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Activities of several membrane and cell-wall hydrolases, ethylene biosynthetic enzymes, and cell wall polyuronide degradation during low-temperature storage of intact and fresh-cut papaya (*Carica papaya*) fruit

Yasar Karakurt, Donald J. Huber *

Horticultural Sciences Department, P.O. Box 110690, University of Florida, Gainesville, FL 32611, USA

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Abstract

Fresh-cut fruit tissue deteriorates more rapidly than its intact counterpart. A study was conducted to determine changes in firmness, cell-wall polyuronides, and the activities of cell-wall and membrane hydrolases and ethylene biosynthetic enzymes in intact and fresh-cut papaya fruit during storage at 5 °C. Processing of papaya fruit was performed under sanitized conditions at 5 °C, and fruit pieces were stored for 8 days at 5 °C. Levels of total and CDTA-soluble polyuronides in intact fruit did not change during storage, but water-soluble polyuronides increased significantly by day 8 (15%). In contrast, total polyuronide content of fresh-cut papaya decreased (9.5%), whereas levels of CDTA- and water-soluble polyuronides increased 45 and 30%, respectively. Firmness and mol mass of polyuronides decreased more rapidly in fresh-cut fruit than in intact fruit. The activities of polygalacturonase (EC 3.2.1.15), alpha- (EC 3.2.1.22) and beta-galactosidases (EC 3.2.1.23), lipoxygenase (EC 1.13.11.12), phospholipase D (EC 3.1.4.4), and ACC synthase (EC 4.4.1.14) and ACC oxidase increased within 24 h in fresh-cut fruit, and remained significantly higher compared with levels in intact fruit throughout the storage period. Pectin methyl esterase (EC 3.2.1.11), and phospholipase C (EC 3.1.4.3) activities showed no consistent differences between fresh-cut and intact fruit. The data suggest that a wound-induced increase in enzymes targeting cell walls and membranes contributes to the rapid deterioration of fresh-cut fruit. The significantly less pronounced changes observed for intact fruit stored under identical conditions indicate that the enhanced deterioration of fresh-cut fruit does not reflect low-temperature injury.

Keywords: Cell wall; Fresh-cut; Lipoxygenase; Membrane; Papaya; Phospholipase; Polyuronides; Wounding

1. Introduction

Fresh-cut fruits and vegetables are those that have been subjected to various degrees of peeling, trimming, coring, slicing, shredding or dicing. The objectives of fresh-cut technology are to deliver to

* Corresponding author. Fax: +1-352-392-6479
E-mail address: djh@mail.ifas.ufl.edu (D.J. Huber).

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consumers a convenient, fresh-like product with extended shelf life, and high nutritional and sensory quality (Reyes, 1996).

The loss of protective epidermal and subepidermal tissues, and the acute physical injury associated with processing, contribute to a high perishability of fresh-cut produce (Reyes, 1996). Fresh-cut tropical fruits including banana (Abe and Watada, 1991), and papaya and kiwifruit (O'Connor-Shaw et al., 1994; Paull and Chen, 1997), for example, were of unacceptable quality after only 2 days storage at 4 °C, primarily due to tissue softening. The biochemical basis of texture loss in fresh-cut fruits is unknown. In view of the physical damage associated with fruit processing, likely candidates for rapid metabolic responses to wounding include the cell wall and membranes. The activities of some cell-wall enzymes, for example, are minimal under normal conditions but are greatly enhanced in response to mechanical wounding (Dumville and Fry, 2000; Huber et al., 2001). The senescence-delaying influence of Ca^{2+} dips on shredded carrots (Picchioni et al., 1996) and the increased juice leakage during storage of fresh-cut watermelon (Cartaxo et al., 1997) are consistent with an important role for altered cell-wall and/or membrane metabolism in the deterioration of fresh-cut produce.

Although fresh-cut commodities initially possess the quality and sensory attributes of the intact commodity, the metabolism of fresh-cut compared with intact fruit diverges rapidly. Fresh-cut products show enhanced respiration and ethylene production, and are significantly more perishable than the intact commodity (Varoquaux and Wiley, 1994). Several studies have emphasized a role for specific enzymes in the deterioration of fresh-cut commodities. Enhanced activities of chlorophyll-degrading enzymes in coleslaw (Heaton et al., 1996) and pyrophosphatase-phosphofructokinase in sliced carrots (Kato-Noguchi and Watada, 1996) have been reported. A wound-induced increase in phenylalanine ammonia lyase was associated with browning of the cut surfaces of head lettuce (Lopez-Galvez et al., 1996) and carrot roots (Leja et al., 1997). Wound-induced phospholipase A_2 in tomato leaves (Narvez-Vasquez et al., 1999), and phospholipase D in Arabidopsis and lipoxygenase in tomato leaves (Heitz et al., 1997) have been shown to be essential for the synthesis of and cellular responses to jasmonic acid (JA), a wound signalling compound (Wang et al., 2000). During the first hours after wounding, plants accumulate phosphatidic acid and unesterified fatty acids that are released from lipids, presumably by the action of wound-inducible phospholipases of types D and A_2 (Conconi et al., 1996; Lee et al., 1997; Narvez-Vasquez et al., 1999; Ryu and Wang, 1996), thus producing substrates for lipoxygenases (Bruxelles and Roberts, 2001). Limited studies have addressed enzymes contributing to deterioration of fresh-cut fruit, especially those with high chill sensitivity.

The objectives of this study were to determine changes in the activities of several catabolic and wound-induced enzymes, including ethylene biosynthetic enzymes, and in cell wall polyuronides in fresh-cut papaya fruit stored at low temperature. To distinguish between changes induced by wounding versus those induced by exposure to low temperature, intact papaya fruit stored under identical conditions were included as controls.

2. Materials and methods

2.1. Plant material and processing procedures

Papaya (*Carica papaya*, var. Sunrise Solo) fruit were obtained from Brooks Tropicals, Homestead, FL. Following transfer to the postharvest facilities in Gainesville, the fruit were sorted, washed with tap water, chlorinated water (150 μl l^{-1} free chlorine, pH 7.0) and rinsed. The fruit were then stored at 20 °C until they reached 60–70% yellow surface color. Prior to slicing, the cutting surfaces and cold room interior were rinsed with chlorinated water (150 μl l^{-1} free chlorine). Hairnets, latex gloves, surgical masks, and disposable aprons were worn during cutting and handling to minimize contamination. All operations were performed at 5 °C and fruit were held at this temperature for 12 h prior to processing. Fruit were peeled, and tissue processed into pieces ($\sim$7 cm × 5 cm × 3 cm) with sharp, sterile knives. Knives were re-sterilized in 150 μl l^{-1} free chlorine

during the cutting operations. The tissue pieces were randomized and stored in vented plastic containers for 8 days at 5 °C. Intact fruit stored under identical conditions served as controls.

At the indicated intervals, fruit pieces were removed from storage, immediately frozen in liquid nitrogen, and analyzed as described below. Intact fruit were peeled, cut into pieces as described above, and immediately frozen in liquid nitrogen. Samples were stored at −30 °C until analyzed.

2.2. Firmness of fresh-cut and intact papaya

Twenty cubes of approximately 9 cm^3 were excised from the intact and fresh-cut papaya mesocarp tissue pieces and firmness of individual cubes was measured using an Instron Universal Testing Instrument (Model 4411, Canton, MA). After establishing zero force contact with the cube, a 10-mm diameter convex probe was driven (crosshead speed 10 cm min^{-1}, 5 kg load cell) a distance of 2 mm. The firmness data represent the maximum force (N) recorded during compression.

2.3. Preparation of ethanol-insoluble solids

Partially thawed tissue (100 g) derived from fresh-cut and intact papaya fruit at each sampling interval was homogenized in 400 ml ethanol for 2 min with a Polytron homogenizer (Brinkmann, PT 10-35, Lens Kruenz, Switzerland) set at maximum speed. The homogenate was refluxed in a boiling water bath for 25 min to inactivate enzymes and then filtered through GF/C filter papers (Whatman, Clifton, NJ) in an aspiration flask and washed with 95% ethanol. The residue was transferred to 100 ml chloroform/methanol (1:1, v/v) and incubated with stirring for 30 min. The suspensions were filtered (GF/C) and washed with 100 ml acetone. After partial drying via aspiration, the ethanol insoluble solids (EIS) were placed in a drying oven at 43 °C for 12 h and stored in a desiccator at room temperature.

2.4. Extraction and chromatography of water and chelator-soluble polyuronides

Extraction of water-soluble polyuronides was performed by incubating EIS (20 mg) in 7 ml of deionized water for 4 h at room temperature with stirring. The suspension was filtered through GF/C filter paper and the supernatant retained for gel chromatography. The retained material was suspended in 7 ml of 50 mM Na-acetate, 50 mM *trans* -1,2-cyclohexanediamine-*N*, *N*, *N′*, *N′*-tetraacetic acid (CDTA), pH 6.5 and incubated at room temperature with stirring for 6 h. The suspension was filtered through GF/C filters and the supernatant retained for chromatography. Uronic acids in the filtrates were determined using the procedure of Blumenkrantz and Asboe-Hansen (1973). Total uronic acids in the EIS were measured as described in Ahmed and Labavitch (1977).

Gel chromatography of polyuronides was performed as described by Chun and Huber (2000) on a bed (1.5 cm width, 27 cm length) of CL-4B-200 (Pharmacia, Piscataway, NJ) packed and operated in 200 mM ammonium acetate, pH 5.0. Polyuronides (∼0.5 mg galacturonic acid equivalents in a volume of 2.5 ml) were applied to the column and eluted with the ammonium acetate. Fractions of 2 ml were collected, and 0.5 ml aliquots of these used for the determination of uronic acids. Uronic acids from each treatment were chromatographed in triplicate. The V_o and V_i were determined using Dextran 2000 and glucose (Sigma, St. Louis, MO), respectively.

2.5. Isolation and assay of cell-wall enzymes

Partially thawed mesocarp tissue (20 g) derived from fresh-cut and intact papaya fruit and 40 ml cold ethanol were homogenized for 2 min with a Polytron homogenizer, and the homogenate was centrifuged for 20 min (4 °C) at 12 000 × *g* (Beckman, Model J2-21, Palo Alto, CA). The pellet was washed with 25 ml of 80% cold ethanol and centrifuged at 12 000 × *g* (4 °C) for 20 min. The pellet was suspended in 10 ml of 25 mM Na-acetate, pH 5.0 containing 1.2 M NaCl and incubated at 1 °C for 30 min. After centrifugation at 12 000 × *g* (4 °C) for 20 min, the cell-free

protein extract was filtered through Miracloth (Calbiochem, La Jolla, CA) and used for the determination of polygalacturonase (EC 3.2.1.15), and α- (EC 3.2.1.22) and β-galactosidase activities (EC 3.2.1.23). Total protein was determined using a standard BCA kit (Pierce, Rockford, IL).

Reaction mixtures for the determination of PG activity consisted of 0.1 ml of the cell-free protein extract and 0.5 ml (1 mg) of polygalacturonic acid (citrus, from Sigma) in 50 mM Na-acetate, pH 5.5. The samples were incubated for 2 h in a water bath at 34 °C. Activity was assayed reductometrically (Milner and Avigad, 1967), and expressed as mol galacturonic acid equivalents (kg protein)$^{-1}$ h^{-1}.

Reaction mixtures for α- and β-galactosidase activities contained 0.2 ml of the cell-free protein extract and 0.2 ml of the ρ-NO_2-phenyl derivatives (6.6 mM) of α- and β-D-galactopyranoside (Sigma) in 0.1 M Na-acetate, pH 5.2. The reactions were carried out for 20 min at 37 °C and terminated by addition of 1 ml of 1 N NH_4OH containing 2 mM EDTA. The release of ρ-NO_2-phenol was measured at 400 nm. ρ-NO_2-phenol (Sigma) was used as standard. Activity was expressed as mol ρ-NO_2-phenol (kg protein)$^{-1}$ min^{-1}.

Pectinmethylesterase (PME, EC 3.2.1.11) was extracted and assayed as described by Hagerman and Austin (1986). Activity was expressed as mol H^+ equivalents (kg protein min)$^{-1}$.

2.6. ACC synthase activity

ACC synthase activity (EC 4.4.1.14) was determined as described by Mullins et al. (2000) with slight modifications. Tissue (5 g) derived from fresh-cut or intact papaya fruit was homogenized with a Polytron homogenizer in a buffer containing 100 mM potassium phosphate, pH 8.0, 5% ammonium sulfate, 5 μM pyridoxal phosphate, 4 mM dithiothreitol (DTT) and 3% polyvinylpolypyrrolidine (PVPP). The extract was filtered through a layer of Miracloth (Calbiochem) and centrifuged at 10 000 × g for 10 min. The supernatant was brought to 90% saturation with solid ammonium sulfate, stirred for 90 min at 4 °C and the suspension centrifuged at 25 000 × g for 10 min. The pellet was resuspended in 5 ml of incubation buffer (100 mM potassium phosphate, 0.1 mM DTT and 4 μM pyridoxal phosphate, pH 7.0). For desalting, the sample was dialyzed against incubation buffer overnight. ACC synthase activity was determined in a reaction mixture containing 3.67 ml desalted enzyme extract, 375 μl incubation buffer and 450 μl of 500 μM S-adenosyl-L-methionine (AdoMet). After 3 h at 30 °C, protein was eliminated from the mixture by adding 3.75 ml of phenol/chloroform/isoamyl alcohol (25:24:1, v/v/v) followed by vortexing and then centrifuging for 10 min at 28 500 × g. The aqueous phase was decanted and clarified by centrifuging at 28 500 × g for 10 min. ACC formed was assayed by the method of Lizada and Yang (1979) using 1 μmol of $HgCl_2$ and 1 ml of headspace gas for injection into the GC. Activity was expressed as mol of ACC formed (kg protein)$^{-1}$ h^{-1}.

2.7. ACC oxidase activity

ACC oxidase was extracted and assayed as described by Fernandez-Maculet and Yang (1991), with some modifications. Four g of papaya tissue were homogenized in 12 ml of extraction medium containing 0.1 M Tris (pH 7.4), 10% glycerol and 30 mM sodium ascorbate. The slurry was filtered through four layers of cheesecloth and centrifuged at 28 000 × g for 25 min. Enzyme activity was assayed at 25 °C in 1 ml reaction mixtures containing 0.4 ml of the enzyme extract, 30 mM sodium ascorbate, 0.1 mM $FeSO_4$, 1 mM ACC and extraction buffer in 25 ml vials stoppered with rubber septa. The reaction mixtures were incubated at 25 °C for 2 h, and 1 ml of gas sample withdrawn from headspace was analyzed for ethylene by gas chromatography. ACC oxidase activity was expressed as mol ethylene formed (kg fresh weight h)$^{-1}$.

2.8. Lipoxygenase and phospholipase C and D activities

Lipoxygenase activity (EC 1.13.11.12) was determined as described by Cherif et al. (1997). Phospholipase D (PLD) (EC 3.1.4.4) and phospholipase C (PLC) (EC 3.1.4.3) were extracted as

described by Ryu and Wang (1996) with some modifications. Briefly, papaya mesocarp tissue (10 g) was homogenized at 4 °C in 10 ml of extraction buffer containing 50 mM Tris–HCl (pH 8.0), 0.5 M sucrose, 10 mM KCl, 1 mM EDTA, 0.5 mM PMSF and 2 mM DTT. The homogenate was centrifuged at $15\,000 \times g$ for 30 min, and the supernatant used for enzyme assay. PLC and PLD activities were determined spectrophotometrically as described by Kurioka and Matsuda (1976) and Gupta and Wold (1980), respectively, using ρ-nitrophenylphosphorylcholine (Sigma) as substrate. PLC and PLD activities were expressed as mol p-nitro-phenol (kg protein h)$^{-1}$.

2.9. Statistical analysis

The data were analyzed according to a completely randomized design using GLM program of Statistical Analysis System (SAS) and Duncan's multiple range test at 5% level of significance.

3. Results and discussion

Firmness of fresh-cut papaya fruit decreased nearly 36% after only 2 days and continued a steady decline throughout storage at 5 °C (Table 1). In contrast, tissue derived from intact fruit stored under identical conditions showed little change in firmness through 4 days of storage. At 8 days, the firmness of tissue from fresh-cut and intact fruit had declined approximately 54 and 19%, respectively. The rapid reduction in fresh-cut papaya is consistent with other reports (O'Connor-Shaw et al., 1994; Paull and Chen, 1997).

Total polyuronide content did not change in tissue of intact papaya stored for up to 8 days at 5 °C whereas a small but significant decline (10%) was noted for the fresh-cut fruit (Table 1). In intact fruit, levels of chelator-soluble polyuronides did not change whereas water-soluble polyuronides showed a significant increase after 8 days at 5 °C (Table 1). In fresh-cut fruit, the levels of both water- and CDTA-soluble polyuronides increased significantly within the first 24 h and continued to increase with storage. After 8 days, chelator- and water-soluble polyuronides in fresh-cut papaya were 30 and 45% higher, respectively, compared with levels at day 0 (Table 1). Chelator- and water-soluble polyuronides from intact fruit showed little change in mol mass during storage (Fig. 1). Mol mass downshifts in polyuronides in fresh-cut tissue were first evident at 2 days of storage (not shown), with more extensive downshifts evident at 4 and 8 days (Fig. 1).

The lower mol mass polyuronides from fresh-cut compared with intact fruit could arise from depolymerization or from increased solubility (Table 1) of inherently smaller polymers. Consistent with the participation of depolymerization was the significantly greater levels of PG activity in fresh-cut compared with intact fruit (Fig. 2). Paull et al. (1999) have attributed the mol mass downshifts in papaya fruit during ripening to increases in PG activity. Further evidence for polyuronide depolymerization in fresh-cut fruit was the decline in total uronic acids (Table 1), suggesting that a portion of polyuronides was converted to ethanol-

Table 1
Changes in firmness and cell-wall polyuronide solubility of fresh-cut and intact papaya fruit during storage at 5 °C

Days	Firmness (N)		Total polyuronides (g kg^{-1})		Water-soluble polyuronides (g kg^{-1})		CDTA-soluble polyuronides (g kg^{-1})	
	Intact	Fresh-cut	Intact	Fresh-cut	Intact	Fresh-cut	Intact	Fresh-cut
0	8.7a	8.7a	327.0a	327.0a	62.3b	62.3c	34.7a	34.7c
1	8.7a	7.8a	326.1a	325.5a	64.2ab	72.7b	35.1a	40.9bc
2	8.5a	5.6b	328.7a	321.1a	68.0ab	75.1b	36.1a	44.5ab
4	8.1a	5.0bc	327.1a	312.1a	68.5ab	77.1ab	36.7a	46.7ab
8	7.0b	4.1c	321.4a	295.2b	71.7a	83.1a	37.0a	50.3a

Means followed by the same letter within each column are not significantly different at the 5% level.

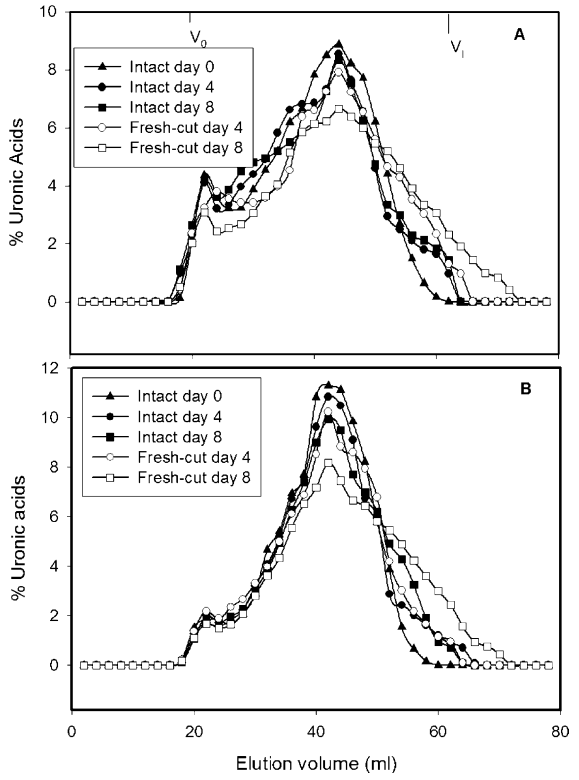

Fig. 1. Mol mass profiles of CDTA (A) and water-soluble (B) polyuronides from intact and fresh-cut papaya fruit stored at 5 °C for 0, 4 and 8 days. Polyuronides (0.5 mg galacturonic acid equivalents) were applied to a CL-4B-200 (1.5 × 27 cm) column operated with a mobile phase of 200 mM ammonia acetate, pH 5.0. Fractions were analyzed for uronic acids. V_o and V_i denote the void and included volumes, respectively.

soluble products (oligouronides) that would not be recovered in EIS preparations. The higher levels of PG activity in fresh-cut fruit might represent a response to increased ethylene production, which has been shown to increase as much as 10-fold in papaya fruit within hours of slicing (Paull and Chen, 1997). Ethylene-induced transcriptional activation of PG synthesis has been demonstrated for tomato (Sitrit and Bennett, 1998) and avocado fruits (Buse and Laties, 1993; Dopico et al., 1993). Consistent with a possible role for ethylene in the increases in PG activity, ACC synthase (ACS) and ACC oxidase (ACO) activities increased markedly in fresh-cut and intact papaya (Fig. 3A,B). After 2 days of storage, levels of ACS activity in fresh-cut and intact fruit were 250 and 63% higher, respectively, compared with those of fruit prior to storage (day 0). The differences in ACO activity between fresh-cut and intact fruit (Fig. 3B) were not as dramatic as noted for ACS. ACO activity in intact fruit reached a maximum at day 2 (a 31% increase compared with day 0) and then decreased significantly with further storage. In fresh-cut tissue, maximum activity was at day 4, at which time the activity was 47% higher than the activity at day 0. Despite the differences in timing and maximum activities between intact and fresh-cut papaya, ACO activity does not show a clear pattern with fresh-cut in papaya and is probably not rate limiting at the activity levels observed (Lelieure et al., 1997).

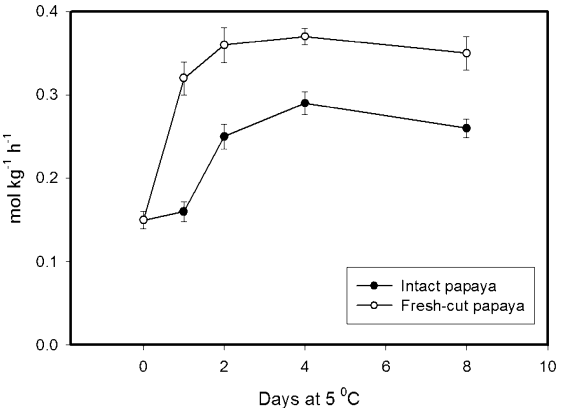

Fig. 2. Polygalacturonase activity of intact and fresh-cut papaya fruit stored at 5 °C for 0 1, 2, 4 and 8 days. Data expressed as D-galacturonic acid equivalents produced per kg of protein and are the means of six replications. Vertical bars represent S.D.

Other enzymes involved in pectin metabolism and depolymerization, including galactosidases and pectinmethylesterase (PME), were also examined in fresh-cut papaya. PME was not different in fresh-cut compared with intact fruit (Fig. 4), with activity increasing through 2 days of storage in both tissues and thereafter remaining constant. Although pectin methyl de-esterification declines during papaya ripening (Paull et al., 1999), our data suggest that levels of extractable PME do not parallel the softening and deterioration of fresh-cut compared with intact papaya fruit. Analysis of pectin mol mass in tomato fruit homogenates, however, provide evidence that pre-existing levels

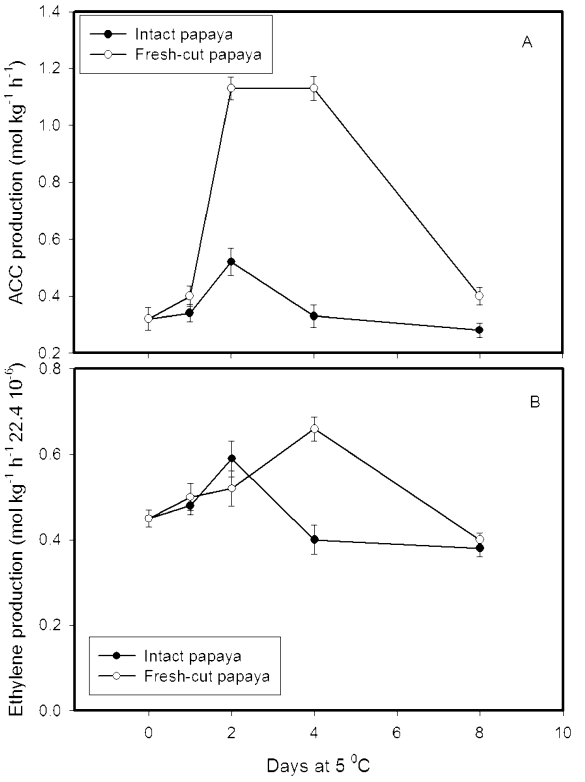

Fig. 3. ACC synthase (A) and ACC oxidase (B) activities of intact and fresh-cut papaya fruit stored at 5 °C for 0 1, 2, 4 and 8 days. Data represent ACC produced per kg protein (A) and mol of ethylene produced per kg tissue fresh weight (B) and are the means of six replications. Vertical bars represent S.D.

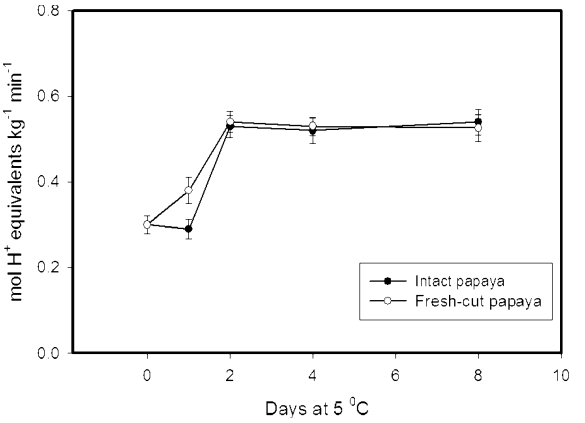

Fig. 4. Pectinmethylesterase activity of intact and fresh-cut papaya fruit stored at 5 °C for 0 1, 2, 4 and 8 days. Data expressed as mol H^+ equivalents produced per kg protein and are the means of six replications. Vertical bars represent S.D.

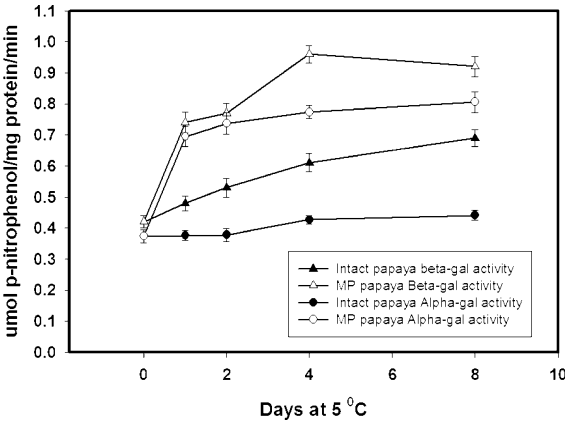

Fig. 5. Alpha- and β-galactosidase activities of intact and fresh-cut papaya fruit stored at 5 °C for 0 1, 2, 4 and 8 days. Data expressed as mol nitrophenol equivalents produced per kg protein and are the means of six replications. Vertical bars represent S.D.

of enzymes (PG and PME) may be sufficient to explain the rapid (within 5 min) and extensive depolymerization in response to tissue disruption (Huber et al., 2001).

Alpha- (α-Gal) and beta-galactosidase (β-Gal) activities were enhanced in fresh-cut compared with intact papaya (Fig. 5). In fresh-cut tissue, α- and β-Gal activities increased 86 and 76%, respectively, after only 24 h compared with day 0 fruit. After 4 days of storage, activity levels remained significantly higher in fresh-cut tissue than levels in intact fruit. Over the entire storage period, α- and β-gal activities increased 147 and 116%, respectively, in fresh-cut fruit compared with 18 and 64% in intact fruit. Over the 8-day storage period, the enhanced activity due to fresh-cut (wounding) was 83 and 33%, respectively, for α- and β-Gal. As for PG, the increased activities of galactosidases might represent a response to enhanced ethylene production. Galactosidase activities were suppressed in ACS-antisense tomato fruit, which had greatly reduced ethylene production, but accumulated in fruit exposed to exogenous ethylene (Sozzi et al., 1998). Although the functions of galactosidases in pectin metabolism in particular and cell wall metabolism in general are not fully understood (Huber et al., 2001), the trends in the activities of

both enzymes paralleled the firmness declines in the fresh-cut and intact papaya. A correlation between α- and β-Gal activities increases and firmness decline has been reported for ripening papaya fruit (Lazan and Ali, 1993; Lazan et al., 1995). Furthermore, Tucker et al. (1999) reported that cold-break pastes from tomato fruit expressing an antisense-gene for a β-galactanase exhibited higher viscosity compared with pastes from normal fruit, providing indirect evidence for a role for these enzymes in the degradation of pectins, and possibly other structural polymers.

Another factor of potential importance in the rapid softening and deterioration of fresh-cut fruit is increased cellular leakage (Cartaxo et al., 1997; Lopez-Galvez et al., 1996; Hodges et al., 2000) resulting from wound-induced degradation of membrane lipids. An increase in electrolyte efflux in response to fresh-cut has been noted for papaya fruit (Ergun and Huber unpublished). Wu et al. (1999) reported that increased lipoxygenase (LOX) activity was associated with firmness loss in peach mesocarp tissue. As shown in Fig. 6, LOX activity accumulated dramatically in response to fresh-cutting, increasing more than 3-fold in the initial 24 h of storage and reaching a maximum at day 4. In contrast, LOX activity increased slightly in intact fruit during storage. PLC activity increased markedly (32%) in fresh-cut fruit within 24 h (Fig. 7). A parallel but delayed increase was also noted for intact papaya on day 2. After day 2, PLC activity decreased in both fresh-cut and intact tissue through 8 days of storage. PLD activity in fresh-cut tissue increased (48%) within 24 h and then remained relatively constant during the rest of the storage period. However, PLD activity increased significantly within 4 days in intact fruit and then remained constant throughout the storage (Fig. 7). Generally, the activity of lipolytic enzymes including phospholipases and LOXs increases during senescence (Todd et al., 1990; Wang, 2001), the former activities resulting in the release of membrane unsaturated fatty acids that can serve as substrates for LOX. In response to physical wounding, LOX could be involved either positively, through its role in the production of defense-related signaling molecules (Creelman et al., 1992; Albrecht et al., 1993; Laudert et al., 1996), or negatively through participation in autocatalytic peroxidation reactions (Hildebrand, 1989). LOX hydroperoxides can contribute to tissue damage through inactivation of protein synthesis and deterioration of cellular membranes. Dumville and Fry (2000) and Schweikert et al. (2000) have posited a role for radical-based mechanisms in polysaccharide breakdown in plant development, providing a possible relationship

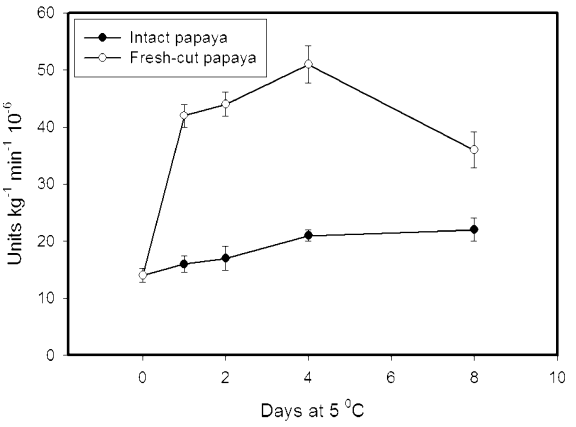

Fig. 6. Lipoxygenase activity of intact and fresh-cut papaya fruit stored at 5 °C for 0 1, 2, 4 and 8 days. Data are the means of six replications. One unit is defined as a change of 0.001 absorption units (234 nm) min^{-1}. Vertical bars represent S.D.

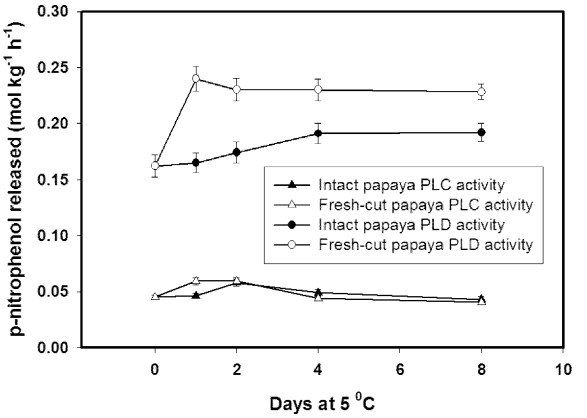

Fig. 7. Phospholipase C (A) and D (B) activity of intact and fresh-cut papaya fruit stored at 5 °C for 0 1, 2, 4 and 8 days. Data expressed as mol nitrophenol equivalents produced per kg protein and are the means of six replications. Vertical bars represent S.D.

between peroxidative lipid metabolism and tissue softening.

In summary, fresh-cut and intact papaya stored at 5 °C showed dramatic differences in tissue firmness and in the activity trends of a number of hydrolases. The generally higher and more rapid accumulation of enzyme activities in fresh-cut tissue might represent a general or global response to wounding. The rapidity of these increases, typically observed within 24 h of tissue wounding, argues that microbial proliferation, which becomes more problematic during long-term storage of fresh-cut fruit (Cartaxo et al., 1997), did not contribute to the enhanced activities of cell wall and membrane hydrolases. Furthermore, the differences noted between intact and fresh-cut fruit, which were stored under identical conditions, support the notion that the firmness and enzyme trends in fresh-cut papaya are not a direct consequence of low-temperature stress. The rapid softening and deterioration of fresh-cut papaya likely involve membrane and cell wall catabolism accelerated or otherwise altered in response to physical wounding. Wound-induced changes in permeability and solute efflux could modify Ca^{2+} binding (Ferguson et al., 1980) and other apoplastic conditions (Almeida and Huber, 1999), contributing to accelerated cell wall and membrane catabolism. Enhanced polyuronide degradation in response to tissue damage (Brummell and Labavitch, 1997; Fry, 1998; Huber et al., 2001), for example, is consistent with a role for wound-induced modification of apoplastic conditions in the deterioration of fresh-cut fruits. Collectively, the data suggest that enhanced hydrolase activity and accelerated senescence are involved in the rapid softening and deterioration of fresh-cut papaya fruit.

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