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Cell wall modification in 1-methylcyclopropene-treated post-climacteric fresh-cut and intact papaya fruit

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Abstract Papaya is a climacteric fruit in which ripening is greatly regulated by ethylene often associated with stress responses such as wounding. The changes in cell wall compositions in papaya fruit at an advanced stage of ripening under stress conditions including chilling temperature of 5°C and wounding employed as fresh-cut and how these changes were affected by an ethylene action inhibitor of 1-methylcyclopropopene (1-MCP) were examined in the study. The recovery of ethanol-insoluble solids, total soluble sugars, water-soluble polyuronides, neutral hemicelluloses, and neutral sugars of rhamnose, arabinose, mannose and glucose were not affected by 1-MCP or fresh-cut processing. The fresh-cut processing, however, caused a higher loss of total polyuronides and the neutral sugar galactose while increasing the recovery of chelator-soluble polyuronides. Few significant differences due to 1-MCP application were recorded in the recoveries of alkali-soluble polyuronides, hemicellulosic polyuronides extracted with 4% KOH, and the neutral sugar xylose. Modifications of cell wall polyuronides and hemicelluloses in ripe fresh-cut papaya fruit exhibited mostly similar patterns to those in intact ripe papaya fruit under the chilling temperature of 5°C while minimally affected by 1-MCP.

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Introduction

Fresh-cut fruits are extremely fragile and perishable and exhibit significant differences in terms of physiological behaviors relative to their intact counterparts despite having similar quality and sensory attributes. They demonstrate enhanced ethylene and respiration rates, wound-healing processes (synthesis of secondary compounds, suberization and lignification), biochemical changes (membrane and cell wall changes, browning and degreening), and physical changes (softening, watersoaking and water loss; Rolle and Chism 1987; Miller 1992; Brecht 1995; Jeong and Huber 2004). These processes can greatly influence the quality maintenance of fresh-cut fruits mostly in the form of rapid texture loss. The rapid texture loss in fresh-cut fruits has not been fully clarified yet; however, a number of studies have shown that the rapid texture loss possibly results from cell wall and/or membrane damages (Cartaxo et al. 1997; Dumville and Fry 2000; Huber et al. 2001; Karakurt and Huber 2003).

Textural modifications are largely attributed to the changes in cell wall structure (Huber 1983; Tucker and Grierson 1987), resulting mostly from restructuring and recomposition of pectic polymers (Seymour et al. 1987) possibly with the help of expansin. Solubilization and depolymerization of both polyuronides (Fischer and Bennett 1991) and hemicelluloses (Lashbrook et al. 1997) during ripening due primarily to enzymatic activities are frequently associated with cell wall loosening and disintegration. These changes are brought about by various hydrolases such as polygalacturonase (PG; EC 3.2.1.15), pectin methylesterase (PEM; EC 3.1 .1 .11), xyloglucanendotransglucosyl/hydrolase (EC 2.4.1.207), and pectate lyases (EC 4.2.2.2).

Non-enzymic processes, such as pH or ionic force of solution encircling the cell wall, could also involve in softening (Fishman et al. 1989). In addition to the depolymerization of both polyuronides and hemicelluloses, fruit softening is accompanied by a loss of neutral sugars, primarily galactose and arabinose, from pectic and hemicellulosic polysaccharides (Tucker 1993).

Studies on papaya cell wall have indicated that softening is accompanied by polyuronide hydrolysis and modification of hemicelluloses (Karakurt and Huber 2003; Zhao et al. 1996; Paull et al. 1999; Manrique and Lajolo 2004) and a continuous or temporary increase in the activities of PG (Karakurt and Huber 2003; Paull and Chen 1983), xylanase (Paull and Chen 1983) (EC 3.2.1.8), α-galactosidase (α-Gal; EC 3.2.1.22), and β-galactosidase (Ali et al. 1998; Gallon et al. 2009) (β-Gal; EC 3.2.1.2), and cellulase (Gallon et al. 2009) (EC 3.2.1.4). The levels of water-soluble (Zhao et al. 1996; Paull et al. 1999; Manrique and Lajolo 2004), chelator-soluble (Paull et al. 1999; Lazan et al. 1995), and alkali-soluble polyuronides have also been reported to increase during papaya fruit ripening (Zhao et al. 1996; Lazan et al. 1995; Ali et al. 1998; Paull et al. 1999). Additionally, Manrique and Lajolo (2004) have reported a loss of galactose with a simultaneous release of rhamnose during papaya ripening. The authors further postulated that variations in the levels of glucose, xylose, and mannose were the consequence of gradual breakdown of hemicellulose–cellulose linkages. A recent study claimed that papaya subtilase gene, which encodes subtilisin-like serine proteases, may involve in papaya fruit softening (Othman and Nuraziyan 2010). The subtilase is probably associated with ripening through its role in latex component breakdown.

1-Methylcyclopropene (1-MCP), an ethylene action inhibitor (Sisler and Serek 1997), was shown to suppress softening in a number of fruit including papaya during ripening (Ergun and Huber 2004; Ergun et al. 2006; Manenoi and Paull 2007; Fabi et al. 2007; Sanudo-Barajas et al. 2009). 'Sunrise Solo' papaya fruit treated with 1-MCP, later processed into fresh-cut or remained at intact and stored at 20 or 5°C, expressed delayed softening from an early ripening stage to an advanced ripening stage (Ergun and Huber 2004; Ergun et al. 2006). 1-MCP, moreover, suppressed endoxylanase gene expression, protein, and enzymatic activity in ripe 'Sunset' variety (Manenoi and Paull 2007) and delayed uronic acid loss in ripe 'Maradol' variety (Sanudo-Barajas et al. 2009).

In the present study, we examined the changes in cell wall polysaccharides in response to fresh-cut processing in post-climacteric papaya fruit pre-treated with 1-MCP and then stored at 5°C.

Materials and methods

Plant material and 1-MCP treatment

Papaya fruits (*Carica papaya* L. 'Sunrise Solo'), originated from Brazil, were purchased from C-Brand Tropicals Inc., Homestead, FL. The fruit were shipped from Brazil to Homestead by airfreight within 3 days after harvest and stored at 13°C. The fruit were transferred to the Postharvest Horticulture Laboratory at the University of Florida within 24 h of arrival at the packinghouse. The fruit were maintained at 20°C until the majority of the fruit reached the post-climacteric ripening stage (three-quarter ripe, 70–80% yellow surface color; Wills and Widjanarko 1995; Ergun et al. 2006). The average firmness, ethylene production, and respiration rate of the fruit were 6.8 N, 0.8 μL kg^{-1} h^{-1} and 22 mL CO_2 kg^{-1} h^{-1}, respectively, at 20°C. The fruit were gently brushed, dipped in chlorinated water (200 μL L^{-1}) for 1 min, air-dried, and placed in metal chambers (174 L) for 1-MCP treatment. The fruit were treated four times at 6-h intervals with 2.5 μL L^{-1} of 1-MCP generated from commercial powder formulation (0.14%; AgroFresh, Philadelphia, PA) for 24 h at 20°C. Control fruit were maintained under identical conditions with the exception of 1-MCP gassing. 1-MCP was measured using a gas chromatograph (Hewlett Packard-5890 II; Avondale, PA) equipped with a 80- to 100-mesh Chromosorb PAW stainless steel column (1.8 m × 3.18 mm i.d.; Supelco, Bellefonte, PA) with injector, oven, and detector temperatures (FID) set at 150, 150, and 200°C, respectively. Isobutylene gas, which has an FID response similar to that of 1-MCP (Jiang et al. 1999), was used as a standard. The fresh-cut processing was performed at 5°C. After 1 h at 5°C to allow temperature equilibration, the blossom and pedicel ends of each fruit were removed and the fruit longitudinally cut using a bread slicer (Coupe-Pain, China) into 1.5-cm thick slices. The two outermost slices were peeled and cut into pieces (1.5 cm × 3.5 cm × 4 cm) weighing 15–20 g. The slices afterward were rinsed with sterile isotonic mannitol (500 mM) using a squeeze bottle, and then, the slices were placed in vented plastic containers (1.7 L; FridgeSmart, Tupperware Co., St. Paul, Minn). The treatments included the following: fresh-cut fruit pre-treated with 1-MCP (FCM), fresh-cut fruit pre-treated with air (FCC), intact fruit pre-treated with 1-MCP (IM), and intact fruit pre-treated with air (IC). The fresh-cut and intact fruit were stored for 0, 2, 6, and 10 days at 5°C.

At the indicated intervals, fresh-cut and intact fruit were removed from storage and stored at −30°C until analyzed. Prior to freezing, intact fruit were peeled and cut into slices as described above.

Ethanol-insoluble solids

Approximately 80 g of partially thawed mesocarp tissue from each treatment was placed in 420 mL of 95% ethanol and homogenized with a Polytron homogenizer (Kinematica, Kriens-Luzen, Switzerland) for 2 min. The homogenate was refluxed in a boiling water bath for 20 min and filtered through glass fiber filter paper (Whatman GF/C) in an aspiration flask and then washed with 95% cold ethanol. The residue was transferred to 200 mL of chloroform/methanol (1:1 v/v) and incubated with stirring for 30 min. The suspensions were filtered (GF/C) and washed with 300 mL of acetone. The ethanol-insoluble solids (EIS) were oven-dried at 43°C for 5 h and stored in a desiccator at room temperature.

Total soluble sugars and polyuronides

Partially thawed mesocarp tissue (2 g) from each treatment in 20 mL of 95% ethanol was homogenized (Polytron homogenizer) for 30 s. The homogenate was held at −20°C for a minimum of 2 h and then centrifuged at 3,000×*g* for 5 min. Aliquots of the supernatant (0.5 mL) were used for the determination of total soluble sugars (TSS) as described (Dubois et al. 1956). Total polyuronide content in the EIS samples (7 g) was determined using the hydroxydiphenol assay (Blumenkrantz and Asboe-Hansen 1973).

Sequential fractionation of cell wall materials

Water-, CDTA (1,2 cyclohexylenedinitrilotetraacetic acid)- and Na_2CO_3-soluble polyuronides were extracted by suspending 30 mg of EIS in 7 mL of distilled water, 50 mM CDTA plus 50 mM Na-acetate, pH 6.5, and 50 mM Na_2CO_3, sequentially, at room temperature. Suspensions incubated on an oscillating shaker (1.4 cycle s^{-1}) for 4 h were filtered through a Whatman GF/C filter paper in an aspiration flask. Polyuronides in aliquots were determined as described (Blumenkrantz and Asboe-Hansen 1973).

Polyuronides recovered with water, CDTA, and Na_2CO_3 (0.5 mg of galacturonic acid equivalent) were run on a Sepharose CL-4B column (1.5 cm × 28 cm; Sigma Chemical Co., St. Louis, Mo.) equilibrated with 200 mM ammonium acetate at pH 5.0. Fractions of 2 mL were collected at a flow rate of 40 mL h^{-1}, and 0.5 mL of each fraction was used for the determination of polyuronide content (Blumenkrantz and Asboe-Hansen 1973). Blue Dextran (2,000 kDa.) and glucose (Sigma St. Louis, Mo.) were used to determine the void (V_o) and total (V_t) volumes of the column, respectively.

Hemicellulosic polysaccharide extraction

For pectin removal, approximately 200 mg of EIS in 500 mL Na-phosphate buffer (40 mM, pH 6.8) was heated in a boiling water bath for 20 min, cooled, filtered through Miracloth, and washed with 1 L distilled water, sequentially. After the removal of excess water, the residue was transferred into 200 mL of 80% ethanol, filtered through Miracloth, transferred into 200 mL of 50% chloroform/50% methanol and then filtered through Miracloth. The residue was then transferred into 200 mL of acetone to remove chloroform/methanol and filtered through GF/C under aspiration (Buchler funnel system) with additional acetone wash. The residue was oven-dried at 43°C for 5 h.

For the extraction of hemicellulosic fractions, the dried residues (50 mg) were suspended in 5 mL of 4% KOH/0.02% $NaBH_4$ overnight at room temperature. The suspension was centrifuged at 1,300×*g* at room temperature for 10 min, and the supernatant was removed from the pellet and saved at 5°C. The pellet was resuspended in 1 mL 4% KOH/0.02% $NaBH_4$ and centrifuged as described above. The supernatant was combined with the previous supernatant. The remaining pellet was subjected to the same procedure described above using 24% KOH/0.02% $NaBH_4$ instead of 4% KOH/0.02% $NaBH_4$. The supernatants were neutralized over ice with concentrated acetic acid. Hemicellulosic polysaccharides in the neutralized samples were determined using the hydroxydiphenol and phenol–sulfuric acid assays.

Compositional analysis of cell wall polymers

EIS (2 mg) were used for glycosyl composition analysis using a gas chromatography (Hewlett-Packard 5490 II, Avondale, PA) packed with a 25-m cross-linked 5% phenylmethyl silicone capillary column (Hewlett Packard, 0.2 mm i.d., 0.33 μM film thickness). *Myo*-inositol was used as internal standard. The EIS samples were hydrolyzed in 2 N trifluoroacetic acid for 1 h at 120°C. The cooled hydrolytes were then reduced and acetylated as described below (Blakaney et al. 1983). The resulting monosaccharides were reduced with 0.66 M sodium borohydride in 1 N ammonium hydroxide overnight at 25°C. The samples were acidified with Dowex 50 W (Sigma, St. Louis, MO), and the resin was removed by filtration through a syringe fitted with GF/C filter paper. The samples were dried and subsequently washed three times with methanol and once with ethanol before derivatization. The sugars were converted into acetyl derivatives in the presence of 0.2 mL of acetic acid anhydride and 0.2 mL of pyridine for 1 h at 100°C. After cooling to room temperature, the samples were dried under a gentle stream of air, washed with toluene three times, solubilized in methylene chloride, and injected into a gas chromatography (Hewlett Packard 5890 II, Avondale Pa). The chromatography was run at 210°C for 5 min, and then, the temperature was increased to 230°C within 10 min and finally held at 230°C for 5 min.

Statistical analysis

The experimental design was a randomized complete block design in which treatments (FCC, FCM, IC and IM) were blocks with 5 replicates. A total of 160 fruit (40 fruit for each treatment) were employed in the study. Numerical data were analyzed by general linear model program of SAS (SAS institute, Carey, NC) and Duncan's multiple range test.

Results

Ethanol-insoluble solids and total soluble sugars

The EIS remained statistically constant in all treatments with storage duration, showing no variations among treatments (Table 1). Assay of TSS was used primarily to quantify glucose, sucrose, and xylose in the ethanol homogenate. TSS significantly decreased only from day 0 to day 2 in all treatments, and then remained unchanged (Table 1), having reduction percentages of approximately 28, 28, 25 and 21% for IC, IM, FCC and FCM, respectively, over the 10-day storage period.

Polyuronides and their sequential fractions

The water-soluble polyuronide fraction constituted the majority of polyuronides followed by CDTA and Na_2CO_3-soluble fractions, respectively (Table 2). The pectic polymers recovered in solutions of water, chelator, and dilute alkali compromised more than 50% of total polymers recovered from EIS at day 0, 2, 6 or 10. Water-soluble polyuronides, representing more than 30% of the total polyuronides, increased in all treatments over time and by day 10 the levels of water-soluble polyuronides augmented

Table 1 Changes in ethanol-insoluble solids and total soluble sugars of three-quarter ripe intact and fresh-cut papaya fruit pre-treated with 1-MCP during a 10-day period of storage at 5°C

Day	IC	IM	FCC	FCM
Ethanol-insoluble solids (EIS; mg g^{-1} fw)				
0	28.03[a] a	26.43[a] a	27.44[a] a	27.12[a] a
2	25.94[a] a	25.69[a] a	25.54[a] a	26.36[a] a
6	25.24[a] a	25.43[a] a	25.76[a] a	26.18[a] a
10	26.34[a] a	25.53[a] a	25.65[a] a	25.65[a] a
Total soluble sugars (TSS; mg g^{-1} fw)				
0	147.31[a] a	144.45[a] a	140.58[a] a	142.12[a] a
2	106.27[b] a	104.92[b] a	105.86[b] a	112.50[b] a
6	98.81[b] a	96.19[b] a	99.86[b] a	116.98[b] a
10	102.58[b] a	98.03[b] a	93.12[b] a	94.76[b] a

IC, intact control fruit; *IM*, intact 1-MCP-treated fruit; *FCC*, fresh-cut control fruit; *FCM*, fresh-cut fruit with 1-MCP

[a] Means in the same column with same letters were not significantly different at $P \leq 0.05$

a: means in the same row with same letters were not significantly different at $P \leq 0.05$

Table 2 Changes in polyuronic acid composition of three-quarter ripe intact and fresh-cut papaya fruit pre-treated with 1-MCP during a 10-day period of storage at 5°C

Day	IC	IM	FCC	FCM
Water-soluble polyuronides (μg mg^{-1} EIS)				
0	127.78[c] a	128.24[c] a	132.25[c] a	131.63[c] a
2	137.16[b] a	130.05[bc] a	137.21[b] a	139.84[b] a
6	135.33[b] a	136.54[b] a	138.66[b] a	137.87[b] a
10	149.29[a] a	146.34[a] a	149.10[a] a	142.76[a] a
CDTA-soluble polyuronides (μg mg^{-1} EIS)				
0	54.65[a] a	53.70[a] a	56.75[b] a	53.01[b] a
2	51.14[a] b	49.55[a] b	63.41[a] a	63.57[a] a
6	54.33[a] b	49.70[a] b	60.47[a] a	61.43[a] a
10	53.53[a] b	52.21[a] b	61.14[a] a	64.65[a] a
Na_2CO_3-soluble polyuronides (μg mg^{-1} EIS)				
0	24.70[b] a	25.88[b] a	23.10[b] a	26.74[b] a
2	23.73[b] b	32.30[a] a	25.80[b] b	30.29[a] a
6	30.00[a] ab	33.17[a] a	28.10[ab] b	30.77[a] ab
10	32.04[a] a	32.80[a] a	29.58[a] a	31.72[a] a
Total polyuronides (μg mg^{-1} EIS)				
0	432.67[a] a	428.65[a] a	425.25[a] a	422.23[a] a
2	381.35[b] a	387.35[b] a	368.15[b] ab	362.50[b] b
6	363.94[b] ab	373.06[b] a	357.69[b] ab	356.92[b] b
10	363.31[b] a	365.33[b] a	345.35[b] ab	339.67[b] b

IC, intact control fruit; *IM*, intact 1-MCP-treated fruit; *FCC*, fresh-cut control fruit; *FCM*, fresh-cut fruit with 1-MCP

[a] Means in the same column with same letters were not significantly different at $P \leq 0.05$

a: means in the same row with same letters were not significantly different at $P \leq 0.05$

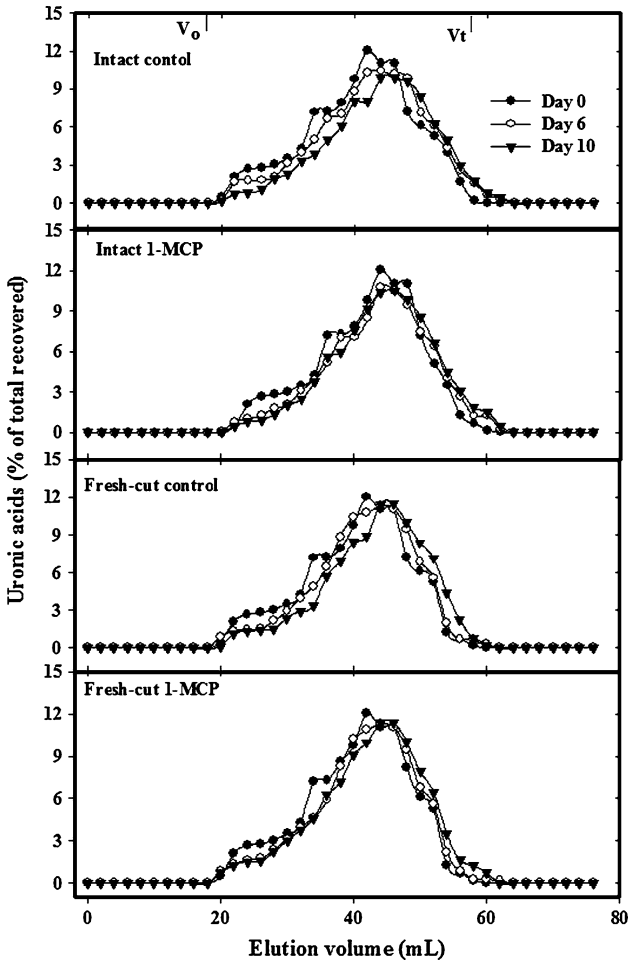

Fig. 1 Molecular mass distribution of water-soluble polyuronides of three-quarter ripe intact and fresh-cut papaya fruit pre-treated with 1-MCP at day 0 (*open circle*), 6 (*filled circle*), and 10 (*filled inverted triangle*). Polyuronides (0.5 mg galacturonic acid equivalents) were applied to CL-4B-200 (1.5 × 28 cm) column operated with a mobile phase of 200 mM ammonia acetate, pH 5.0. Individual fractions were analyzed for polyuronides. Data for each fraction were expressed as a percentage of the total polyuronides. V_o void volume, V_t total volume

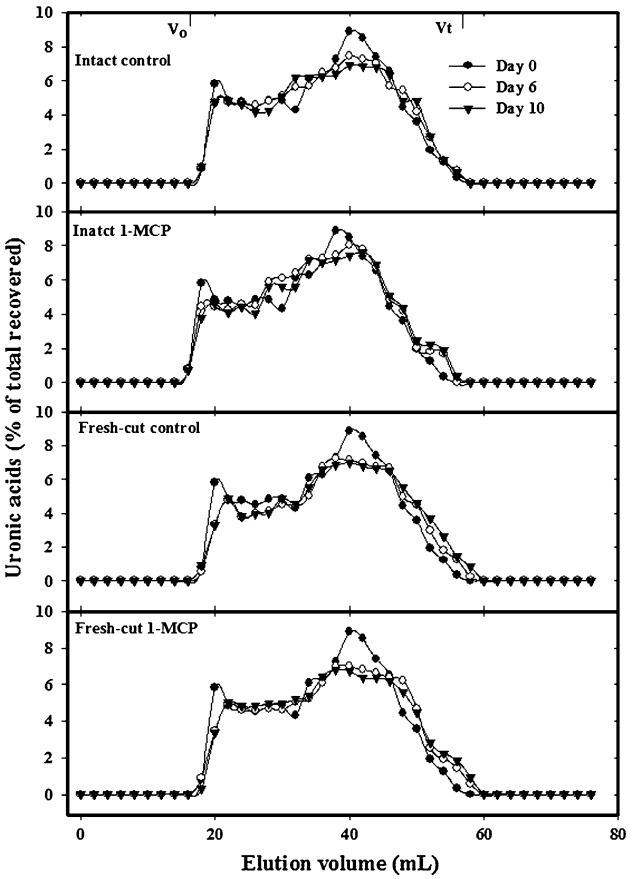

Fig. 2 Molecular mass distribution of CDTA-soluble polyuronides of three-quarter ripe intact and fresh-cut papaya fruit pre-treated with 1-MCP at day 0 (*open circle*), 6 (*filled circle*), and 10 (*filled inverted triangle*). Polyuronides (0.5 mg galacturonic acid equivalents) were applied to CL-4B-200 (1.5 × 28 cm) column operated with a mobile phase of 200 mM ammonia acetate, pH 5.0. Individual fractions were analyzed for polyuronides. Data for each fraction were expressed as a percentage of the total polyuronides. V_o void volume, V_t total volume

by 17, 14, 13 and 8% in IC, IM, FCC and FCM, respectively, compared to the levels at day 0. The increase in water-soluble polyuronides was similar for both the fresh-cut and intact fruit, regardless of 1-MCP treatment. The level of the CDTA-soluble polyuronides in the intact fruit (IC and IM) did not show a marked change during the storage period, while in the fresh-cut fruit, the level increased by 8% for FCC and by 22% for FCM after 10-day of storage, and the increase from day 0 to day 2 was statistically significant. From day 2 through 10, the fresh-cut fruit (FCC and FCM) yielded significantly higher chelator-soluble polyuronides compared with the intact fruit (IC an IM). The alkali-soluble polyuronides augmented significantly in the both intact (IC, 30%; IM, 27%) and fresh-cut (FCC, 28%; FCM, 19%) fruit over the 10-day storage period. At day 2, IM and FCM showed significantly higher alkali-soluble polyuronide contents relative to IC and FCC treatments. Total polyuronide levels significantly decreased from day 0 to day 2 in all treatments (IC, 12%; IM, 10%; FCC, 13%; and FCM, 15%) while FCC and FCM exhibited significantly higher decreases compared with both IC and IM.

Gel permeation chromatography of water-soluble polyuronides from all treatments is shown in Fig. 1. Water-soluble polyuronides from IM and FCM showed negligible changes in molecular mass over time while IC and FCC demonstrated slightly higher changes, especially in the levels of intermediate molecular mass polymers. Significant changes in the molecular mass of chelator-soluble polyuronides were also recorded in all treatments during storage (Fig. 2), with IC, FC and FCM showing more changes compared with IM, and IC and IM exhibiting less degradation to small molecular mass products (oligomers)

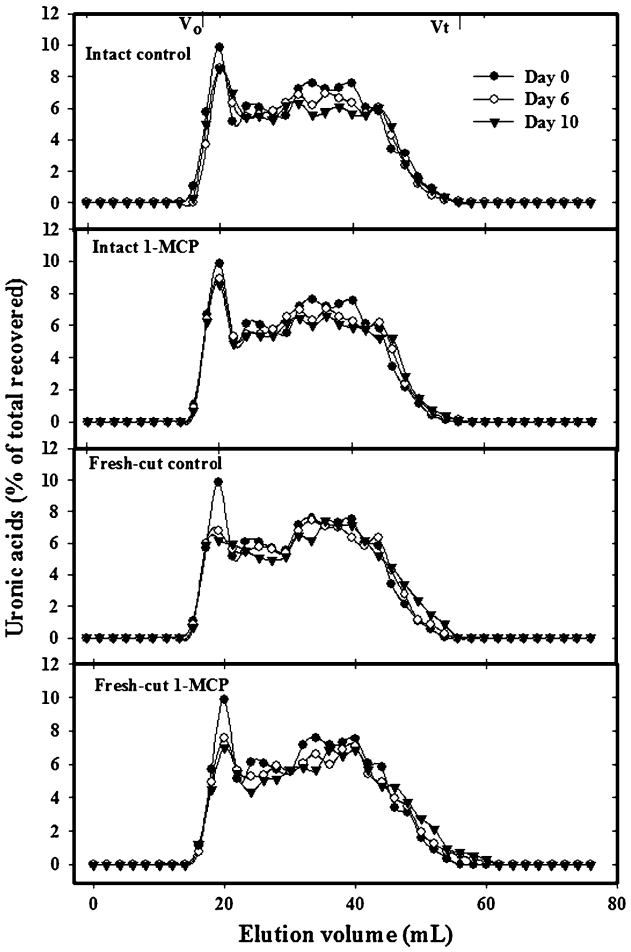

Fig. 3 Molecular mass distribution of Na_2CO_3-soluble polyuronides of three-quarter ripe intact and fresh-cut papaya pre-fruit treated with 1-MCP at day 0 (*open circle*), 6 (*filled circle*), and 10 (*filled inverted triangle*). Polyuronides (0.5 mg galacturonic acid equivalents) were applied to CL-4B-200 (1.5 × 28 cm) column operated with a mobile phase of 200 mM ammonia acetate, pH 5.0. Individual fractions were analyzed for polyuronides. Data for each fraction were expressed as a percentage of the total polyuronides. V_o void volume, V_t total volume

as compared to FC and FCM. Alkali-soluble polyuronides also exhibited molecular mass downshifts involving a decrease in the levels of both high and intermediate mass polymers in all treatments but more extensive downshifts especially in the high molecular mass polymers were observed in fresh-cut fruit over time (Fig. 3).

Hemicellulosic polysaccharides

The levels of neutral hemicelluloses and polyuronides extracted with 4 and 24% KOH are shown in Table 3. Weakly bound neutral hemicelluloses (extracted with 4% KOH) significantly declined (IC, 24%; IM, 16%; FCC, 27%; and FCM, 26%) in all treatments during the period of storage while no differences were recorded among treatments. Hemicellulosic polyuronide levels extracted with 4% KOH from each treatment also demonstrated significant fall offs over time which were 5% for IC, 35% IM, 28% FCC, and 28% FCM.

Strongly bound hemicelluloses (extracted with 24% KOH) showed no differences among treatments and no significant changes during the period of storage. Polyuronide levels in the 24% KOH extract, however, declined significantly in all treatments over time (IC, 22%; IM, 24%; FCC, 15%; and FCM, 15%). The decrease in the polyuronide content of intact fruit (IC and IM) at day 10 was more prominent than that of fresh-cut fruit (FCC and FCM).

Compositional analysis of cell wall polymers

Non-cellulosic neutral sugar composition of EIS is shown in Table 4. Analysis of the neutral sugars in EIS revealed that the predominant non-cellulosic neutral sugar in ripe papaya fruit is galactose, followed by glucose, xylose, rhamnose, mannose, and arabinose, respectively. Proportional rhamnose quantity increased significantly in IC and FCC fruit after 10 days of storage, but there was no significant variation among treatments. Arabinose level of each treatment increased significantly (30% for IC, 21% for IM, 32 for FCC, and 26% for FCM) in the course of storage while unaffected by the 1-MCP or fresh-cut processing. Proportional quantity of xylose, however, declined by 30% for IC, 24% for IM, 27% for FCC, and 26% for FCM with time in storage. Xylose content was significantly affected by 1-MCP treatment in the form of lower xylose content from day 2 through 6. Remaining constant over time, mannose levels did not show any significant differences among treatments. All treatments had a significant decline in proportional galactose quantity (IC, 26%; IM, 20%; FCC, 30%; and FCM, 30%) during storage. From days 2 to 10, galactose levels in fresh-cut fruit (FC and FCM) were lower than those measured for IC and IM. Glucose levels also decreased significantly over time, and by day 10, the reductions in glucose quantity were 13, 10, 17, and 17% for IC, IM, FCC and FCM, respectively, compared with the day 0 values. Proportional quantity of glucose was unaffected by either the 1-MCP treatment or the fresh-cut processing.

Discussion

Fresh-cut fruit ripens and deteriorates more rapidly than intact fruit due to the stress factors inflicted on the fruit via fresh-cut processing (wounding) (Karakurt and Huber 2003). One of the consequences of fresh-cut processing is enhanced ethylene production, which has been shown to

Table 3 Changes in neutral hemicellulose and pectin residue composition of three-quarter ripe intact and fresh-cut papaya fruit pre-treated with 1-MCP during a 10-day period of storage at 5°C

Fraction	Day	IC	IM	FCC	FCM
4% KOH					
Neutral hemicelluloses (μg mg^{-1} EIS)					
	0	10.49[a] a	10.01[a] a	11.11[a] a	11.03[a] a
	2	10.30[a] a	10.89[a] a	10.01[a] a	10.05[a] a
	6	9.89[b] a	10.50[a] a	9.09[b] a	9.73[ab] a
	10	7.99[b] a	8.40[b] a	8.16[b] a	8.19[b] a
Polyuronides (μg mg^{-1} EIS)					
	0	5.13[a] a	5.20[a] a	4.95[a] a	5.01[a] a
	2	5.21[a] a	4.17[b] b	4.80[a] a	4.32[b] b
	6	4.54[b] a	4.07[b] b	4.36[b] ab	4.06[b] b
	10	3.86[c] a	3.35[c] a	3.57[c] a	3.59[c] a
24% KOH					
Neutral hemicelluloses (μg mg^{-1} EIS)					
	0	24.90[a] a	23.12[a] a	22.34[a] a	22.00[a] a
	2	22.76[a] a	22.95[a] a	24.88[a] a	24.38[a] a
	6	22.24[a] a	22.24[a] a	22.73[a] a	23.00[a] a
	10	21.48[a] a	22.32[a] a	22.78[a] a	21.18[a] a
Polyuronides (μg mg^{-1} EIS)					
	0	2.85[a] a	2.95[a] a	3.12[a] a	3.04[a] a
	2	2.26[b] a	2.41[b] a	2.80[ab] a	2.71[ab] a
	6	2.30[b] a	2.35[b] a	2.75[b] a	2.71[ab] a
	10	2.21[b] b	2.26[b] b	2.68[b] a	2.65[b] a

IC, intact control fruit; *IM*, intact 1-MCP-treated fruit; *FCC*, fresh-cut control fruit; *FCM* fresh-cut fruit with 1-MCP

[a] Means in the same column with same letters were not significantly different at $P \leq 0.05$

a: means in the same row with same letters were not significantly different at $P \leq 0$

increase approximately tenfold in papaya fruit in response to fresh-cut processing (Paull and Chen 1997). Wounding induces ACC synthase and ethylene production and generates a number of hormonal and other signals, which activates defense and stress responses (Leon et al. 2001). These signals induce the activation of suites of defense and stress genes and lead to altered mRNA and protein expression (Karakurt and Huber 2007). An ethylene action inhibitor, 1-MCP, is effective at very low concentrations and has been demonstrated to delay ripening and enhance shelf life of both intact and fresh-cut fruit (Antunes et al. 2008). However, studies have indicated that the response of fresh-cut fruit to 1-MCP treatment depends on the maturity or ripening stage of the fruit used for fresh-cut processing and when 1-MCP was applied to the fruit (Toivonen and Brummell 2008).

In this study, we have applied 1-MCP to intact and fresh-cut papaya fruit at an advanced stage of ripening to determine its effect on the cell wall polysaccharides. The EIS exhibited no significant changes in quantity with storage, and they were unaffected by either fresh-cut processing or 1-MCP treatment. The insignificant changes of EIS content in the present study were possibly due to the storage temperature (5°C) employed, which arrest/limit the pronounced physiochemical changes. It is also possible that the fruit were already ripe at the start of the experiment presented here and that 10-day storage period was not long enough to observe marked changes in EIS content at 5°C. Similarly the levels of TSS decreased significantly during storage in the present study, irrespective of the 1-MCP treatment or fresh-cut processing, which is a trend different from fruit at an early ripening stage which demonstrates an increase in the levels of total soluble sugars (Paull et al. 1999; Manrique and Lajolo 2004).

Total polyuronide levels in EIS decreased significantly after 10 days of storage in both the intact and fresh-cut fruit irrespective of 1-MCP treatment. However, the decrease in total polyuronide content was more prominent in the fresh-cut fruit confirming the findings of Karakurt and Huber (2003), who reported a significant reduction in total polyuronide content of fresh-cut papaya fruit (60–70% yellow surface color) during storage at 5°C. The decrease in total polyuronides suggests their depolymerization to ethanol-soluble monomer and small oligomers that are not recovered in the EIS preparations. Water-soluble polyuronides constituted the majority of polyuronides in EIS followed by the CDTA- and Na_2CO_3-soluble fractions, and all fractions increased during storage except for the CDTA-soluble polyuronides of intact fruit (IC and IM). The changes in all the three pectic fractions of fresh-cut fruit suggest a possible role of polyuronide metabolism in the rapid tissue softening reported for fresh-cut papaya fruit (Karakurt and Huber 2003). The increase in water-, chelator- and alkali-soluble polyuronides could

Table 4 Changes in neutral sugar composition of three-quarter ripe intact and fresh-cut papaya fruit pre-treated with 1-MCP during a 10-day period of storage at 5°C

Day	IC	IM	FCC	FCM
Rhamnose (μg mg^{-1} EIS)				
0	35.89[b] a	38.25[a] a	36.65[b] a	36.46[a] a
2	35.74[b] a	36.79[a] a	35.60[b] a	36.84[a] a
6	38.31[b] a	37.50[a] a	34.80[b] a	38.42[a] a
10	38.62[a] a	41.24[a] a	37.71[a] a	39.24[a] a
Arabinose (μg mg^{-1} EIS)				
0	18.65[b] a	21.37[b] a	19.19[b] a	17.48[b] a
2	17.45[b] a	20.34[b] a	20.46[b] a	17.66[b] a
6	22.60[ab] a	21.68[b] a	24.47[a] a	18.27[b] a
10	24.08[a] a	25.78[a] a	25.26[a] a	22.06[a] a
Xylose (μg mg^{-1} EIS)				
0	88.46[a] a	85.23[a] a	90.56[a] a	85.27[a] a
2	73.40[b] a	61.23[b] b	69.00[b] a	55.19[b] b
6	70.46[b] a	64.32[b] b	68.26[b] a	54.95[b] c
10	62.04[c] a	65.33[b] a	66.33[b] a	49.08[b] b
Mannose (μg mg^{-1} EIS)				
0	24.52[a] a	25.89[a] a	23.97[a] a	24.49[a] a
2	22.71[a] a	23.92[a] a	24.76[a] a	22.81[a] a
6	22.06[a] a	24.94[a] a	24.63[a] a	25.58[a] a
10	25.12[a] a	27.72[a] a	26.32[a] a	25.42[a] a
Galactose (μg mg^{-1} EIS)				
0	114.21[a] a	110.42[a] a	108.08[a] a	107.16[a] a
2	85.04[b] a	88.33[b] a	75.48[c] b	75.38[c] b
6	87.74[b] a	89.54[b] a	80.79[b] b	80.48[b] b
10	88.76[b] a	86.96[b] a	83.36[b] b	81.45[b] b
Glucose (μg mg^{-1} EIS)				
0	102.27[a] a	104.04[a] a	106.18[a] a	107.00[a] a
2	96.80[ab] a	99.32[a] a	95.35[b] a	97.24[b] a
6	95.74[b] a	98.54[a] a	90.79[b] a	88.48[b] a
10	89.25[b] a	94.07[b] a	88.84[b] a	89.72[b] a

IC, intact control fruit; *IM*, intact 1-MCP-treated fruit; *FCC*, fresh-cut control fruit; *FCM*, fresh-cut fruit with 1-MCP

[a] Means in the same column with same letters were not significantly different at $P \leq 0.05$

a: means in the same row with same letters were not significantly different at $P \leq 0$

be the result of structural modifications, due largely to enhanced PG and PME activities (Karakurt and Huber 2003; Manrique and Lajolo 2004). In addition to the increase in polyuronide solubility in the intact and fresh-cut fruit, polyuronides exhibited molecular mass down-shifts during storage especially in fresh-cut fruit. The lower molecular mass of polyuronides from fresh-cut fruit compared with the intact fruit could arise from depolymerization or largely from increased solubility of inherently smaller polymers. Polyuronide solubility and depolymerization in papaya fruit could also be due to other mechanisms such as free radical-induced polyuronide degradation (Dumville and Fry 2000). 1-MCP treatment failed to inhibit polyuronide solubility and depolymerization in stored intact and fresh-cut papaya fruit. This observation suggests that polyuronide solubility and depolymerization in papaya fruit is possibly ethylene independent at the final stages of ripening in papaya fruit or that 1-MCP is ineffective in delaying the changes in polyuronides in fruit that are already ripe or at an advanced ripening stage. This has also been reported for kiwifruit treated with 1-MCP before slicing as well as for fresh-cut pineapple and watermelon (Budu and Joyce 2003; Mao et al. 2006).

Significant changes were also evident in neutral hemicelluloses and hemicellulosic polysaccharides during storage at 5°C. Both neutral hemicelluloses and polyuronides in 4% KOH-soluble hemicelluloses decreased significantly during storage in all treatments, with 1-MCP having no effects on the hemicelluloses and feeble effects on the polyuronides. The reduction in neutral hemicelluloses of weakly bound hemicelluloses possibly results from enhanced activities of galactosidases/galactanases. Consistent with the involvement of galactosidases/galactanases in depolymerization and solubility of hemicelluloses, galactose, glucose and xylose, the predominant neutral sugars detected in papaya fruit, decreased significantly with storage. Twenty-four percentage of KOH-soluble neutral hemicelluloses did not demonstrate a significant change

with storage but polyuronides showed a marked decline which might result from wound-induced polygalacturonase action. Polygalacturonases and galactosidases/galactanses have been linked with polyuronide and hemicellulose solubility and depolymerization in papaya (Lazan et al. 1995; Rose et al. 1998; Karakurt and Huber 2003).

The effects of 1-MCP treatment and fresh-cut processing on both weakly bound and strongly bound hemicelluloses were in general insignificant even though few differences were recorded suggesting that 1-MCP was ineffective in inhibiting the activity of cell wall hydrolases including PG and galactosidases in fruit at an advanced ripening stage. In fact it has been reported that 1-MCP did not have any effects on the activities of α- and β-Gal in ripe 'San Castrese' apricot fruit (Botondi et al. 2003) and of exo- and endo-PG in 'Tegan Blue' Japanese plum (Khan and Singh 2009). Similarly, the changes in neutral sugars in response to 1-MCP treatment in our study were minimal in the form of suppressing the loss of xylose, but unaffecting the levels of glucose, galactose, mannose and arabinose.

The effects of fresh-cut processing were evident in the marked loss of xylose and galactose, suggesting a significant change in xyloglucans, which may indicate that the enzymes contributing to degalactosidation of polyuronides and hemicelluloses are upregulated by wounding (Karakurt and Huber 2003). Both α- and β-Gal activities have been reported to increase significantly in fresh-cut papaya fruit with storage (Karakurt and Huber 2003). Polyuronide solubilization may also result from the loss of galactosyl residues in the form of galactose-rich side chains of rhamnogalacturonans (Seymour et al. 1990; Redgwell et al. 1992). Loss of galactans has been demonstrated to accompany increased solubilization of polyuronides (Kim et al. 1991). Proportional quantity of glucose also demonstrated a significant reduction during storage of papaya fruit irrespective of the 1-MCP treatment or fresh-cut processing in the present study, suggesting a possible role of deglucosidation in the hemicellulosic polysaccharide solubility and depolymerization. Huber et al. (2001) has reported a significant role of deglucosidation in the degradation and softening of fresh-cut fruit.

It is concluded that modifications of cell wall polyuronides and hemicelluloses in fresh-cut ripe papaya fruit showed similar patterns to those in intact ripe papaya fruit, although few differences were observed between the fresh-cut and intact fruit. In fresh-cut papaya fruit, degalactosidation and deglycosylation possibly play a significant role in the solubility and depolymerization of hemicellulosic polysaccharides. The effect of 1-MCP is minimal in inhibiting the solubility and depolymerization of polyuronides and hemicelluloses of both fresh-cut and intact papaya fruit during the final stages of ripening at 5°C. Thus, it is suggested that ripening stage at the time of fresh-cut processing strongly affects the changes in cell wall polysaccharides, and the effects of 1-MCP in inhibiting the changes in cell wall polysaccharides are largely dependent on the maturity or the ripening stage of the fruit at the time of fresh-cut processing (Toivonen and Brummell 2008).

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