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ARTICLE

Changes in Cell Wall Composition Associated to the Softening of Ripening Papaya: Evidence of Extensive Solubilization of Large Molecular Mass Galactouronides

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Papaya (*Carica papaya*) is a climacteric fruit that undergoes dramatic pulp softening. Fruits sampled at three different conditions (natural ripening or after exposition to ethylene or 1-methylcyclopropene) were used for the isolation of cell wall polymers to find changes in their degradation pattern. Polymers were separated according to their solubility in water, CDTA, and 4 M alkali, and their monosaccharide compositions were determined. Water-soluble polymers were further characterized, and their increased yields in control and ethylene-treated fruit, in contrast to those that were treated with 1-MCP, indicated a strong association between fruit softening and changes in the cell wall water-soluble polysaccharide fraction. The results indicate that the extensive softening in the pulp of ripening papayas is a consequence of solubilization of large molecular mass galacturonans from the pectin fraction of the cell wall. This process seems to be dependent on the levels of ethylene, and it is likely that the releasing of galacturonan chains results from an endo acting polygalacturonase.

KEYWORDS: 1-MCP; plant cell wall; polygalacturonase; ethylene; fruit softening; *Carica papaya*; fruit ripening

INTRODUCTION

Fruit softening is a well-regulated phenomenon that involves degradation of cell wall polysaccharides. Plant cell wall is a dynamic structure made up of complex polysaccharides such as β-glucans, galacturonans, arabinans and galactans and some additional minor components as glycoproteins, enzymes, minerals and phenolic compounds (*1*). This structure bestows rigidity and protection to the cell, being largely responsible for the texture of plant-based food, especially vegetables and fleshy fruits.

Cell walls of fleshy fruit pulp are composed of cellulose, hemicellulose and large amounts of pectins, mainly galacturonans, and the degradation and solubilization of these components contributes to reduce cell adhesion, resulting in tissue softening during ripening (*2*). In climacteric fruits, this process is stimulated by the presence of ethylene, and there is a direct correlation between the increments in activity of ethylene-responsive enzymes and pulp softening (*3*). Despite our current knowledge, the molecular and biochemical mechanisms that regulate cell wall disassembly are still not completely understood, partly because softening may also be mediated by a complex series of nonenzymatic events, which include chemical breakage involving hydroxyl radicals (*OH) and reactive oxygen species (ROS) (*4*, *5*).

Papaya (*Carica papaya*) is a commercially relevant fruit crop whose shelf life is limited by the severe softening of the pulp, a process that is markedly affected by the presence of ethylene (*6*). To overcome this problem and improve the shelf life and commercial trading, some post-harvest techniques have already been tested, such as the use of 1-methylcyclopropene (1-MCP), an ethylene antagonist that blocks the ethylene receptor. However, the optimum conditions for the commercial use of this inhibitor are far from being standardized, and molecular and enzymatic mechanisms that regulate papaya pulp softening are still unknown (*7*, *8*).

The characterization of the changes in carbohydrate composition in ripening papaya may reveal important information regarding the degradation pattern of the cell wall and may contribute to a better understanding of the role played by enzymatic and nonenzymatic processes in fruit softening. In this way, the present manuscript presents data on the changes in the composition of cell wall carbohydrates from ripening papayas. Unripe fruit were let to ripe naturally or after exposure to exogenous ethylene or 1-MCP, and fruit sampled from these three different conditions were used for the isolation of soluble cell wall-derived monosaccharides and cell wall polymers.

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Extraction of the polymers from cell wall material was based on their solubility in water, trans-1,2-cyclohexanediaminetetraacetic acid (CDTA) and 4 M alkali. The results reveal substantial ethylene-dependent changes in water-soluble pectin linked to papaya softening.

MATERIALS AND METHODS

Plant Material. Papaya fruit (*Carica papaya* cv. Golden) were obtained directly from a producer in Espírito Santo State, Brazil. Papayas were harvested at color break to 1/4 yellow (around 150 days after anthesis; DAA). Soon after the arrival in the laboratory (2 days after harvest; DAH), fruits were randomly divided into three groups. Papayas from the control group were left to ripen normally in a 240 dm^3 chamber with controlled temperature and humidity (25 °C ± 0.1 °C and 95%, respectively). The other two groups were treated with ethylene or 1-MCP, as previously described by Fabi et al. (*6*). For the ethylene-treated group, papayas were exposed to a concentration of 100 ppm (100 $\mu L\ L^{-1}$) ethylene in a synthetic air mixture in a constant flow-through system during 2 h (for gas saturation) and 10 h in a closed system. For the 1-MCP-treated group, papaya fruits were exposed to 1-MCP (EthylBloc - Floralife, Inc.) at a concentration of 100 ppb (100 nL L^{-1}), for 12 h in a closed system. After both treatments, the fruits were ventilated and left to ripen in separate chambers under the same conditions of temperature and humidity described for the control group.

Pulp Firmness. The measurement of texture was performed in a TA-XT2 texturometer powered by a "blade with knife" tool (Stable MicroSystems) according to the procedure previously described by Fabi et al. (*6*).

Respiration and Ethylene Production. A minimum number of 4 fruit from each group was individually placed in airtight 1.7 L jars equipped with a rubber stopper, and left at 25 °C for 1 h. The respiration and ethylene production were measured as previously described by Fabi et al. (*6*).

Cell Wall Isolation. Frozen papaya pulp was ground in a mortar with liquid N_2. Four grams of powdered plant material were incubated three times with 200 mL $CHCl_3$: MeOH (1:1, v/v) at 60 °C for 20 min and with boiling EtOH 80% for 10 min for pigment and fat removal and enzyme inactivation in a modified procedure of Carrington et al. and Irving et al. (*9*, *10*). Suspensions were centrifuged at 9000 *g* for 15 min at each step, and the supernatants were discarded. The residue was sequentially washed with three volumes of 200 mL of 80% EtOH and with three volumes of acetone, and after these steps the powder was dried. The remaining defatted material was extracted three times (under constant stirring) with 20 mL of distilled water and pelleted by centrifugation at 10 000 *g* for 10 min. The supernatants were pooled and dialyzed through SpectraPor dialysis membrane, MWCO 3.5 kDa (Spectrum, U.S.A.) for 48 h against distilled water, and the freeze-dried material was named water-soluble cell wall polymers (WSP). The residue was fractionated according to Shiga and Lajolo (*11*) to obtain polymers immobilized by calcium (CDTA fraction), alkali soluble polymers (extracted with 4 M NaOH) and the cellulose-rich residues.

Soluble Sugars Analysis. Papaya pulp was ground as previously described and extracted three times with 80% ethanol at 80 °C. After centrifugation, the supernatants were combined and the ethanol was evaporated under vacuum. The residues were reconstituted with water and filtered through a 0.45 μm membrane. All sugar composition (derived from intracellular material as well as from cell wall material) was analyzed on a CarboPac PA10 (Dionex Corp., Sunnyvale, U.S.A.) pellicular anion-exchange analytical column (250 × 4 mm) with the correspondent guard column using a DX 500 HPAEC-PAD system (Dionex, U.S.A.) equipped with a GP40 gradient pump (Dionex, U.S.A.) and an AS50 autosampler (Dionex, USA). The carbohydrates were detected by a PAD system mode, using a gold working electrode and an Ag/AgCl reference electrode.

Running of neutral sugars was performed isothermally and isocratically (32 °C; H_2O; at 1.0 mL min^{-1} flow rate) for 40 min followed by a cleaning sequence with 300 mM NaOH for 10 min and another 10 min of re-equilibration. Postcolumn 300 mM NaOH was also used for detection.

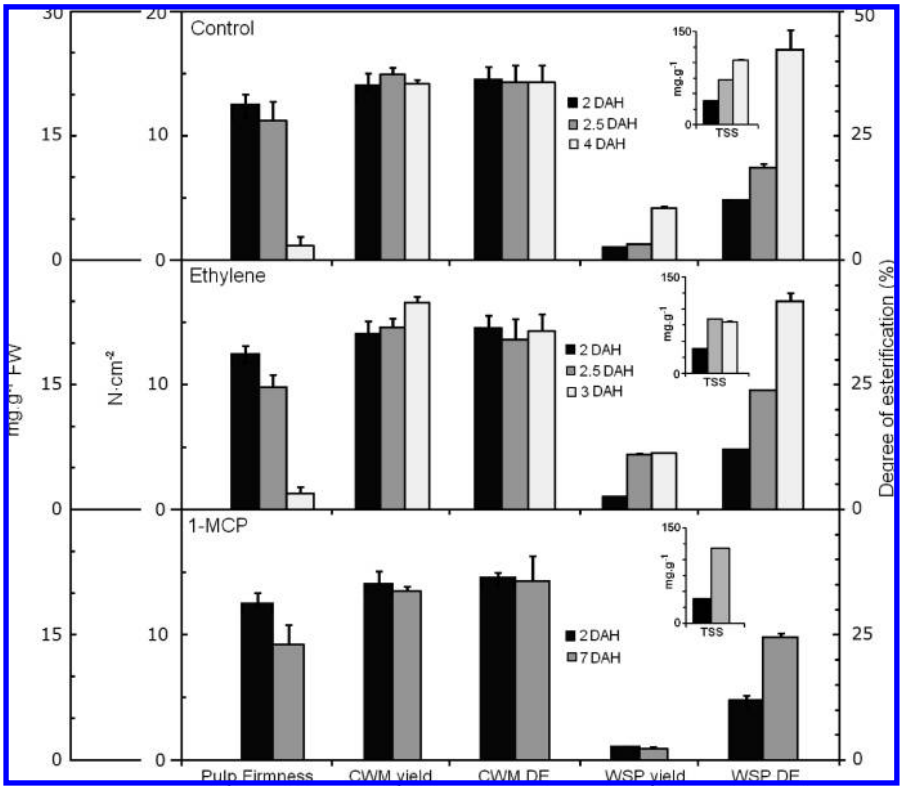


Figure 1. Changes in papaya pulp firmness, total cell wall material (CWM) yield and degree of esterification (CWM DE), and water-soluble polysaccharide (WSP) yield and degree of esterification (WSP DE) in a fresh weight (FW) basis, of fruit let to ripen naturally (control) or after treatment with ethylene or 1-MCP, sampled at different days after harvest (DAH). The evolution of total soluble sugar (TSS) composition during ripening was included. Values of humidity were about 86%, with no expressive change during ripening (values obtained were means of three determination). Results are means of at least three determinations. Error bars indicate the standard deviation.

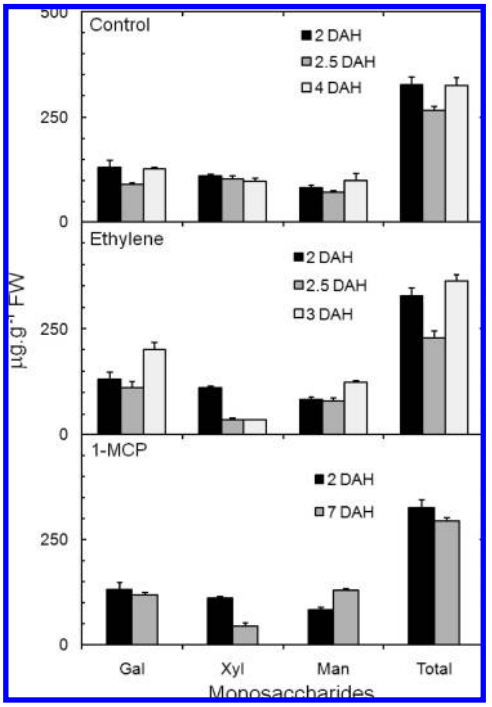


Figure 2. Free monosaccharides present in the pulp of papaya fruit in a fresh weight (FW) basis. Galactose (Gal), xylose (Xyl), mannose (Man) and total monosaccharides were extracted from the pulps of papaya fruit let to ripen naturally (control) or after treatment with ethylene or 1-MCP, sampled at different days after harvest (DAH). Results are means of at least three determinations. Error bars indicate the standard deviation.

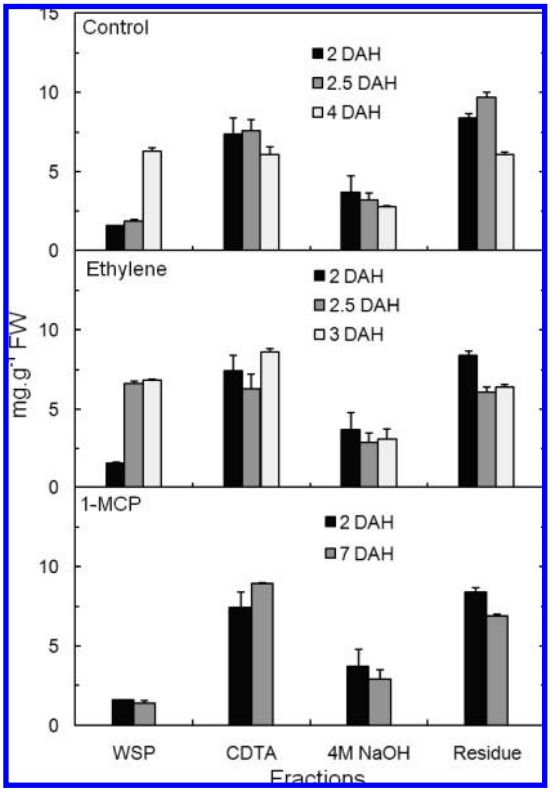


Figure 3. Yield of cell wall fractions in the pulp of ripening papayas, based on a fresh weight basis (FW). On the basis of the differential solubility approach, the fractions corresponding to water-soluble polymers (WSP), polymers solubilized by chelating agent (CDTA), polymers immobilized by ester linkages released by saponification (4 M NaOH), and the insoluble residue were extracted from the cell wall material isolated from the pulps of papaya fruit let to ripen naturally (control) or after treatment with ethylene or 1-MCP, sampled at different days after harvest (DAH). Results are means of at least three determinations. Error bars indicate the standard deviation.

Galacturonic and glucuronic acids were run with 150 mM NaOH with a four-step linear gradient, 0−25; 25−50, 50−95 and 95−220 mM sodium acetate at 8, 20, and 30 min. An additional cleaning step with 500 mM sodium acetate for 10 min was also included. Calibration curves were prepared using external sugar standard from 0.1 to 2.0 $\mu g \cdot 25\ \mu L^{-1}$.

Polysaccharide Composition. About 1 mg of the polymers obtained by extraction was hydrolyzed with 1 mL of 2 M trifluoroacetic acid (TFA) at 120 °C for 60 min in a screw-capped conical vial. The hydrolysates were centrifuged at 2,000 *g* for 5 min and the supernatants were transferred to new vials and dried under N_2 stream. After resuspension in water the material was analyzed in the HPAEC-PAD system in the same way as the soluble sugars.

The cellulose-rich residues obtained after 2M TFA hydrolysis were dried under N_2 stream and rehydrolyzed with exact 0.9 mL of 2M H_2SO_4 at 120C for 90 min more. At the end, the hydrolysates were neutralized with 0.1 mL of NaOH 50% (w/w) and analyzed in the HPAEC-PAD system in the same way as mentioned above.

Size Exclusion Chromatography. Size exclusion chromatography was performed on a C16/70 column, 16 mm × 70 cm (Pharmacia, Sweden), and packed with Sephacryl S-400 (Pharmacia). The column was equilibrated and eluted with 20 mM phosphate buffer (pH 5.8; 0.02% (w/v) NaN_3; 1% (w/v) NaCl). The void and included volumes were determined using, respectively, blue dextran (Pharmacia) and glucose. The molecular mass standards used were dextran polymers (10, 50, 150, 410, and 770 kDa, Fluka, Switzerland). The flow was fixed in 12 mL h^{-1} and 2.5 mL fractions were collected. Peaks were detected by colorimetric assay for total sugars (*12*) and uronic acid (*13*).

HPSEC-MALLS Analysis. High pressure size exclusion chromatography (HPSEC) was carried out on polysaccharide solution at 25 °C, using a multidetection equipment in which a Waters 2410 differential refractometer (RI) and a Wyatt Technology Dawn F multiangle laser light scattering (MALLS) detector were connected online. Four Waters Ultrahydrogel 2000/500/250/120 columns were connected in series and coupled to the multidetection equipment. The mobile phase consisted of a 0.1 mol L^{-1} $NaNO_2$ solution containing 0.5 g L^{-1} NaN_3 (vacuum-filtered through a 0.22 μm membrane filter) at a flow rate of 0.6 mL min^{-1}. For HPSEC analyses, samples were solubilized in 0.1 mol L^{-1} $NaNO_2$ solution containing NaN_3 (0.5 g L^{-1}) under magnetic stirring. The sample, previously filtered (0.22 μm; Millipore), was injected (100 μL loop) at 1.0 mg mL^{-1}. HPSEC data were collected and analyzed by a Wyatt Technology ASTRA program.

Determination of Degree of *O*-Methyl Esterification. Fourier transform-infrared (FT-IR) spectra were collected at the absorbance mode in the frequency range of 4000−400 cm^{-1} using a Bomem MB-100 spectrophotometer (Hartman & Braun, Canada), at 4 cm^{-1} resolution. Spectroscopic grade KBr powder was used, and discs were prepared using a 90:10 salt/sample proportion. Previous to FT-IR analysis, KBr was dehydrated at 120 °C for 24 h, and the pectin samples were desiccated under vacuum in an Abderhalden equipment containing P_2O_5. From each sample, two independent analyses were taken and their FT-IR spectra were recorded and the areas of interest were measured. The band areas were determined using the Win-Bomem Easy software at 1749 and 1630 cm^{-1}, corresponding to methyl esterified and free uronic acids, respectively. A calibration curve previously obtained using the same conditions (*14*) was used to determined the degree of *O*-methyl esterification that corresponds to pectins degree of esterification (DE).

RESULTS

Fruit Softening, Soluble Sugars and Cell Wall Composition. Exposure to 1-MCP impaired the severe pulp softening that normally occurs during ripening of papaya (**Figure 1**). The amount of total cell wall material (CWM) and degree of ester-

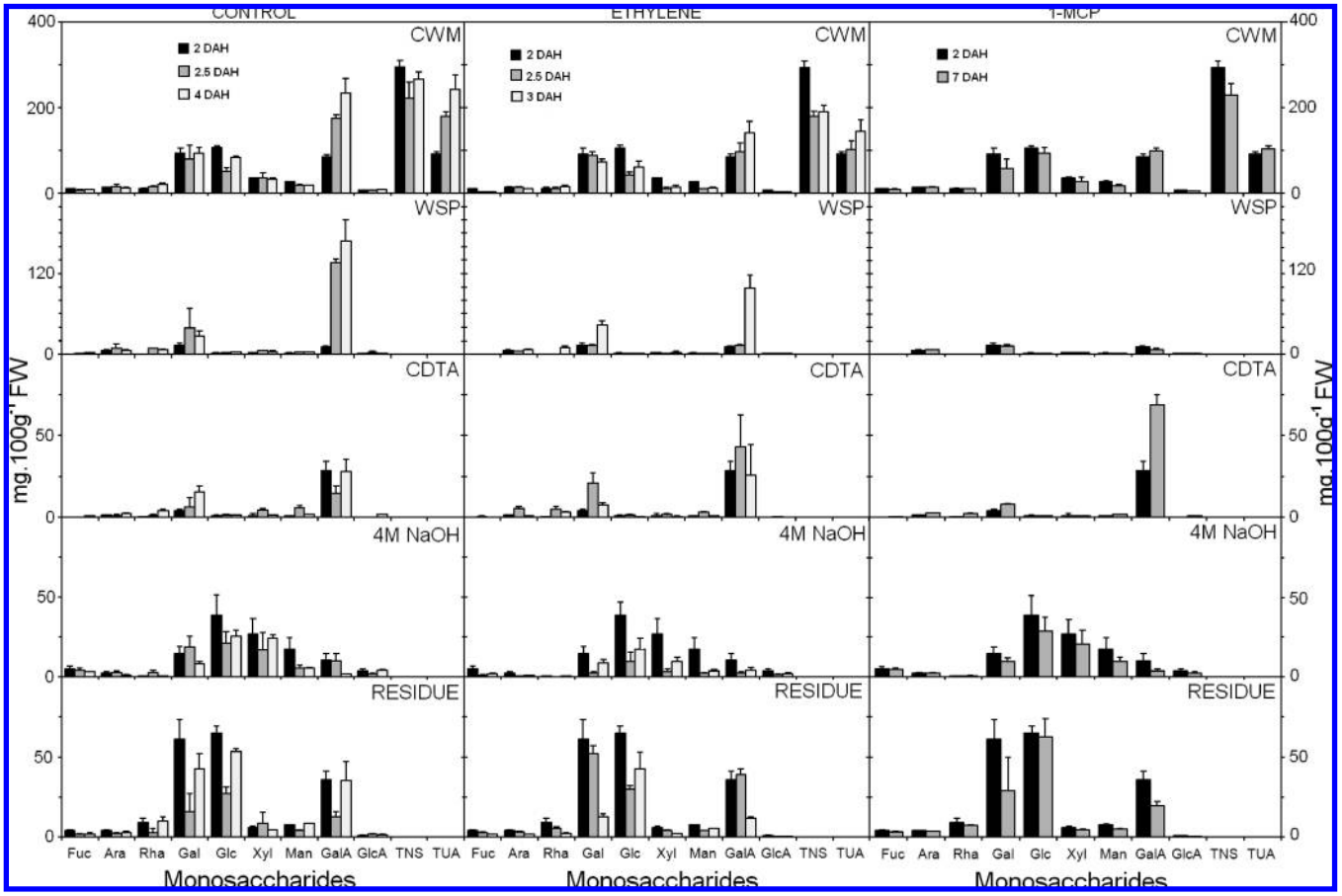


Figure 4. Monosaccharide composition of cell wall fractions isolated from ripening papaya. Fucose (Fuc), arabinose (Ara), rhamnose (Rha), galactose (Gal), glucose (Glc), xylose (Xyl), mannose (Man), galacturonic acid (GalA), glucuronic acid (GlcA), total neutral sugars (TNS) and total uronic acids (TUA) were isolated from the total cell wall material (CWM), water-soluble polymers (WSP), polymers solubilized by chelating agent (CDTA), polymers immobilized by ester linkages, released by alkali (4 M NaOH), and the cellulose-rich residue from the pulps of papaya fruit let to ripe naturally (control) or after treatment with ethylene or 1-MCP, sampled at different days after harvest (DAH). Values are expressed in a fresh weight (FW) basis. Results are means of at least three determinations. Error bars indicate the standard deviation.

ification (DE) were similar for the three groups, with no expressive changes during ripening, but higher yields in water-soluble polysaccharides (WSP) were seen during ripening of control and ethylene-treated papayas. The DE of control and ethylene WSP at 4 and 3 DAH were about 40% higher than 1-MCP-treated samples (**Figure 1**). Free galactose, xylose and mannose, but not free galacturonic acid, were detected in the pulp of the fruit (**Figure 2**). The galactose, xylose and mannose ratios did not change (1:1:1) during ripening of control fruit, but higher proportions of galactose and mannose (6:1:3 and 3:1:3, respectively) were detected in fruit treated with ethylene or 1-MCP.

The CWM from the pulp of unripe papaya was composed of 7% acidic water-soluble pectins (WSP), 35% calcium pectate (CDTA), 18% ester linked pectins and hemicellulose (4 M NaOH) and 40% cellulose-rich polysaccharides (Residue). According to **Figure 3**, there was a significant increase of WSP in pulp of the fruit from control and ethylene during ripening but not in that of the 1-MCP group. In relation to the other fractions, no important changes were noted for those soluble in CDTA or 4 M NaOH, and the amount of the cellulose-rich residue decreased during ripening, regardless the treatment.

Composition analysis of CWM fractions of unripe fruit (**Figure 4**) indicated that WSP was composed of comparable amounts of galacturonic acid and galactose and lower amounts of arabinose, while the polymers released by CDTA were primarily composed of galacturonic acid, with lower amounts of galactosyl and arabinosyl residues. Glucosyl, xylosyl, galactosyl and fucosyl units predominated in polymers released by 4 M alkali, and the residue was essentially made up of high amounts of glucose and galactose. Ripening resulted in significant accumulation of galacturonic acid in WSP fraction of control and ethylene-treated fruits, but not in 1-MCP group. In contrast, the amount of this sugar acid increased in the CDTA fraction of CWM from 1-MCP treated fruit.

Characterization of the Water-Soluble Fraction of Cell Wall. The apparent molecular mass profile was obtained using dextran standard from 10 to 770 KDa. Galacturonans of WSP from unripe fruit ranged from 80 KDa to values higher than 770 KDa, while that of pectins composed predominantly of neutral sugars was about 10 KDa (**Figure 5**). The amount of galacturonans, as well as their polydispersity, increased markedly during ripening of control and ethylene-treated fruit. The molecular mass of polygalacturonans from noninduced ripe papaya ranged from 50 KDa to values higher than 770 KDa, while the values for the ethylene-group were between 150 KDa to values higher than 770 kDa. Solubilization of polygalacturonans from the pulp of 1-MCP-treated fruit occurred to a much lesser extent. Composition analysis of fractions obtained by size-exclusion chromatography revealed the absence of neutral polysaccharides and the predominance of uronic acids in WSP (**Figure 5**).

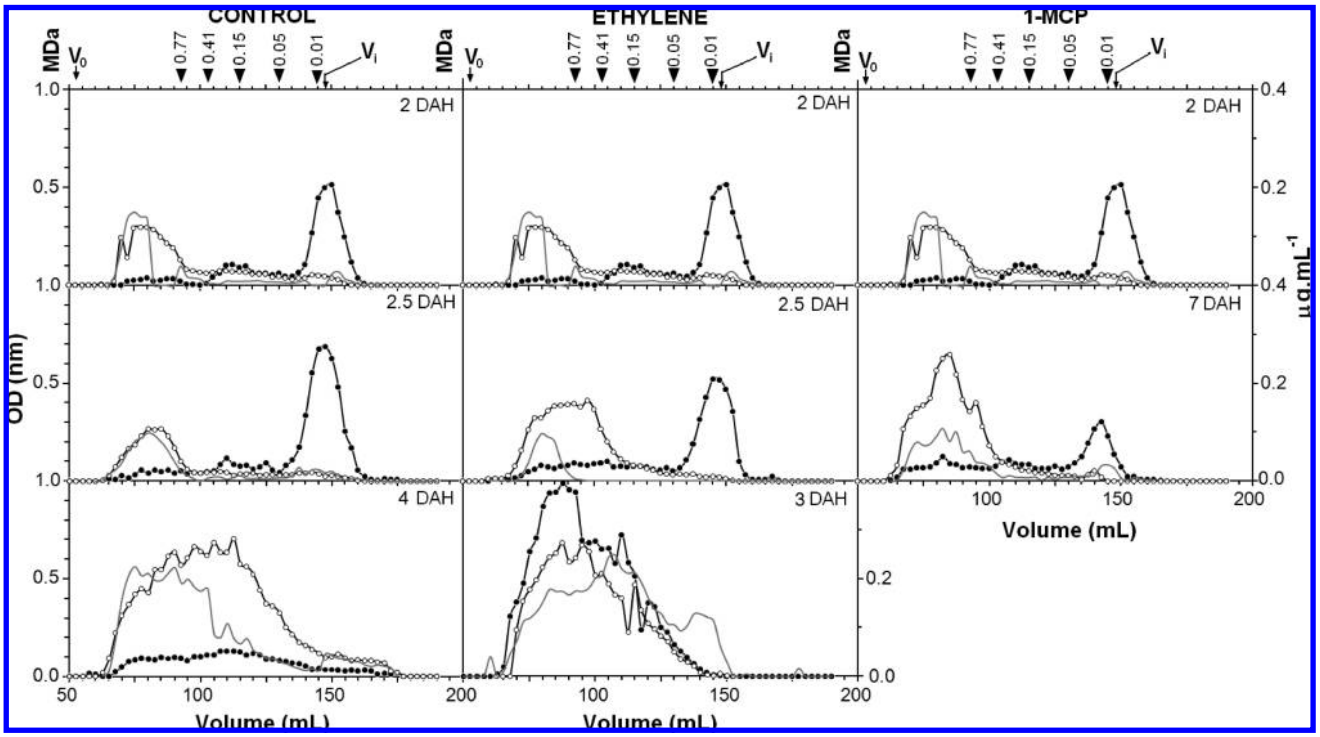


Figure 5. Molecular mass profile of water-soluble polymers (WSP) from the pulps of papaya fruit allowed to ripen naturally (control) or after treatment with ethylene or 1-MCP, sampled at different days after harvest (DAH). Fractions eluted from the size-exclusion chromatographic column were assayed for total sugars, (closed symbol) and uronic acid (open symbol), at 490 and 525 nm, respectively, as described in the methods section. Uronic acid content of the size-fractioned WSP was also determined by HPAEC-PAD analysis of the eluted fractions (continuous line). Void (Vo) and included (Vi) volumes were determined using blue dextran and glucose. Numbers positioned at the top indicate the size of the column calibrating markers, in mega Daltons (MDa).

HPSEC-MALLS/RI is a powerful technique for analyzing molecular mass distribution. Four columns with different exclusion limits connected in series were used to provide a qualitative profile of the molecular mass of WSP fractions. According to the HPSEC-MALLS/RI analysis (**Figure 6**), the WSP from unripe fruit contain a mixture of two polysaccharides of high molecular mass, eluted at ~38 and ~47 min, both detected by RI and MALLS. RI gives a signal proportional to the concentration, whereas the MALLS response to a given concentration is proportional to the product: concentration × molecular mass. Thus, for molecular mass below a few thousand, relatively high concentrations may be required so that molecules produce a detectable scattered light signal (*15*).

Ripening significantly decreased the amount of higher molecular mass polysaccharides. After 4 days after harvest (DAH), the peak at ~38 min detected by RI is shifted to higher elution times. On the other hand, the RI signal of the lower molecular mass peak at ~47 min becomes more intense and polydispersed. The peaks detected by RI in WSP from unripe fruit become almost undistinguished with the broadening of the curves in 4 DAH. A significant amount of smaller molecular species were detected by RI for 4 DAH sample as shown by the tail toward to higher elution times. This intense and broad peak is not detected by MALLS, confirming the low molecular mass of the polymers.

The profile obtained by MALLS at 90° almost did not change with ripening comparing to the RI signal. However, 2.5 DAH a small peak is detected by MALLS at ~42 min, which was not present in WSP from unripe fruit, indicating the extraction of a new population of molecules with lower molecular mass than that eluted at ~ 38 min.

The same profile was obtained for WSP fraction from ethylene-treated fruits 3 DAH. However, after 2.5 DAH, the population of higher molecular mass increased in ethylene-treated fruits related to the control group. As expected, 1-MCP caused the opposite effect, precluding the break of chains of high molecular mass polysaccharides. After 7 DAH, WSP from 1-MCP treatment showed almost the same molecular mass profile of the control group after 2 DAH. In this case, the peak at ~38 min, corresponding to high molecular mass polymers, becomes more intense and less polydispersed than the control group (2 DAH).

DISCUSSION

Although the use of exogenous ethylene induced ripening, and 1-MCP inhibited the rapid softening of papaya pulp, the polysaccharide yields in CWM did not change significantly, suggesting limited depolymerisation of the cell wall constituents. This idea is corroborated by the absence of free galacturonic acid or sharp increments in galactose, xylose and mannose, which possibly are derived from the cell wall. In this regard, the amount of alcohol soluble sugar derived from the cell wall changed similarly during ripening, even in the 1-MCP-group. In the case of extensive cell wall depolymerisation, high amounts of monosaccharides would be produced. Although fast monosaccharide mobilization could also account for the small amounts of free galactose, xylose and mannose, it is likely that cell wall rigidity could be affected by a less extensive or a component-restricted depolymerisation of the cell wall carbohydrates. This has been observed previously in tomatoes (*16*).

To check which components were affected by the treatments, cell wall fractions were individually analyzed. The increased yields of WSP in control and ethylene-treated fruit revealed a larger dependence of fruit softening on this cell wall fraction. Monosaccharide composition allowed the calculations of galacturonans degree of branching (Rha/GalA ratio), which revealed that WIP in ripe fruits were composed predominantly of branched galacturonans when compared to unripe fruits whereas WSP is

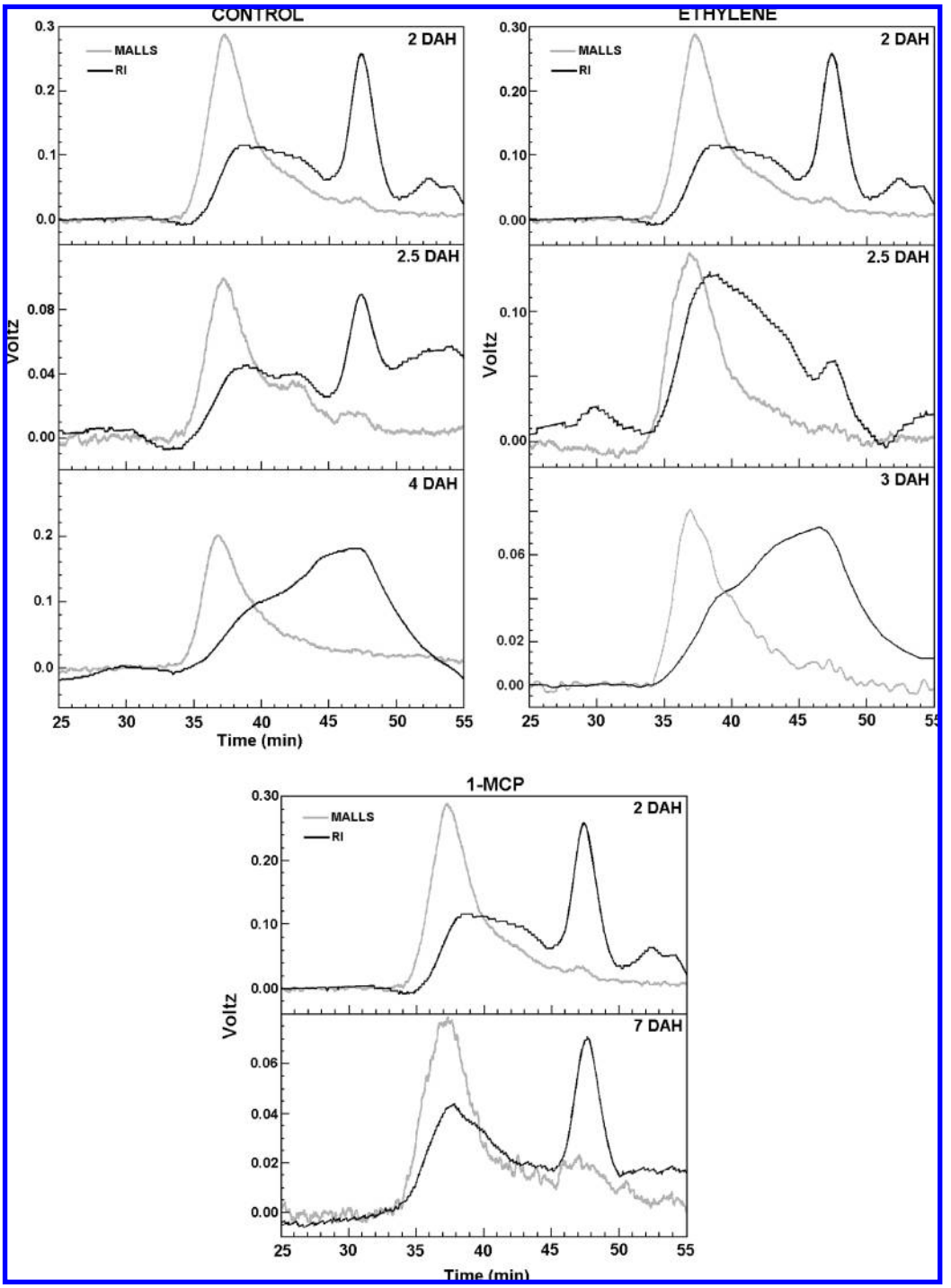
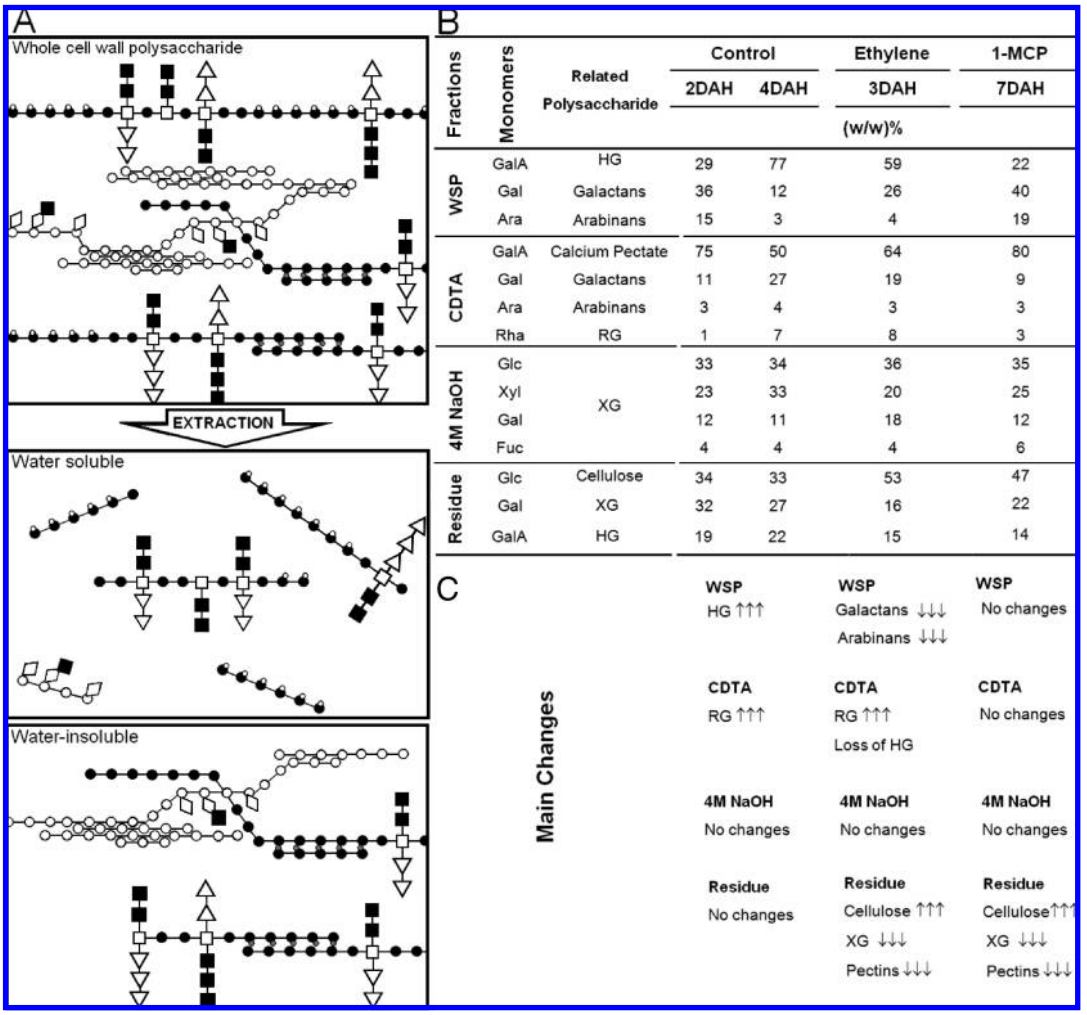


Figure 6. HPSEC analysis with RI and light scattering detector at 90° for water-soluble polymers (WSP) from the pulps of papaya fruit allowed to ripen naturally (control) or after treatment with ethylene or 1-MCP, sampled at different days after harvest (DAH).

composed of low branched polysaccharides. This could be evidence that homogalacturonans are the main polysaccharide hydrolyzed by endopolygalacturonase (EPG). In relation to the calcium-chelated low-branched galacturonans released by CDTA, no evident differences were noted during ripening, except for the 1-MCP-treated fruits that showed depolymerisation of galacturonic acid. The monosaccharide composition of the polymers released by 4 M alkali denoted the predominance of xyloglucan, which remained unchanged regardless of the ripening stage or the treatment. The same results were seen for the insoluble residue probably composed of cellulose, hemicellulose, and highly cross-linked pectins.

The elution profiles from HPSEC-MALLS-RI analysis suggest a reduction in polymer molecular mass during ripening. The changes in the molecular mass distribution of WSP could be due to loss of side chain of pectins. The decrease in the molecular mass of polysaccharides probably increased the solubility of the molecules, resulting in higher yields of WSP. On the basis of the results from the ethylene or 1-MCP treatment, the release of high molecular mass water-soluble galacturonans seems to be dependent on the levels of this plant hormone.

Despite the many reports on xyloglucans solubilization in fruits (*17*−*19*), no evidence of extensive hemicellulose (xyloglucans) depolymerization was observed in papaya, as already



Fractions	Monomers	Related Polysaccharide	Control 2DAH	Control 4DAH	Ethylene 3DAH	1-MCP 7DAH
					(w/w)%	
WSP	GalA	HG	29	77	59	22
	Gal	Galactans	36	12	26	40
	Ara	Arabinans	15	3	4	19
CDTA	GalA	Calcium Pectate	75	50	64	80
	Gal	Galactans	11	27	19	9
	Ara	Arabinans	3	4	3	3
	Rha	RG	1	7	8	3
4M NaOH	Glc	XG	33	34	36	35
	Xyl		23	33	20	25
	Gal		12	11	18	12
	Fuc		4	4	4	6
Residue	Glc	Cellulose	34	33	53	47
	Gal	XG	32	27	16	22
	GalA	HG	19	22	15	14

Figure 7. Summarized information on the changes in cell wall carbohydrate composition associated with the softening of ripening papaya. (A) Scheme of the solubilization of polysaccharides in the CWM by the putative endo PG activity, and the resulting fractions of water-soluble polymers (WSP) and water-insoluble polymers (WIP). Symbols are representative of glucose (○), galacturonic acid (●), esterified metil group (small ○), calcium bridge (gray circle), rhamnose (□), galactose (■), xylose (lozenge), arabinose (△), as the main monosaccharide units of the polymers. (B) Changes in the main monosaccharides from the water-soluble polymers (WSP), polymers solubilized by chelating agent (CDTA), polymers immobilized by ester linkages, released by alkali (4 M NaOH), and the cellulose-rich residue from the pulps of papaya fruit allowed to ripen naturally (control) or after treatment with ethylene or 1-MCP, sampled at different days after harvest (DAH). The main polysaccharides related to each fraction are indicated on the column at the right side (Homogalacturonans, HG; Rhamnogalacturonan RG; Xyloglucans, XG). (C) Changes in the main polysaccharides related to fractions isolated from papaya cell wall material.

observed by Manrique and Lajolo (*22*). The HPAEC-PAD analysis of the fractions eluted from size-exclusion chromatography revealed the absence of neutral polysaccharides and the predominant presence of uronic acids in papaya WSP.

The results presented here indicate a massive solubilization of large molecular mass galacturonans, but not an extensive depolymerisation of this cell wall component. This observation would be in agreement to the action of polygalacturonase (PG) at endo positions of the polysaccharide chain, corroborating the fact observed by Fabi et al. (*21*) when a papaya EPG gene was up-regulated after ethylene treatment. Since only minute amounts of monomers were detected in alcohol soluble extract, the exopolygalacturonase activity would be less prevalent in the pulp of ripening papayas. As the increased yield in WSP was accompanied by an increase in its degree of esterification, in spite of the constant degree of esterification of the CWM, it would indicate that the released water-soluble pectin would be still methylesterified, suggesting that previous de-esterification by pectin methylesterase (PME) seems to not be necessary for the EPG action, or its site of action is preferably nearby the methylesterified regions.

Manrique and Lajolo (*22*), working with the same cultivar, did not observed any alteration in the DE of the WSP; nevertheless, they noticed a decrease in the DE for the imidazol-soluble and carbonate-soluble polysaccharides. Higher values of free galacturonic acid found in papaya pulp (*20*) as well as differences in DE could be attributed to the de-esterifying property of carbonate, differences in extraction procedures, or the use of colorimetric analysis for uronic acid determination, which may cross-react with phenolic compounds.

The relatively small amount of pulp softening after 1-MCP treatment reveals that while the majority of the softening is ethylene dependent there is a component that occurs even in the presence of MCP. **Figure 7** summarizes the results obtained, which allow us to conclude that the extensive softening undergone by the pulp of ripening papayas is a consequence of the extensive solubilization of large molecular mass galacturonans from the pectin fraction of the cell wall. This process seems to be largely dependent on the levels of ethylene, and it is likely that the releasing of galacturonan chains results from an endo acting polygalacturonase.

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