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Compositional Changes in 'Bartlett' Pear (*Pyrus communis* L.) Cell Wall Polysaccharides As Affected by Sunlight Conditions

María D. Raffo,[†] Nora M. A. Ponce,[‡,⊗] Gabriel O. Sozzi,[§,⊗] Ariel R. Vicente,[⊥,⊗] and Carlos A. Stortz[*,‡,⊗]

[†]Instituto Nacional de Tecnología Agropecuaria (INTA), EEA Alto Valle de Río Negro, Ruta Nac. 22 Km. 1190, 8332 Allen, Argentina

[‡]Departamento de Química Orgánica-CIHIDECAR, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón 2, 1428 Buenos Aires, Argentina

[§]CONICET, Av. B. Rivadavia 1917, 1033 Buenos Aires, Argentina

[⊥]Centro de Investigación y Desarrollo en Criotecnología de Alimentos, Facultad de Ciencias Exactas, CONICET-UNLP, 47 y 116, and Cátedra de Agroindustrias, Facultad de Ciencias Agrarias y Forestales, Calle 60 y 119, La Plata, 1900 Argentina

[⊗]Research Member of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

ABSTRACT: Preharvest conditions can have a great impact on fruit quality attributes and postharvest responses. Firmness is an important quality attribute in pear, and excessive softening increases susceptibility to bruising and decay, thus limiting fruit postharvest life. Textural characteristics of fruits are determined at least in part by cell wall structure and disassembly. Few studies have analyzed the influence of fruit preharvest environment in softening, cell wall composition, and degradation. In the current work 'Bartlett' pears grown either facing the sun (S) or in the shade (H) were harvested and stored for 13 days at 20 °C. An evaluation of fruit soluble solids, acidity, color, starch degradation, firmness, cell wall yield, pectin and matrix glycan solubilization, depolymerization, and monosaccharide composition was carried out. Sun-exposed pears showed more advanced color development and similar levels of starch degradation, sugars, and acids than shaded fruit. Sunlight-grown pears were at harvest firmer than shade-grown pears. Both fruit groups softened during storage at 20 °C, but even after ripening, sun-exposed pears remained firmer. Sunlight exposure did not have a great impact on pectin molecular weight. Instead, at harvest a higher proportion of water-solubilized uronic acids and alkali-solubilized neutral sugars and a larger mean molecular size of tightly bound glycans was found in sun-exposed pears. During ripening cell wall catabolism took place in both sun- and shade-grown pears, but pectin solubilization was clearly delayed in sun-exposed fruit. This was associated with decreased removal of RG I-arabinan side chains rather than with reduced depolymerization.

KEYWORDS: quality, pectin, hemicellulose, softening, preharvest, pome

■ INTRODUCTION

European pears (*Pyrus communis* L.) are temperate-zone fruits cultivated throughout the world. There are over 2000 pear varieties, but only a few of them are relevant in terms of volume of production and commercialization. Given its good organoleptic properties and despite its relatively short postharvest life, rapid softening, and bruising susceptibility, 'Bartlett' is the most common cultivar in Argentina. The fruit industry faces an important problem derived from a large variability in fruit quality and responses during storage, which can be greatly affected by environmental conditions prevailing during the growing season.[1] Sunlight exposure is one of the most important environmental factors influencing fruit growth and development and shows large variations depending on the region, orchard orientation, and even within a single tree. Direct sunlight can result in temperatures 15−20 °C higher than those of shade-growing fruit and even higher than the local temperature.[2,3] Temperature and radiation interception may account for part of the variability observed in postharvest responses.[3] Temperature during fruit development also plays an important role in pear-ripening behavior.[4]

Fruit texture may be affected by direct exposure to sunlight. Higher firmness was reported in the sun-exposed side of avocado,[2] apple,[5] and kiwifruit.[6] The latter fruits could be stored

longer after preharvest sun exposure. Changes in fruit texture occur at least in part due to modifications in the chemistry of primary cell wall polysaccharides.[7] There are three major categories of wall polysaccharides, namely, cellulose, hemicelluloses, and pectins. Cellulose microfibrils are hydrogen-bonded assemblies of $(1-4)$-β-D-glucan chains. The most abundant hemicellulosic compound in dicotyledonous species is xyloglucan (XyG), which has a backbone of 4-linked β-D-glucopyranoses with regular branches at O-6 of α-D-xylopyranosyl units, which can be further decorated with galactose and fucose.[7] Pectins are a family of acidic polymers rich in 4-linked α-D-galacturonic acid moieties. The most abundant pectic polysaccharide is homogalacturonan (HG), a linear homopolymer of α-1,4-linked galacturonic acid.[7] Rhamnogalacturonan I (RG-I) is a heteropolysaccharide containing a backbone of alternating 4-linked α-D-galactopyranosyluronate residues and 2-linked α-L-rhamnopyranosyl residues, carrying variable amounts of side chains of α-L-arabinofuranose and β-D-galactopyranose.[7] The most structurally complex polyuronide, RG-II, contains a backbone of

4-linked α-D-galacturonic acid residues, and a complex branching pattern containing up to 12 different types of sugars. Despite the existence of these common building blocks, plant cell wall architecture and disassembly show differences depending on fruit species and cultivar.[7−9] Work analyzing changes in pear cell walls has been mainly devoted to describing polysaccharide degradation in fruit ripened on or off the tree,[9−13] as well as changes occurring during storage.[14,15] To the best of our knowledge, no work has been carried out relating cell wall composition modifications with preharvest environmental conditions. The aim of this research was to evaluate the effect of preharvest sunlight exposure on compositional changes, softening, and disassembly of cell wall polysaccharides from 'Bartlett' pears.

■ MATERIALS AND METHODS

Plant Material and Experimental Design. The pear trees used for the experiments were located in the Río Negro Upper Valley, Argentina (39° 01′ 00″ S, 67° 40′ 00″ W, 242 m above sea level). Fruits from the outer canopy, fully exposed to sunlight (S) or located in the inner canopy of the southern side of the tree (H), from three different trees for each condition were tagged and followed during the growing season. Fruit surface temperature was measured on the fruit half facing the outer side of the canopy with a hand-held Raytek infrared thermometer (Instarg S.A., La Plata, Argentina) under different meteorological conditions, 80−88 days after bloom. Thirty measurements were done for each condition. Quanta sensors coupled to Cavadevice data loggers (Cavadevices.com, Buenos Aires, Argentina) were used to determine photosynthetic active radiation (PAR) levels in both treatments. Measurements were taken every 10 min during the growing season.

Eighty fruits without wounds, blemishes, or other defects from sunexposed or shaded sites of the canopy were harvested (113 days after bloom) and used to evaluate firmness, soluble solids, acidity, starch, and cell wall analysis. For each condition the pears were divided in two batches (40 fruits each). One of the batches was used to perform the evaluations at harvest time, and the remaining batch was packed in cardboard boxes with trays inside, covered with polyethylene bags (30 μm thick), and stored at 20 °C for 13 days. In every case, the sunexposed side and nonexposed side of S pears were marked and worked up separately. The results shown for S pears are exclusively for the sunexposed side.

Firmness. Firmness was measured at harvest and after 13 days of storage a 20 °C for each condition on 30 randomly selected fruits. Compression tests were done in an Instron Universal Testing Machine model 4442 (Instron Corp, Canton, MA). Fruit with removed skin was placed on a stationary steel plate and punctured to a depth of 10 mm with a 7.9- mm diameter probe at a speed of 0.8 mm/s. The maximum force during the test was registered, and results were expressed in newtons (N).

Starch Degradation, Soluble Solids, Titratable Acidity, and Surface Color. The starch test was performed on 40 fruits for each condition. Slices of 1−1.5 mm thickness from the equatorial zone of each fruit were taken and dipped into a solution of iodine.[16] Starch degradation was determined by comparison with varietal tables. Surface color measurements from the equatorial region of intact fruit were taken with a Minolta chroma meter CR-300 (Minolta, Osaka, Japan) using CIE illuminant C lighting conditions. Twenty pears per condition were evaluated. For soluble solids (SS) measurements, juice samples obtained by squeezing longitudinal wedges from five pears were evaluated in a hand-held temperature-compensated refractometer (Atago Co., Tokyo, Japan). Measurements were done in triplicate. Titratable acidity (TA) was determined by titrating a 10 mL juice sample with 0.05 mol/L NaOH to an end point of pH 8.2 as indicated by phenolphthalein.

Results were expressed as millimoles of H^+ per liter of juice. Three replications per condition were evaluated.

Alcohol-Insoluble Residue (AIR). After removal of the skin and core, the pulp was sliced into pieces, frozen in liquid nitrogen, and stored at −50 °C until use. Cell wall preparation was performed as previously described in pears by Hiwasa et al.[9] to obtain the AIR. The AIR was air-dried in a vacuum desiccator overnight and then weighed. AIR content was expressed in grams per 100 g of fresh fruit. The starch content of the AIR was estimated using an enzymatic method[17] involving α-amylase, amyloglucosidase, and o-dianisidine using a kit provided by Sigma (St. Louis, MO). Results were calculated in grams of starch per 100 g of fruit.

Cell Wall Fractionation. AIR fractionation was performed as previously described[8] with minor modifications. Briefly, 1 g of AIR was stirred for 16 h at room temperature with 100 mL of 0.02% (w/v) thimerosal aqueous solution and filtered. The suspension was filtered, and the filtrate was saved and designated the water-soluble fraction (W-F). Sequential extraction of the pellet with 0.05 mol/L CDTA in 0.05 M NaOAc/HOAc buffer, pH 6, containing 0.02% (w/v) thimerosal (24 h), 0.1 mol/L Na_2CO_3 containing 0.02 mol/L $NaBH_4$ (24 h), 1 mol/L KOH containing 0.02 mol/L $NaBH_4$ (24 h), and 4 mol/L KOH containing 0.02 mol/L $NaBH_4$ (24 h) yielded the CDTA-soluble fraction (CDTA-F), Na_2CO_3-soluble fraction (Na_2CO_3-F), 1 mol/L and 4 mol/L KOH-soluble fractions (1 M KOH-F and 4 M KOH-F), respectively. The supernatants were recovered after centrifugation at 13100g for 10 min. In the case of the KOH-soluble fractions, the pH was adjusted to 5 with glacial acetic acid. All fractions were dialyzed (MW cutoff 6000−8000 Da) against tap water for 2 days and against distilled water for another day at 4 °C. The fractions were recovered by lyophilization.

Uronic Acid, Total Carbohydrate, and Neutral Sugar Measurements. Uronic acids were quantitated according to the m-hydroxybiphenyl method[18] using galacturonic acid as standard and expressed as anhydro units. Total carbohydrates were determined by the phenol−H_2SO_4 method[18] using glucose as standard. The proportion of neutral sugars was determined after subtracting the uronic acid content from that of total carbohydrates. For this purpose, the phenol−H_2SO_4 reaction was also carried out with a galacturonic acid standard, which showed an absorbance ratio of 0.28 against the same glucose weight.[8]

Size Exclusion Chromatography (SEC). To examine the size distributions of polymers in CDTA-F and Na_2CO_3-F, ca. 3 mg of lyophilized samples from each fraction was dissolved in 0.8 mL of 0.4 mg/mL imidazole to which 0.2 mL of 1 mol/L ammonium acetate (pH 5) had been added. Solutions were centrifuged and then chromatographed on a low-pressure SEC by employing a 300 mm × 9 mm i.d. Sepharose CL-2B column (Sigma Chemical Co., St. Louis, MO) eluted at room temperature with 0.2 M ammonium acetate, pH 5. Fractions were collected, and aliquots were assayed for total carbohydrates.[18] Samples from the W-F and 1 and 4 M KOH-F were dissolved in 0.1 mol/L NaOH, cleaned up by centrifugation, and chromatographed on a 300 mm × 9 mm i.d. Sepharose CL-6B column (Sigma Chemical Co.) eluted at room temperature with 0.1 mol/L NaOH. Fractions were collected, and aliquots were assayed for total carbohydrates.[18]

Neutral Sugar Composition. Each fraction (ca. 3 mg) was hydrolyzed with 1 mL of 2 mol/L TFA for 90 min at 120 °C in closed-cap vials. The TFA was eliminated by evaporation, and the resulting monosaccharides were reduced to alditols using $NaBH_4$, converted to alditol acetates as previously reported,[18] and subsequently analyzed using a Hewlett-Packard 5890 gas chromatograph (Agilent Technologies Inc., Santa Clara, CA) fitted with a capillary column 30 m × 0.25 mm i.d., 0.20 μm, SP-2330 (Supelco Inc., Bellefonte, PA) and equipped with a FID operated at 240 °C. The injector temperature was 240 °C, and the oven temperature was kept isothermally at 220 °C. Nitrogen was used as the carrier gas at a flow rate of 1 mL/min. Samples were injected with a split ratio of 80:1. *myo*-Inositol was used as the
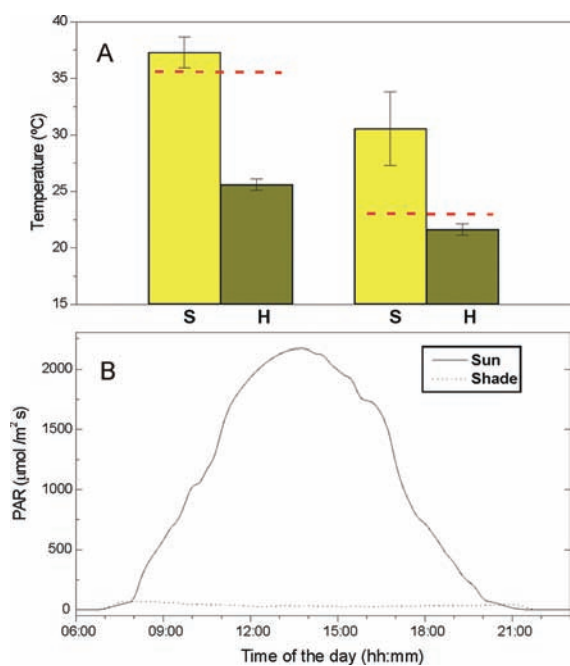
Figure 1. (A) Skin temperature of 'Bartlett' pears growing under two different sunlight conditions (sun-exposed, S; and shade, H). Measurements were carried out on two days (80−88 days after bloom with different average air temperatures (---). Values represent the mean ± SD ($n = 30$). (B) Photosynthetic active radiation (PAR) profile for 'Bartlett' pears growing under two different sunlight conditions (S and H), over a representative day during December (summer) with 25.9 °C average air temperature.

Figure 2. (A) Soluble solids content and acidity and (B) color of 'Bartlett' pears growing under two different sunlight conditions (sun-exposed and shade), at harvest. Values represent the mean ± SD ($n = 15$ for SSC; $n = 3$ for acidity; $n = 20$ for color). Means with different letters are significantly different ($P \le 0.05$).
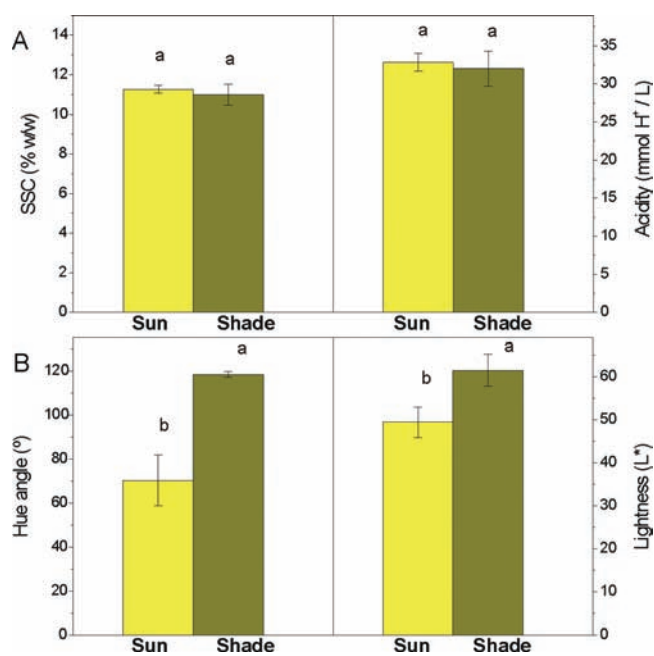
internal standard, and the different alditol acetates were identified by comparison with authentic standards. The percentage of the different monosaccharides was calculated by considering that the FID responses are proportional to the molecular weight of the alditol acetates.

Statistical Analysis. For firmness, uronic acid content, and neutral sugar content, statistical significance was determined by one-way ANOVA with the PC-SAS software package (SAS Institute Inc., Cary, NC). The model assumptions of homogeneity of variance and normality were probed by means of the Levene and Shapiro−Wilk tests, respectively. When these assumptions were not satisfied, data were transformed into ranks for further analysis. When a significant F value was found, treatment means were compared using Tukey's studentized range test ($P < 0.05$).

■ RESULTS AND DISCUSSION

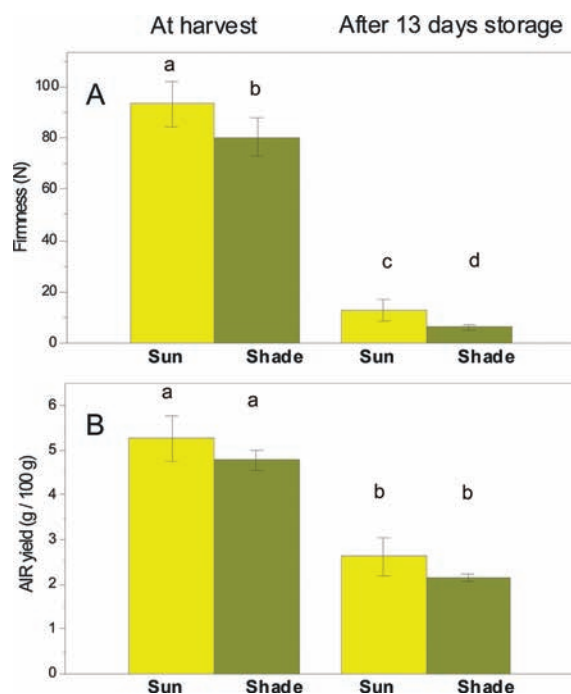**Preharvest Conditions, Fruit Color, Starch, and Firmness.** The temperature of the skin of the sun-exposed (S) and shaded (H) fruit was measured on two days with sharply different air temperatures (Figure 1A). In both cases the surface temperature of sun-exposed fruit was >30 °C. Fruit temperature on a hot day (air temperature ca. 36 °C) was close to 37 °C, whereas on a milder day (ca. 23 °C) it was 31 °C. The temperature of the shaded pears was, during these same days, lower than that found in sun-exposed fruit. Fruit surface temperature of shaded fruit was close to 25 °C and showed only slight variation between days despite the large difference (>10 °C) in air temperature. High fruit temperatures measured in the field have been strongly associated with direct exposure to sunlight. Temperatures over 40 °C have been reached in temperate climates.[3] Temperature is

a major factor in determining fruit growth rates and quality.[1] The PAR profiles were determined over the whole growing season to characterize light conditions in which pears were developing. Figure 1B shows the average PAR of a typical day during December (summer season) received by sun-exposed and shaded fruit. H pears received only 8% of the PAR relative to that of S pears. The history of light exposure of a fruit has been suggested to be a major source of variation in both at-harvest and postharvest quality.[1] Lower postharvest scald incidence was found in apples receiving longer periods below 10 °C before harvest. The effect of preharvest temperature was even more pronounced than that of the maturity stage.[19] The quantity and quality of sunlight during development can also affect fruit flavor, texture, appearance, and nutritional value.[2,20] In the present work, we evaluated the effect of sunlight exposure on surface color, acidity, soluble solids, starch content, and degradation and firmness. S pears showed lower hue and lightness (L^*) values on the exposed sides than H fruit (Figure 2B), indicating surface red blush. In other fruits such as grape, sunlight exposure has been associated with higher anthocyanin content.[21] Low light intensity reduced color development in red apple cultivars,[22] a usual condition for fruit located well within the tree canopy. In our work the nonexposed side of S pears had about the same hue angle and lightness values as H fruit (data not shown). Pear fruit bagging promoted degradation of chlorophyll and prevented anthocyanin synthesis.[23] The effect was associated with the interception of UV light rather than with differences in fruit temperature.[23] UV radiation has been shown to induce both phenylalanine ammonia-lyase (a key enzyme in the biosynthesis of phenolic compounds) and chalcone synthase catalyzing the first committed step in the flavonoid biosynthetic pathway.[24] Sunlight induction of anthocyanin synthesis seems to be regulated by a myeloblast transcription factor.[25]

Figure 3. (A) Firmness (N) and (B) alcohol-insoluble residue (AIR) yield of the exposed side of 'Bartlett' pears growing under two different sunlight conditions (sun-exposed and shade), at harvest or after 13 days of storage at 20 °C. Values represent the mean ± SD (n = 30 for firmness; n = 2 for AIR yield). Means with different letters are significantly different ($P \leq 0.05$).

Table 1. Uronic Acid Content of Cell Wall Fractions (Grams per 100 g Fraction) from 'Bartlett' Pears Growing under Two Different Sunlight Conditions (Sun-Exposed and Shade), at Harvest or after 13 Days of Storage at 20 °C[a]
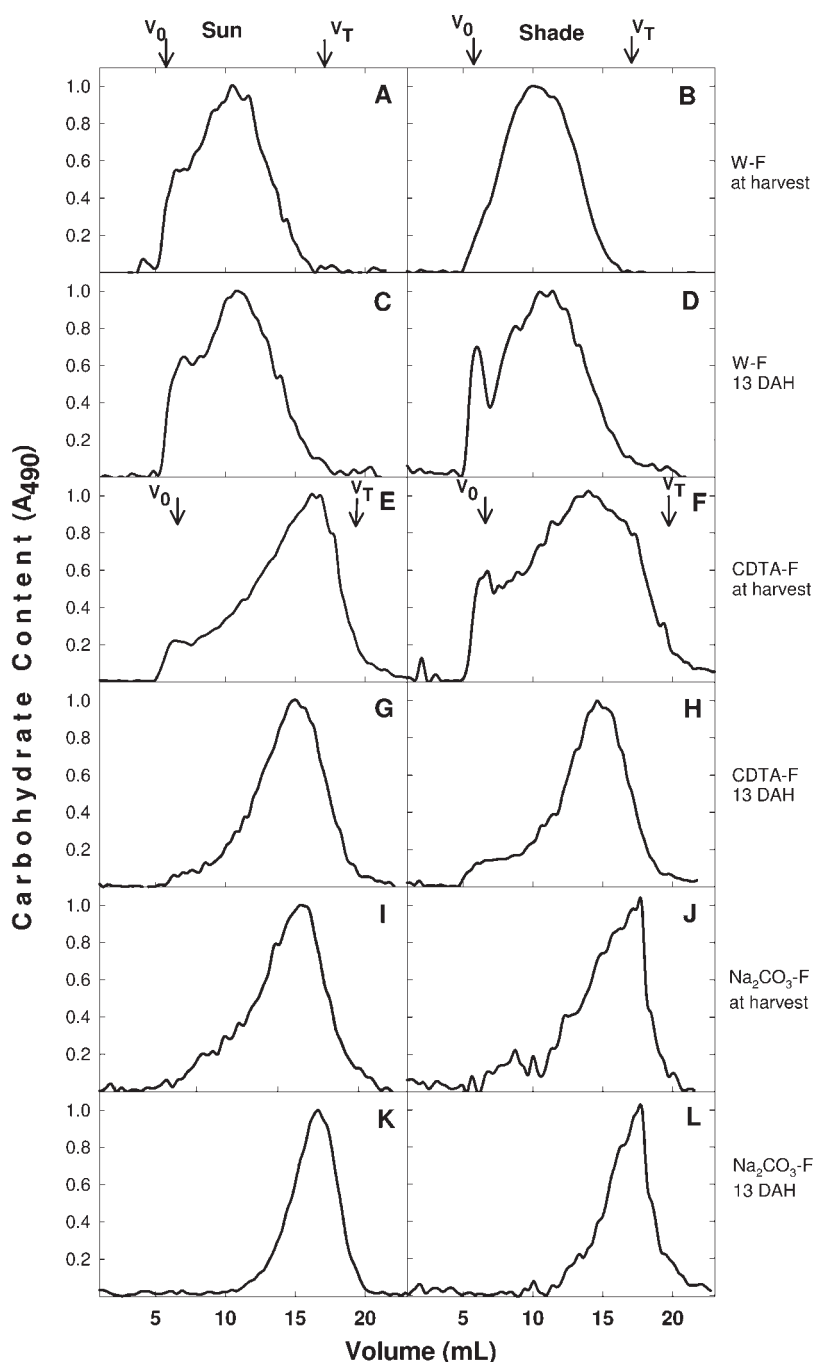
	at harvest		13 days after harvest	
cell wall fraction	sun	shade	sun	shade
W-F	26.0 ± 0.4 b	4.1 ± 0.2 c	27.0 ± 0.3 b	41.6 ± 0.3 a
CDTA-F	8.2 ± 1.6 c	35.1 ± 1.5 a	25.6 ± 0.2 b	29.9 ± 0.6 ab
Na_2CO_3-F	59.4 ± 1.4 a	59.0 ± 0.6 a	42.5 ± 0.2 b	20.2 ± 0.6 c
1 M KOH-F	4.7 ± 0.0 a	0.7 ± 0.6 b	4.9 ± 0.1 a	5.6 ± 0.5 a
4 M KOH-F	1.7 ± 0.3 a	1.1 ± 0.4 a	0.0 ± 0.6 b	2.7 ± 0.1 a

[a] Values represent the mean ± SD (n = 3). Different letters within each cell wall fraction (row) indicate significant differences at $P \leq 0.05$.

induced in the fruit by sunlight exposure are beyond being a change in the timing at which ripening occurred.

Cell Wall Yield and Fractionation. Modifications in fruit firmness by preharvest exposure to sunlight have been previously reported.[3] Greater firmness was found on exposed sides of avocado fruit.[2] Despite many papers suggesting that fruits in shaded areas are not as firm as fruit produced in outer regions of the canopy, no attempt to relate this to differing cell wall composition, cell number, or cell turgor pressure has been made. To determine if the differences in firmness were related to changes in cell wall components, the AIR was obtained. At harvest, the yield of AIR was around 5% in both sun-exposed and shade fruits (Figure 3B). The cell wall residue decreased during storage in both S and H fruits and after 13 days at 20 °C AIR was 2.5%. Results show that degradation of cell wall components proceeded in both control fruits during ripening. This reduction on a fruit weight basis largely exceeded the loss of starch, which might be partly present in the AIR. Decreases in AIR content have reported during ripening of various fruits.[8,9] As pear ripening progresses, extensive loss of arabinose from cell wall polysaccharides occurs.[10,11] The hydrolytic cleavage of arabinose would yield sugar residues that are readily soluble in ethanol during the retrieval of the AIR. Significant losses of other wall monosaccharides have been also reported.[10,11]

Solubilization and Depolymerization of Uronic Acid Containing Polymers. Given that the differences in firmness detected between S and H pears did not seem to be related to changes in the global yield of cell wall material, we further characterized the AIR to evaluate the structure and composition of specific wall polymers. Previous studies showed that the main changes in the cell wall of pears during ripening occurred in noncellulosic polysaccharides,[9] usually represented in dicots and noncomellinoid monocots by xyloglucans (XyG), homogalacturonans (HG), and RG-I and RG-II.[7] At harvest the uronic acids solubilized in water relative to CDTA was higher in S pears (Table 1). Solubilization of pectin by CDTA allegedly results from the disruption of ionic bridges between calcium and nonesterified galacturonate residues by the chelator. The larger proportion of water-soluble pectin in S fruit at harvest could have resulted from a higher degree of esterification, thus reducing the ability of polyuronides to form ionic bridges. Alternatively, it can be explained by a higher prevalence of RG-I side chains, which are believed to compromise pectin cross-linkage by steric hindrance. Further studies would be required to analyze this issue in more detail. The Na_2CO_3

In the present work no significant differences in soluble solids or acidity were detected between S and H fruit at harvest (Figure 2A). High light intensity has been reported to increase dry matter in avocado[2] and SS in various fruits,[20] but several studies have also found no significant differences in some cases.[26,27] Because similar size was found for pears grown under both conditions, the lack of differences in SS content cannot be justified just by a compensation of increased sugar translocation occurring together with higher growth rates in S fruit. Although leaves and fruits located in illuminated regions received more PAR, this should not necessarily result in higher fruit sugars. High temperatures in the fruit could have a negative impact on assimilate translocation.[28]

Starch content at harvest was around 1.0 and 0.7% in S and H fruit, respectively (data not shown). Starch degradation determined with iodine at harvest was around 20% in both S and H fruit. After 13 days at 20 °C, the starch content in both S and H fruit was lowered to 0.01%. Interestingly, the firmness at harvest was clearly higher in S pears (Figure 3A). After ripening, both groups of fruits softened, but differences were still clearly detected. The resistance to penetration of S pears was 12 N at the end of the storage period, but only 5 N in H fruit. Some of the differences observed between S and H fruits such as firmness and starch could be associated with a delay in ripening in sunlight fruit as the primary effects. However, in this case higher SS and lower acidity would have been expected, and this did not occur. In addition, some changes not necessarily associated with pear ripening such as anthocyanin biosynthesis in the peel were induced in S fruit. Interestingly, in other fruits it has been shown that sunlight exposure can accelerate rather than delay ripening.[29] Results suggest that at least some of the modifications

Figure 4. Size exclusion chromatography profiles from sun-exposed (A, C, E, G, I, K) or shade-grown 'Bartlett' pears (B, D, F, H, J, L) at harvest time (A, B, E, F, I, J) or after 13 days of storage at 20 °C (C, D, G, H, K, L): (A−D) W-F on a Sepharose CL-6B column; (E−H) CDTA-F on a Sepharose CL-2B column; (I−L) Na_2CO_3-F on a Sepharose CL-2B column. V_0, void volume; V_t, total volume.

soluble uronic acids represented the most abundant fraction of pectins, and no differences were found between S and H fruits. Polyuronides are usually extracted in water, CDTA, or Na_2CO_3, the fractions representing loosely, ionically, or tightly bound polymers,[8] respectively. However, some UA could remain associated with other wall components and be extracted only in harsher media. The higher level of UA extractable in 1 M KOH in sun-exposed pears at harvest as compared to shade fruit (Table 1) suggests stronger association between UA and cross-linking glycans. Despite the original suspected independence between pectic and hemicellulosic matrices, covalent linkages are known to occur between both types of polymers. Thompson and Fry[30] suggested that hemicelluloses in the cell walls of suspension-cultured rose cells exist in covalently linked complexes with acidic pectins. During ripening the proportion of water plus CDTA-soluble uronic acids increased (Table 1). Murayama et al.[13] also reported higher solubility of polyuronides in ripe pears, and this occurs with a reduction of Na_2CO_3-soluble uronate.[12] In the present work, the proportion of tightly bound pectin decreased in both S and H pears during ripening,

Table 2. Neutral Sugar Composition (Moles per 100 mol) of Pectic Fractions of 'Bartlett' Pears under Different Sunlight Conditions[a]

fraction	condition	Rha	Fuc	Ara	Xyl	Man	Gal	Glc
W-F	sun at harvest	11	2	79	1	1	4	2
	shade at harvest	4	3	55	9	3	14	12
	sun + 13, 20	4	2	56	10	2	13	13
	shade + 13, 20	4	1	79	7		5	4
CDTA-F	sun at harvest	4	1	42	8	3	29	13
	shade at harvest	7	1	76	2		12	3
	sun + 13, 20	4	1	67	7	2	13	6
	shade + 13, 20	4	1	81	6		5	3
Na_2CO_3-F	sun at harvest	8	tr	84	2		6	
	shade at harvest	3	tr	79	1		17	
	sun + 13, 20	5	1	72	3		18	1
	shade + 13, 20	9	2	59	10	2	16	2

[a] "Sun + 13, 20" and "shade + 13, 20" indicate fruit evaluated after 13 days of storage at 20 °C.

Table 3. Neutral Sugar Composition (Moles per 100 mol) of Glycan Matrix Fractions of 'Bartlett' Pears under Different Sunlight Conditions[a]

fraction	condition	Rha	Fuc	Ara	Xyl	Man	Gal	Glc
1 M KOH-F	sun at harvest	1	2	14	15	2	8	58
	shade at harvest	1	1	16	18	3	9	52
	sun + 13, 20	3	2	45	21	2	13	14
	shade + 13, 20	3	2	35	37	3	7	13
4 M KOH-F	sun at harvest	tr	3	4	19	3	9	62
	shade at harvest	1	3	6	23	5	11	51
	sun + 13, 20	1	3	12	35	6	13	30
	shade + 13, 20	1	5	7	39	7	11	30

[a] "Sun + 13, 20" and "shade + 13, 20" indicate fruit evaluated after 13 days of storage at 20 °C.

the reduction being much more dramatic in shade-grown fruit (Figure 4).

As depicted in Table 2, the composition of loosely (W-F), ionically (CDTA-F), and tightly bound (Na_2CO_3-F) pectins proved to be especially rich in arabinose and, to a lesser extent, other sugars, such as galactose, xylose, rhamnose, and glucose. Substantial amounts of arabinose are lost during ripening of 'Bartlett' pears.[10] The clearest difference detected during ripening was found in the proportion of arabinose, which decreased in the tightly bound pectins of H fruit. This was paralleled by an enrichment of arabinose in the water-soluble fraction. After 13 days of storage, this sugar represented 79% of NS on a molar basis of the W-F of H fruit and only 56% in S fruit. No great differences in pectin size were found between H and S fruits in the W-F at harvest (Figure 4A,B). The CDTA-soluble polyuronides showed slightly higher molecular weight in H fruit than in S fruit (Figure 4E,F),

Table 4. Neutral Sugar Content[a] of Cell Wall Fractions (Grams per 100 g Fraction) from 'Bartlett' Pears Growing under Two Different Sunlight Conditions (Sun-Exposed and Shade), at Harvest or after 13 Days of Storage at 20 °C[b]

cell wall fraction	at harvest		13 days after harvest	
	sun	shade	sun	shade
W-F	1.8 ± 0.4 c	3.9 ± 0.9 c	16.8 ± 0.2 b	24.3 ± 0.2 a
CDTA-F	3.5 ± 0.6 b	4.2 ± 0.7 b	13.6 ± 0.2 a	12.8 ± 0.2 a
Na_2CO_3-F	14.8 ± 0.7 c	47.1 ± 0.9 a	32.9 ± 0.2 b	8.7 ± 0.4 d
1 M KOH-F	34.0 ± 0.5 a	16.0 ± 0.8 c	10.9 ± 0.2 d	23.1 ± 0.4 b
4 M KOH-F	45.8 ± 1.0 a	28.7 ± 0.6 c	25.8 ± 0.3 cd	31.2 ± 0.4 b

[a] Obtained after subtracting the content of uronic acids from that of total carbohydrates (see Materials and Methods). [b] Values represent the mean ± SD (n = 3). Different letters within each cell wall fraction (row) indicate significant differences at $P \leq 0.05$.

whereas the opposite behavior was observed for the Na_2CO_3-soluble pectins (Figure 4I,J).

Pectin depolymerization during ripening in either S or H fruit was limited. The only clear difference occurred in the water-soluble fraction, in which the proportion of larger polyuronides represented by the shoulder eluting at lower volumes decreased more in H fruit. A slight decrease in larger polyuronides with ripening occurred for the CDTA-F and Na_2CO_3-F fractions (Figure 4E−L), both without great differences between sun-exposed and shaded fruits. Overall, preharvest sunlight conditions did not have great impact on pectin size at harvest or after ripening. A larger proportion of UA associated with hemicelluloses and more water-extractable polyuronides was found in sunlight-exposed pears at harvest. Upon ripening, the solubilization of pectic polymers was evidently reduced in sun-exposed 'Bartlett' pears, and this seemed to be associated with decreased removal of RG-I arabinan side chains rather than with reduced depolymerization.

Solubilization, Composition of Neutral Sugars Polysaccharides, and Cross-Linking Glycan Depolymerization. Glucose was the main monosaccharide of the 1 M KOH-F and 4 M KOH-F at harvest (Table 3). Xylose, arabinose, and galactose were the main accompanying sugars. The abundance of arabinose and xylose and the presence of less glucose in the 1 KOH-F after ripening suggest the presence of a significant proportion of arabinoxylans as previously reported.[15] The extractability of neutral sugars from S and H pears with different solvents is shown in Table 4. The largest difference in the extraction of NS at harvest was found in the fractions obtained at high pH. S fruit presented at harvest a larger proportion of NS extractable with 1 and 4 M KOH than H fruit, which showed a higher prevalence of Na_2CO_3-soluble NS. The small differences in starch content previously mentioned as well as the similar proportion of monosaccharides in the KOH fractions of S and H fruits at harvest show that the differences in extractability were related to wall polymers. From these results, it can also be inferred that no preferential enrichment in any specific NS-rich polysaccharide took place in S fruit (Table 3). For instance, whereas the presence of arabinan-rich RG-I could account for the increased proportion of KOH-soluble UA and NS in S fruit at harvest, this should have necessarily resulted also in an enrichment of galactose and rhamnose, which was not observed (Table 3). Taken together,

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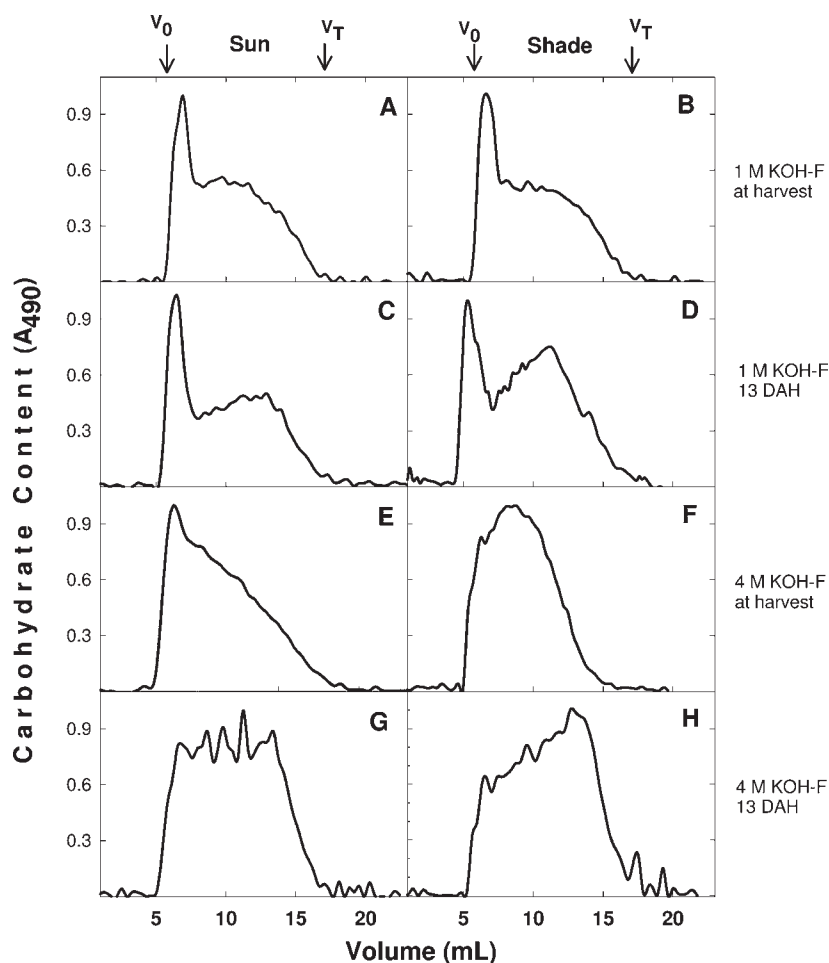
Figure 5. Size exclusion chromatography profiles from sun-exposed (A, C, E, G) or shade-grown (B, D, F, H) 'Bartlett' pears at harvest time (A, B, E, F) or after 13 days of storage at 20 °C (C, D, G, H): (A−D) 1 M KOH-F and (E−H) 4 M KOH-F on a Sepharose CL-6B column. V_0, void volume; V_t, total volume.

these results suggest that the harsher conditions required for solubilization of cell wall NS in S pears at harvest might have resulted from increased association of homogalacturonans, and not of RG-I with hemicelluloses. Upon ripening, the solubility of NS changed in both S and H fruits. The Na_2CO_3-F of shaded pears decreased 4-fold. In S pears a clear reduction of KOH-soluble NS was observed, but a large proportion (35%) remained in the Na_2CO_3-F.

SEC revealed that matrix glycans extracted in the 1 M KOH-F (Figure 5A−D) were different with regard to their MW profile from those extracted in the 4 M KOH-F (Figure 5E−H). The 1 M KOH-F at harvest shows a very high MW peak, together with some midsized matrix glycans, not highly dependent on the sunlight conditions or the ripening stage. Interestingly, at harvest the 4 M KOH fraction (tightly bound glycans) of S fruit showed a higher mean apparent MW than H fruit. Depolymerization progressed during storage at 20 °C in both groups of fruit but after ripening; the S fruit maintained a lower proportion of smaller cross-linking glycans than H fruit. Although no major changes in cellulose have been reported to occur in pear during ripening, we do not know if preharvest sunlight conditions could have also affected this polymer. It would be of interest to evaluate whether or not orchard environment also affects cellulose content, degree of polymerization, and/or degree of crystallinity. Despite that, results

from this work show that preharvest sunlight conditions influence 'Bartlett' pear texture and provide some insight regarding the way environmental conditions can modulate cell wall plasticity. Sun-exposed pears are firmer than shaded fruit at harvest, differences that remain after ripening and that are not a consequence of a delay of overall ripening. Sun-exposed fruit showed higher proportions of water- and alkali-soluble UA and neutral sugars (NS) at harvest and larger mean molecular size of 4 M KOH soluble polymers. During ripening, cell wall degradation proceeded in both fruit groups, but pectin solubilization was clearly delayed in sunlight-grown 'Bartlett' pears. This was associated with decreased removal of RG-I arabinan side chains rather than with reduced depolymerization.

■ AUTHOR INFORMATION

Corresponding Author

*Phone/fax: (+5411)-4576-3346. E-mail: stortz@qo.fcen.uba.ar.

■ ABBREVIATIONS USED

AIR, alcohol-insoluble residue; CDTA, *trans*-1,2-diaminocyclohexane-*N*,*N*,*N'*,*N'*-tetraacetic acid; CDTA-F, CDTA-soluble fraction; DAH, days after harvest; H, shade-grown fruit; NS, neutral sugars; 1 M KOH-F, 1 M KOH-soluble fraction; 4 M KOH-F, 4 M KOH-soluble fraction; Na$_2$CO$_3$-F, Na$_2$CO$_3$-soluble fraction; PAR, photosynthetic active radiation; RG-I, rhamnogalacturonan-I; S, sun-grown fruit; SEC, size exclusion chromatography; SS, soluble solids; UA, uronic acid; W-F, water-soluble fraction.

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