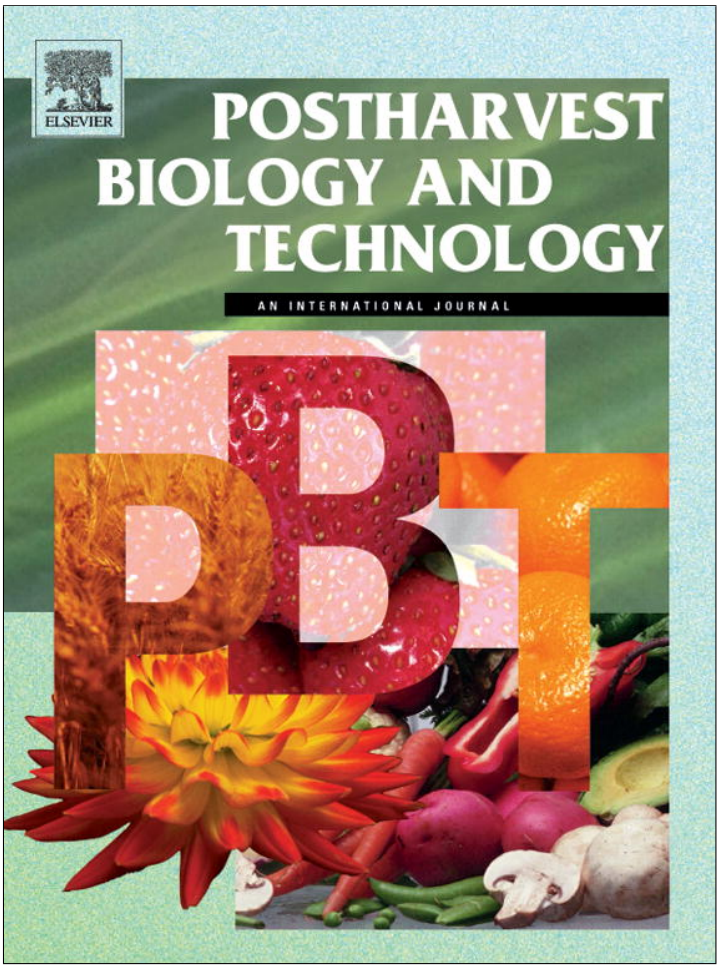

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Developmental changes in cell wall polysaccharides from sweet cherry (*Prunus avium* L.) cultivars with contrasting firmness

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ABSTRACT

Firmness is a major quality attribute of fresh cherries, and is also a main factor affecting susceptibility to bruising and postharvest rots. In order to identify the factors determining the textural differences between genotypes, we evaluated the solubilization, depolymerization and monosaccharide composition of pectin and hemicelluloses from two cultivars with contrasting firmness ('Sweetheart', firm and 'Newstar', soft) at four different developmental stages. Firm 'Sweetheart' cherries had higher contents of cell wall material than soft 'Newstar' fruit. Moderate depolymerization of hemicellulose and tightly bound pectins was detected irrespective of cultivar firmness. The general pattern and extent of uronic acid solubilization was quite similar in both cultivars. Rhamnogalacturonan I (RG-I) seemed to be preferentially solubilized in firm 'Sweetheart' fruit as opposed to tightly bound homogalacturonans (HG) in soft cherries. Pectic polymers with higher neutral sugar to uronic acids ratio were found from early development in soft 'Newstar' fruit. Overall, soft 'Newstar' fruit had reduced wall content and higher branching of tightly bound pectins than firm 'Sweetheart' fruit. These factors may be associated with the varietal differences in cherry firmness.

1. Introduction

Cherry fruit firmness is a main quality attribute, and also is an important determinant of tolerance to handling and physical damage and decay (Looney et al., 1996; Mattheis and Fellman, 2004). Firmness loss during fruit development is usually associated with cell wall polysaccharide turnover (Brummell and Harpster, 2001). Although the role of pectin depolymerization in cherries is not fully understood, Batisse et al. (1996a) showed that soft 'Bigarreau Burlat' cherries had lower polymerization degrees than firm fruit. However, in other cases, softening occurred without extensive endo-polygalacturonase action and pectin dismantling (Batisse et al., 1994). Polyuronides became more soluble (Fils-Lycaon and Buret, 1990; Batisse et al., 1996b), rhamnogalacturonan I (RG-I) side chains were removed (Kondo and Danjo, 2001) and some cell wall-degrading enzymes such as pectin methyl esterase, polygalacturonase, and β-galactosidase increased as the cherry ripened (Fils-Lycaon and Buret, 1990; Barrett and Gonzalez, 1994; Andrews and Shulin, 1995). However, the specific contribution of each of these changes to the softening process is still far from being understood.

Although texture has been suggested as one important goal for cherry improvement (Srinivasan et al., 2005), since this early work, few studies have attempted to characterize further the biochemical changes associated with cherry softening. The relationship between firmness loss and hemicellulose characteristics has received little attention in cherry (Choi et al., 2002a). Few general structural changes have been suggested to contribute to the wide range of firmness existing in different cherry cultivars, although Choi et al. (2002a) reported that soft varieties presented lower total cell wall contents. Part of the genotypic variations in fruit firmness have been linked with differences in the patterns of cell wall disassembly (Batisse et al., 1994). Soft cherry cultivars have more water soluble pectin upon ripening than firm cultivars (Choi et al., 2002a), but in some cases the differences in texture among cultivars are already recorded at initial stages of development and persist throughout ripening (Rosli et al., 2004), suggesting that early structural differences in wall architecture may be relevant. The

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general structural features of the polysaccharides that make up the plant cell wall are highly conserved among species (Carpita and McCann, 2000; Brummell, 2006). However, their degree of substitution, branching and integration in the cell wall may differ and have great impact in tissue properties (Harholt et al., 2010; Zhang et al., 2011). Batisse et al. (1996a) found that firm immature cherries had higher neutral sugar/uronic acid ratios and suggested that this results from polyuronides with higher branching. However, it is not known whether or not these features may contribute to the differences in firmness among cultivars.

In order to further characterize the cell wall changes occurring on cherry ontogeny and to identify putative factors associated with firmness variation in cherry fruit genotypes, we performed a detailed evaluation of the changes in cell wall composition of two sweet cherry cultivars: 'Newstar' and 'Sweetheart', with soft and firm flesh, respectively at four different developmental stages.

2. Materials and methods

2.1. Plant material

Cherry fruit (cvs. Sweetheart and Newstar) were randomly picked from trees located in the Río Negro Upper Valley, Argentina (39°01′32″S, 67°44′22″W, 240 m above sea level). Forty-four fruit were collected at four different developmental stages: I (cell division/first growth phase), II (endocarp lignification), III (cell expansion/second growth phase), and IV (commercial maturity). Out of the 44 fruit initially collected per stage, 24 were used for firmness, divided into sublots and used to determine SSC in triplicate. The extra 20 fruit were used for cell wall analysis. Immediately after removing the endocarp and peduncle, fruit was frozen in liquid nitrogen, and stored at −18 °C until use. For the determination of firmness, size and soluble solids content, fruit from both cultivars were harvested weekly during development.

2.2. Fruit weight, firmness, and soluble solids

Twenty-four fruit were evaluated weekly during development for fresh weight, equatorial diameter, firmness and subsequently for soluble solids. Fruit weight was determined with an electronic scale (Ohaus TA3001 with a readability of 0.1 g). Firmness was determined by measuring the force required to compress the fruit 2 mm in an Instron Universal Testing Machine Model 342 (Instron Corp., Canton, MA, USA) equipped with a 3-mm diameter convex probe, at a speed of 20 mm min^{-1}. Each fruit was measured twice on opposite sides of the equatorial plane and the registered forces were averaged and considered a replicate. For soluble solids content (SSC), juice samples obtained by squeezing eight cherries were evaluated with a hand-held temperature-compensated refractometer (Atago Co., Tokyo, Japan). Three replicates were evaluated for each variety and developmental stage.

2.3. Cell wall preparation and fractionation

Cell wall preparation was performed as previously described (Ponce et al., 2010): 20–80 g of fruit pulp were put into of ice-cold 80% ethanol (4 mL/g fruit) and homogenized in a Waring blender and in an Omni Mixer homogenizer (Omni International, Kennesaw, GA, USA). The homogenate was boiled for 30 min, then cooled, and filtered through glass filter paper (Whatman GF/C). The retentate was thoroughly washed with 95% ethanol. The solids were then resuspended in chloroform:methanol (1:1, 3 mL/g fruit), stirred for 15 min and filtered. The retentate was washed with the same solvent mixture (2 mL/g fruit). The insoluble material was washed with acetone, yielding the crude cell wall extract (alcohol insoluble residue, AIR). The AIR was air-dried in a hood and in a vacuum desiccator overnight and then weighed. Cell wall fractionation was performed as previously described (Raffo et al., 2011). Briefly, 1 g of AIR were stirred for 24 h at room temperature with 100 mL of 0.02% (w/v) thimerosal aqueous solution, and filtered. The filtrate was saved, and defined as water-soluble fraction (WSF). Sequential extraction of the pellet with 0.05 M CDTA (trans-1,2-diaminocyclohexanetetraacetic acid monohydrate) in 0.05 M NaAcO/HOAc buffer, pH 6.0, containing 0.02% (w/v) thimerosal (24 h), 0.1 M Na_2CO_3 in 0.1 mM $NaBH_4$ (24 h), 4% KOH in 0.1% (w/v) $NaBH_4$ (24 h) and 24% KOH in 0.1% (w/v) $NaBH_4$ (24 h), yielded the CDTA-soluble fraction (CSF), Na_2CO_3-soluble fraction (NSF) and 4% and 24% KOH-soluble fractions (4KSF and 24KSF), respectively. The supernatants were recovered after centrifugation at 13,100 × *g*. In the case of the KOH-soluble fractions, pH was adjusted to 5.0 with glacial acetic acid. All fractions were dialyzed (M_w cut-off 6000–8000 Da) exhaustively against tap water for 2 days, and against distilled water for another day at 4 °C. Samples were freeze dried and stored until used for size exclusion chromatography and to assay neutral sugars and uronic acids and for neutral sugar composition analysis.

2.4. Uronic acids, total carbohydrate and total neutral sugars

Uronic acids (UA) were quantified according to the *m*-hydroxybiphenyl method (Filisetti-Cozzi and Carpita, 1991) using galacturonic acid (GalA) as the standard, and expressed as anhydro units. Total carbohydrates were determined by the phenol–H_2SO_4 method (Dubois et al., 1956) using glucose (Glc) as the standard. The proportion of neutral sugars (NS) 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 GalA standard, which showed an absorbance ratio of 0.28 against the same Glc weight.

2.5. Size-exclusion chromatography (SEC)

To examine the polymer-size distributions, samples from the CSF and NSF were dissolved in 0.8 mL of 0.4 mg/mL imidazole to which 0.2 mL of 1 M NH_4AcO (pH 5) were added. Solutions were cleaned up by centrifugation, and chromatographed by low-pressure SEC on a 300 mm × 9 mm i.d. Sepharose CL-2B column (Sigma Chemical Co., St. Louis, MO, USA) eluted at room temperature with 0.2 M NH_4AcO, pH 5.0 (Ponce et al., 2010). Samples from the WSF, 4KSF and 24KSF were dissolved in 0.1 M NaOH, cleaned up by centrifugation, and chromatographed by SEC, by means of a 300 mm × 9 mm i.d. Sepharose CL-6B column (Sigma Chemical Co., St. Louis, MO, USA) eluted at room temperature with 0.1 M NaOH. Fractions were collected and aliquots were assayed for total carbohydrates.

2.6. Monosaccharide composition

Aliquots from the different cultivars, developmental stages and wall fractions (containing *ca.* 3 mg of carbohydrates) were hydrolyzed with 1 mL of 2 M trifluoroacetic acid (TFA) for 90 min at 120 °C in closed-cap vials. The TFA was eliminated by evaporation, and the resulting monosaccharides were redissolved in 1 M NH_4OH (0.5 mL) and treated overnight with $NaBH_4$ (5 mg) to generate the alditols. After addition of Amberlite IR-120 resin (H^+) and treatment with MeOH (5× 0.5 mL) with sequential evaporation in order to eliminate the boric acid, the acetylation was performed by treating the samples with Ac_2O/pyridine (1:1, 1 mL) for 45 min at 100 °C. The resulting mixture was partitioned between $CHCl_3$ and H_2O, the organic solution washed out with saturated $NaHCO_3$ solution and finally with water. The alditol acetates (dissolved in $CHCl_3$) were analyzed using a Hewlett Packard 5890 gas chromatograph

(Agilent Technologies, Inc., Santa Clara, CA, USA) fitted with a capillary column 30 m × 0.25 mm i.d. 0.20 μm, SP-2330 (Supelco Inc., Bellefonte, PA, USA) and equipped with a flame ionization detector (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^{-1}. Samples were injected with a split ratio of *ca.* 80:1. *Myo*-inositol was used as the internal standard, and the different alditol acetates were identified by comparison with authentical 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.

2.7. Statistical analysis

Results were analyzed by ANOVA with the PC-SAS software package (SAS Institute Inc., Cary, NC, USA). The model assumptions of homogeneity of variance and normality were probed by means of Levene's and Shapiro–Wilk's tests, respectively. Mean comparisons were performed by a LSD test ($P < 0.05$).

3. Results and discussion

3.1. Fruit weight, firmness and soluble solids of the fruit during ontogeny

The initial growth phase (stage I) occurred 31 days after anthesis (DAA) in 'Sweetheart' and 28 DAA in 'Newstar' fruit. During the first month of development the growth rate was similar for both cultivars, reaching a fresh weight of about 1.7 g. After that, 'Newstar' showed an accelerated growth rate (Fig. 1A and B). Stone fruit usually show a double sigmoid shape: in stage I, active cell division and increased fruit weight occur and during stage II, a lag associated with endocarp lignification and development of the embryo occurs, and the growth rate is reduced. This stage occurred 54 DAA in 'Sweetheart', and though present (36 DAA) was not clearly detected without stone inspection in 'Newstar'. It has already been found (Flore and Layne, 1999; Choi et al., 2002b) that this stage is not evident in early harvested cultivars. The secondary growth phase (stage III) was reached 62 DAA in 'Sweetheart' and 44 DAA in 'Newstar'. Finally, commercial maturity, took 59 days in 'Newstar' and 75 days in 'Sweetheart' fruit (Fig. 1E).

The average weight of 'Sweetheart' fruit at full maturity was higher than that of 'Newstar' (8.8 g and 7.2 g, respectively, Fig. 1A). The equatorial diameter at harvest was close to 26 mm with slightly higher values in 'Sweetheart' (Fig. 1B). Cherries, together with berry fruit, are quite peculiar, since in contrast to many other species in which growth and ripening can be clearly distinguished, they show an overlap between these two processes. The changes in soluble solids (SSC) during development are depicted in Fig. 1C. In 'Sweetheart', SSC increased between stages I and II, but showed a slight reduction at stage III. Afterwards, soluble solids accumulated rapidly reaching levels of 18% at harvest. In 'Newstar', SSC increased significantly between days 44 and 51, reaching at harvest 16% (Fig. 1C).

Excessive softening is one of the main changes associated with cherry fruit deterioration (Manganaris et al., 2007), and texture has been highlighted as one of the main targets for the selection of genetic material (Srinivasan et al., 2005). Firmness was recorded from *ca.* 45 DAA to harvest. The maximal compression force required to cause tissue failure was significantly higher in 'Sweetheart' than in 'Newstar' throughout development (Fig. 1D). 'Sweetheart' was consequently defined as "firm" as opposed to "soft" 'Newstar' cherries. It is important to note that no worldwide accepted ranges have been defined to classify cherry genotypes,

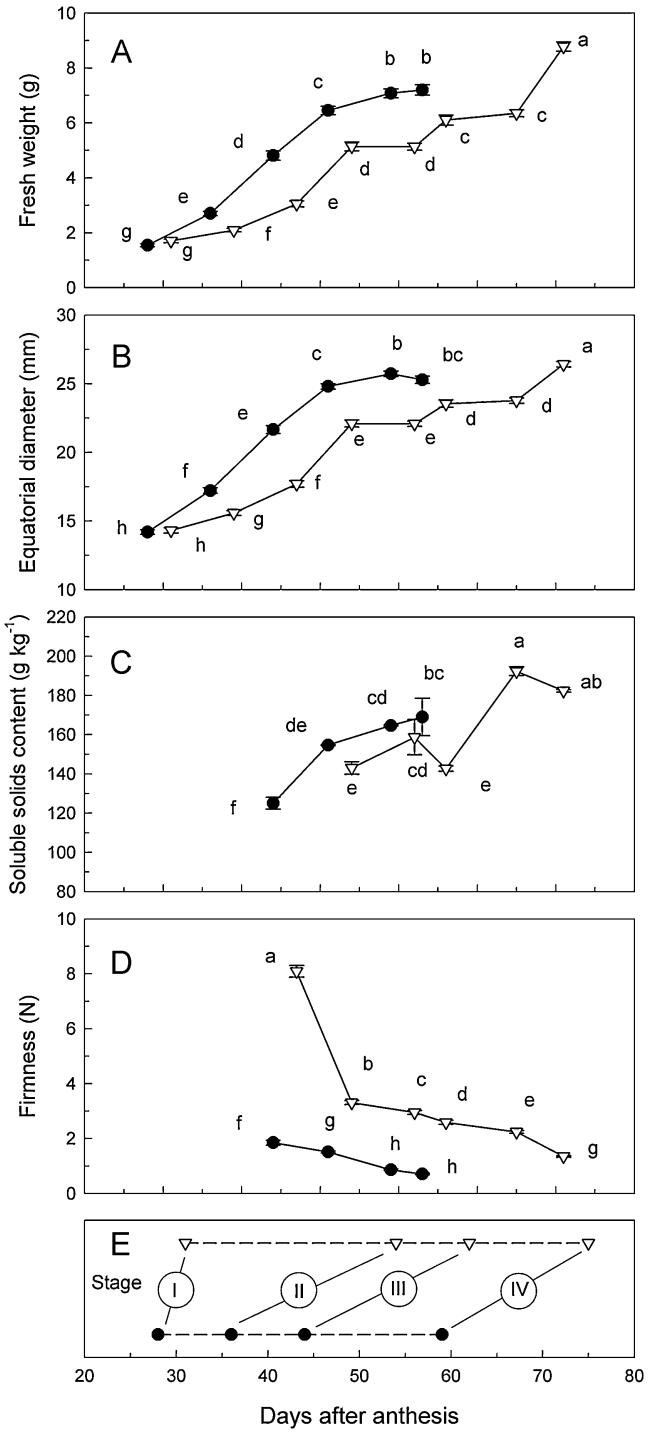

Fig. 1. Fresh weight (A, $n = 24$), equatorial diameter (B, $n = 24$), soluble solid content (C, $n = 3$) and firmness (D, $n = 24$) of 'Sweetheart' (hollow triangle) and 'Newstar' (solid circle) cherry fruit during development. The standard deviation is shown. Different letters indicate significant differences based on an LSD test at a level of significance of $P < 0.05$. Panel E shows the timings for the four developmental stages used for cell wall analysis, using the same symbols.

and the terms soft or firm only refer to the comparative firmnesses of the two cultivars analyzed. Previous studies have associated firmer fruit with late-ripening cherry phenotypes (Christensen, 1995). However, for a given growth pattern, large variations in softening are also found among cultivars (Choi et al., 2002a,b). Though many factors (skin thickness, tissue architecture, turgor pressure) are known to affect texture, the structure, composition

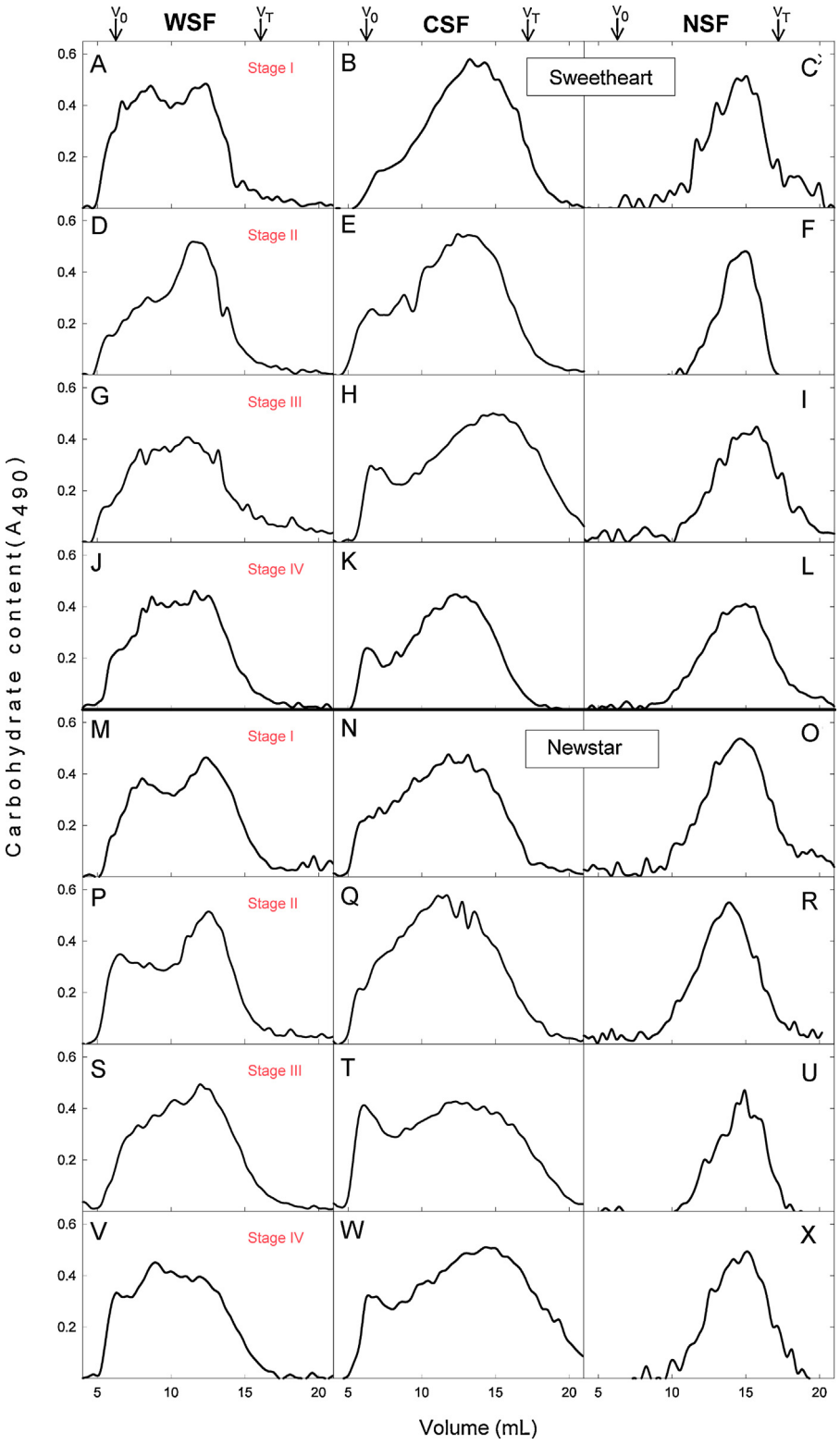

Fig. 2. Size exclusion chromatography profiles of the water (WSF) (first column), CDTA (CSF) (second column), and Na_2CO_3 (NSF) (third column) fractions of 'Sweetheart' (A–L) and 'Newstar' (M–X) cherry fruit during development (I: first growth; II: endocarp lignification; III: second growth; IV: commercial maturity) fractionated on Sepharose CL-6B (WSF) and CL-2B (CSF and NSF) columns. V_0, void volume; V_T, total volume.

and disassembly of the cell wall has been suggested as one of the main factors (Brummell, 2006). Previous studies characterized the changes in pectin metabolism at early and late ripening stages of cherry (Fils-Lycaon and Buret, 1990; Batisse et al., 1994, 1996a,b). No marked polyuronide depolymerization was found and substantial solubilization was reported. In addition, loss of pectin side chains was associated with low firmness (Batisse et al., 1996a). The dismantling of hemicelluloses and cellulose has received much less attention (Choi et al., 2002a) and thus, the biochemical determinants accounting for these differences remain obscure.

3.2. Cell wall yield

In order to identify some features associated with the differences in firmness among the cultivars, we performed a chemical characterization of the cell wall structure and composition of 'Sweetheart' and 'Newstar' cherries throughout the four developmental stages (Fig. 1E). In agreement with previous reports (Choi et al., 2002a; Facteau, 1982), firmer 'Sweetheart' fruit showed higher alcohol insoluble residue (AIR) contents than soft 'Newstar' cherries (Table 1). A reduction in the AIR on a fresh weight (FW) basis of around 65% was found in both cultivars throughout development (Table 1). When expressed on fruit unit basis, the AIR content actually increased throughout development, suggesting that cell wall deposition continues even until the final developmental stages, agreeing with previous reports (Mitcham et al., 1989; Batisse et al., 1996a).

Table 1
Alcohol-insoluble residue (AIR) yields of 'Sweetheart' and 'Newstar' cherry fruit during development (I: first growth; II: endocarp lignification; III: second growth; IV: commercial maturity).

Stage	'Sweetheart'		'Newstar'	
	g/100 g FW	mg/fruit	g/100 g FW	mg/fruit
I	4.78	81.2	4.59	70.7
II	3.54	73.9	3.28	88.6
III	1.85	94.7	1.75	84.1
IV	1.77	112.2	1.53	108.6

3.3. Neutral sugar composition of the cell wall polysaccharides

Differences in firmness between several fruit cultivars have been correlated with their pattern of cell wall disassembly (Stewart et al., 2001; Rosli et al., 2004). Consequently, we fractionated the

Table 2
Neutral sugar composition (mol/100 mol) of pectin and matrix-glycans fractions of the 'Sweetheart' and 'Newstar' cherry during development (I: first growth; II: endocarp lignification; III: second growth; IV: commercial maturity).

Fraction	Stage	Neutral sugar composition (mol/100 mol)						
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc
'Sweetheart'								
WSF	I	6	1	36	10	11	27	10
	II	5	1	31	8	14	29	14
	III	7	1	39	6	9	30	10
	IV	8	1	36	5	7	32	10
CSF	I	11	tr	70	3	tr[a]	16	tr
	II	11	1	62	3	4	15	3
	III	12	1	72	2	2	10	2
	IV	13	1	65	2	2	13	3
NSF	I	7	tr	85	1	–	7	–
	II	8	tr	78	1	–	11	1
	III	10	1	79	1	tr	7	1
	IV	12	1	78	1	–	7	1
4KSF	I	5	2	53	16	3	11	11
	II	5	1	63	10	3	11	7
	III	4	1	60	11	4	10	10
	IV	6	1	64	9	3	9	9
24KSF	I	3	2	25	20	10	13	27
	II	3	3	24	21	10	15	25
	III	3	2	23	18	11	16	27
	IV	3	2	29	17	10	14	26
'Newstar'								
WSF	I	6	–	41	10	14	20	10
	II	6	1	33	9	13	28	11
	III	6	1	44	7	8	25	9
	IV	6	1	46	6	6	28	7
CSF	I	8	1	67	8	3	11	3
	II	9	1	74	3	3	9	2
	III	8	1	78	1	1	10	tr
	IV	9	1	79	1	1	8	1
NSF	I	8	1	74	4	–	13	tr
	II	8	1	87	1	–	3	–
	III	11	1	80	1	tr	6	1
	IV	11	1	80	1	tr	6	1
4KSF	I	5	1	52	16	3	13	9
	II	5	2	45	20	5	13	12
	III	5	1	60	11	4	10	10
	IV	5	1	51	14	5	11	12
24KSF	I	3	3	22	21	10	15	27
	II	3	3	21	21	12	14	27
	III	2	2	15	21	14	17	29
	IV	2	3	15	22	12	17	29

[a] Traces (<0.5 mol/100 mol).

AIR from the different developmental stages, by successive extractions with H_2O, CDTA, Na_2CO_3, 4% KOH and 24% KOH, to yield the loosely bound pectins (soluble in water, WSF), ionically bound pectins (soluble in a Ca^{2+} chelating agent, CSF), covalently bound pectins (soluble in sodium carbonate, NSF), fractions rich in weakly (4KSF) and strongly attached (24KSF) cross-linking glycans, respectively. In the pectic fractions WSF, CSF and NSF, arabinose was the most abundant neutral sugar, accounting in both cultivars for 30–40, 60–70 and 70–80% of the total on a molar basis respectively, followed by galactose and rhamnose (Table 2). In 'Newstar', all pectic fractions showed a trend toward increasing slightly the proportion of Ara during development. In contrast, in 'Sweetheart' the proportion of Ara decreased slightly as fruit developed in the CSF and NSF. The relative contents of Ara and Gal in the different fractions suggest that a galactan-rich RG-I was predominantly present in the WSF, while the arabinan-rich RG-I was prevalent in the NSF. Galactose showed higher solubilization in soft 'Newstar' fruit. The 4KSF presented high proportions of Ara in both cultivars, suggesting the association of RG-I with hemicelluloses. As expected Glc and Xyl were among the most abundant monosaccharides in the 24KSF, although Ara still represented a significant proportion.

3.4. Cell wall depolymerization

The WSF did not show marked variations in polymer size in any of the cultivars (Fig. 2, first column). In the CDTA-soluble fraction (CSF) there was an increase in the polyuronide size from stage II (Fig. 2E and Q). An interesting point is to determine whether or not the increase of large pectin molecules resulted from separation of polyuronides associated to cellulose or from *de novo* biosynthesis of cross-linking glycans. Some depolymerization was observed for NSF (both cultivars), but the shift was relatively low compared to that observed in other fruits. Overall, irrespective of the firmness of the cultivars, cell wall depolymerization for most fractions only showed moderate changes throughout development, thus agreeing with the results reported by Batisse et al. (1994). Previous studies focused on pectin turnover and changes in hemicelluloses have received little attention (Choi et al., 2002a). The 4KSF presented a bimodal distribution with high proportion of both large and small molecules, as has already been observed (Ponce et al., 2010; Raffo et al., 2011; Alayón-Luaces et al., 2012). The peak with higher molecular weight showed some depolymerization without major changes between cultivars (Fig. 3, first column). The xyloglucan-rich fraction (24KSF) consisted of a single peak at all stages, for both cultivars, and showed a downshift mainly between stages II and III (Fig. 3D, F, L and N).

3.5. Cell wall solubilization

The tightly bound pectins (NSF) represented the most abundant fraction (Fig. 4), for both cultivars at stages I, II and III. This fraction also shows the larger modifications, decreasing drastically as ripening progressed. At commercial maturity (stage IV) the proportion of NSF was similar to that of the CSF fraction (Fig. 4). The uronic acids in NSF are lost without a concomitant accumulation in WSF or CSF (Fig. 4). This suggests that the reduction of tightly bound pectins may have resulted from the action of exoenzymes which would result in small GalA oligomers, lost during wall preparation. Clear distinctions in UA solubilization have been reported between immature and ripe stages (Batisse et al., 1996a) as well as among cultivars with contrasting firmness (Choi et al., 2002a). In the present study the general pattern of UA dismantling was similar in both 'Sweetheart' and 'Newstar' cultivars despite of their clear textural differences.

Extensive reduction of the NS content in NSF (main fraction) was evident in 'Sweetheart', but did not occur in 'Newstar'. This

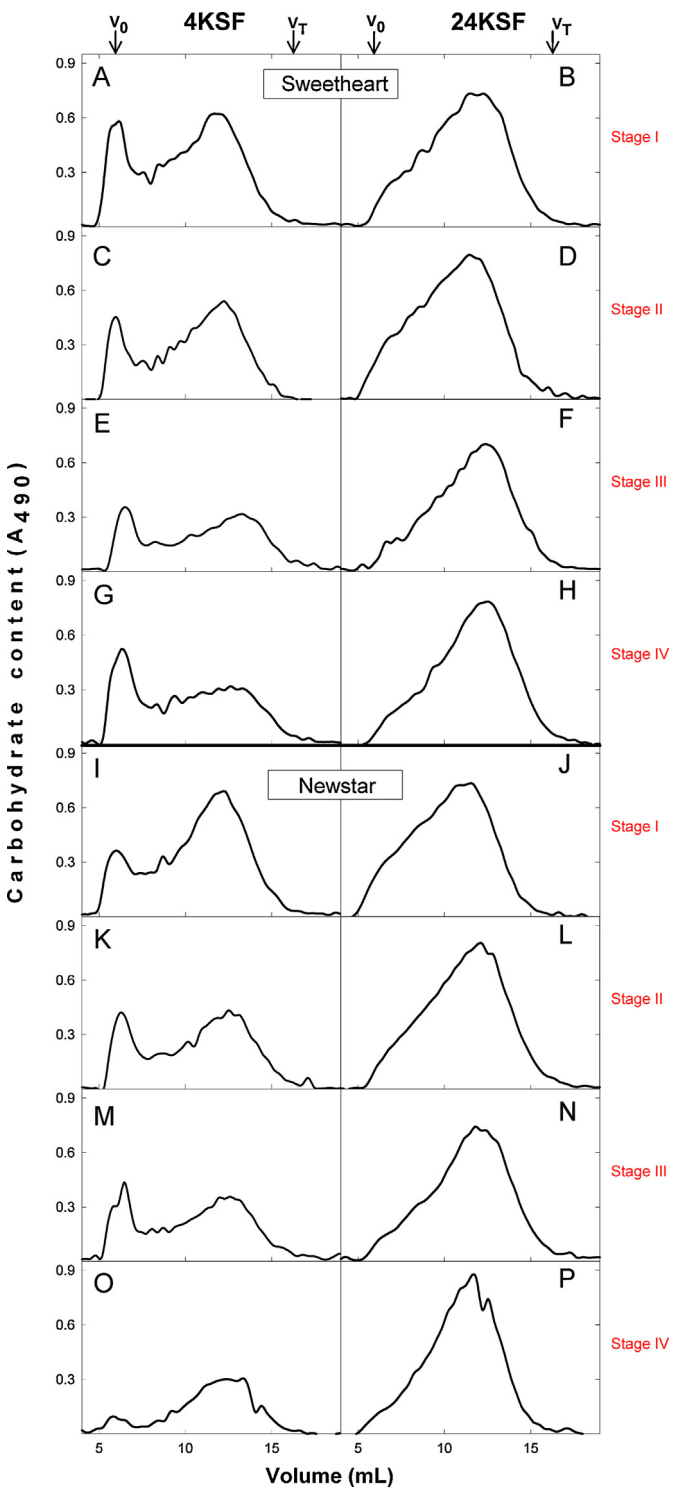

Fig. 3. Size exclusion chromatography profiles of 4% KOH (4KSF) (first column), and 24% KOH (24KSF) (second column) fractions of 'Sweetheart' (A–H) and 'Newstar' (I–P) cherry fruit during development (I: first growth; II: endocarp lignification; III: second growth; IV: commercial maturity) fractionated on Sepharose CL-6B column. V_0, void volume; V_T, total volume.

suggests that debranching of tightly bound pectin is more limited in soft 'Newstar' fruit. A trend for preferential disassembly of Na_2CO_3-soluble homogalacturonan in soft cherries occurs, as opposed to that of RG-I in firm 'Sweetheart' fruit. In 'Sweetheart', the increase in NS in the water-soluble fraction seemed to result from turnover of tightly bound polysaccharides. Instead, in 'Newstar', the increase of water- and CDTA-soluble NS originated more likely from 24%

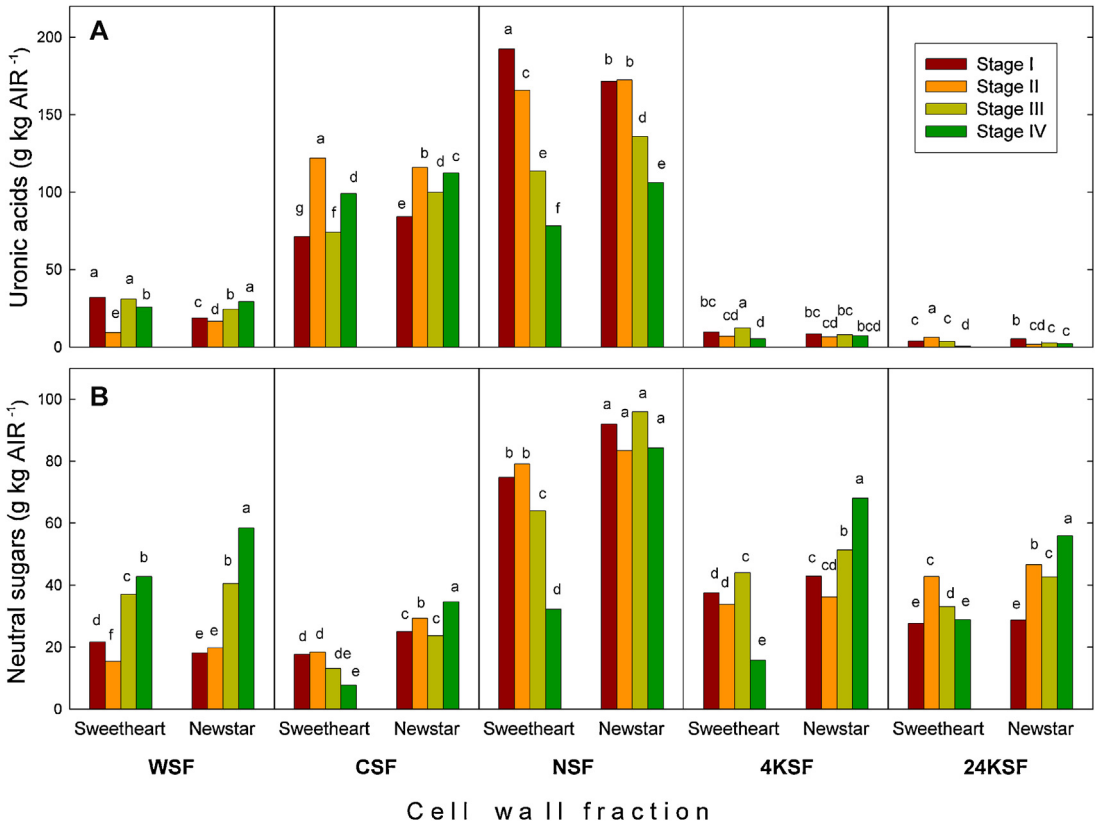

Fig. 4. Uronic acids (A) and neutral sugars (B) (g kg^{-1} AIR) in the water (WSF), CDTA (CSF), Na_2CO_3 (NSF), 4% KOH (4KSF), and 24% KOH (24KSF) fractions of 'Sweetheart' and 'Newstar' cherry fruit during development (I: first growth; II: endocarp lignification; III: second growth; IV: commercial maturity). Different letters indicate significant ($P < 0.05$) differences between cultivars and stages ($n = 3$).

KOH-insoluble material, probably cellulose-associated arabinogalactans. In 'Sweetheart', the NS content in the 4KSF decreased through development while in 'Newstar' they accumulated in 4KSF and 24KSF. It may be speculated that the degradation of the usually disregarded 24KSF-insoluble fraction has an important role in cherry softening and this may require further evaluation.

Given that differences between cultivars were already observed at stage I, it could be hypothesized that early structural characteristics in the wall (*i.e.*, besides variations in its disassembly) have a major role. It is worth noting that from stage I the NS to UA ratio of the most abundant pectic fraction (NSF) was higher in soft 'Newstar' fruit. Since Ara was by far the most abundant neutral sugar, and given that rhamnose content was similar in both cultivars (Table 2), the differences in the NS/UA ratio may be originated in variations in pectin branching. The higher NS/UA ratios in soft 'Newstar' fruit suggest more prevalence of RG-I side chains in the soft cultivar. Our results differ from those reporting that more pectin branching was associated with higher firmness (Batisse et al., 1996a). Although the results might depend on several different factors, the presence of calcium may contribute to reinforce the pectic matrix (Seske, 1995). In cherry, calcium has been repeatedly associated with improved firmness (Brown et al., 1995; Rupert et al., 1997) indicating that ionic bridges could be particularly important for tissue texture. Chen et al. (2009) suggested that pectin orientation may differ in firm and soft Chinese cherries (*Prunus pseudocerasus* L.). Since pectin arabinan side chains are spatial regulators of the proximity of HG domains (Harholt et al., 2010), they may also affect ionic interactions, and thus a larger branching of RG-I may prevent or hinder the formation of Ca^{2+} bridges, resulting in softer genotypes. The susceptibility to cracking, which will be the subject of further research, may also be related with the difficulties for ionic interactions.

4. Conclusions

In this work, we characterized the changes in cell wall composition, solubilization and depolymerization of two different sweet cherry cultivars with contrasting firmness. Firm 'Sweetheart' cherries showed higher contents of total wall material on a fruit mass basis than soft 'Newstar' fruit. The general pattern of wall dismantling consisting on substantial pectin solubilization and moderate depolymerization of both pectin and hemicelluloses was similar in firm and soft cultivars. However, Na_2CO_3-soluble rhamnogalacturonan I (RG-I) seemed to be the polyuronide preferentially solubilized in firm 'Sweetheart' fruit as opposed to tightly bound homogalacturonans (HG) in 'Newstar' cherries. Soft fruit had lower wall contents together with higher neutral sugar rich-pectin side chains. These factors may be involved in the differences in firmness among cherry cultivars.

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