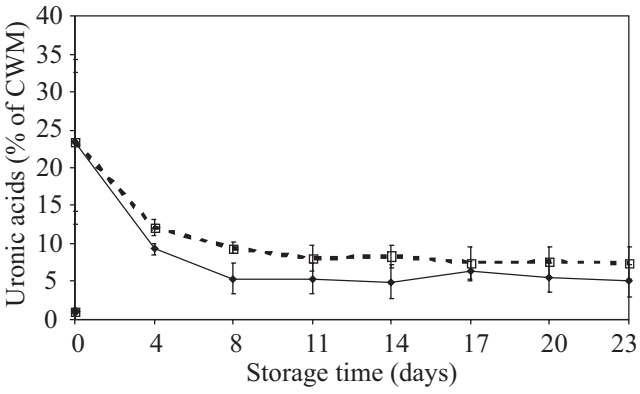

Figure 3. Changes in uronic acids concentration from isolated cell wall material (CWM) during postharvest storage of sapodilla treated with 300 nL L^{-1} 1-MCP (□) and (♦) control, at 25 ± 2°C and 70 ± 5% RH. *n* = 3 ± SD.

Cell wall hydrolases: Cell wall PME activities were high in sapodilla (Figure 5A). In control fruits, PME activity increased gradually until day 11 reaching ca. 480 activity units and then started to decrease. When sapodilla was treated with 1-MCP, PME maximum activity was delayed up to day 14 and reached a lower level (ca. 440 activity units) showing that, although PME activity was not suppressed by the treatment with 1-MCP, its induction was restricted.

Activity of PG increased during storage reaching a maximum (ca. 9 activity units) on days 8 and 14 for control and 1-MCP-treated sapodilla, respectively (Figure 5B) and then decreased. The 1-MCP treatment delayed the PG peak, although the activity levels were significantly lower for both treatments.

On harvest, there was no detectable β-GAL activity in sapodilla, but as ripening of control fruit started there was a significant increase (Figure 6). The 1-MCP treatment delayed β-GAL activity, thus in control fruits while the activity was 352.5 U min^{-1} g^{-1} at day 4, in 1-MCP-treated fruits it only reached 136.33 U min^{-1} g^{-1} by day 11. Consistent with the marked initial suppression of β-GAL levels in 1-MCP-treated sapodilla, softening was significantly delayed (Figure 1). The solubilization and degradation of uronic acid were also delayed and reduced in 1-MCP-treated fruit (Figure 3) and among the neutral sugars analyzed, galactose showed great reduction in concentration (Figure 4C), probably metabolized in the respiratory process.

DISCUSSION

The delay in softening observed in 1-MCP-treated sapodilla indicates the importance of ethylene for ripening of climacteric fruits. The ability of the 1-MCP-treated fruits to soften at the end of storage suggests that new ethylene cell membrane receptors were synthesized and cells regained their sensibility to this hormone, as sapodilla ripened regularly (Blakenship, 2001). These results support the observation that sapodilla 'Itapirema-31' stored for 8 d at 24ºC with no postharvest treatment, exhibited a firmness loss from 78.6 N to 5.4 N (Araujo-Neto et al., 2001). It was also observed that 1-MCP-treated fruits keep firmer for a longer period as found in peaches, plums, apricot and avocado (Jeong et al., 2002; Lurie and Weksler, 2005); a delay was also observed in softening of bananas treated with 1-MCP at 100 nL L^{-1} (Lohani et al., 2004).

The reduction in firmness observed as fruits ripen is mostly a consequence of modifications of cell wall carbohydrate metabolism and of its structure. Middle lamellae and cell wall hydrolysis is the main process responsible for loss of firmness in fruits. Thus, the delay in fruit softening is probably due to inhibition of cell wall hydrolytic enzymes, which are activated by ethylene that is inhibited by 1-MCP (Majumder and Mazumdar, 2002). Fruit firmness is considered one of the main quality attributes and often limits postharvest shelf life. In the case of sapodilla, when the fruit is apt for consumption it is so soft that it is very susceptible to mechanical damage and pathogen attack.

For 1-MCP-treated sapodilla, the lower degradation rate of hemicellulose is consistent with the delay in firmness loss. The role of hemicelluloses in the softening process during ripening has not yet been clearly elucidated, due to the irregular behavior that these polymers show among fruit species (Manrique and Lajolo, 2004). Hemicellulose is a group of heterogeneous polysaccharides that bind to cellulose fibers and consist of a variety of neutral sugars. They form chains reuniting cellulose fibers in a close net or act as sliding covers that impede direct contact between cellulose fibers (Redgwell and Fischer, 2002). During ripening of 'Simmonds' avocado at 20°C, hemicelluloses exhibited a gradual molecular mass downshift that was not significantly affected by 1-MCP treatment (Jeong et al., 2002).

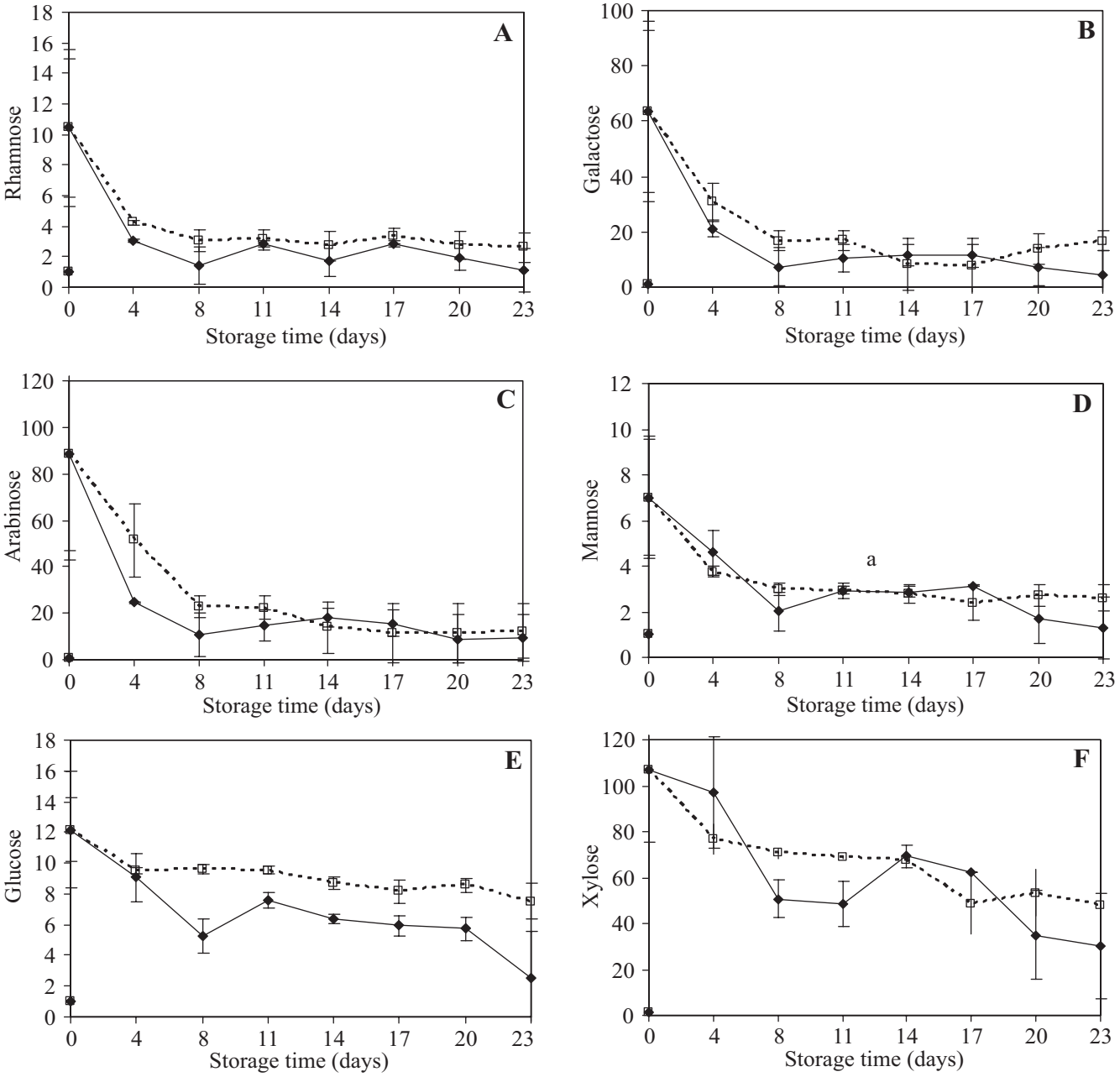

Figure 4. Changes in the concentrations of neutral sugar residues: rhamnosyl (**A**), galactosyl (**B**), arabinosyl (**C**), mannosyl (**D**), glucosyl (**E**) and xylosyl (**F**) from isolated cell wall material (CWM) during postharvest storage of sapodilla treated with 300 nL L^{-1} 1-MCP (□) and (♦) control, at 25 ± 2°C and 70 ± 5% RH. Concentrations are expressed as mg g^{-1} of CWM. $n = 3 \pm$ SD.

The lower uronide levels found in control compared to 1-MCP-treated sapodilla indicate that uronic acid solubilization is coordinated at some level by ethylene. The reduction in firmness observed as fruits ripen is mostly a consequence of modifications on primary cell wall carbohydrate metabolism and structure. Solubilization of cellulose-associated pectins in primary cell wall and in middle lamellae and as well as solubilization of non-cellulosic hemicellulosic neutral sugars are the main changes observed in cell wall composition during fruit softening (Ali et al., 2004; Manrique and Lajolo, 2004). Increases in pectin

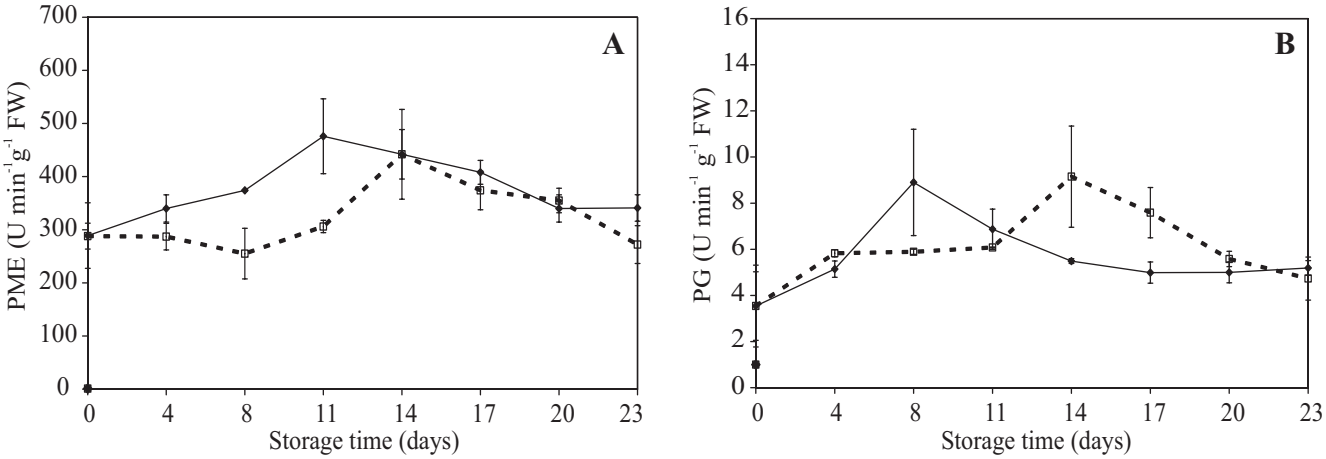

Figure 5. Changes in pectinmethylesterase, PME (**A**) and polygalacturonase, PG (**B**) activities during postharvest storage of sapodilla treated with 300 nL L^{-1} 1-MCP (□) and (♦) control, at 25 ± 2°C and 70 ± 5% RH. *n* = 3 ± SD.

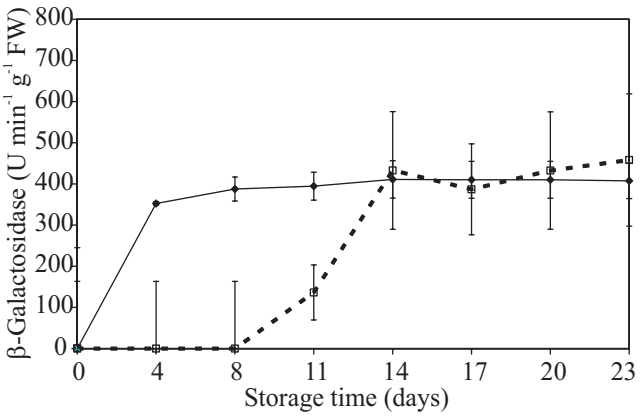

Figure 6. Changes in β-Galactosidase activity during postharvest storage of sapodilla treated with 300 nL L^{-1} 1-MCP (□) and (♦) control, at 25 ± 2°C and 70 ± 5% RH. *n* = 3 ± SD.

solubilization were accompanied by a loss in total polyuronides in mango, tomato, carambola, banana, papaya and guava (Ali et al., 2004). In avocados, 1-MCP treatment decreased uronic acids solubilization and degradation as a result of the inhibition of the hydrolytic enzymes (Jeong et al., 2002).

The distribution of main neutral sugars among different polysaccharides fractions during fruit ripening is indicative of the nature of the polymers present in the fractions and their levels are indicative of the probable changes these polymers undergo (Manrique and Lajolo, 2004). The neutral sugars, rhamnose, mannose, glucose, galactose, arabinose and xylose found in the sapodilla cell wall were released from pectic and hemicellulosic polymers of the cell wall and declined during postharvest storage of sapodilla, as also reported previously in studies with other species (Fischer et al., 1994). The concurrent reduction of rhamnose, arabinose and galactose concentrations are indicative of the linearization of rhamnogalacturonan pectic chains, since rhamnose residues are hydrolytic points of degradation of such a polymer (Bonnas et al., 2004). Filgueiras et al. (1996) concluded that a high correlation between arabinose and galactose concentrations and cell wall-bound galacturonic acid concentration is indicative of the importance of these neutral sugars for the integrity of polyuronides.

The high galactose and arabinose concentrations found for sapodilla suggest the predominance of arabinogalactans polymers, while the glucose and xylose concentration suggest the presence of xyloglucans chains, although the possible presence of xyloglucomanans cannot be entirely rejected, in spite of the low concentration of mannosyl residues. Fucosyl residues were found only in a negligible amount and thus this data was not shown. Our results on neutral sugars concentrations are in accordance with those reported for pineapple (Bonnas et al., 2004).

The release of these sugars during solubilization of pectin and hemicellulose polymers and their decreasing levels, during storage, suggest their rapid metabolism and probable use in glycolysis (Manrique and Lajolo, 2004). 1-MCP postharvest treatment reduced

depolymerization of pectin and hemicellulose and thus decreased the free neutral sugars from sapodilla cell wall. In 1-MCP-treated sapodilla, the glucose levels were unique since they differed markedly from control fruits, although they did present a similar behavior with a linear decline. Neutral sugars are responsible for the linkages between pectin and hemicellulose chains (Redgwell and Fischer, 2002). These results indicate that 1-MCP treatment slowed down the degradation of cell wall, probably by inhibiting hydrolytic enzymes.

Pectinmethylesterase catalyzes the demethylesterification of galacturonic acid of pectin chains and since PG depolymerizes these galacturonic acid chains, PG activity is dependent on PME for making substrate available (Tieman and Handa, 1994). In sapodilla, the high pectin demethylesterification activity catalyzed by PME is probably required not only for subsequent PG activity, which was very low, but also to modify pH and cation exchange properties of the cell wall, which might impact on other cell wall enzymes. In an earlier study, Ali et al. (2004) reported similar activity patterns for PME in papaya and carambola showing that PME activity was significant, but its role in softening of tropical fruit was still unclear. In bananas, 1-MCP treatment did not delay PME peak activity; nevertheless, the levels were markedly lower indicating that cell wall hydrolases are largely dependent on ethylene production and perception (Lohani et al., 2004).

The 1-MCP treatment delayed PG peak activity and in spite of the low activity levels for both treatments, the increase observed for PG activity was consistent with the decline of uronic acid concentrations. The low activity values presented here for PG were similar to those observed for sapodilla stored under ambient temperature and modified atmosphere (Miranda et al., 2001). During ripening of avocado and banana, PG activity was very low in the pre-climacteric stage, and then increased as the climacteric proceeded and continued increasing during the postclimacteric phase (Jeong et al., 2002; Lohani et al., 2004). In both cases, PG activity was preceded by PME. When avocado fruits were treated with 1-MCP, PG activity was not recovered although firmness reached values similar to the control (Jeong et al., 2002). In 1-MCP-treated banana, PG activity increased gradually throughout the period of 7 d reaching lower levels than those of control fruits (Lohani et al., 2004).

For some time, changes in firmness observed during ripening were mainly credited to pectin hydrolysis by PG. Now, there is evidence that other mechanisms are also involved in fruit softening (Redgwell and Fischer, 2002). Polygalacturonase activity is not the main factor responsible for tomato softening, since in transgenic tomatoes the inhibition of PG activity had very little effect on firmness loss (Giovannoni, 2001). Similarly in tomato, a high PG activity did not result in higher pectin solubilization indicating that other factors are probably involved in this process (Filgueiras et al., 1996). In sapodilla, the low PG activity reported here and in a previous study also indicates that it is not the main factor responsible for softening (Miranda et al., 2001).

The delay in β-GAL activity observed in treated sapodilla indicates the importance of ethylene for the activity of this cell wall hydrolase. Studies on gene expression in tomatoes have shown an increase in expression of the β-GAL gene during the climacteric, as reported by Moctezuma et al. (2003). These authors suggested the use ethylene antagonists such as 1-MCP to slow the activity of β-GAL and prolong the postharvest life of tomatoes. A previous study showed that β-GAL activity increased as sapodilla softened and the loosening of the cell wall, to which β-GAL is associated, could be observed through microscopic analysis (Miranda et al., 2001).

During ripening of melons, the lack of PG activity, the reduction of galactosyl residues from the cell wall and the concomitant increase in β-GAL activity led to the idea that β-GAL is responsible for the changes in structural polysaccharides that result in softening (Ranwala et al., 1992). The galactosyl residues result from the action of β-GAL, that is able to catalyze the cleavage of cross-linking β(1-4) galactans that connect pectin rhamnogalacturonans to the xyloglucan-cellulose microfibril complex (Ali et al., 2004). β-Galactosidase is able to modify both pectin and hemicellulose, and thus should be a significant influence on cell wall disassembly during ripening of sapodilla.

CONCLUSIONS

The postharvest treatment with 1-MCP delayed softening of sapodilla for 11 d by hindering cell wall polysaccharide metabolism. Treatment with 1-MCP reduced depolymerization of pectin and hemicellulose

slowing down the degradation of the sapodilla cell wall by inhibition of hydrolytic enzymes. These results suggest the importance of ethylene in coordinating the activity of cell wall hydrolytic enzymes during fruit ripening; and the softening of sapodilla can be attributed to modification of both pectin and the xyloglucan-cellulose microfibril network. Thus, 1-MCP seems to be a powerful tool in retarding fruit ripening and in helping understand the involvement of ethylene in cell wall modifications.

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