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Cell Wall Polysaccharides of Near-Isogenic Lines of Melon (*Cucumis melo* L.) and Their Inbred Parentals Which Show Differential Flesh Firmness or Physiological Behavior

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Supporting Information

ABSTRACT: We characterized differences in cell wall material and polysaccharide structures, due to the quantitative trait loci associated with higher flesh firmness in a nonclimacteric near-isogenic line (NIL) SC7-2, and with the climacteric behavior of the NIL SC3-5-1, using their nonclimacteric inbred parentals, "Piel de Sapo" (PS) and PI 161375 (SC). PS was firmer and had a higher ripening index and greater hemicellulosic content than SC, with its lower wall material yield, and uronic acid, neutral sugar, cellulose and free sugar content and higher pectic content. SC3-5-1 showed lower uronic acid values, a higher soluble solid content, and similar flesh firmness to PS. SC3-5-1 yielded mainly high molecular weight polysaccharides in the imidazole-soluble fraction than PS. SC7-2 showed greater flesh firmness, a higher neutral sugar (especially galactose and mannose) and uronic acid content, together with a larger cellulose and α-cellulose residue than PS. SC7-2 also contained more polysaccharides of low molecular weight in the first pectic fraction and shifted toward higher molecular weights in the main peak of the 4 M potassium-soluble fraction compared with PS.

KEYWORDS: *Cucumis melo* L, chemical fractionation, glycosidic composition, fruit ripening, flesh firmness, climacteric behavior

INTRODUCTION

The cell wall is a complex and dynamic structure which acts as a structural and mechanical support for the plant body and plays an important role in fruit textural changes,[1,2] being a key but not unique component of fruit texture characteristics.[3] The degree of primary cell wall degradation is an important factor in fruit tissue integrity that is affected by the composition and structure of middle lamella polysaccharides, the cuticle and the turgor generated within the cell walls by osmosis.[4−7]

The pectins, hemicellulose and cellulose, are the main cell wall polysaccharides susceptible to solubilization, depolymerization and de-esterification by different cell-wall-modifying enzymes during fruit ripening and storage.[8−10] Overall during melon ripening there is a shift to a lower-molecular-mass distribution of hemicellulose polymers and a substantial degree of solubilization and depolymerization of pectins, especially the water-soluble pectins.[5] Cell wall changes associated with fruit softening during ripening can be divided into two sequential stages. The first occurs during early fruit ripening and is associated with the disassembly of xyloglucan polymers through the action of cell wall hydrolases and, especially, expansin proteins. The second stage occurs in the late stage of ripening and is related to disassembly of the pectin network. These processes occur in a temporal overlap, but in some climacteric melons, such as "Charentais", pectin disassembly occurs late in ripening and after xyloglucan disassembly, while polygalacturonases appear to be present during pectin disassembly, albeit at very low levels.[5,11]

Texture is one of the most important quality parameters and is partly responsible for consumer preferences of edible fruit,[12] while softening is a determining factor in the quality and postharvest life of fruit.[4,9] The softening process has ethylene-dependent and independent components,[13,14] but rapid melon softening often correlates with autocatalytic ethylene production and the degradation of cell wall polymers.[15] Endopolygalacturonase (PG, EC 3.2.1.15), one of the enzymes responsible for polyuronide depolymerization and solubilization during ripening, shows the genetic expression of different subunits that are either dependent or independent of ethylene action.[13,16] Cell wall-modifying enzymes are synthesized by a large family of genes that plays an important role in fruit metabolism during melon ripening. For example, pectin methylesterase (PME) contributes to the action of polygalacturonase, converting methyl-esterified pectins into substrates upon which these enzymes could act.[17]

Candidate genes for several quantitative trait loci (QTL) associated with fruit softening have been identified in melon.[18] Previous results indicate that certain near-isogenic lines (NILs) of this collection undergo a similar softening process, although the initial value of whole fruit firmness and variability in the senescence end point are critical.[18−20] Therefore, study of the cell wall composition of both NILs and its parentals at this point may help us to characterize the possible effect of the QTL on the cell wall during ripening. Our null hypothesis is that melon QTLs associated with differences in texture and climacteric affect cell wall composition.

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MATERIALS AND METHODS

Plant Material and Experimental Design. The plant materials used were melon near-isogenic lines or NILs and their nonclimacteric parentals, the Spanish melon *Cucumis melo* L. cv. "Piel de Sapo" (PS) and the Korean accession PI 161375 "Shongwan Charmi" (SC).[21] The NILs evaluated were the climacteric NIL SC3-5-1 and the nonclimacteric NIL SC7-2. Both NILs contain introgressions of SC into PS genetic background according to the first number, which refers to the linkage group with the introgression[18,21] (A. J. Monforte, Institute of Molecular and Cellular Biology of Plants, Valencia, Spain, personal communication). The fruits were harvested in two consecutive seasons (2008 and 2009). Crop management, harvest practices and other details have been reported previously.[14,22] The field was divided into blocks in a completely randomized design within each block. The distance between rows was 2 m and between plants 1.5 m. Each replicate consisted of three plants separated 1.5 m from the other replicates (in 2008) or six plants (three in two adjacent rows) with the same separation between replicates (in 2009). Fruit were harvested throughout both seasons according to the maturity indices previously described.[14] The quality traits reported below were evaluated in both seasons using at least two fruits per replicate as follows: In season 2008, SC $n = 10$, in PS $n = 21$, in SC3-5-1 $n = 7$ and in SC7-2 $n = 6$. In season 2009, SC $n = 6$, in PS $n = 9$, in SC3-5-1 $n = 9$ and in SC7-2 $n = 7$.

Physiological Behavior Evaluation. Respiration rate and ethylene production were measured at harvest (optimum stage of maturity) in at least five individual fruits of different replicates according to Fernández-Trujillo et al.[23]

Sampling and Sample Preparation for Extraction of Cell Wall Material (CWM). In cell wall experiments, four lines were analyzed, with three replicates per line and year. Depending on the fruit size, two to eight selected fruits per replicate were used for obtaining the fresh melon flesh as follows. Fruits were peeled, and the seeds and placental tissue were removed with a stainless steel knife. The flesh was cut into pieces, weighed and stored in zip closure bags in a freezer at −25 °C.

Cell Wall Material Isolation. CWM was obtained by adapting the methodology of Selvendran[24] to melon fruit. Samples were first extracted with 96% v/v ethanol (four volumes per unit of flesh fresh weight). Samples were ground with a mixer (Orione Mod 0.7 kg, Sirman spa, Venice, Italy), homogenized (Ultraturrax T25 Janke and Kunkel, Ika-Labortechnik, Funkentsört, Germany) and filtered, and the remaining pellet was treated again with 80% v/v ethanol. Subsequently, the pellet was washed with acetone until it became white and finally air-dried and stored at −20 °C until analysis. An aliquot of the ethanol-soluble fractions was kept at 4 °C until subsequent analysis.

Extraction of Polysaccharide Components. Cell wall polysaccharides were fractionated into four groups and the final α-cellulose residue (α-CEL). The first two extracts were two pectic fractions: the imidazole-soluble fraction (ISF) and carbonate-soluble fraction (CSF). The next two were the hemicellulosic fractions: the 1 M potassium-soluble fraction (K1SF) and 4 M potassium-soluble fraction (K4SF). One gram of CWM was sequentially extracted according to Jiménez et al.[25,26] with slight modifications in the solutions and concentrations used: (a) 0.5 M imidazole/hydrochloric acid, 10 mM in metabisulfite (Fluka puriss, Buchs, Switzerland) (two times, 250 mL) for 4 h at room temperature in the first extraction and 18 h in the second, and washed with distilled water (250 mL) (ISF); (b) 0.05 M sodium carbonate (Sigma, Barcelona, Spain), 20 mM in sodium borohydride (Panreac, Barcelona, Spain) (two times, 250 mL) for 18 h at 4 °C and 4 h at room temperature and washed (CSF); (c and d) 1 and 4 M potassium hydroxide (Merck, Darmstadt, Germany), 10 mM in sodium borohydride (Panreac) (two times, 250 mL) for 18 at 4 °C, 4 h at room temperature and washed (K1SF and K4SF). The fractions CSF, K1SF and K4SF were neutralized with glacial acetic acid (Merck) to pH 6. The final α-CEL obtained and the four fractions (ISF, CSF, K1SF and K4SF) were dialyzed in dialysis tubing of molecular weight cutoff (MWCO) 12000 Da (cellulose membrane, Sigma Chemical CO., St. Louis, MO) against water. Conductivity was measured (Hanna Instruments HI 8819N, Ann Arbor, MI) checking that the washing water reached a conductivity below 100 μS/cm. Fractions were frozen at −25 °C and lyophilized (Flexi-Dry MP Freeze-Dryer, FTS Systems Inc., Stone Ridge, NY) for glycoside analysis.

Polysaccharide Analysis. The glycosidic composition of the different fractions was determined by acid hydrolysis with 2 N trifluoroacetic acid (Sigma-Aldrich, Chemie GmbH, Steinheim, Germany) (121 °C, 1 h),[27] derivatization to alditol acetates and quantification by gas chromatography.[28]

A HP 6890 Plus+ gas chromatograph (Hewlett-Packard, Palo Alto, CA) fitted with a 30 m × 250 μm × 0.20 mm capillary column (SP-2330, Supelco, Bellefonte, PA) was used. The carrier gas was helium with a constant flow equal to 2.2 mL/min, pressure 21.5 psi (148.24 kPa). Injection was performed in splitless mode. The oven temperature was held at 50 °C for 2 min after injection, then programmed to 180 at 35 °C/min, held at 180 °C for 5 min, and then immediately increased to 220 at 5 °C/min, and held at 220 °C for 22 min. Total run was 40.7 min. The injector temperature was 250 °C, flame ionization detector (FID), 300 °C. Neutral sugars (NS) L-rhamnose (Rha), D-fucose (Fuc), L-arabinose (Ara), D-xylose (Xyl), D-mannose (Man), D-galactose (Gal) and D-glucose (Glc) were identified. *myo*-Inositol was used as internal standard.

The residue of the α-cellulose fraction after trifluoroacetic acid hydrolysis was treated with 72% H_2SO_4 (Panreac) for 2 h according to Jiménez et al.[26] to quantify its cellulose content. The glucose in the hydrolysates was colorimetrically quantified by the anthrone assay.[29] Uronic acids (UA) were quantified by the *m*-hydroxybiphenyl method.[30] The absorbance values of standards and samples were measured at 630 and 540 nm, respectively, in a microplate reader (MPM 600; Bio-Rad Laboratories, Inc., Hercules, CA).

High-Performance Size Exclusion Chromatography (HPSEC). Analysis of the Molecular Weight (MW) of the Polysaccharides. The method used for HPSEC analysis was that described by Jiménez et al.[25] with slight modifications. Polysaccharidic fractions (ISF, CSF, K1SF and K4SF) from the 2009 season were analyzed for MW determination after desalting with PD-10 columns (Supelco). The MW was measured in Jasco equipment (LC-Net II ADC, Kyoto, Japan) with a refractive index detector (Jasco RI-1530) and injection valve (Rheodyne, loop 20 μL, Cotati, CA). Two different columns in sequence were used: TSKgel GMPWxl and TSKgel G3000PWxl (300 × 7.8 mm i.d., Tosoh Bioscience LLC, King of Prussia, PA) after calibration with 250, 110, 70, 40, 6 kDa and glucose (Fluka, Buchs, Switzerland). Blue dextran was used to test the void volume (V0) of the column. The elution buffer was 0.05 M tris−hydrochloric acid and 0.2 M sodium chloride at a flow rate 0.4 mL/min. Fractions of 250 μL were collected using a Redifrac fraction collector (Pharmacia Biotech, Uppsala, Sweden). Fractions were assayed for NS by the Dische method,[29] and absorbance values were measured at 630 nm in a microplate reader (MPM 600, Bio-Rad). Peaks above 250 kDa or below 6 kDa were considered to be of high and low MW, respectively, throughout the manuscript.

Quality Traits. The weight, equatorial and longitudinal diameter of each individual fruit was measured according to Fernández-Trujillo et al.[31] and Obando et al.[22] Whole fruit hardness (in N/mm) was measured at a selected point of the equator of the fruit by a compression test of 2 mm deformation according to Tijskens et al.[20] Flesh firmness was measured in cylinders (20 × 15 mm) by using a 1.6 mm wide probe adapted to a testing machine.[31] Flesh juiciness, juice density and total soluble solids (in °Brix) were measured according to Obando et al.[22] Flesh juiciness and the pellet content of the juice after centrifugation are expressed in grams of juice per kg fresh weight or juice, respectively.

Table 1. Fruit Quality Traits of the Parental Lines and NILs of Melon Obtained from Two Consecutive Seasons (2008 and 2009)[a]

parental line or NIL	soluble solids (°Brix)	flesh firmness (N)	whole fruit hardness (N/mm)	pellet content of the juice (g/kg)	flesh juiciness (g/kg)
			Season 2008		
PS	9.60 a	7.30 a	83.36 ac	28.94 a	355.77 a
SC	7.76 a	6.97 ab	24.79 bc	26.33 a	326.93 b
SC3-5-1	10.81 b*	6.23 b	28.91 b	40.95 b*	356.15 a
SC7-2	9.15 a	11.51 c*	58.19 c	30.04 a	327.34 a
			Season 2009		
PS	11.92 a	5.66 a	64.04 a	22.82 a	295.81 a
SC	5.32 b	6.21 a	13.99 b	39.53 b	435.44 b
SC3-5-1	13.53 c*	6.22 a	32.55 b	46.83 b*	276.36 a
SC7-2	12.01 a	9.21 b*	54.98 c	30.54 a	161.12 c*

[a] Results were statistically analyzed per individual season. Means of each attribute followed by different letters in the same column (a−d) are significantly different according to the LSD test (p-value = 0.05). The NIL means followed by an asterisk are significantly different from PS according to the Dunnett test (p-value = 0.05).

Statistical Analysis. Quality traits and cell wall components data were subjected to univariate analysis of variance using cultivar as a single factor. If means within the cultivars were significantly different, they were separated by a Tukey−Kramer HSD test (quality traits) and LSD test (cell wall components) at $p = 0.05$. All the comparisons of quality traits of the NILs were made with PS, using JMP 5.1 software (SAS Institute Inc., Cary, NC), according to a Dunnett test ($p = 0.05$), because they share the same genetic background.

RESULTS

Quality, Physiological Behavior and Cell Wall Material of the Parental Lines (PS and SC). PS showed higher soluble solid levels and a greater pellet content proportion in the juice compared with SC. The same was true for whole fruit hardness (>70% higher in PS than SC), but flesh firmness values were similar (Table 1). The respiration rate and ethylene production pointed to nonclimacteric behavior in both parentals (data not shown).

The CWM of the replicates of all lines was combined every year for subsequent extraction because of the sparse differences detected between replicates (data not shown). Cell wall components and CWM yield were higher in PS than in SC (Table 2).

Glucose and galactose were the main neutral sugars in cell wall material in both parentals while the highest values of the rest of sugars corresponded to xylose and arabinose. PS showed higher values of Glc and Gal and lower values of Ara and Rha than SC (Table 3). The characterization of pectic and hemicellulosic fractions reported below outlines the main differences between parentals (Tables 4 and 5).

Cell Wall Fractions. *Pectic Fraction and Parental Lines.* The ISF and CSF of the parental lines were rich in pectic polysaccharides (UA, Gal and Ara). Overall, the parental lines showed similar profiles in the pectic fractions and similar HPSEC results, with some exceptions.

In ISF, PS had a higher fucose (25−50%) and xylose (18−49%) content than SC (Figure 1). Polysaccharides with a molecular weight above 250 kDa or below 6 kDa in ISF were lower (or undetectable) in PS than in SC (Figure 2). In CSF, the parentals had high UA values and significant amounts of arabinose, galactose and rhamnose, homogalacturonans being the main components of this fraction. SC showed higher Rha and Ara values than PS (Figure 3). In the CSF, a dense gel was formed when the HPSEC elution buffer was added.

Hemicellulosic Fraction and Parental Lines. The K1SF and K4SF showed higher percentages of glucose and xylose (Figures 4 and 6). In general, in both lines the percentage of uronic acids (30−53% in K1SF and 14−18% in K4SF) was lower in

Table 2. Cell Wall Material (CWM) Composition of the Parental Lines and NILs of Melon Obtained from Two Consecutive Seasons (2008 and 2009)[a]

parental line or NIL	yield of CWM[b]	free sugars[b]	NS[c]	UA[c]	CEL[c]
			Season 2008		
PS	0.86 acd	5.90 a	68.11 abd	201.15 a	271.67 a
SC	0.67 b	5.22 a	61.51 b	159.53 b	209.30 b
SC3-5-1	0.80 c	5.95 a	47.16 c	170.80 c	222.78 c
SC7-2	0.95 d	4.84 a	77.52 d	220.59 d	288.23 a
			Season 2009		
PS	0.93 a	9.27 a	56.30 a	157.68 a	178.01 a
SC	0.54 b	3.83 b	42.93 b	108.72 b	132.43 b
SC3-5-1	1.01 a	10.01 a	68.05 c	144.43 a	210.10 c
SC7-2	1.04 a	9.98 a	95.70 d	179.62 c	255.97 d

[a] Results were analyzed per individual season. [b] Results are expressed in g/100 g of fresh weight and are the average value of three replicates. [c] Results are expressed in mg/100 g of fresh weight and are the average value of three replicates. Means of each attribute followed by different letters in the same column (a−d) are significantly different according to the LSD test (p-value = 0.05). The NIL means followed by an asterisk are significantly different from PS according to the Dunnett test (p-value = 0.05).

Table 3. Neutral Sugar Composition Cell Wall Material of the Parental Lines and NILs of Melon Obtained from Two Consecutive Seasons (2008 and 2009)[a]

	neutral sugars[b]						
parental line or NIL	Rha	Fuc	Ara	Xyl	Man	Gal	Glc
				Season 2008			
PS	2.9 ab	2.6 a	5.2 a	8.4 ab	4.5 ac	25.4 a	18.1 a
SC	3.3 ab	2.5 a	8.6 b	9.6 ab	4.2 a	18.7 b	11.4 b
SC3-5-1	2.5 a	1.9 a	4.1 a	7.2 a	3.1 b	14.9 b	13.4 b
SC7-2	4.0 c	4.0 b	5.2 a	10.6 b	5.0 c	32.4 c	16.3 ab
				Season 2009			
PS	1.7 a	1.4 a	5.3 a	9.4 a	3.5 a	13.1 a	21.8 a
SC	2.0 a	1.0 a	7.7 b	8.4 a	4.7 ab	10.3 b	7.8 b
SC3-5-1	1.9 a	1.2 a	5.4 a	9.5 a	4.5 ab	15.7 c	31.9 c
SC7-2	3.9 b	2.1 b	7.5 b	16.8 b	5.8 b	27.5 d	30.5 c

[a] Results were analyzed per individual season. [b] Results are expressed in mg/100 g of fresh weight and are the average value of three replicates. Means of each attribute followed by different letters in the same column (a−d) are significantly different according to the LSD test (p-value = 0.05).

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Table 4. Relative Percentage (%) of Polysaccharides in Pectic [Imidazole-Soluble Fraction (ISF) and Carbonate-Soluble Fraction (CSF)] and Hemicellulosic [1 M Potassium-Soluble Fraction (K1SF) and 4 M Potassium-Soluble Fraction (K4SF)]. Fractions of Cell Wall Material and Percentages of the Sum of Pectins and Hemicelluloses (ΣP + ΣH) in the Parental Lines and NILs of Melon Obtained from Two Consecutive Seasons (2008 and 2009)

	pectic polysaccharides		hemicellulosic polysaccharides	
parental line or NIL	ISF + CSF (%)[a]	ΣP + ΣH (%)[b]	K1SF + K4SF (%)[a]	ΣP + ΣH (%)[b]
		Season 2008		
PS	13.8	44.0	17.6	56.0
SC	5.1	54.3	4.3	45.7
SC3-5-1	10.8	40.4	16.0	59.6
SC7-2	9.3	40.5	13.7	59.5
		Season 2009		
PS	7.0	45.4	8.4	54.6
SC	9.8	57.7	7.2	42.4
SC3-5-1	7.4	39.2	11.4	60.8
SC7-2	5.4	39.2	8.4	60.9

[a] Relative percentage of the total fractions quantified from cell wall material (ISF, CSF, K1SF, K4SF, and α-CEL). [b] Relative percentage of the noncellulosic fractions quantified from cell wall material.

Table 5. Relative Percentage of Polysaccharides (%) in Imidazole-Soluble Fraction (ISF), Carbonate-Soluble Fraction (CSF), 1 M Potassium-Soluble Fraction (K1SF) and 4 M Potassium-Soluble Fraction (K4SF) of the Noncellulosic Polysaccharides Extracted from Cell Wall Material in the Parental Lines and NILs of Melon Obtained in Two Consecutive Seasons (2008 and 2009)

	pectic polysaccharides		hemicellulosic polysaccharides	
parental line or NIL	ISF (%)	CSF (%)	K1SF (%)	K4SF (%)
		Season 2008		
PS	32.3	11.7	22.4	33.6
SC	41.0	13.2	23.7	22.1
SC3-5-1	19.0	21.4	26.5	33.1
SC7-2	21.1	19.4	23.9	35.6
		Season 2009		
PS	24.3	21.1	31.0	23.6
SC	25.0	32.7	24.3	18.0
SC3-5-1	27.9	11.3	41.3	19.5
SC7-2	30.5	8.6	30.6	30.3

hemicellulosic fractions (especially in K4SF) than in the pectic fractions (44−55% in ISF and 55−81% in CSF) (Figures 1, 3, 4 and 6).

In K1SF, uronic acids were still the main component but to a lesser extent in SC (in 2008). Xylose was the most abundant neutral sugar in both parentals. PS showed lower Gal (14−34%) and Man (13−48%) values and a very low Ara content compared with SC (81−172% higher in SC than PS). Rha content was lower in PS than SC only in 2009 (Figure 4). The peak below 6 kDa present in SC was absent from PS, and this parental had a group of intermediate molecular weight polysaccharides (110 to 70 kDa) absent from SC (Figure 5). In K4SF, Glc, UA, Xyl and Gal were the main components, with no differences between parentals, while Rha and Man had a lower and higher content in PS than in SC, respectively. Fucose was present in substantial percentages (in some samples higher than 5% of CWM in K4SF) (Figure 6). The main peak ranged between 70 and 110 kDa in both parentals. In PS this peak was wider than in SC and broadened due to a higher molecular weight. PS had more polysaccharides between 6 and 40 kDa with a higher peak close to 6 kDa than SC (Figure 7).

Cellulosic Fraction and Parental Lines. No differences between parentals were found as regards the predominance of the α-CEL fraction in the cell wall structure (77−87% overall). In this fraction, cellulose was the major component (>86%). Galactose, glucose and uronic acids were the main noncellulosic components, indicating the presence of xyloglucans. PS showed more Gal (around 24%) and Man (52−66%) and less Ara (38−104%) and Glc (around 25%) than SC. Both parentals had significant percentages of Rha (>7%) (Figure 8).

Table 4 presents a summary of the soluble fractions (pectic and hemicellulose) and showed the differences between parental lines. The pectic fraction was defined as the sum of ISF and CSF, and the hemicellulosic fraction as K1SF plus K4SF. The percentages of pectic and hemicellulosic fractions were around 44% and 55%, respectively, for PS; while the same fractions for SC were around 56% and 44%, respectively. Therefore, PS showed a higher hemicellulosic fraction and a lower pectic fraction than SC. The ISF showed a higher content of low molecular weight polysaccharides in SC than PS (Figure 2).

Fruit Quality Traits of the Climacteric NIL SC3-5-1 versus PS. Cell Wall Material and Fractions. SC3-5-1 showed lower flesh firmness than PS in 2008 but not in 2009. The juice of SC3-5-1 had a higher soluble solid content and similar flesh juiciness and pellet content of the juice than PS. The hardness of SC3-5-1 was 50−65% lower than that of PS (Table 1). At harvest, the respiration rate and ethylene production were significantly higher in the climacteric NIL SC3-5-1 than in PS (data not shown).

In SC3-5-1, α-CEL (77%), was the main component of the cell wall structure, in agreement with its inbred parentals. Overall, SC3-5-1 had 9−16% lower uronic acids content than PS (Table 2).

After cell wall fractionation, study of the ISF showed that the pectic sugars (UA, Gal and Ara) were present in SC3-5-1, as they were in PS. The imidazole-soluble fraction from the climacteric NIL had a noticeably higher content of nonpectic sugars (especially in 2008) than PS. Glucose and fucose were 23−77% and 42−121% higher in SC3-5-1 than PS, respectively. In the season 2008, Man and Xyl values were higher (111% and 75% respectively) in SC3-5-1 than PS (Figure 1). By HPSEC (Figure 2), it was seen that the climacteric NIL showed more peaks in the ISF of high and low molecular weight than PS. The main peak of SC3-5-1 at 70 kDa was wider than the same peak in PS and showed a broadening of the eluted peak to a higher molecular weight. High MW polysaccharides (250 kDa) were detected in SC3-5-1 and were more abundant than in PS. Homogalacturonans were the main component in carbonate-soluble fraction of SC3-5-1, as in PS (Figure 3).

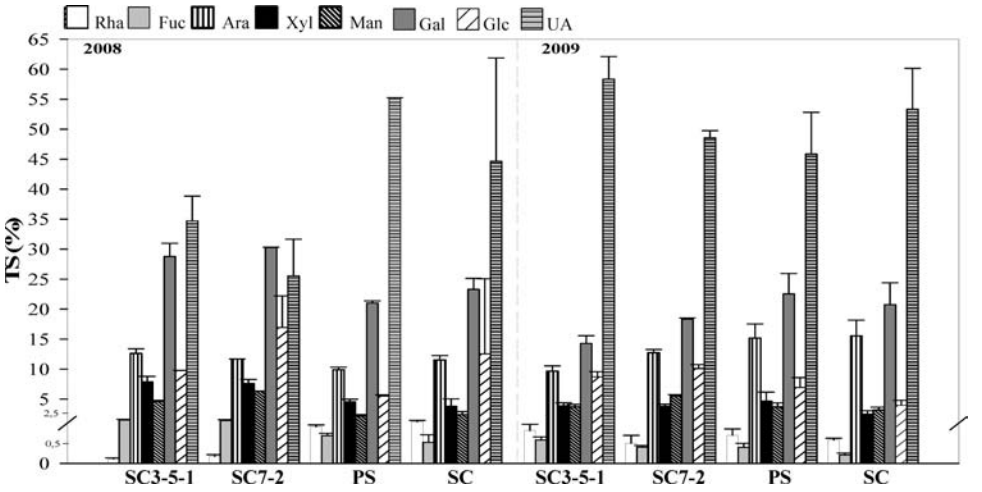

Figure 1. Composition of neutral sugars (NS) and uronic acids (UA) in the pectic imidazole-soluble fraction (ISF) of the fruit flesh of different melon lines harvested in two consecutive seasons, 2008 and 2009. NS and UA are expressed in percentage of total sugar (TS) recovered from this fraction. Error bars with standard deviation (σ) are included in columns.

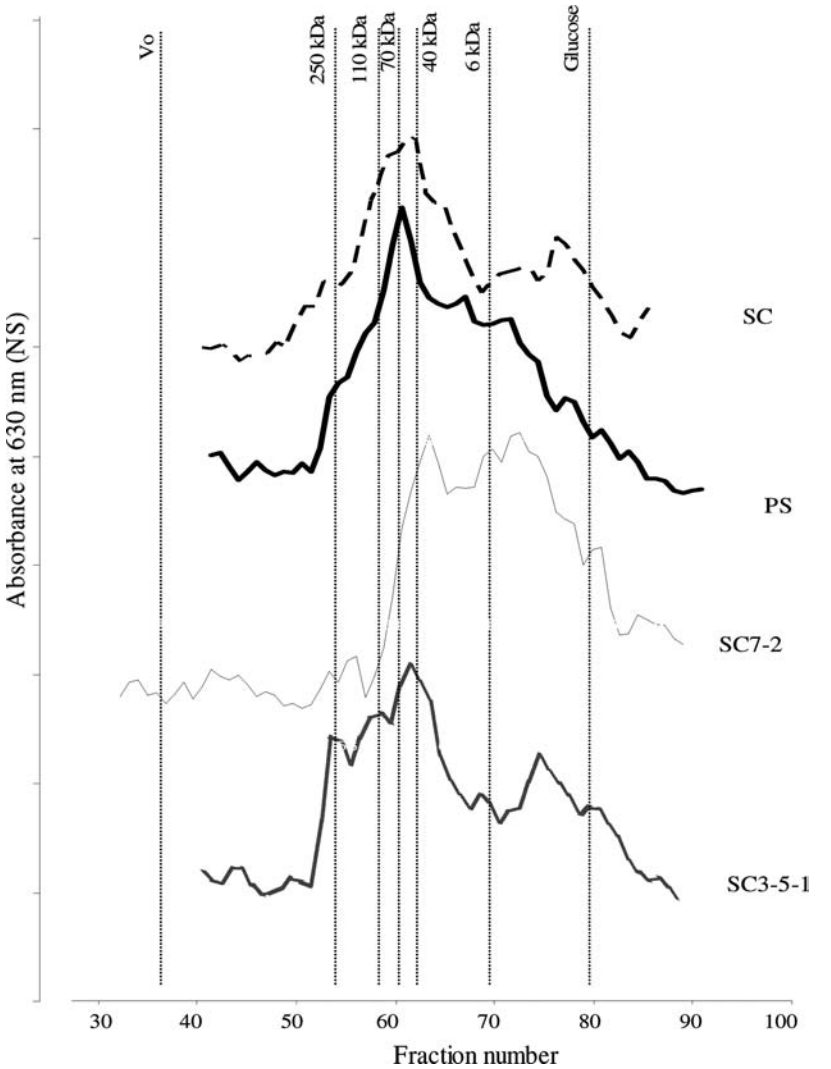

Figure 2. Molecular-mass distribution of the neutral fraction from the pectic imidazole-soluble fraction (ISF) of the fruit flesh of different melon lines. T250, T110, T70, T40, T6 and glucose were the molecular size standards, and V0 was the void volume.

In the K1SF hemicellulosic fraction, uronic acids and xylose were the main sugars in PS and in SC3-5-1, but this climacteric NIL showed around 39% lower values in Ara than in PS (Figure 4). In HPSEC (Figure 5), SC3-5-1 did not show the

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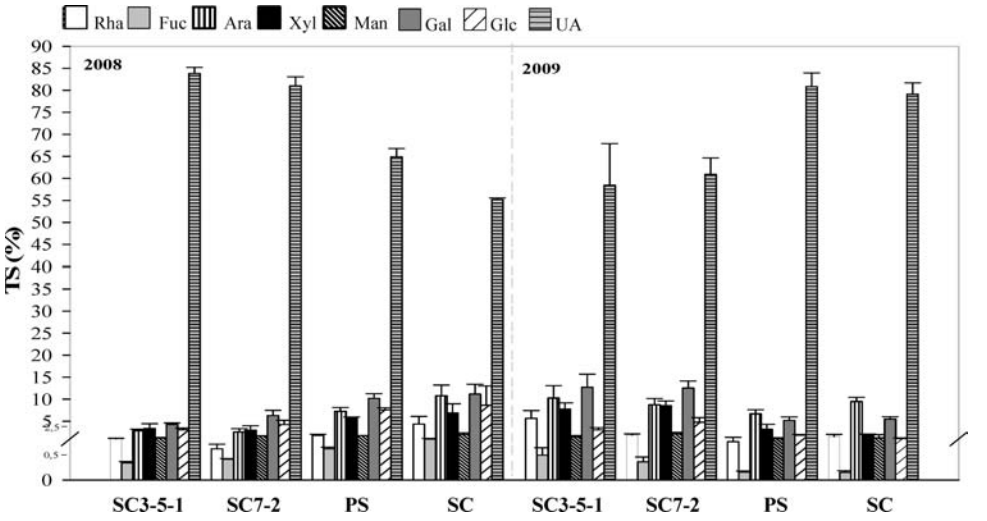

Figure 3. Composition of neutral sugars (NS) and uronic acids (UA) in the pectic carbonate-soluble fraction (CSF) of the fruit flesh of different melon lines harvested in two consecutive seasons, 2008 and 2009. NS and UA are expressed in percentage of total sugar (TS) recovered from this fraction. Error bars with standard deviation (σ) are included in columns.

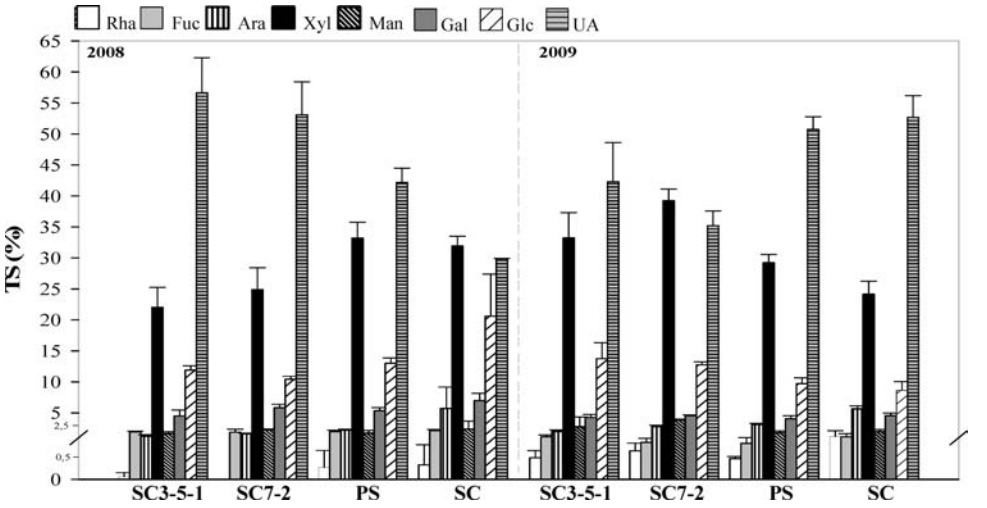

Figure 4. Composition of neutral sugars (NS) and uronic acids (UA) in the hemicellulosic 1 M potassium-soluble fraction (K1SF) of the fruit flesh of different melon lines harvested in two consecutive seasons, 2008 and 2009. NS and UA are expressed in percentage of total sugar (TS) recovered from this fraction. Error bars with standard deviation (σ) are included in columns.

peak at 70 kDa present in PS, although other polysaccharides seemed to be similar. In the K4SF, the high values of glucose, xylose, galactose and fucose suggested the presence of xyloglucans in SC3-5-1, as in PS (Figure 6). SC3-5-1 had less Glc (7−46%), Rha (63−77%) and Fuc (38%) and more UA (23−50%) than PS (higher values in 2009). After HPSEC analysis (Figure 7), SC3-5-1 showed a principal polysaccharide peak close to 40 kDa, which shifted toward 70 kDa in PS.

In the α-cellulose residue of SC3-5-1, CEL was the major component and Gal, Glc and UA were abundant, as in PS. SC3-5-1 had more Ara and less Man (especially in 2009), Fuc and Xyl than PS (Figure 8).

The pectic and hemicellulosic fractions of SC3-5-1 were 11% lower and 9% higher respectively, compared with PS. The main difference was in the hemicellulosic fractions of SC3-5-1 containing around 27% more K1SF than PS (Table 5).

The climacteric NIL SC3-5-1 showed the highest values in galactose in the CSF, K4SF, and especially in α-CEL (Figures 3, 6 and 8).

Fruit Quality Traits of the Nonclimacteric NIL SC7-2 versus PS. Cell Wall Material and Fractions. The trends in respiration rate and ethylene production were similar in the nonclimacteric NIL SC7-2 and PS (data not shown). SC7-2 had 60% higher flesh firmness, 15−30% lower hardness and lower flesh juiciness (particularly in 2009) but a similar soluble solid content to PS (average value 10.7 °Brix) (Table 1).

Overall, SC7-2 had more neutral sugars, uronic acids and cellulose than PS (Table 2). Neutral sugars Gal, Rha, Man, Xyl and Fuc were higher in SC7-2 than PS (Table 3), differences that were more pronounced in 2009.

In the ISF, the flesh of SC7-2 had higher levels of hemicellulosic sugars Glc and Man than PS (Figure 1). In SC7-2, the main peak (present at 70 kDa in PS) shifted to a molecular weight below 40 kDa, and polysaccharides of low MW were more abundant than in PS (Figure 2). In the CSF, homogalacturonans were present in both, SC7-2 and PS (Figure 3).

In the K1SF, uronic acids and xylose were the main components in SC7-2, as in PS, and xylans were the main hemicellulosic polysaccharides. SC7-2 had less Ara and Fuc but slightly higher Man levels than PS (Figure 4). In HPSEC analysis, SC7-2 did not show the peak at 70 kDa present in PS (Figure 5). In PS, the main peak could be formed by the overlapping of two others, one of molecular weight between 40 and 6 kDa and another of around 6 kDa. However, in SC7-2 this second peak below 6 kDa was reduced to a small shoulder, so that the main peak was visible between 40 and 6 kDa. In the K4SF, uronic acids, glucose, xylose and galactose were the main components, so that xyloglucans were probably the most abundant polysaccharides, as in PS (Figure 6). The NIL SC7-2 had a main peak close to 110 kDa with a shift toward lower molecular weight (110 to 70 kDa) in PS, as indicated by HPSEC analysis (Figure 7).

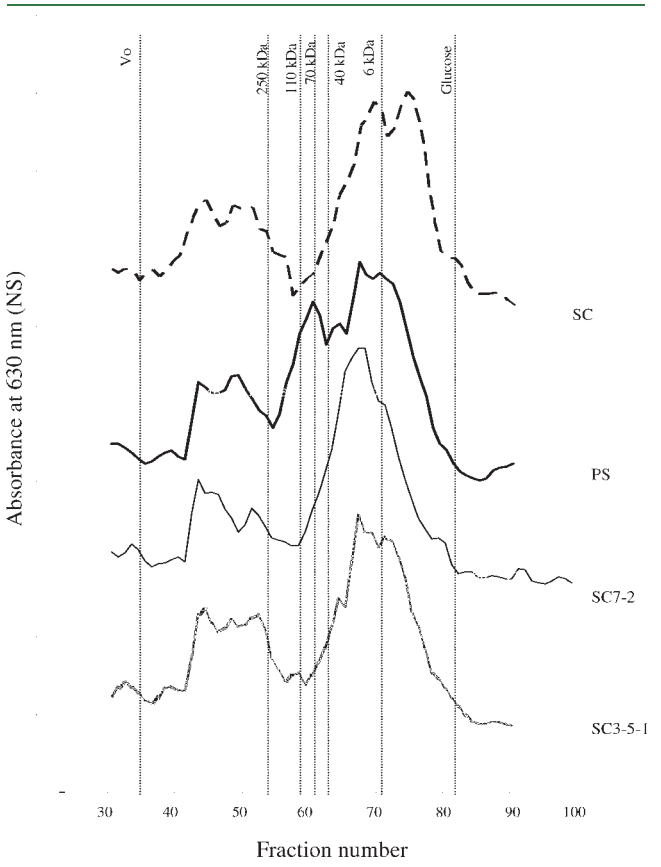

Figure 5. Molecular-mass distribution of neutral fraction from the hemicellulosic 1 M potassium-soluble fraction (K1SF) of the fruit flesh of different melon lines. T250, T110, T70, T40, T6 and glucose were the molecular size standards, and V0 was the void volume.

Cellulose was the major constituent in α-CEL of SC7-2, and noncellulosic sugars Gal, Glc and UA were the main components, as in PS. SC7-2 showed more Gal (24−58%), and less Man (around 25%) and UA (18%) than PS (Figure 8).

Compared with PS the cell wall structure of SC7-2 had a slightly higher α-CEL content (>7%, data not shown) and less pectin and more hemicellulose (Table 4). The greatest differences were the higher values in the K4SF (Table 5), unlike in SC3-5-1, where K1SF was the main hemicellulosic fraction.

Differences between Seasons. The fruit from season 2008 grew outside their optimum summer conditions and attained a lower maturity index at harvest, with lower levels of soluble solids and free sugars. Uronic acids, cellulose and flesh firmness were higher in 2008 than in 2009 (Tables 1 and 2). As shown, both NILs showed a higher content of nonpectic sugars and lower content of UA in ISF of 2008 than 2009 (Figure 1) and in CSF of 2009 than 2008 (Figure 3).

The glucose/galactose ratio was less than 1 in 2008 and more than 1 in 2009 (Table 3). In 2009, the higher values of Gal and Rha in SC3-5-1 (Figure 3) would have contributed to the higher content of rhamnogalacturonans than observed in PS.

SC fruits harvested in 2009 only attained 5.3 °Brix and 3.8 g/100 g of free sugars (55.4 and 41.4% lower than PS, respectively). The pectic components (UA, Gal and Rha) and hemicellulosic components (Glc, Xyl and Man) were higher and lower in 2008 than in 2009, respectively (Table 3).

DISCUSSION

The locations and structures of cell wall polysaccharides are related to several texture parameters such as flesh firmness and

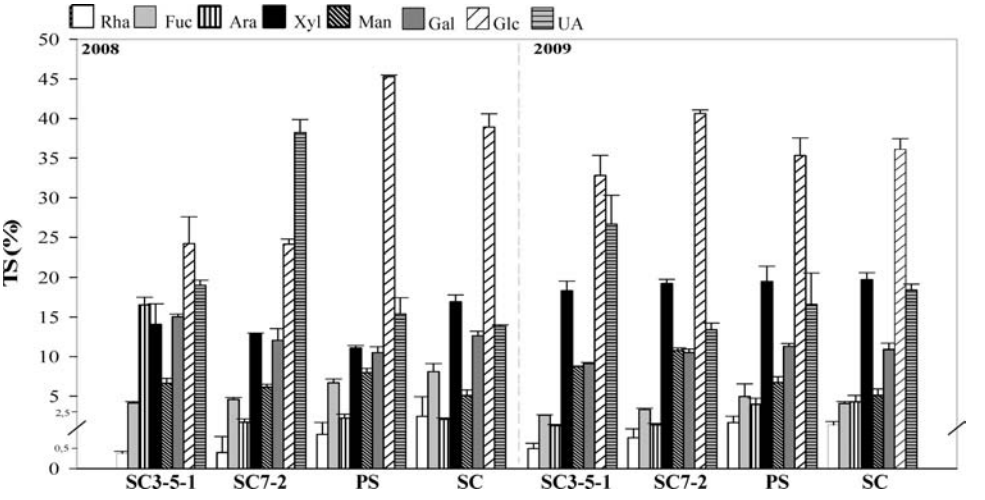

Figure 6. Composition of neutral sugars (NS) and uronic acids (UA) in the hemicellulosic 4 M potassium-soluble fraction (K4SF) of the fruit flesh of different melon lines harvested in two consecutive seasons, 2008 and 2009. NS and UA are expressed in percentage of total sugar (TS) recovered from this fraction. Error bars with standard deviation (σ) are included in columns.

whole fruit hardness (Table 1). In general, the main changes that occur during fruit ripening, including softening, have been associated with modifications in the cell wall pectic structure. However, investigations have revealed that the hemicellulosic matrix of the cell wall is at least as important as the pectin matrix in determining the structural integrity of the wall.[32,33] From the results of the present work, it can be proposed that no individual polysaccharide group was fully responsible for the value of fruit texture in any of the melons lines analyzed, but the relative amounts of each group were important for texture.

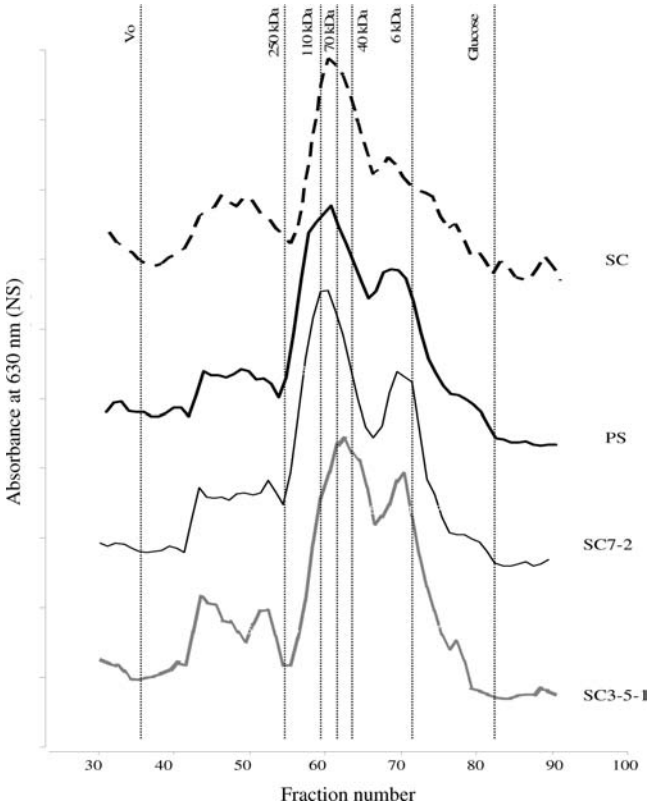

Figure 7. Molecular-mass distribution of neutral fraction from the hemicellulosic 4 M potassium-soluble fraction (K4SF) of the fruit flesh of different melon lines. T250, T110, T70, T40, T6 and glucose were the molecular size standards, and V0 was the void volume.

Parental Lines (PS and SC). This is the first study that demonstrates that parental melons PS and SC showed marked differences in the intrinsic characteristics and composition of the cell wall structure irrespective of the other quality traits studied (Tables 1 and 2) and according to previous studies conducted for QTL mapping of quality traits.[18,22] In general, PS had higher cell wall material yield, total uronic acids, neutral sugars and cellulose content and higher molecular weight, in the hemicellulose fractions than SC. These values, together with the different percentages of pectic and hemicellulosic components, contributed to the differences with PS, but did not explain why SC had similar flesh firmness to PS. The variability in firmness, texture and juiceness characteristic of ripe fruit of different types is in relation with differences in cell wall composition and marked diversity exists in cell wall changes between species and even different cultivars of the same species.[34] However, this information particularized on melons is lacking.

Pectic fractions of parental lines (and NILs) showed a typical melon composition found in several cultivars,[5,7] with high content in UA, Gal and Ara. In parentals (and NILs), values of the typically hemicellulosic sugars (Xyl, Glc, Fuc and Man) pointed to depolymerization of these polysaccharides, whose fragments were collected in this fraction such as in other previous studies on "Prince" and "Wasada-Uri" melons.[8] The presence of hemicellulosic sugars in SC could be related to the presence of polysaccharides of low molecular weight (<6 kDa) indicating the onset of increased hydrolytic activity in this parental because depolymerization of hemicelluloses has been observed throughout ripening in "Charentais" melons.[5]

In the carbonate-soluble fraction, homogalacturonans were the most abundant polysaccharide constituents in parental and NILs, accompanied by lower levels of rhamnogalacturonans and arabinogalactans in parentals. The solubilization problem of this fraction was present in the parentals and NILs analyzed. In CSF in green bean pods, solubilization problems have been reported previously[2] and suggest a low degree of esterification of the pectin extracted.

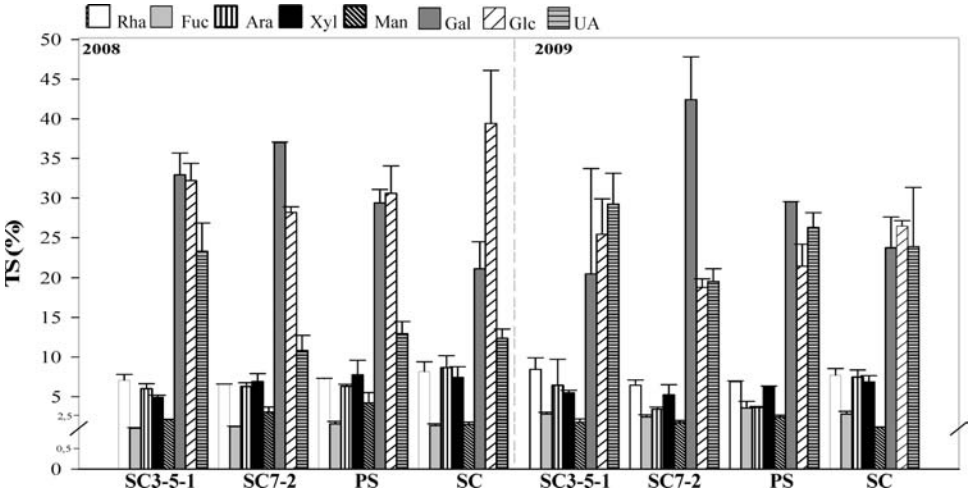

Figure 8. Composition of neutral sugars (NS) and uronic acids (UA) in α-cellulose residue (α-CEL) of the fruit flesh of different melon lines harvested in two consecutive seasons, 2008 and 2009. NS and UA are expressed in percentage on total noncellulosic sugar (TS) recovered from this fraction. Error bars with standard deviation (σ) are included in columns.

In hemicellulosic fractions, lower values of uronic acids than in pectic fractions were in agreement with results already reported in melon.[5] However, in K1SF, xylose values suggested that xylans are the main polysaccharides, although the high content of uronic acids also quantified in this fraction indicated that significant amounts of pectins were still bound to the wall (in parental and NILs). The xylans are hemicellulosic components present in small quantities in the primary and secondary cell walls of dicotyledonous plants such as melon.[10]

Differences in texture between parentals could be explained in part by the intermediate molecular weight polysaccharides of K1SF present in PS, which could contribute to the increased firmness of this parental.

Xyloglucans could be the main component of K4SF of both parentals (and NILs) as indicated the values of Glc, Xyl and Fuc (Figure 6). These results are consistent with observations made for the monosaccharide composition of the hemicellulosic fraction of other cultivars such as Galia melons with their high levels of glucose, xylose and galactose.[35]

α-Cellulose residue was the main component in parentals (and NILs), which agrees with the results reported for Galia melon.[35] Cellulose and xyloglucans presents in α-CEL contributed to texture values through their interactions.[36] Rhamnose values indicate that rhamnogalacturonans of low degree of branching were bound to cellulose microfibers, probably due to rhamnogalacturonan/galactan-type structure associations, as reported in papaya fruit.[37]

Cellulose and hemicellulose exhibit a distinctive network of microfibrils via hydrogen bonds, which enhance cell wall rigidity and resistance to tearing. Pectin and hemicellulose confer plasticity and the ability to stretch. In the middle lamella, pectin plays a primary role in intercellular adhesion.[32] Therefore, the differences observed in the percentages of pectins and hemicelluloses found in parental cell walls could have an important effect on fruit texture. There is extensive evidence to support the contribution of the matrix of noncellulosic polymers in cell wall disassembly during normal fruit ripening; however, cellulose microfibrils, in most cases, are not significant contributors to disassembly.[17]

NILs (SC3-5-1 and SC7-2). The genetic nature of the climacteric NIL SC3-5-1, that can somehow separate some ripening events such as climacteric ripening (ethylene-dependent) and part of the softening process (a partly ethylene-independent and partly ethylene-dependent process[4,13]), could explain differences in the cell wall composition (especially UA) and the reason for SC3-5-1 showing similar flesh firmness values despite different ripening index values at harvest compared to PS. The action of ethylene would determine in part the lower values of uronic acids in SC3-5-1 than in PS, probably due to the extensive depolymerization of pectic components as a result of enhanced endopolygalacturonase activity. In fact, ethylene-treated watermelon fruit with a reduced total UA content showed increased PG activity.[38]

On the other hand, the expression of polygalacturonase genes, which act in cell wall degradation and the loss of cell membrane semipermeability during ripening, has components that are both dependent and independent of ethylene action.[16] Juice pellet content, juice, density and flesh juiciness values in the climacteric line SC3-5-1 could also help explain this fact, since, according to several authors,[9,12] tissue firmness is associated with several attributes, including those mentioned.

The higher content in nonpectic sugars in imidazole-soluble fraction of SC3-5-1 than PS was possibly a result of different ripening index. Also, the NS content from side chains of pectins was higher in SC3-5-1 than in PS. The presence of NS from pectins is a common indication of ripening in all fruit species including melons.[7,9,39]

The climacteric behavior of SC3-5-1 was in agreement with previous results in other climacteric NILs versus PS.[18,23] The climacteric behavior of SC3-5-1 could be responsible for the differences in the high molecular weight polysaccharides extracted and could be related to the higher content of hemicellulosic polysaccharides present in ISF. The greater loss of high MW polymers in SC3-5-1 than in PS could affect the structure of the wall and consequently textural values during the subsequent postharvest phase, but particularly juiciness.

In K4SF, the lower molecular weight of the main peak in SC3-5-1 could be correlated with the QTLs contained in the introgression of SC present in this NIL and other similar NILs.[18,21,23]

Climacteric NIL SC3-5-1 had the highest values in galactose. The increase in Gal indicates an increased content of pectic galactan side chains.[40] Studies in muskmelon (*Cucumis melo* L.) and other fruits point to a decrease in the level of the Gal as a result of ripening.[36,39] Indeed, galactose does not decrease in ripening-impaired tomato such as Cnr tomato mutants unable to soften[41] or transgenic tomatoes with suppressed β-galactosidase/exogalactanase activity.

The existence of melon varieties with climacteric and nonclimacteric behavior make this species a suitable system for studying the genetic control of climacteric ripening. In a recent study with near-isogenic lines of melon five quantitative trait loci were associated with flesh firmness.[18] The QTL *eth3.5* involved in ethylene production and climacteric response was located in an introgression of approximately 50 cM in SC3-5b.[21] In this introgression, the *CmACS5* and *CmEIL4* genes belonging to ACC synthase (ACS) multigenic family involved in ethylene biosynthesis and signaling have been mapped.[18] In the climacteric NIL SC3-5b, a QTL (*ff3.5*) associated with lower firmness values than the control PS was identified, suggesting that these values might be due to *eth3.5*, a climacteric QTL located in the same introgression as *ff3.5*. In our investigation, lower values of the cell wall components than PS, especially uronic acids, were associated with QTL *eth3.5*. An expansin *CmEXP2* has been mapped in this interval.[18] Expansin enzymes are influenced directly by ethylene and cooperate with endopolygalacturonases to disassemble the polymer networks of cell wall including the cleavage of the α-1,4-linkages between the galacturonic acid residues of homogalacturonans,[17] thereby contributing to fruit softening, especially in the early stage of fruit softening.[42] Although the function of expansin enzymes may not cause the hydrolysis of pectin, it may enhance polymer degradation by affecting PG activity.[43] In tomato fruit, underexpression of an expansin gene resulted in a moderate increment in firmness due to a lower degree of pectin depolymerization.[44]

Flesh juiciness and firmness values of nonclimacteric NIL SC7-2 were lower and higher than those of PS, respectively. Flesh juiciness is considered a main feature in fruit texture, but melon fruit with firmer flesh is usually less juicy and few QTLs associated with juiciness have been mapped so far in melon.[22] In fact, SC7-2 showed lower juice leakage than PS during storage in fresh-cut format.[19]

Enhanced CEL and NS contents (as in SC7-2 versus PS melons) and a reduced UA content in raw and cooked squash (*Cucumis maxima*) fruit have been associated with higher firmness.[36] The opposite trend in the UA content found by the

authors[36] compared with that observed in SC7-2 suggests that reduced UA is a cultivar-dependent effect associated with flesh firmness or that it is not so essential for flesh firmness. In fact, higher values of UA may be due to the better maintenance of cell wall integrity in the covalently bound pectin polymers of SC7-2. A higher content in galactose has been related with higher flesh firmness in several fruits,[41] and a reduction in Gal levels has also been proposed as an index of progression of flesh softening in peach.[45]

In SC7-2, the higher ISF content of nonpectic sugars (as in the climacteric NIL SC3-5-1) than in PS indicates that, irrespective of the differences between NILs in firmness or climacteric behavior, pectins that were more branched and less joined to the wall structure were more solubilized in NILs than in PS. Also, hemicellulosic components were more abundant especially in less mature and firmer fruits harvested in 2008, and this can be an indication of the involvement of hemicellulose disassembly in fruit softening.

Mannose is a nonpectic sugar especially important for cross-linkages between xyloglucan polymers in hemicelluloses and cellulose microfibers, and the higher Man content in the pectic fractions (ISF and CSF) can been associated with firmer SC7-2 melons, as previously reported by Nishizawa and Ito.[8]

Hemicellulose was higher in NILs than PS. In SC7-2, K4SF was the main hemicellulosic fraction and K1SF in SC3-5-1. This fact could be consistent with the texture values of both NILs (Table 1). According to several authors,[5,40,46] K1SF is characterized by solubilizing glycans barely involved in the wall structure, and contained only small amounts of xyloglucans, whereas in K4SF glycans tightly bound to the wall structure are released by breaking hydrogen bonds and swelling without dissolution of the cellulose microfibrils. This indicates greater involvement of K4SF polysaccharides in the maintenance of the wall structure, and, therefore, a higher percentage of this fraction could be related to a harder fruit texture.

The highest values of hemicellulose and α-CEL in SC7-2 could be related to the 1.6-fold higher flesh firmness observed in this nonclimacteric NIL than in PS. The fruit flesh firmness QTL, *fh7.2,* was located in an introgression of approximately 27 cM from SC in linkage group VII, and resulted in a fruit hardness value 1.8-fold higher than the value found for PS.[18,22] Previous analyses of the change in hardness of fresh fruit[20] and in the flesh firmness of fresh-cut cubes of SC7-2 and PS during storage[19] indicated that the value at harvest is critical and that SC7-2 is also firmer than PS. The QTL of SC7-2 is located in linkage group VII.[21] The effect of this QTL on cell wall composition according to our results was a higher level of hemicellulosic and lower level of pectic components, and higher uronic acid, neutral sugar (especially galactose), mannose (only in pectic fractions), cellulose and α-cellulose residue contents. A pectin methylesterase *CmPME2* has been mapped in linkage group VII.[18] PME de-esterification of polyuronides removes the methyl groups from the C6 position of galacturonic acid residues of high molecular weight pectin during fruit ripening.[37,46] A higher yield of galactose and uronic acids may be due to increased content of pectic galactan side chains, and, consequently, the greater interaction with cellulose microfibers[40] was probably associated with decreased PME (EC 3.1.1.11). Pérez-Almeida and Carpita[33] proposed that the galactose side chains on the wall would decrease the wall's porosity, blocking the access of hydrolases such as PME to the wall components and preventing the depolymerization of structural polysaccharides. In this context, a reduced β-galactosidase level would also limit pectin methylesterase activity although this mechanism is not clear.[45]

As regards the structural integrity of the cell wall, is important to highlight the role of the matrix of hemicellulose.[32,33] The highest values of hemicellulose in SC7-2 (Tables 4 and 5) can be related to slower ripening process. A large number of studies have demonstrated that the depolymerization of hemicelluloses in ripening fruits, including melon, is closely correlated with fruit softening.[17] In melon, modifications of hemicelluloses occurred through all ripening stages, but only few changes in the molecular weight of these compounds during ripening have been described.[5] The role of cellulosic fraction is important because cellulose microfibers interact with xyloglucans through cross-bridges, which contributes to maintaining and strengthening the network structure observed microscopically.[36]

The presence of more hemicellulosic and cellulosic fractions and reduced flesh juiciness can compromise the edibility of melon fruit containing the QTL of SC7-2. The importance of the abundance of cellulosic polysaccharides and/or of the covalently bound pectin polymers in cell wall integrity together with the galactose and mannose content of different fractions agrees with previous results of experiment in long shelf life melons stored at 20 °C.[8] The enhanced cellulose fractions in SC7-2 may extend shelf life of this NIL but could compromise the fruit's nutritional value.

In summary, the biochemical effect of the QTLs of the climacteric NIL SC3-5-1 was associated with extensive pectin degradation and, consequently, polyuronide degradation, with no always significant effect on flesh firmness at harvest. In the nonclimacteric NIL SC7-2, the effect of the QTL which resulted in higher values of flesh firmness was associated with higher levels of cell wall components (especially galactose) and α-cellulose residue.

Differences between Seasons. The less advanced solubilization and depolymerization processes of the cell wall components in 2009 than in 2008 explained the differences in UA, CEL and flesh firmness values between seasons. Menezes et al.[35] reported higher values of UA in less mature fruits of Galia melon. The higher soluble solids and NS content measured in 2009 was associated with fruit attaining a higher ripening index in 2009 than in 2008 for environmental and crop management reasons. In 2009, SC fruits were harvested less mature because the plants suffered from *Fusarium* sp. in their roots. These differences provoked a different level of solubilization and depolymerization of pectic and hemicellulosic components that reflected fruit ripening.[9,39,40]

Sucrose accumulation accompanies fruit development and ripening in sweet melons[47] and was clearly dependent on environmental conditions because fruit from season 2009 accumulated more sucrose in the flesh than those from 2008 (data not shown).

This research will serve as the basis for studies on the dynamic of changes in texture attributes (particularly softening) and cell wall degradation caused by physical and enzymatic processes during melon ripening, as affected by QTLs.

■ ASSOCIATED CONTENT

Supporting Information. Supplementary Figure 1, preparation of cell wall material (CWM) from the fresh melon flesh. Supplementary Figure 2, preparation of polysaccharide fractions from the fresh melon flesh. This material is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS USED

Ara, L-arabinose; α-CEL, α-cellulose residue; CEL, cellulose; CSF, carbonate-soluble fraction; CWM, cell wall material; *ff*, flesh firmness QTL; *fh*, flesh hardness QTL; Fuc, D-fucose; Gal, D-galactose; Glc, D-glucose; ISF, imidazole-soluble fraction; K1SF, 1 M potassium-soluble fraction; K4SF, 4 M potassium-soluble fraction; Man, D-mannose; NIL, near-isogenic line; MW, molecular weight; NS, neutral sugars; PG, endopolygalacturonase; PME, pectin methylesterase; PS, "Piel de sapo"; QTL, quantitative trait loci; Rha, L-rhamnose; SC, accession PI 161375 cultivar "Shongwan Charmi"; UA, uronic acids; Xyl, D-xylose

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