Sugar Composition of Pectin and Hemicellulose Extracts of Peach Fruit during Softening over Two Harvest Seasons

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Additional index words. *Prunus persica*, cell wall, polysaccharide, pectin-associated polysaccharide, hemicellulose-associated polysaccharide

Abstract. Changes in cell wall polysaccharides associated with peach fruit softening were characterized over two harvest seasons. Enzymically inactive cell walls were prepared from mesocarp tissues of peach fruit harvested at three stages of softening. Pectin-associated and hemicellulose-associated polysaccharides were extracted from the cell walls sequentially, and glycosyl residue compositions were determined by GLC. Pectin extracts from both years were richest in galacturonosyl, arabinosyl, and rhamnosyl residues. Hemicellulose extracted with 1 M potassium hydroxide contained a high mole percentage of xylosyl, glucosyl, and fucosyl residues. Hemicellulose extracted with 4 M potassium hydroxide contained a substantial amount of pectin-associated sugar residues in addition to hemicellulose-associated sugar residues. During softening in both years, sugar compositions for cell walls, aqueous phenol-soluble polysaccharides, and imidazole extracts reflected a decrease in galacturonosyl residues and a concomitant increase in arabinosyl residues on a mole percent basis. The degree of change for galacturonosyl residues in these fractions depended on season, with greater variation exhibited from fruit at earlier stages of softening. With the notable exception of the seasonal variation exhibited for galacturonosyl residues in cell walls, the relative stability of other glycosyl compositional changes over seasons indicates conserved changes for pectins and hemicelluloses occur during peach fruit softening.

Softening is one of the most dramatic processes that occurs during fruit ripening. Softening of fruit tissues is associated with changes in degree of polymerization and sugar composition of polysaccharides resulting in alterations in cell wall structure and leading to decreased tissue cohesion (Fischer and Bennett, 1991). Particular attention has been given to modifying pectic polysaccharides during fruit softening. In tomato pericarp, softening involved solubilization of polyuronides, galactose, and arabinose (Carrington et al., 1993; Gross and Wallner, 1979; Seymour et al., 1990). During softening of many fruit, including muskmelon (McCollum et al., 1989), avocado (Huber and O'Donoghue, 1993), persimmon (Cutillas-Iturralde et al., 1993), kiwifruit (Redgewell et al., 1991), and pear (Ahmed and Labavitch, 1980), pectins usually depolymerized and became more soluble. During softening of nectarines, pectic polymer depolymerization was accompanied by galactan side chain loss (Dawson et al., 1992). In addition to changes in pectic polymers, softening-associated changes in hemicellulosic polymers have been reported; hemicelluloses undergo changes in molecular weight distribution during the softening of tomato (Huber, 1983; Tong and Gross, 1988), strawberry (Huber, 1984), hot pepper (Gross et al., 1986), and muskmelon (McCollum et al., 1989).

Softening of peaches has been attributed to the conversion of protopectin to soluble forms (Chapman and Horvat, 1990; Postylmayr et al., 1966; Pressey and Avants, 1978; Shewfelt, 1965). Pressey et al. (1971) reported a positive correlation between polygalacturonase activity and an increase in water-soluble pectin

and fruit softening. Pressey and Avants (1973) resolved the peach fruit polygalacturonase into exo-acting and endo-acting forms, and in 1978, they noted the occurrence of exo- and endo-acting enzymes in freestone peaches but only an exo-acting enzyme in ripe clingstone peaches (Pressey and Avants, 1978). Endopolygalacturonase activity increased during softening of freestone peach fruit (Pressey et al., 1971). Exo-polygalacturonase in freestone peach fruit also appeared to dramatically increase in abundance with fruit ripening (Downs and Brady, 1990). Pectin methyl esterase activity exhibited no direct relationship to softening in peaches (Shewfelt, 1965).

Peach fruit softening also has been studied by following changes in the sugar composition and apparent molecular weight of pectic substances during on-tree and shelf-ripening. Weight percentage of cell walls, firmness, and pectin content decreased markedly between the 21st and 22nd week after flowering and between the third and sixth day of storage for melting flesh peaches compared to nonmelting flesh peaches (Fishman et al., 1991). The molecular weight of chelator-soluble pectins and alkali-soluble pectins was greater in melting flesh peaches than in nonmelting flesh peaches (Fishman et al., 1993). Callahan et al. (1992) compared the RNA accumulation of pch313 (pTOM 13 Homolog, which codes for the endopolygalacturonase enzyme) during fruit softening of two phenotypically different peach cultivars. The cultivar that softened faster, 'Bailey', had a significantly higher amount of pch313 RNA accumulate during softening than the slower-softening cultivar 'Suncrest'. Interestingly, peach fruit only accumulated one-fiftieth the amount of endopolygalacturonase during ripening compared to tomato fruit.

Apart from changes in pectic polymers, little is known about cell wall modifications during softening of peach fruit. Modifications of other cell wall components, such as hemicelluloses, also may occur during softening. To our knowledge, no information is available on year-to-year variation in cell wall polymer changes during softening of peaches. Our objective was to characterize

Received for publication 12 Oct. 1995. Accepted for publication 7 June 1996. Approved for publication by the Director, Oklahoma Agricultural Expt. Station. This research was supported by the Oklahoma Agricultural Expt. Station under project H-2161 and by U.S. Dept. of Agriculture grant 92-34150-7190. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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pectin and hemicellulose glycosyl composition during softening of peach fruit and to compare glycosyl compositions across years.

Materials and Methods

Plant material. ‘Belle of Georgia’ peach [*Prunus persica* (L) Batsch] fruit were obtained from ≥30 trees in the same area of an orchard in 1991 and 1992 and were transported to the laboratory in ice chests. Mesocarp firmness was measured after removal of exocarp on opposite cheeks using an Effegi penetrometer (Effegi, Alfonsine, Italy) with a standard 8-mm probe. Fruit were sorted into firmness groups of 40 to 53 (firm, represented as 47 N), 24 to 33 (medium soft, represented as 30 N), and 9 to 20 N (soft, represented as 15 N), using the average of both penetrometer measurements, and were used immediately for cell wall preparation.

Preparation of fruit cell walls. Enzymically inactive cell walls were prepared from fresh mesocarp tissue essentially as described by Huber (1991). Mesocarp was separated, placed on ice, diced into small pieces, and homogenized for 6 min in three 2-min bursts on ice with three volumes (w/v) of Tris-saturated phenol using a Omnimixer homogenizer (Omni International, Waterbury, Conn.) set at speed 6. The homogenate was filtered through two layers of Miracloth (Calbiochem-Nova-biochem Corp., La Jolla, Calif.). The residue was washed with water to remove phenol, transferred to a scintered glass funnel, and washed with 1 L 1 chloroform : 1 methanol (v/v) and acetone (1 to 2 L) until a fluffy consistency was obtained. The residue was dried at 60 °C and stored in a brown bottle.

Aqueous phenol-soluble polysaccharide recovery. Polysaccharides were recovered from the aqueous phase of the initial Tris-saturated phenol filtrate by precipitation with hot ethanol (Dick and Labavitch, 1989). Recovered polysaccharides were dialyzed against deionized water and lyophilized.

Extraction of cell walls. Cell walls, processed in the same year they were obtained, were extracted sequentially to obtain pectin associated polysaccharides and then hemicellulose associated polysaccharides as described by Selvendran et al. (1985) with some modifications. Cell walls (1 g) were hydrated in 100 ml of 500 mM imidazole, pH 7, plus 0.05 % sodium azide in vacuo and then was extracted for 12 h at 1 °C. Samples were centrifuged and the supernatant was recovered. The extraction was repeated for 2 h at 20 to 22 °C. Combined extracts were dialyzed against deionized water, lyophilized, and weighed. Imidazole was substituted for CDTA or EDTA to extract pectins because imidazole, in contrast to CDTA or EDTA, readily dialyses away against deionized water (Mort et al., 1991). The imidazole extracted residues then were extracted with 100 ml of 50 mM sodium carbonate plus 20 mM sodium borohydride for 16 h at 1 °C. The extraction was repeated for 3 h at 20 to 22 °C. The supernatants were adjusted to pH 5 with acetic acid, dialyzed against deionized water, and lyophilized. Extraction with sodium carbonate at 1 °C deesterifies the pectins, thus minimizing depolymerization by β-elimination during subsequent alkaline extractions (Selvendran et al., 1985).

The “depectinated” residues were extracted with 1 M KOH plus 10 mM sodium borohydride for 2 h at 1 °C and then for 2 h at 20 to 22 °C. Further extraction was performed with 4 M KOH plus 10 mM sodium borohydride for 2 h at 20 to 22 °C and then with 4 M KOH plus 3% to 4% boric acid for 2 h at 20 to 22 °C. Supernatants were adjusted to pH 5 with acetic acid and then dialyzed against deionized water and lyophilized. The final residue was lyophilized and weighed. Two replications were done each year for pectin and hemicellulose extractions.

Sugar composition analysis. Samples (100 to 200 µg) were methanolyzed and trimethylsilylated for gas liquid chromatography as described by Komalavilas and Mort (1989). One microliter of the trimethylsilylated sugars was injected onto a DB-1-fused silica capillary column (30 m × 0.25 mm i.d.; J & W Scientific, Rancho Cordova, Calif.) installed in a gas chromatograph (model 6000; Varian Associates, Walnut Creek, Calif.) equipped with a cool, on-column injector and FID detector, using helium as carrier gas. The sample was injected at 105 °C, and the temperature was raised to 160 °C and held for 4 min before being raised to 200 °C at 1 °C per min. Sugars were identified according to comparison with authentic standards and were quantified using inositol as an internal standard.

Results and Discussion

In this study, we characterized changes in polysaccharides during softening of peaches for two successive seasons. The rapidly softening ‘Belle of Georgia’ peach was used. We used cell wall preparation procedures designed to inactivate bound hydrolases to eliminate the capacity

Table 1. Sugar composition of cell walls and aqueous phenol-soluble polysaccharides for cell walls of peach fruit differing in firmness.

Resistance to puncture (N) ^z	Extraction recovery (%) ^y	Sugar composition (mole %)								
		Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
<i>Cell wall (1991)</i>										
47	22	4	1	9	14	3	5	38	4	
30	29	5	2	13	10	3	3	31	4	
15	31	4	2	12	13	4	5	24	5	
<i>Cell wall (1992)</i>										
47	34	5	2	12	12	2	3	28	3	
30	32	4	1	10	9	2	5	35	2	
15	38	4	2	12	14	3	4	22	2	
<i>Aqueous phenol soluble (1991)</i>										
47	6	24	3	1	5	17	2	6	38	4
30	3	29	4	1	7	23	2	7	24	3
15	3	43	5	2	6	19	3	6	16	1
<i>Aqueous phenol soluble (1992)</i>										
47	5	34	5	1	5	17	2	3	29	4
30	1	30	2	1	10	27	7	8	10	4
15	1	36	3	1	7	32	4	5	8	4

^zFruit were sorted into firmness groups of 47 (firm), 30 (medium soft), and 15 N (soft) by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.

^yExtraction recovery was the percentage of the weight recovered from the aqueous phase of the buffered phenol filtrate to the initial weight of cell wall obtained.

of resident active bound hydrolases to alter cell wall components during cell wall preparation as well as during subsequent extraction steps. Tris-saturated phenol effectively inactivates cell wall hydrolases in other fruit (Huber, 1991). In preliminary trials, we confirmed use of buffered phenol for inactivation of peach cell wall hydrolytic activity (data not shown).

In both years, cell walls contained considerable percentages of galacturonosyl, arabinosyl, galactosyl, and xylosyl residues (Table 1). As fruit softened, cell walls exhibited a consistent increase in arabinosyl residues and a somewhat inconsistent decrease in galacturonosyl residues on a mole percent basis. In 1991, a gradual decline in galacturonosyl residues was observed in cell walls from progressively softer fruit. In 1992, the galacturonosyl residue content increased slightly from firm to medium soft fruit and then declined in soft fruit to a level below that of firm fruit. This seasonal variation could be due to a slight difference in softening mechanism that is under environmental control. It also may indicate that cleavage of homogalacturonan may be less strongly associated with tissue softening during early softening stages (firm to medium soft fruit) than during latter softening stages (medium soft to soft). Some evidence to support this assumption has been demonstrated for tomato. Using antisense endopolygalacturonase transgenic lines in which endopolygalacturonase enzyme population was reduced by 99%, Carrington et al. (1993) demonstrated that tomato pericarp softening rate was only reduced during latter softening stages (red), with little to no effect on softening rate during earlier softening stages. Since the cell wall target for

endopolygalacturonase is thought to be homogalacturonan, homogalacturonan's role in tomato fruit softening appears to be greater during latter rather than in earlier stages of pericarp softening. For our peaches, in both years, galacturonosyl residue composition did decline by the latter stage of softening, perhaps indicating selective galacturonic acid loss by latter stages of softening as a similarity between peach and tomato in regard to the contribution of changes in homogalacturonan to tissue softening.

Aqueous phenol-soluble polysaccharides contained high amounts of pectin-associated sugar residues (Table 1). This fraction, presumably soluble in fruit in vivo, was enriched in arabinosyl and galactosyl residues as fruit softened, indicating that arabinose and galactose residues (or polysaccharides containing high amounts of these sugars as sidechains) may be selectively cleaved during softening of peach fruit in vivo. We expected that the aqueous phenol-soluble polysaccharide fraction from soft fruit would contain an increasing mole percentage of galacturonosyl residues to account for the decreased galacturonosyl residue content of cell walls. Ripening tomato fruit pericarp water-soluble polysaccharides exhibited this expected increase in galacturonosyl residue yield (Carrington et al., 1993). Our observed decrease in aqueous phenol-soluble polysaccharide galacturonosyl residue content may have been due to increased exo-polygalacturonase activity during ripening. Exo-polygalacturonase is known to dramatically increase in abundance during freestone peach fruit ripening (Downs and Brady, 1990), and the resulting monomer galacturonic acid enzyme product would fail to precipitate with ethanol and be lost

Table 2. Sugar composition of pectin and hemicellulose extracts and insoluble residue for cell walls of peach fruit differing in firmness (harvested in 1991).

	Resistance to puncture	Extraction recovery	Sugar composition (mole %)									
Variable	(N) ^z	(%) ^y	Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA	
500 mM	47	11	15 ± 1 ^x	4 ± 0.4	1 ± 0.1	2 ± 0.8	8 ± 6	2 ± 0.6	3 ± 0.9	62 ± 6	3 ± 0.4	
Imidazole	30	5	24 ± 2	5 ± 2	1 ± 0.1	3 ± 0.1	5 ± 2	2 ± 0.4	3 ± 0.6	55 ± 5	2 ± 0.6	
	15	5	30 ± 2	6 ± 0.1	1 ± 0.1	3 ± 0.3	10 ± 0.3	4 ± 0.7	5 ± 1	38 ± 0.4	2 ± 0.1	
50 mM	47	7	29 ± 6	6 ± 0.9	1 ± 0.2	3 ± 1	8 ± 0.8	1 ± 0.4	2 ± 1	49 ± 2	2 ± 0.1	
Na ₂ CO ₃	30	10	48 ± 8	10 ± 2	1 ± 0.3	5 ± 1	6 ± 2	1 ± 0.1	3 ± 0.2	24 ± 0.1	3 ± 0.7	
1 °C	15	6	40 ± 2	7 ± 0.1	1 ± 0.6	5 ± 2	9 ± 2	1 ± 0.2	4 ± 3	32 ± 1	2 ± 0.3	
50 mM	47	5	36 ± 1	9 ± 2	2 ± 0.4	6 ± 4	13 ± 3	1 ± 0.5	4 ± 1	28 ± 3	1 ± 0.3	
Na ₂ CO ₃	30	2	41 ± 5	8 ± 0.1	1 ± 0.2	7 ± 2	7 ± 1	2 ± 0.2	6 ± 2	26 ± 9	2 ± 0.6	
20 to 22 °C	15	2	46 ± 7	8 ± 0.7	1 ± 0.2	5 ± 0.5	9 ± 0.1	1 ± 0.5	3 ± 0.3	27 ± 5	2 ± 1	
1 M KOH	47	2	4 ± 0.2	1 ± 0.3	5 ± 0.7	47 ± 3	15 ± 1	7 ± 0.2	19 ± 3	1 ± 0.1	1 ± 0.2	
1 °C	30	2	7 ± 3	1 ± 0.1	4 ± 2	41 ± 5	10 ± 0.5	5 ± 0.8	22 ± 0.1	9 ± 1	1 ± 0.4	
	15	3	4 ± 0.7	1 ± 0.4	3 ± 1	49 ± 8	11 ± 1	8 ± 2	20 ± 9	3 ± 1	1 ± 0.3	
1 M KOH	47	4	27 ± 6	6 ± 0.2	2 ± 0.1	15 ± 5	13 ± 4	2 ± 0.7	10 ± 3	22 ± 2	3 ± 0.6	
20 to 22 °C	30	4	24 ± 2	5 ± 0.2	3 ± 1	21 ± 4	12 ± 1	4 ± 1	14 ± 5	14 ± 0.2	2 ± 0.4	
	15	3	26 ± 11	5 ± 2	3 ± 0.7	25 ± 11	12 ± 1	4 ± 1	11 ± 3	13 ± 2	1 ± 0.9	
4 M KOH	47	9	24 ± 9	4 ± 1	4 ± 0.1	20 ± 0.6	11 ± 2	4 ± 1	21 ± 8	8 ± 4	3 ± 2	
20 to 22 °C	30	14	20 ± 2	3 ± 0.1	4 ± 1	18 ± 2	18 ± 4	6 ± 1	23 ± 5	12 ± 7	2 ± 1	
	15	13	22 ± 2	3 ± 0.6	4 ± 1	23 ± 4	13 ± 2	5 ± 0.2	19 ± 5	10 ± 5	1 ± 0.5	
4 M KOH +	47	5	34 ± 5	5 ± 0.6	2 ± 0.1	9 ± 1	12 ± 4	11 ± 6	13 ± 1	13 ± 3	2 ± 1	
boric acid	30	2	35 ± 8	4 ± 0.1	2 ± 1	10 ± 2	13 ± 1	12 ± 1	16 ± 4	7 ± 0.1	1 ± 0.1	
20 to 22 °C	15	7	31 ± 1	4 ± 1	2 ± 0.2	10 ± 3	14 ± 0.4	14 ± 2	16 ± 1	8 ± 3	1 ± 0.2	
Insoluble	47	22	29 ± 1	5 ± 2	3 ± 1	9 ± 0.1	8 ± 2	2 ± 1	34 ± 2	8 ± 3	1 ± 0.3	
Residue	30	27	37 ± 3	5 ± 1	1 ± 0.3	10 ± 0.3	10 ± 2	2 ± 0.1	26 ± 7	9 ± 2	1 ± 0.2	
	15	26	29 ± 10	4 ± 1	3 ± 2	8 ± 2	10 ± 4	2 ± 0.1	34 ± 2	9 ± 4	2 ± 0.4	

^zFruit were sorted into firmness groups of 47 (firm), 30 (medium soft), and 15 N (soft) by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.

^yExtraction recovery was the percentage of the weight of the lyophilized supernatant (or insoluble residue) to the initial weight of cell wall used for extraction.

^xData are the means of two replications ± SE.

Table 3. Sugar composition of pectin and hemicellulose extracts and insoluble residue for cell walls of peach fruit differing in firmness (harvested in 1992).

Variable	Resistance to puncture (N) ^z	Extraction recovery (%) ^y	Sugar composition (mole %)								
			Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
500 mM	47	6	12 ± 3 ^x	2 ± 1	1 ± 0.1	3 ± 0.1	8 ± 2	2 ± 1	5 ± 0.1	64 ± 6	4 ± 0.6
Imidazole	30	7	16 ± 3	3 ± 0.1	1 ± 0.1	3 ± 0.1	6 ± 0.1	2 ± 0.1	4 ± 0.1	64 ± 3	2 ± 0.1
	15	6	25 ± 0.7	4 ± 0.1	1 ± 0.1	4 ± 0.4	12 ± 0.2	3 ± 0.7	5 ± 0.5	43 ± 0.4	3 ± 0.6
50 mM	47	5	19 ± 1	3 ± 1	1 ± 0.1	2 ± 0.5	9 ± 2	1 ± 0.1	2 ± 0.1	61 ± 11	3 ± 0.7
Na ₂ CO ₃	30	6	23 ± 6	4 ± 0.6	1 ± 0.1	1 ± 0.4	9 ± 0.1	1 ± 0.1	2 ± 0.7	58 ± 5	2 ± 0.7
1 °C	15	14	37 ± 1	5 ± 0.7	1 ± 0.1	7 ± 0.5	10 ± 2	1 ± 0.3	2 ± 0.7	24 ± 3	3 ± 0.7
50 mM	47	5	44 ± 5	6 ± 1	1 ± 0.3	2 ± 0.6	10 ± 3	1 ± 0.1	2 ± 0.8	33 ± 9	2 ± 0.2
Na ₂ CO ₃	30	6	50 ± 4	7 ± 0.4	1 ± 0.3	2 ± 0.2	19 ± 1	1 ± 0.1	2 ± 0.1	28 ± 6	1 ± 0.1
20 to 22 °C	15	3	50 ± 0.6	8 ± 0.1	1 ± 0.1	4 ± 0.6	13 ± 2	1 ± 0.1	5 ± 0.1	21 ± 2	2 ± 0.6
1 M KOH	47	3	5 ± 2	1 ± 0.1	1 ± 0.6	36 ± 4	15 ± 0.8	9 ± 0.1	27 ± 3	4 ± 1	1 ± 0.1
1 °C	30	3	6 ± 1	1 ± 0.1	3 ± 0.3	33 ± 2	16 ± 1	8 ± 1	26 ± 1	7 ± 1	1 ± 0.4
	15	4	5 ± 1	1 ± 0.4	5 ± 0.1	37 ± 4	15 ± 1	8 ± 1	23 ± 3	6 ± 2	1 ± 0.4
1 M KOH	47	3	34 ± 6	4 ± 0.6	2 ± 0.2	13 ± 4	13 ± 1	3 ± 0.3	11 ± 0.1	17 ± 2	2 ± 0.1
20 to 22 °C	30	2	30 ± 4	4 ± 1	2 ± 0.3	13 ± 1	15 ± 1	3 ± 0.2	14 ± 2	17 ± 0.1	3 ± 1
	15	5	32 ± 4	4 ± 1	3 ± 1	18 ± 5	14 ± 0.1	4 ± 0.3	12 ± 3	13 ± 1	1 ± 0.1
4 M KOH	47	7	30 ± 3	4 ± 0.4	4 ± 0.6	15 ± 1	16 ± 1	5 ± 1	16 ± 3	11 ± 2	1 ± 0.2
20 to 22 °C	30	9	28 ± 5	3 ± 1	3 ± 0.6	15 ± 2	16 ± 0.3	5 ± 1	17 ± 4	12 ± 0.1	1 ± 0.2
	15	10	27 ± 6	3 ± 1	3 ± 0.1	17 ± 0.3	16 ± 0.1	7 ± 2	18 ± 5	8 ± 0.4	1 ± 0.2
4 M KOH + boric acid	47	3	39 ± 6	4 ± 1	1 ± 0.4	6 ± 3	24 ± 1	2 ± 0.1	6 ± 0.2	13 ± 1	2 ± 0.6
	30	2	32 ± 6	6 ± 1	1 ± 0.1	6 ± 2	15 ± 1	5 ± 1	7 ± 0.2	18 ± 0.1	2 ± 0.3
20 to 22 °C	15	2	33 ± 6	5 ± 0.2	2 ± 1	8 ± 2	16 ± 3	9 ± 2	12 ± 4	13 ± 2	2 ± 0.1
Insoluble	47	24	32 ± 7	4 ± 1	1 ± 0.4	9 ± 3	13 ± 0.1	2 ± 0.4	31 ± 4	8 ± 3	1 ± 0.1
residue	30	23	26 ± 8	4 ± 1	1 ± 0.4	9 ± 1	15 ± 1	3 ± 0.1	32 ± 9	9 ± 1	1 ± 0.1
	15	25	28 ± 8	3 ± 2	1 ± 0.6	6 ± 1	11 ± 4	2 ± 0.3	44 ± 8	4 ± 1	1 ± 0.1

^zFruit were sorted into firmness groups of 47 (firm), 30 (medium soft), and 15 N (soft) by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.

^yExtraction recovery was the percentage of the weight of the lyophilized supernatant (or insoluble residue) to the initial weight of cell wall used for extraction.

^xData are the means of two replications ± SE.

during recovery of the aqueous phenol-soluble polysaccharide fraction. Quantitative loss of precipitable galacturonosyl residue would be greater for soft fruit compared to firm fruit, perhaps explaining why overall recovery of soluble polysaccharides was lower for softer fruit (Table 1).

Imidazole extracts from both years were enriched in galacturonosyl and arabinosyl residues (Table 2 and 3). As fruit softened, there was a decrease in mole percentage of galacturonosyl residue, a substantial increase in mole percentage of arabinosyl residues, and a slight increase in rhamnosyl and galactosyl residues. As was noted for cell walls, the magnitude of decrease in galacturonosyl residues for this extract was more pronounced in 1991 than in 1992. The seasonal variation in galacturonosyl residue content probably was due mostly to, and was a reflection of, differences in cell wall glycosyl composition. The galacturonosyl : rhamnosyl residues ratio decreased as softening progressed. Calcium is thought to form ionic bridges between unesterified pectic polymers, thereby conferring rigidity to the cell wall. Our cell wall preparation procedure has extracted little calcium from walls (Huber, 1991), and subsequent extraction with 0.5 M imidazole (presumably by chelation of calcium; Mort et al., 1990) should result in extraction of the ionically bound homogalacturonan polymers representative of those present in the native walls plus any associated covalently bound polymers. The homogalacturonan region is thought to consist of blocks of consecutive galacturonosyl residues, interrupted at fairly regular intervals by 1,2-linked rhamnosyl residues (McNeil et al., 1984). During fruit softening,

endopolygalacturonase is thought to function in hydrolysis of the galacturonic acid-galacturonic acid linkages (but not galacturonic acid-rhamnose linkages). Rhamnosyl residues are reported to occur once in every 25 galacturonosyl residues for apple, citrus, and sunflower pectins (Powell et al., 1982); once in every 70 residues for carrots (Konno et al., 1986); and once in every 72 to 100 residues for beet (Thibault et al., 1993). In our study, based on yields from very firm fruit, the interval for peach appears to be once in every 24 residues. This interval appeared to decrease for softening fruit, averaging 16 for medium soft and eight for soft fruit (Table 2 and 3). This information supports a selective cleavage of the galacturonosyl residues in the homogalacturonan region during fruit softening or increased extraction of the rhamnogalacturonan regions in softer fruit.

The imidazole insoluble pectic polysaccharide was further solubilized using sodium carbonate to hydrolyze weak ester cross-links (Waldron and Selvendran, 1992). Sodium carbonate extracts from both years were enriched in galacturonosyl, arabinosyl, and rhamnosyl residues (Table 2 and 3). Arabinosyl and rhamnosyl residues were particularly enriched in these fractions compared to imidazole extracts. Extraction with sodium carbonate at 1 °C deesterifies the pectins, thus minimizing the degradation by β elimination, and extraction at room temperature is known to solubilize certain pectic substances that are in association with hemicellulosic polymers (Selvendran and O'Neil, 1987). This was true in peaches because warm sodium carbonate extracts contained a higher mole percentage of hemicellulose associated sugar resi-

dues (extracted along with pectin associated sugar residues) compared to cold sodium carbonate extracts (Table 2 and 3). Similar results have been reported for other fruit (Cutillas-Iturralde et al., 1993; Rydan and Selvendran, 1990; Seymour et al., 1990).

As fruit softened, aqueous phenol-soluble polysaccharides (Table 1) and imidazole and sodium carbonate extracts (Tables 2 and 3) exhibited a decrease in galacturonosyl residues, a substantial increase in arabinosyl residues, and a decrease in the galacturonosyl : rhamnosyl residue ratio. Similarly in nectarines, as ripening progressed, uronide content of the pectic fractions (i.e., CDTA and sodium carbonate) decreased (Lurie et al., 1994) and arabinosyl residues increased (Dawson et al., 1992). Assuming arabinosyl residues were present as a covalently linked pectin side chain, a selective galacturonosyl residue loss, as would occur through combined endo- and exo-polygalacturonase enzyme action, would result in the observed increase in arabinosyl residue on a mole percent basis. The concomitant increase of arabinosyl residues with a decrease in galacturonosyl and galacturonosyl : rhamnosyl residue ratio supports a mechanism of increased arabinosyl residue content as a consequence of concentration of pectic arabinan sidechains in extracts of soft fruit.

'Depectinated' cell wall material was extracted further with increasing concentrations of KOH to solubilize the hemicelluloses. Alkali solubilizes the hemicellulose-associated polysaccharides by disrupting hydrogen bonding between hemicellulose and cellulose microfibrils (Selvendran et al., 1985). More loosely bound hemicellulose extracts (1 M KOH) were particularly enriched in xylosyl (13 to 49 mole percent) and glucosyl residues (10 to 27 mole percent) in both years compared to imidazole and sodium carbonate extracts (Table 2 and 3). The 1 M KOH extracts also were enriched in fucosyl residue in fruit of all firmness groups in both years compared to cell walls, imidazole, or sodium carbonate extracts. Fucose is thought to be present in cell walls predominantly as a xyloglucan side chain (Rydan and Selvendran, 1990). Extracts at 1 °C had higher mole percentages of xylosyl and glucosyl residues compared to extracts at 20 to 22 °C. The amount of pectin-associated sugar residues (galacturonic acid, arabinose, and rhamnose) vs. hemicellulose-associated sugar residues (xylose, glucose, and fucose) increased substantially in warm extracts compared to cold extracts, and these differences were stable across years. In both years, the xylosyl : glucosyl residues ratio was higher for cold extracts than warm extracts. Cold 1 M KOH appeared to extract a more highly purified xyloglucan fraction with less pectic contamination than warm 1 M or 4 M KOH. The 4 M KOH plus 10 mM sodium borohydride extracts resembled 1 M KOH warm extracts more than 1 M KOH cold extracts in terms of sugar composition, but extraction yields were higher for 4 vs. 1 M KOH extracts (Table 2 and 3). Xylosyl, glucosyl, and fucosyl residues were enriched in this fraction compared to cell walls. The pectin and hemicellulose content of this extract was essentially equal for fruit of all firmness groups, with 35 to 45 mole percent of pectin-associated sugar residues and 38 to 46 mole percent of hemicellulose associated sugar residues. In fruit of all firmness groups, the xylosyl : glucosyl residue ratio was closer to 1:1 than for other pectin and hemicellulose extracts. The 4 M KOH plus boric acid extracts contained higher percentages of pectin-associated sugar residues than hemicellulose-associated sugar residues in fruit of all firmness groups (Table 2 and 3). Warm 1 and 4 M KOH extracts extracted hemicelluloses and pectins. There were similar results with apples (Stevens and Selvendran, 1984). A higher proportion of pectin-associated sugar residues in more tightly bound hemicellulose extracts in fruit of all firmness groups indicates that pectin

and hemicellulose are in some way associated in the cell wall of peach fruit. This association could be by covalent linkage, as indicated for apple (Renard et al., 1991) and tomato (Seymour et al., 1990) or by physical entrapment of pectin by hemicellulose without covalent linkage as suggested for onion (McCann et al., 1990).

In both years, the insoluble residue from fruit of all firmness groups contained a high mole percentage of arabinosyl and glucosyl residues (Table 2 and 3). Residue recovery was similar between firmness groups in both years, ranging from 27% to 22% of starting weight.

Season to season difference in sugar composition in cell walls may be influenced by factors external to the fruit, like atmospheric condition, temperature, stress situations, and cultural factors. We assume that methods used for cell wall preparation and pectin and hemicellulose extraction would prevent additional polysaccharide cleavage so that solubility of the polymers could be related to changes in cell wall associations occurring before cell wall preparation and not during extraction. Season-to-season difference in sugar composition of pectin and hemicellulose extracts was probably influenced primarily by the differences in cell wall composition and to a lesser extent inconsistent selectivity in extractant solubilization of clearly defined polymer mixtures. In pectin extracts from both years, the increase in arabinosyl residues and decrease in galacturonosyl residues and galacturonosyl : rhamnosyl residue ratio for soft peaches could be due to the combined action of endo- and exo-polygalacturonase, assuming arabinosyl residues were present as a covalently linked pectin side chain. The most loosely bound hemicellulose fraction extracted principally xyloglucan with a low pectin-associated sugar residue yield. More tightly bound hemicellulose fractions extracted pectin- and hemicellulose-associated sugar residues indicating that they are in some way associated in the cell wall of peach fruit. Although some seasonal variation in glycosyl composition of cell walls and pectin extracts was evident, the relative stability over years of sugar compositional changes in cell walls, aqueous phenol-soluble polysaccharides, pectin, and hemicellulose extracts indicates conserved changes for pectins and hemicelluloses occur during peach fruit softening. These results also may indicate that a wide range of pectolytic and nonpectolytic enzymes may be involved in peach fruit softening.

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