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Changes in Apparent Molecular Mass of Pectin and Hemicellulose Extracts during Peach Softening

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Abstract. **Pectin and hemicellulose were solubilized from cell walls of peach [*Prunus persica* (L.) Batsch] fruit differing in firmness by extraction with imidazole and sodium carbonate (pectin extracts), followed by a graded series of potassium hydroxide (hemicellulose extracts). The extracts were subjected to size exclusion chromatography. In imidazole extracts, as fruit softened, there was an increase in proportion of a large apparent molecular mass peak, with a galacturonosyl to rhamnosyl residue ratio resembling a rhamnogalacturonan-like polymer. A smaller apparent molecular mass peak was enriched in galacturonic acid and probably represented a broad polydisperse peak derived from more homogalacturonan-like polymers. In sodium carbonate extracts, a homogalacturonan-like polymer appeared to elute primarily as a higher apparent molecular mass constituent, which increased in quantity relative to other constituents as fruit softened. In cold 1 mol·L^{-1} KOH extracts three peaks predominated. A xyloglucan-like polymer appeared to elute predominantly in the second peak and fucose was strongly associated with it. In 4 mol·L^{-1} KOH extracts (tightly bound hemicellulose) the higher apparent molecular mass peak was predominantly acidic and presumably of pectic origin. The smaller apparent molecular mass peaks were composed primarily of neutral sugars, the second peak became smaller and the third peak larger as fruit softened. The ability to separate pectin and xyloglucan-like polymer as two separate fractions based on charge suggests that the nature of any pectin-hemicellulose interaction in this fraction is probably one of physical entrapment of pectin fractions by hemicellulose and not principally by covalent crosslinking between the two polysaccharide classes in peach. Flesh firmness serves as an important determinant of quality in peaches. Our results indicate that apparent molecular mass of both pectins and hemicelluloses changed as peaches softened, resulting in alteration of cell wall structure and associated with decreased tissue cohesion.**

Ripening of most fleshy fruit is often characterized by softening of edible tissues. It is assumed that modifications of cell wall polysaccharide components underlie the process of softening. Pectic polysaccharides are a major constituent of primary cell walls, coexisting with other polysaccharides such as hemicellulose and cellulose, forming a cross-linked matrix network. Physical interconnections between adjacent cells are thought to occur primarily through interlocking of pectin originating from adjoining cells and forming the middle lamella, and the degree of interconnection affects the rigidity of interaction, or firmness, on a tissue-wide basis (McCann et al., 1990). It is thought that the breakdown of the bonds holding the cross linked matrix structure leads to loosening of the stability of this network, which results from cleavage of cell wall components, causing loss of tissue firmness. Ripening-associated modifications in sugar composition and apparent molecular mass of pectins and hemicelluloses have been reported in many fruit.

In muskmelon (*Cucumis melo* L.), molecular mass of pectic polymers and hemicelluloses extracted from fruit mesocarp cell walls shift from larger to smaller polymers during ripening (Ranwala et al., 1992). In avocado (*Persea americana* Mill.), hemicelluloses (4 mol·L^{-1} alkali-soluble) exhibit a very broad distribution of polymer sizes and an overall decrease in apparent molecular mass during softening (O'Donoghue and Huber, 1992). There is a marked change in molecular mass distribution of cell wall hemicelluloses of tomato (*Lycopersicon esculentum* Mill.) fruit (Huber, 1983). During ripening a progressively lower proportion of high molecular mass polysaccharides coincides with a higher proportion of low molecular mass polymers. Similarly, a hemicellulose fraction extracted from hot pepper (*Capsicum annuum* L.) fruit cell walls is modified during maturation and ripening, resulting in a shift from higher to lower molecular mass (Gross et al., 1986). In muskmelon, McCollum et al. (1989) reported an increase in solubility and a decrease in molecular mass of polyuronides in the cell walls of fruit as softening progresses and Ranwala et al. (1992) observed molecular mass of pectin and hemicellulose polymers extracted from fruit mesocarp cells shift from larger to smaller polymers during ripening. In kiwifruit [*Actinidia deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson var. *deliciosa*], three distinct molecular mass classes of hemicelluloses occur, with a proportional increase in the smaller polymers with fruit ripening (Redgewell et al., 1991). Ripening of nectarines [*Prunus persica* var. *nectarina* (Ait) maxim] results in solubilization of pectic polymers of high molecular mass class and concurrent galactan side chain removal from pectic polymers. Solubilized pectic polymers are depolymerized to lower molecular mass class as ripening progresses (Dawson et al., 1992).

Peach softening during ripening has been attributed to the enzymic degradation of pectic polymers (Pressey et al., 1971). In the fruit mesocarp of the melting flesh cultivar 'Redskin', molecular mass of chelator-soluble pectin (extracted with CDTA) and alkaline-soluble pectin (extracted with sodium carbonate plus CDTA) increases considerably during ripening and storage. In the mesocarp of the nonmelting flesh peach cultivar 'Suncling', molecular mass of chelator-soluble pectin and alkaline-soluble fractions are relatively constant during on-tree ripening and storage (Fishman et al., 1993). Although changes in other polysaccharides may be involved, the solubilization of pectin has received the most attention because of the preponderance of this polysaccharide in the middle lamella. From our previous studies with peach cell

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walls, we know that both pectin and hemicellulose sugar compositions change during fruit softening (Maness et al., 1993; Hegde and Maness, 1996). This study was undertaken to develop an understanding of how the apparent molecular mass of these polymers are modified during peach softening. The main objective was to characterize apparent molecular mass changes of pectins and hemicelluloses during peach softening.

Materials and Methods

Plant material. 'Belle of Georgia' peaches were obtained from at least 30 trees in the same area of a commercial orchard and 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 an 8 mm diameter probe. Fruit were sorted into firmness groups of 40 to 53 N (firm, represented as 47 N), 24 to 33 N (medium soft, represented as 30 N) and 9 to 20 N (soft, represented as 15 N), using the average of both measurements, and utilized immediately for cell wall preparation.

Preparation of fruit cell walls. Enzymically inactive cell walls were prepared essentially as described by Huber (1991). The mesocarp was separated, placed on ice, diced into small pieces and homogenized for 6 min in three 2-min bursts on ice with 3 volumes (w/v) of Tris-saturated phenol using an Omni Mixer homogenizer (Omni International, Waterbury, Conn.). The homogenate was stirred for 1.5 h and then filtered through two layers of Miracloth (Calbiochem-NovaBiochem Corp., La Jolla, Calif.). The residue was washed with water to remove phenol, transferred to a scintered glass funnel and washed with chloroform to methanol (1:1, v/v, 1 L) and acetone (1 to 2 L) until a fluffy consistency was obtained. The residue was dried at 60 °C and stored in a brown-colored glass bottle.

Extraction of cell walls. Cell walls 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 were stirred using a magnetic stirrer during extraction. Cell walls (1 g) were hydrated in 100 mL of 500 mmol·L^{-1} imidazole, pH 7 plus 8 mmol·L^{-1} sodium azide *in vacuo* and then extracted for 12 h at 1 °C. Samples were centrifuged and 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 dialyzes away against deionized water (Mort et al., 1991). The imidazole extracted residues were extracted with 100 mL of 50 mmol·L^{-1} sodium carbonate plus 20 mmol·L^{-1} 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 and 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 mol·L^{-1} KOH plus 10 mmol·L^{-1} sodium borohydride for 2 h at 1 °C and then for 2 h at 20 to 22 °C. Further extraction was carried out with 4 mol·L^{-1} KOH plus 10 mmol·L^{-1} sodium borohydride for 2 h at 20 to 22 °C and then with 4 mol·L^{-1} KOH plus 0.5 mol·L^{-1} boric acid for 2 h at 20 to 22 °C. Supernatants were adjusted to pH 5 with acetic acid, then dialyzed against deionized water and lyophilized. Two replications were done for pectin and hemicellulose extractions.

Size exclusion chromatography. Size exclusion chromatography was carried out on a column (1 × 50 cm) of Toyopearl HW55S (Supelco, Inc., Supelco Park, Bellefonte, Pa.) with 300 mmol·L^{-1} ammonium acetate, pH 5.2 as eluent. The stated separation range for globular proteins for this packing was 1,000 to 600,000 U. Samples (10 mg) were dissolved in 1 mL of 1 mol·L^{-1} imidazole, pH 7.0, before injection. A subsample of 400 μL (4 mg) was injected and eluted at a flow rate of 0.5 mL·min^{-1}. Peaks were detected using a refractive index detector (model R401; Waters Associates, Inc., Farmingham, Mass.) at an attenuation of 8×. The column was calibrated with pullulan standards (Polymer Laboratories Inc., Amherst, Mass.) of molecular mass 186,000, 100,000, 48,000, 12,200, 5,800 and glucose, and with pectic acid (Aldrich Chemical Co., Milwaukee, Wis.). Fractions were collected at 1 min intervals and pooled based on the elution time of peaks of interest. Pooled samples were dried under vacuum and rinsed repeatedly with water to remove most of the volatile elution buffer salts, and analyzed for sugar composition by gas chromatography (Komalavilas and Mort, 1989).

Anion exchange chromatography. Anion exchange separations were conducted for 4 mol·L^{-1} KOH plus 10 mmol·L^{-1} sodium borohydride extracts using Accell Plus QMA Sep-Pak cartridges (Waters Associates, Milford, Mass.). The Sep-Pak was preconditioned using 4 mL of 1 mol·L^{-1} ammonium acetate pH 5.2, followed by 4 mL of 50 mmol·L^{-1} ammonium acetate, pH 5.2. Five milligrams of the sample was suspended in 250 μL of 1 mol·L^{-1} imidazole, pH 7.0. When the samples were completely suspended, 250 μL of 1 mol·L^{-1} ammonium hydroxide was added to completely solubilize the sample. Then 100 μL (≈1 mg) of the supernatant was diluted to 1 mL with 50 mmol·L^{-1} ammonium acetate and applied to a preconditioned Sep-Pak. The effluent (neutral sugar fraction) was recovered and then 2 mL of 50 mmol·L^{-1} ammonium acetate was rinsed through the Sep Pak onto the effluent. Bound sugars were recovered into a separate container by elution with 2 mL of 1 mol·L^{-1} ammonium acetate (acidic sugar fraction). The nonbound and bound fractions were utilized for further analyses.

Sugar composition analysis. Desalted fractions 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, Inc., 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. Sugar residues were identified by comparison with authentic standards and quantified using inositol as internal standard.

Results and Discussion

The three stages of fruit ripening chosen for cell wall preparation for this study represented the commercial maturity stages of threshold mature (47 N), firm ripe (30 N), and soft ripe (15 N; Byrne et al., 1991). These key developmental stages represent three economically important developmental stages: harvest maturity (threshold maturity), retail market maturity (firm ripe) and fresh market consumption maturity (soft ripe). Recoveries of the pectin and hemicellulose extracts for cell walls of peaches with three different firmness levels is represented in our previous research (Hegde and Maness, 1996). Mass of pectin and hemicellulose fraction separated by size exclusion chromatography could

Table 1. Sugar composition of imidazole extracts on size exclusion column for cell walls of peaches differing in firmness.

Fruit	Sugar composition (mole percent)								
firmness[z]	Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
Firm (47 N)									
Peak[y] 1	17	3	1	5	8	1	3	61	1
Peak 2	5	1	1	1	3	1	1	85	2
Medium soft (30 N)									
Peak 1	17	3	1	4	26	1	1	40	7
Peak 2	10	2	1	1	10	1	4	66	6
Soft (15 N)									
Peak 1	46	8	1	3	16	1	1	18	6
Peak 2	13	3	1	1	8	1	4	63	6

[z]Fruit were sorted into firmness groups of 47 N (firm), 30 N (medium soft), and 15 N (soft) by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.
[y]Peak 1 (24 to 32 min) and peak 2 (33 to 56 min) were the apparent molecular size peaks of the imidazole extracts separated on Toyopearl HW55S column.

not be accurately determined due to the precipitation of volatile elution buffer salts, even after repeated rinsing with water. Aqueous-phenol soluble polysaccharides were recovered during peach cell wall preparation and their sugar composition was determined in our earlier work (Hegde and Maness, 1996). Because of very low recovery of aqueous phenol-soluble polysaccharides, we could not analyze it by size exclusion chromatography.

Imidazole extracts separated into two apparent molecular mass peaks, a high apparent molecular mass peak, represented by peak 1, and a lower apparent molecular mass broad polydisperse peak, represented by peak 2 (Fig. 1). The X-axis represents the elution time in minutes and the Y-axis represents the relative response of the R.I. detector relative to the composition of the effluent. High apparent molecular mass peaks eluted close to the void volume of the column and in the included volume was the salt peak, mostly originating from the 1 mol·L^{-1} imidazole, pH 7.0 buffer used to solubilize samples before injection. As fruit softened there was a proportional increase in size of peak 1 relative to peak 2 (Fig. 1). Peak 1 contained a higher mole percentage of arabinosyl, rhamnosyl and galactosyl residues, and lower mole percentage of galacturonosyl residues, than peak 2 (Table 1). These sugar compositional differences were accentuated in soft versus firm fruit. The galacturonosyl to rhamnosyl residue ratio decreased substantially in peak 1 as fruit softened from 20:1 in firm fruit, 13:1 in medium soft fruit, to 2:1 in soft fruit, but the ratio of rhamnosyl to arabinosyl residue remained the same, averaging 6:1. Imidazole is known to extract calcium and solubilize the calcium crosslinked homogalacturonan (HG) region (Mort et al., 1991). The HG region is known to consist primarily of a homopolymer of galacturonic acid, interrupted at fairly regular intervals (every 25 to 40 galacturonic acid residues) with rhamnose. The degree of decrease in galacturonosyl to rhamnosyl residue ratio in peak 1 for softening fruit, and the large apparent molecular mass for peak 1 (elution time greater than a 100,000 molecular mass pullulan polysaccharide standard), is not consistent with the HG region as a predominant source of the peak. The rhamnogalacturonan (RG) region is thought to consist of a backbone rich in galacturonic acid and rhamnose up to 100 sugar residues in length, and bears numerous sidechains rich in arabinose and galactose (McNeil et al., 1984). The low galacturonosyl to rhamnosyl residue ratio in peak 1, combined with a relatively constant rhamnosyl to arabinosyl residue ratio and the large apparent molecular size supports progressive cosolubilization of a relatively large rhamnogalacturonan-like region during peach softening, with little degradation of putative arabinan sidechains. In other fruit like pears, two major pectic polysaccharides obtained from progressively ripening fruit are a homogalacturonan (HG) region and a rhamnogalacturonan 1 (RG1) like polymer with a high arabinose content (Dick and Labavitch, 1989). The two pectic polymers are apparently not linked in pear. RG1 in ripening pears appears to be degraded with

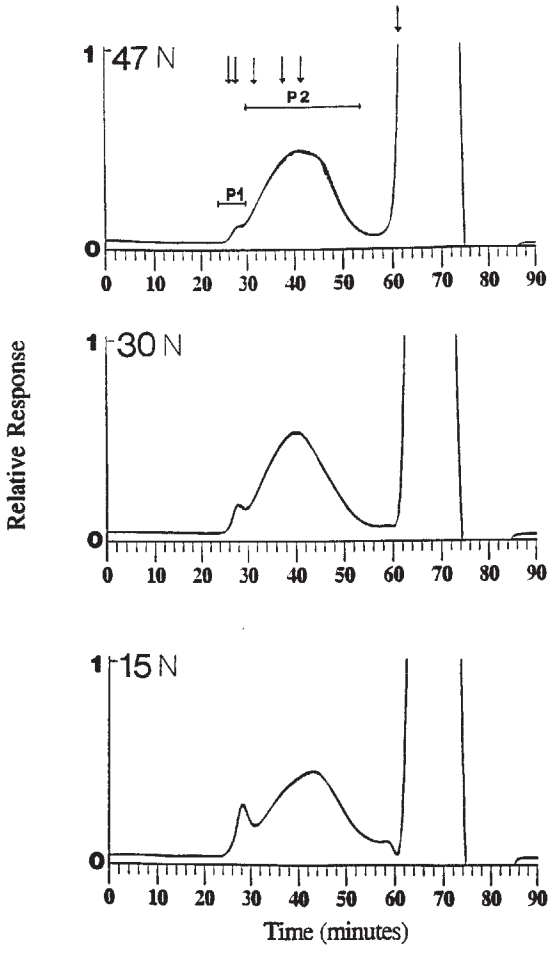

Fig. 1. Toyopearl HW55S apparent molecular size profiles of imidazole extract for cell walls of peaches differing in firmness (47 N firm, 30 N medium soft, and 15 N soft). The X-axis represents time in minutes and the Y-axis represents response of the R.I. detector relative to the composition of the effluent. P1 and P2 represent peak 1 and peak 2. Arrows at the top of the figure represent the elution positions of (from left to right) pullulan standards of molecular mass of 186,000, 100,000, 48,000, 12,200, 5,800, and glucose. Sugar composition of P1 and P2 are shown in Table 1.

Table 2. Sugar composition of sodium carbonate (cold) extracts on size exclusion column for cell walls of peaches differing in firmness.

Fruit	Sugar composition (mole percent)								
firmness[z]	Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
Firm (47 N)									
Peak[y] 1	21	4	3	4	9	5	7	46	1
Peak 2	25	5	1	1	9	1	1	56	1
Medium soft (30 N)									
Peak 1	17	4	1	5	16	9	5	42	1
Peak 2	24	4	1	2	17	1	4	46	1
Soft (15 N)									
Peak 1	31	2	1	3	18	8	3	33	1
Peak 2	23	6	1	1	15	2	1	50	1

[z]Fruit were sorted into firmness groups of 47 N (firm), 30 N (medium soft), and 15 N (soft), by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.
[y]Peak 1 (24 to 29 min) and peak 2 (30 to 50 min) were the apparent molecular size peaks of sodium carbonate (cold) extracts separated on Toyopearl HW55S column.

the initial loss of much of its arabinose. Our data for peach supports increased solubility of rhamnogalacturonan-like region with softening, with little selective loss of putative arabinose side chains. Peak 2 exhibited a higher mole percentage of galacturonic acid residues than peak 1, a substantially higher galacturonosyl to rhamnosyl residue ratio and probably represented a broad polydisperse peak more representative of the homogalacturonan-like region. As fruit softened, the galacturonosyl to rhamnosyl residue ratio decreased from 85:1 for firm fruit, 33:1 in medium soft fruit, to 21:1 in soft fruit. Arabinose contents of peak 2 were one half to one quarter of the contents found in peak 1. Some cosolubilization of rhamnogalacturonan-like region and homogalacturonan-like regions may have occurred during softening, as indicated by a slight increase in arabinose residue and in the galacturonosyl to rhamnosyl residue ratio. If rhamnogalacturonan-like region was present in peak 2, it was certainly less prominent than in peak 1.

In contrast to the results of Ranwala et al. (1992) and Huber and O'Donoghue (1993) we did not observe a distinct increase in proportion of the small apparent molecular mass peak as compared to the large apparent molecular mass peak for softening peaches. In persimmon (*Diospyros kaki* L.) fruit, the EDTA-soluble extract separates into large and small molecular mass classes and as fruit soften, there is a decrease in proportion of the large molecular mass class peak, but there is no change in the small molecular mass class peak (Cutillas-Iturralde et al., 1993). Peach imidazole soluble polysaccharides appeared to follow an intermediate pattern of change in molecular mass during softening, with increased solubility of a large rhamnogalacturonan-like polymer in softened fruit.

Sodium carbonate extracts exhibited different patterns of elution, depending on the extraction temperature. In sodium carbonate cold extracts peak 1 was less prominent in extracts of medium soft and soft fruit than in firm fruit, and there was a general redistribution into a smaller apparent molecular size for soft fruit compared to firm fruit (Fig. 2). Galacturonic acid residues decreased in peak 1 as fruit softened (Table 2). Peak 2 had a higher mole percentage of galacturonosyl residues than peak 1. Both peaks were enriched in pectin-associated sugars (galacturonic acid, arabinose and rhamnose) and only minor changes occurred during softening. In comparison with imidazole extracts, mole percent compositions of rhamnosyl and arabinosyl residues were generally higher, and galacturonosyl residues lower. Neutral sugars were also present in greater abundance in the sodium carbonate extracts compared to imidazole extracts. Glucosyl and mannosyl residues were enriched in peak 1, indicating association of neutral sugars with high apparent molecular mass pectic polymers and/or presence of high apparent molecular mass neutral sugar polymers coeluting with pectic polymers.

Sodium carbonate warm extracts separated into three distinct size classes, represented by peaks 1, 2, and 3 in order of elution (Fig. 3). As in imidazole extracts, peak 1 increased in prominence for extracts from softer fruit. Peaks 2 and 3 decreased substantially in prominence for softer fruit. In contrast to imidazole extracts, peak 1 contained the highest proportion of galacturonosyl residues and lowest proportion of arabinosyl and rhamnosyl residues when

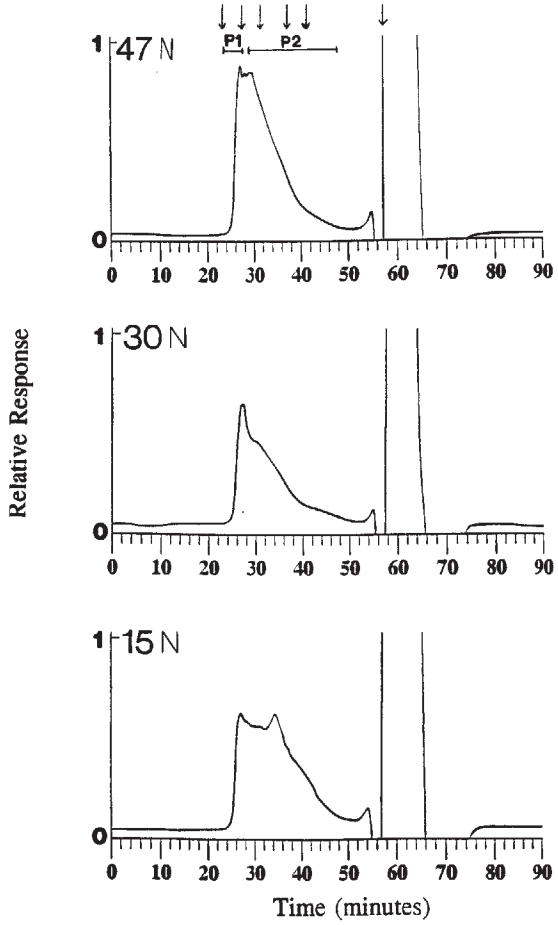

Fig. 2. Toyopearl HW55S apparent molecular size profiles of sodium carbonate (cold) extract for cell walls of peaches differing in firmness. See Fig. 1 for conditions. Sugar composition of P1 and P2 are shown in Table 2.

Table 3. Sugar composition of sodium carbonate (warm) extracts on size exclusion column for cell walls of peaches differing in firmness.

Fruit	Sugar composition (mole percent)								
firmness[z]	Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
Firm (47 N)									
Peak[y] 1	19	2	3	6	19	9	7	34	1
Peak 2	48	7	1	1	28	1	1	12	1
Peak 3	69	6	2	4	7	4	1	6	1
Medium soft (30 N)									
Peak 1	24	6	1	10	18	7	16	17	1
Peak 2	71	8	1	2	10	1	1	5	1
Peak 3	70	9	1	2	8	5	2	2	1
Soft (15 N)									
Peak 1	5	2	1	5	8	11	17	50	1
Peak 2	44	7	1	5	20	3	3	16	1
Peak 3	35	5	1	13	15	1	6	23	1

[z]Fruit were sorted into firmness groups of 47 N (firm), 30 N (medium soft), and 15 N (soft), by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.
[y]Peak 1 (24 to 32 min), peak 2 (33 to 42 min), and peak 3 (43 to 56 min) were the apparent molecular size peaks of sodium carbonate (warm extracts) separated on Toyopearl HW55S column.

compared to peaks 2 and 3 (Table 3). The order of elution for pectic polysaccharides in sodium carbonate warm extracts appeared to differ from imidazole extracts. A higher galacturonosyl to rhamnosyl residue ratio and lower arabinosyl to rhamnosyl residue ratio for peak 1 compared to peaks 2 and 3 (galacturonosyl to rhamnosyl residue ratio of 3:1 to 25:1 for peak 1, compared to 0.2:1 to 4:1 for peaks 2 and 3; arabinosyl to rhamnosyl residue ratio of 2:1 to 9:1 for peak 1, compared to 7:1 to 11:1 for peaks 2 and 3) for sodium carbonate warm extracts is consistent with separation of homogalacturonan-like polymers into large apparent molecular mass and rhamnogalacturonan-like polymers into smaller apparent molecular mass. As was noted for the cold sodium carbonate extracts, glucosyl and mannosyl residues were most strongly associated with peak 1. The substantial increase in predominance of peak 1 over peaks 2 and 3 in soft fruit, together with a high proportion of galacturonosyl residue and low proportions of arabinosyl and rhamnosyl residues in peak 1 of soft fruit, provides evidence that increased homogalacturonan-like polymer solubility was associated with softening in peaches. The coincident presence of relatively high percentages of glucosyl, galactosyl and mannosyl residues in this peak may be indicative of the presence of a gluco-mannan side chain or galacto-mannan side chain, or of coelution of a high apparent molecular mass gluco-mannan or galacto-mannan polymer with the pectic polysaccharides. In other fruit like persimmon, the pectic fraction extracted with warm sodium carbonate is composed of high molecular mass polymers, and shows a considerable shift towards the low molecular mass region as fruit soften. This shift is observed in both uronic acids and noncellulosic neutral sugars (Cutillas-Iturralde et al., 1994). The molecular mass of sodium carbonate extracts does not change appreciably during ripening of nectarine fruit (Dawson et al., 1992). Peach sodium carbonate elution profiles indicate a different pattern of polysaccharide solubility from persimmon or nectarine, with an increase in predominance of a high apparent molecular mass homogalacturonan-like polymer in soft fruit.

Hemicellulose extracts, obtained with increasing concentrations of KOH, exhibited three distinct peaks for fruit of all firmnesses. Loosely bound hemicelluloses were obtained with 1 mol·L^{-1} KOH at cold (4 °C) and then warm (22 °C) extraction temperatures. For cold 1 mol·L^{-1} KOH extracts, there was a decrease in proportion of the high apparent molecular mass peak (peak 1) and a shift in the proportion (shift in elution time) of the small apparent molecular mass peaks (peaks 2 and 3) toward a smaller size as fruit softened (Fig. 4). Peak 3 shifted in elution time from ≈40 min in firm and medium soft fruit to 42 min in soft fruit. As with sodium carbonate extracts, mannosyl residues were principally associated with high apparent molecular mass polymers

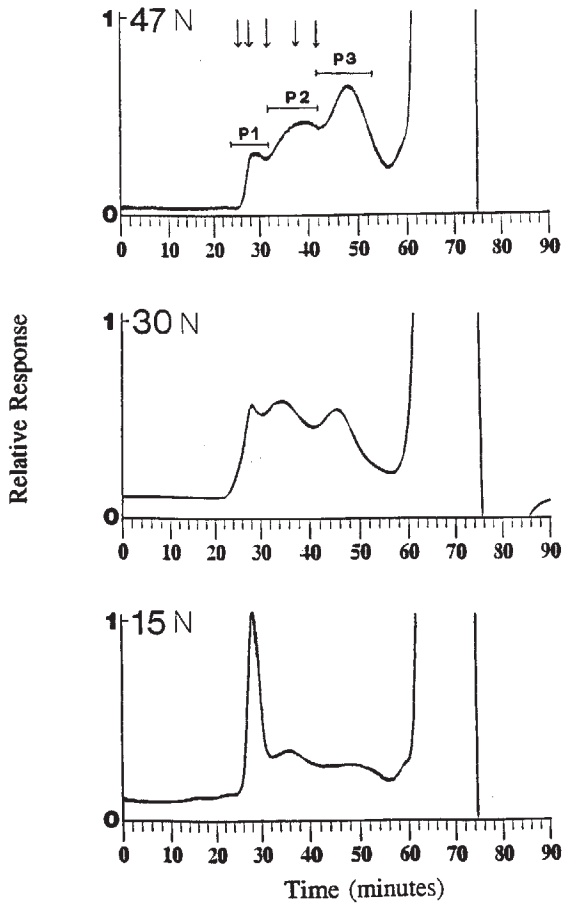

Fig. 3. Toyopearl apparent molecular size profiles of sodium carbonate (warm) extract for cell walls of peaches differing in firmness. P1, P2, and P3 represent peak 1, peak 2, and peak 3. See Fig. 1 for conditions. Sugar composition of P1, P2, and P3 are shown in Table 3.

Table 4. Sugar composition of 1 mol·L^{-1} potassium hydroxide (cold) extracts on size exclusion column for cell walls of peaches differing in firmness.

Fruit	Sugar composition (mole percent)								
firmness[z]	Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
Firm (47 N)									
Peak[y] 1	5	1	1	46	7	12	19	9	1
Peak 2	3	1	6	36	13	3	34	3	1
Peak 3	2	1	2	12	57	1	19	5	1
Medium soft (30 N)									
Peak 1	3	1	1	47	9	13	16	9	1
Peak 2	6	1	6	36	17	2	27	4	1
Peak 3	2	1	1	17	19	1	23	35	1
Soft (15 N)									
Peak 1	9	1	2	18	17	17	17	18	1
Peak 2	4	1	7	20	29	4	29	5	1
Peak 3	4	1	3	21	22	4	26	18	1

[z]Fruit were sorted into firmness groups of 47 N (firm), 30 N (medium soft), and 15 N (soft), by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.
[y]Peak 1 (23 to 30 min), peak 2 (31 to 41 min), and peak 3 (42 to 56 min) were the apparent molecular size peaks of 1 mol·L^{-1} potassium hydroxide (cold) extracts separated on Toyopearl HW55S column.

and mannose increased in proportion to other sugars for softer fruit (Table 4). In contrast to either sodium carbonate (Tables 2 and 3) or imidazole (Table 1) extracts, the pectin-associated sugars galacturonic acid, arabinose and rhamnose were generally only minor constituents of cold 1 mol·L^{-1} KOH extracts. Xylose predominated as the major constituent of peak 1 in firm and medium soft fruit. In peak 1, soft fruit contained a lower xylosyl to glucosyl residue ratio (1:1) compared with medium soft and firm fruit (3:1). Xylosyl and glucosyl residues were associated with higher percentages of fucosyl residues in peak 2 compared with the other two peaks. Xylosyl to glucosyl residue ratio was close to 1:1 in peak 2 for fruit of all firmness groups. Peak 2 appeared to be most xyloglucan-like and fucose was highly associated with it. This peak decreased in apparent molecular mass from medium soft to soft fruit, indicating that a general decrease in apparent molecular mass for xyloglucan-like polymer may have been associated with the later stages of softening in peach. The warm 1 mol·L^{-1} KOH extract exhibited an increase in proportion of peak 1 to peaks 2 and 3 in softer fruit, associated with a shift in proportion of the smaller apparent molecular mass peaks from peak 2 to peak 3 (Fig. 5). Xylosyl to glucosyl residue ratios ranged from slightly >2:1 to slightly <1:1 for all peaks (Table 5). Fucosyl residues were present in highest content in peak 2 for firm fruit, but shifted in elution pattern to peak 1 for medium soft and soft fruit. Assuming fucose is a xyloglucan-associated sugar, the shift in elution pattern from peak 2 in firm fruit to peak 1 in medium soft and soft fruit indicated that at least one fraction of xyloglucan-like polymer extracted as a higher apparent molecular mass polysaccharide during softening in peach. The increase in proportion of peak 1 associated with softening, combined with a constant or increased fucosyl residue mole percent, indicated that the quantity of extractable, high molecular mass xyloglucan-like polymer increased during softening. Warm 1 mol·L^{-1} KOH extracts contained substantially more pectin-associated sugars than cold 1 mol·L^{-1} KOH extracts, and they eluted predominantly as small oligomers in peak 3 from fruit of all firmnesses and were consistently low in the peaks containing more fucose. The high content of xylose and arabinose in peaks 1 and 2 compared with peak 3 indicate the presence of xylans and arabinoxylans. Xylans constitute the major hemicellulose in the primary cell walls of monocots and are found in smaller amounts in the primary cell walls of dicots. Arabinose is the most common side chain of xylans (McNeil et al., 1984).

Taken together, elution patterns for both cold and warm 1 mol·L^{-1} KOH extracts indicated a change in xyloglucan-like polymer elution pattern associated with stage of peach softening, with an increase in solubility of high molecular mass polymers occurring first when fruit were medium soft, followed by a slight

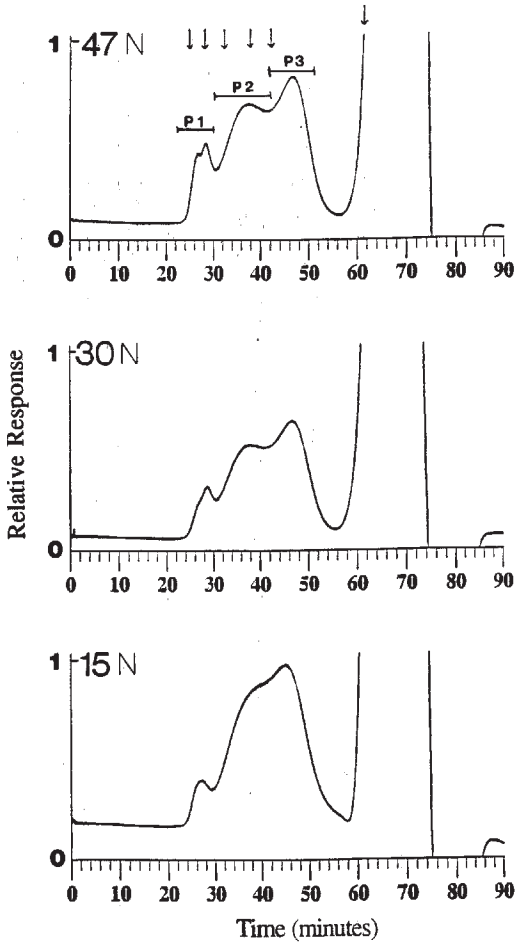

Fig. 4. Toyopearl HW55S apparent molecular size profiles of 1 mol·L^{-1} KOH (cold) extract for cell walls of peaches differing in firmness. P1, P2, and P3 represent peak 1, peak 2, and peak 3. See Fig. 1 for conditions. Sugar composition of P1, P2, and P3 are shown in Table 4.

Table 5. Sugar composition of 1 mol·L^{-1} potassium hydroxide (warm) extracts on size exclusion column for cell walls of peaches differing in firmness.

Fruit	Sugar composition (mole percent)								
firmness[z]	Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
Firm (47 N)									
Peak[y] 1	13	2	1	21	25	4	24	9	1
Peak 2	13	2	9	34	11	2	21	7	1
Peak 3	12	1	1	13	9	1	16	46	1
Medium soft (30 N)									
Peak 1	13	3	12	16	24	3	21	7	1
Peak 2	19	3	4	23	13	2	9	26	1
Peak 3	17	3	1	11	8	1	11	47	1
Soft (15 N)									
Peak 1	18	4	8	30	8	3	22	6	1
Peak 2	17	3	5	13	16	1	14	30	1
Peak 3	11	3	2	11	5	1	13	53	1

[z]Fruit were sorted into firmness groups of 47 N (firm), 30 N (medium soft), and 15 N (soft), by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.
[y]Peak 1 (24 to 31 min), peak 2 (32 to 42 min), and peak 3 (43 to 56 min) were the apparent molecular size peaks of 1 mol·L^{-1} potassium hydroxide (warm) extracts on Toyopearl HW55S column.

decrease in molecular mass of smaller polymers when fruit were soft. In softening persimmon fruit the average molecular mass for xyloglucan present in the 1 mol·L^{-1} KOH hemicellulose fraction increased up to a certain stage and then decreased in the last stage of fruit softening (Cutillas-Iturralde et al., 1994). A general decrease in molecular mass of alkali-soluble hemicellulose, and a shift in proportion from high molecular mass to low molecular mass polymers, has been reported for softening papaya (*Carica papaya* L.) fruit (Lazan et al., 1995).

More tightly bound hemicelluloses were obtained by extraction at 22 °C with 4 mol·L^{-1} KOH plus sodium borohydride followed by 4 mol·L^{-1} KOH plus boric acid. 4 mol·L^{-1} KOH plus sodium borohydride extracts were separated into four apparent molecular size peaks, one large apparent molecular size peak represented by peak 1, and three smaller apparent molecular size peaks represented by peak 2, peak 3, and peak 4. There was an increase in peak 1 relative to peaks 2, 3, and 4 during early stages of softening (in medium soft compared to firm fruit), and a decrease in peak 1 relative to peaks 2, 3, and 4 by later stages of softening (soft fruit) (Fig. 6). The pectin-associated sugars eluted primarily as high apparent molecular mass polymers in peak 1 for fruit of all firmnesses (Table 6), in contrast to elution primarily as small molecular mass polymers in the warm 1 mol·L^{-1} KOH extract (Table 5). Smaller apparent molecular mass fractions were enriched in xylosyl, glucosyl, galactosyl and mannosyl residues. In fruit of all firmnesses, the xylosyl to glucosyl residue ratio was close to 1:1 for peaks 1, 2, and 3, with a sharp decrease in peak 4 to 0.4:1 for firm fruit and 0.2:1 in medium soft and soft fruit. Fucosyl residues were most strongly associated with peak 1 in firm fruit, and with peaks 2 and 3 in medium soft and soft fruit. Using fucose as a xyloglucan-associated sugar, the elution pattern for xyloglucan-like polymer in 4 mol·L^{-1} KOH extracts appeared to follow a general decrease in molecular mass during the early stages of softening in peach. This is in contrast to the pattern of extraction of higher molecular mass xyloglucan in the more loosely bound warm 1 mol·L^{-1} KOH extract (Table 5). There appears to be at least two types of hemicellulose in peach which are altered differently during softening: a more loosely bound high molecular mass fraction which increases in solubility with softening and a more tightly bound fraction which decreases in apparent molecular mass with softening.

The increase in predominance of peak 1 during early softening stages, and the strong association of pectin-associated sugars with peak 1, lead us to further fractionate the 4 mol·L^{-1} KOH fraction into charged and neutral fractions using anion exchange batch chromatography. Anion exchange separation for this extract, followed by size exclusion chromatography of the charged and neutral fractions, revealed that the larger apparent molecular mass

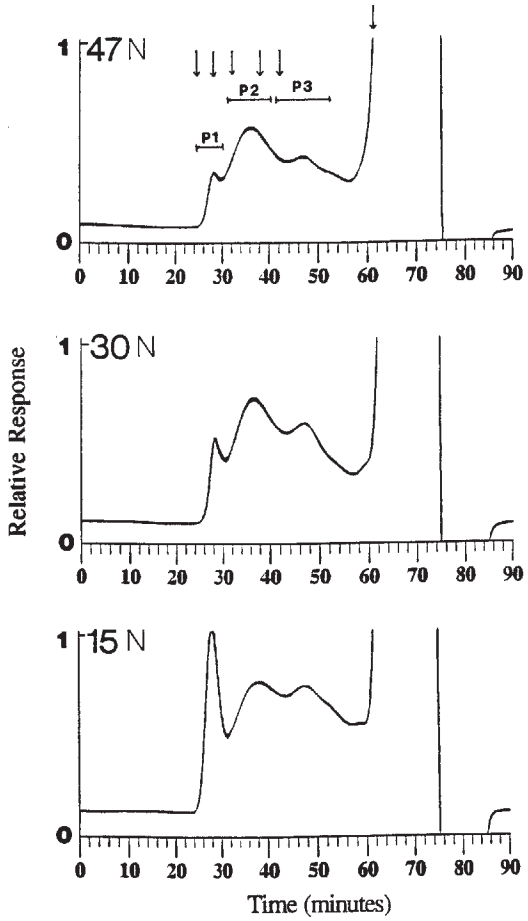

Fig. 5. Toyopearl HW55S apparent molecular size profiles of 1 mol·L^{-1} KOH (warm) extract for cell walls of peaches differing in firmness. P1, P2, and P3 represent peak 1, peak 2, and peak 3. See Fig. 1 for conditions. Sugar composition of P1, P2, and P3 are shown in Table 5.

Table 6. Sugar composition of 4 mol·L^{-1} potassium hydroxide plus sodium borohydride extracts on size exclusion column for cell walls of peaches differing in firmness.

Fruit	Sugar composition (mole percent)								
firmness[z]	Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
Firm (47 N)									
Peak[y] 1	26	5	5	10	16	7	12	18	1
Peak 2	20	4	3	18	23	3	18	10	1
Peak 3	13	2	3	13	20	19	19	10	1
Peak 4	16	2	2	3	9	51	8	8	1
Medium soft (30 N)									
Peak 1	35	7	1	9	16	3	12	16	1
Peak 2	25	5	5	17	19	2	16	10	1
Peak 3	11	2	5	17	18	16	18	12	1
Peak 4	17	1	1	2	8	56	10	4	1
Soft (15 N)									
Peak 1	30	6	1	13	16	4	14	15	1
Peak 2	24	5	4	15	20	3	18	10	1
Peak 3	10	2	5	17	21	23	12	9	1
Peak 4	18	1	1	2	19	46	8	4	1

[z]Fruit were sorted into firmness groups of 47 N (firm), 30 N (medium soft), and 15 N (soft), by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.
[y]Peak 1 (22 to 28 min), peak 2 (29 to 36 min), peak 3 (37 to 46 min), and peak 4 (47 to 53 min) were the apparent molecular size peaks of 4 mol·L^{-1} potassium hydroxide plus sodium borohydride extracts separated on Toyopearl HW55S column.

peak, represented by peak 1 was almost exclusively charged and presumably of pectin origin (Fig. 7). Similar to the original extract, there was an increase in peak 1 during early stages of softening. This fraction contained a high mole percentage of galacturonosyl, arabinosyl and rhamnosyl residues (Table 7). The galacturonosyl to rhamnosyl residue ratio decreased in peak 1 with softening from 3:1 for firm fruit to 2:1 for medium soft and soft fruit. The arabinosyl to rhamnosyl residue ratio remained the same, averaging 5:1. Apparently a rhamnogalacturonan-like region cosolubilized with hemicellulose in the 4 mol·L^{-1} KOH extract with little degradation of putative arabinan side chains. This is similar to the imidazole rhamnogalacturonan-like region in which putative arabinan sidechains appeared to remain intact during softening. Xylosyl to glucosyl residue ratio remained closer to 1:1 in this peak in fruit of all firmness groups and especially in medium soft and soft fruit. The fractions for the second, third and the fourth peak of the original extract for the acidic fraction was also enriched in galacturonosyl, arabinosyl and rhamnosyl residues. Higher mole percentage of galacturonosyl residue and lower mole percentage of arabinosyl and rhamnosyl residues were found in the third and fourth peak, compared to the second peak. KOH at 4 mol·L^{-1} is known to extract hemicelluloses. Coextraction of a rhamnogalacturonan-like region with xyloglucan-like region supports an assumption that pectins and hemicelluloses are associated with each other. Since only a small proportion of sugars present in all peaks from the charged fraction were apparently xyloglucan associated, the nature of the interaction appears not to be by covalent pectin-xyloglucan crosslinking.

The nonbound Sep Pak fraction separated on the size exclusion column (Fig. 8) as peaks 2 and 3 of the native extract (Fig. 6) and was composed of neutral sugars like xylose, glucose and mannose (Table 8). In agreement with apparent molecular mass shifts for the native 4 mol·L^{-1} KOH fraction (Fig. 6), there was a proportional shift in predominance of peak 2 to peak 3 as fruit softened, with a concurrent redistribution of mannose from peak 2 to peak 3. Fucose remained approximately equimolar between the two peaks, indicating that xyloglucan-like polymer was probably equally dispersed between the peaks, and subsequent changes in relative proportion of peak 2 to peak 3 were accompanied by shifts in xyloglucan-like polymer apparent molecular mass. Sugars eluting

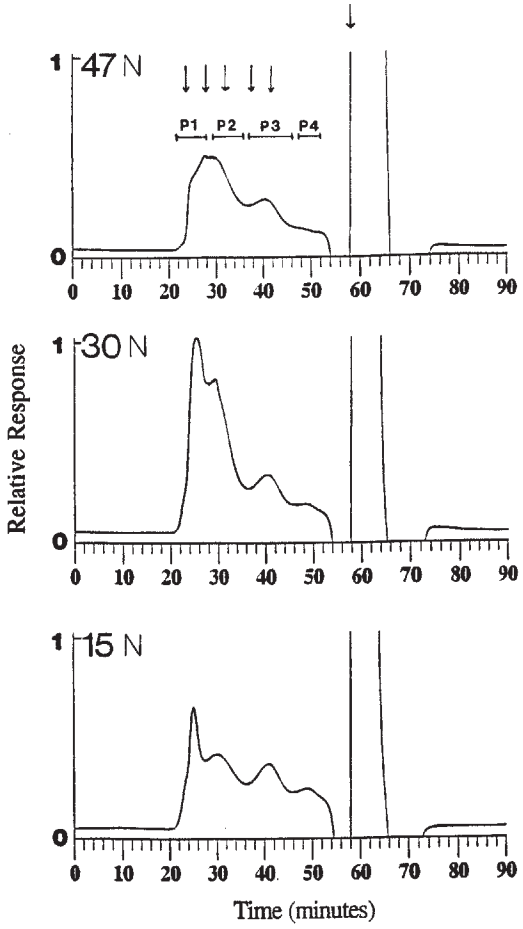

Fig. 6. Toyopearl HW55S apparent molecular size profiles of 4 mol·L^{-1} KOH plus sodium borohydride extract for cell walls of peaches differing in firmness. P1, P2, P3, and P4 represent peak 1, peak 2, peak 3, and peak 4. See Fig. 1 for conditions. Sugar composition of P1, P2, P3, and P4 are shown in Table 6.

Table 7. Sugar composition of acidic fraction of 4 mol·L^{-1} potassium hydroxide plus sodium borohydride extracts on size exclusion column for cell walls of peaches differing in firmness.

Fruit	Sugar composition (mole percent)								
firmness[z]	Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
Firm (47 N)									
Peak[y] 1	38	7	1	4	14	4	7	24	1
Peak 2	37	7	1	3	14	1	3	33	1
Peak 3	28	6	1	3	10	5	4	42	1
Peak 4	27	6	1	3	10	6	8	38	1
Medium soft (30 N)									
Peak 1[y]	43	9	1	3	21	2	2	18	1
Peak 2	39	9	1	3	11	1	2	33	1
Peak 3	19	5	1	3	10	5	4	52	1
Peak 4	17	5	1	3	8	8	5	52	1
Soft (15 N)									
Peak 1	40	8	1	6	14	6	7	17	1
Peak 2	30	8	1	6	14	5	9	26	1
Peak 3	23	5	1	5	11	8	8	38	1
Peak 4	19	4	1	4	11	16	9	35	1

[z]Fruit were sorted into firmness groups of 47 N (firm), 30 N (medium soft), and 15 N (soft), by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.
[y]Peak 1 (23 to 30 min), peak 2 (31 to 40 min), peak 3 (41 to 48 min), and peak 4 (49 to 54 min) are the apparent molecular size peaks of acidic fraction of 4 mol·L^{-1} potassium hydroxide plus sodium borohydride extracts separated on Toyopearl HW55S column.

in the same location as Peak 4 for the whole extract (Fig. 6) contained considerably more arabinosyl and mannosyl residues, and less fucosyl residues, than peaks 2 and 3. It also contained high mole percentage of glucosyl and galactosyl residues.

The most tightly bound hemicellulose fraction, obtained with 4 mol·L^{-1} KOH plus boric acid, exhibited a prominent high apparent molecular mass peak (peak 1) in extracts from fruit of all firmnesses (Fig. 9). There was a shift in predominance from peak 2 to peak 3 as fruit softened, especially between the medium soft and soft developmental stages. A substantial increase in arabinosyl residue content of peak 3 in soft fruit (Table 9) accompanied the increase in peak 3 predominance. In contrast to 4 mol·L^{-1} KOH plus sodium borohydride extracts, this extract contained high mole percentage of pectin-associated sugars in both peaks 1 and 2, and neutral sugars predominated only in peak 3 (as opposed to both peaks 2 and 3). The 4 mol·L^{-1} KOH peach hemicellulose-associated sugars appeared to redistribute from higher to lower apparent molecular mass during softening. This pattern is similar to observations in other fruit, like tomato (Huber, 1983; Tong and Gross, 1988), hot pepper (Gross et al., 1986), strawberry (*Fragaria ×ananassa* Duch.) (Huber, 1984), muskmelon (McCollum et al., 1989), kiwifruit (Redgewell et al., 1991) and avocado (O'Donoghue and Huber, 1992).

The ripening related increase or decrease for a given peak relative to the other peak in an extract could be due to more of the material solubilized in that extract or it could be less of the material in the extract representing that peak. Increased solubilization of rhamnogalacturonan-like region in imidazole extracts during softening could be due to the increased extraction of the RG-like region in soft fruit or could be due to the aggregation of pectin polymers, even though a suitable ionic strength buffer such as imidazole was used. It could be due to the increase in the proportion of monomers present in the backbone of the polysaccharide and also as a consequence of concentration of putative arabinan side chain. The apparent low molecular mass HG-like region appeared as a polydisperse peak in imidazole soluble extracts. The decrease in galacturonic acid in the HG-like region during softening was apparently not due to its loss during dialysis of extracts from walls of soft fruit. It has been reported that oligomers of D-galacturonic acid, with as low a degree of polymerization as 3 in 1,000

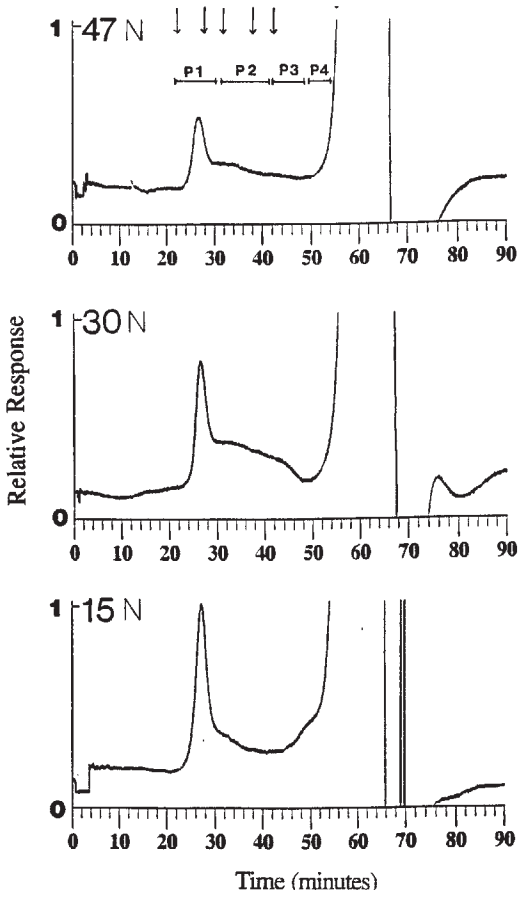

Fig. 7. Toyopearl HW55S apparent molecular size profiles of acidic fraction of 4 mol·L^{-1} KOH plus sodium borohydride extract for cell walls of peaches differing in firmness. P1, P2, and P3 and P4 represent peak 1, peak 2, and peaks 3 and 4. See Fig. 1 for conditions. Sugar composition of P1, P2, P3, and P4 are shown in Table 7.

Table 8. Sugar composition of neutral fraction of 4 mol·L^{-1} potassium hydroxide plus sodium borohydride extracts on size exclusion column for cell walls of peaches differing in firmness.

Fruit	Sugar composition (mole percent)								
firmness[z]	Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
Firm (47 N)									
Peak[y] 2	7	1	5	19	26	9	33	0	0
Peak 3	5	1	4	12	18	40	20	0	0
Peak 4	13	1	2	2	13	55	14	0	0
Medium soft (30 N)									
Peak 2	3	1	8	23	23	7	35	0	0
Peak 3	8	1	7	17	18	31	18	0	0
Peak 4	20	1	2	2	14	49	12	0	0
Soft (15 N)									
Peak 2[y]	3	1	8	29	18	4	37	0	0
Peak 3	3	1	7	11	16	40	22	0	0
Peak 4	16	1	1	2	11	47	22	0	0

[z]Fruit were sorted into firmness groups of 47 N (firm), 30 N (medium soft), and 15 N (soft), by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.
[y]Peak 2 (24 to 38 min), peak 3 (39 to 50 min), and peak 4 (51 to 55 min) were the apparent molecular size peaks of neutral fraction of 4 mol·L^{-1} potassium hydroxide plus sodium borohydride extracts separated on Toyopearl HW55S column.

molecular weight cutoff dialysis tubing or 10 in 12,000 to 14,000 molecular weight cutoff tubing, are retained upon dialysis against distilled water (Mort et al., 1991). We used 6,000 to 8,000 molecular weight cutoff dialysis tubing in our study. The decrease in galacturonic acid in the HG-like region during softening could be due to the selective cleavage of galacturonic acid by exopolygalacturonase enzyme. Solubilization of the RG-like region with softening and some cosolubilization of the RG-like and HG-like region could be due to the combined action of endo- and exo-polygalacturonase enzyme. Free stone peaches like 'Belle of Georgia' contain both endo- and exo-polygalacturonase enzyme. The activity of endopolygalacturonase enzyme (Pressey et al., 1971) and exopolygalacturonase enzyme (Downs and Brady, 1990) increase with softening of peach.

Loss of firmness or softening is one of the most dramatic processes that occur during ripening of fruit. In most of the fleshy fruit, including peaches, flesh firmness serves as an important attribute, affecting the edible quality and shelf life of the fruit. It is probably the most reliable index of maturity and is an economically important attribute. Our results indicate that peach softening is associated with changes in sugar composition (Hegde and Maness, 1996) and in apparent molecular mass for both pectins and hemicelluloses. Increased solubility of a putative rhamnogalacturonan-like region of high apparent molecular mass accompanied softening in imidazole-soluble extracts as a pectin constituent, and again in 4 mol·L^{-1} KOH extracts as a tightly bound hemicellulose constituent. Apparently, putative arabinan sidechains in the pectin-soluble rhamnogalacturonan-like region and hemicellulose-soluble rhamnogalacturonan-like region remained relatively intact during softening. Homogalacturonan-like region appeared primarily as an apparently polydisperse molecular mass class in imidazole soluble extracts, which did not change substantially in magnitude as fruit softened. Changes in hemicellulosic xyloglucan-like region were noted in more loosely bound fractions as both an increase in solubility of high apparent molecular mass polymers, compared to lower molecular mass polymers, as a relatively early event, and a decrease in apparent molecular mass of smaller polymers as a later event (Figs. 4 and 5, Tables 4 and 5). In more tightly bound hemicellulose extracts, the decrease in apparent molecular mass was detected, predominantly as a later event in softening. The occurrence of pectin-associated sugars in tightly bound hemicellulose extracts suggests that pectins and hemicelluloses are in some way associated with each other. Coextraction of the rhamnogalacturonan-like region with more tightly bound hemicellulose supports this concept. The ability to

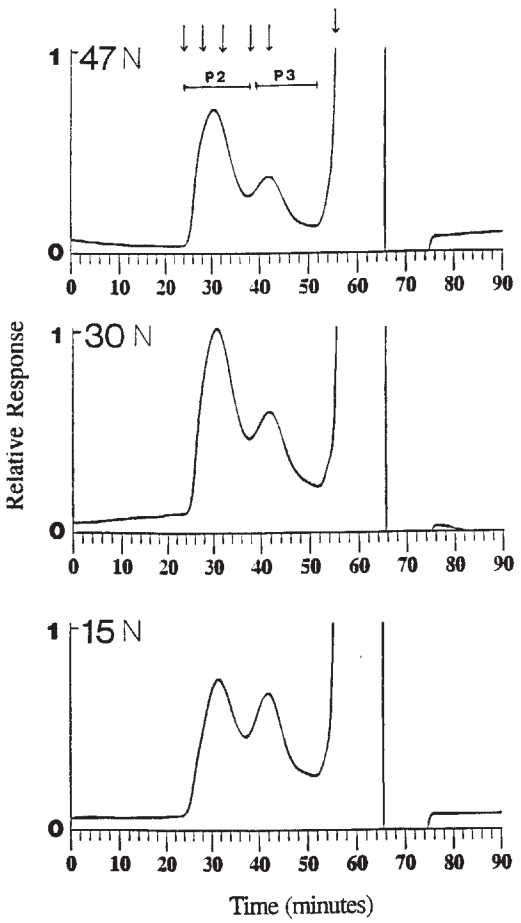

Fig. 8. Toyopearl HW55S apparent molecular size profiles of neutral fraction of 4 mol·L^{-1} KOH plus sodium borohydride extract for cell walls of peaches differing in firmness. P2 and P3 represent peak 2 and peak 3 of the original extract. See Fig. 1 for conditions. Sugar composition of P2, P3, and P4 are shown in Table 8.

Table 9. Sugar composition of 4 mol·L^{-1} potassium hydroxide plus boric acid extracts on size exclusion column, for cell walls of peaches differing in firmness.

Fruit firmness[z]	Sugar composition (mole percent)								
	Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
Firm (47 N)									
Peak[y] 1	22	3	1	7	20	19	11	16	1
Peak 2	28	8	2	5	16	9	6	25	1
Peak 3	20	2	1	2	38	21	9	6	1
Medium soft (30 N)									
Peak 1	33	1	6	9	17	6	9	18	1
Peak 2	30	5	1	6	14	16	9	18	1
Peak 3	11	5	4	4	8	52	9	6	1
Soft (15 N)									
Peak 1	28	4	2	10	19	5	11	20	1
Peak 2	21	4	4	11	16	11	18	14	1
Peak 3	48	1	1	3	14	18	12	2	1

[z]Fruit were sorted into firmness groups of 47 N (firm), 30 N (medium soft), and 15 N (soft), by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.
[y]Peak 1 (23 to 31 min), peak 2 (32 to 41 min), and peak 3 (42 to 56 min) were the apparent molecular size peaks of 4 mol·L^{-1} potassium hydroxide plus boric acid extracts separated on Toyopearl HW55S column.

separate pectin and xyloglucan-like regions as two separate fractions based on charge suggests that the nature of the pectin-hemicellulose interaction is probably one of physical entrapment of pectin fractions by hemicellulose as in onion (*Allium cepa* L.) (McCann et al., 1990). Although some crosslinking may occur, it is not likely that significant covalent bonding important for regulating softening processes between the polysaccharide classes occurs in peach. The complexity of apparent molecular mass changes in pectin and hemicellulose extracts for peaches appears to implicate the action of a number of different enzymes capable of degrading both pectin and hemicellulose cell wall components during softening.

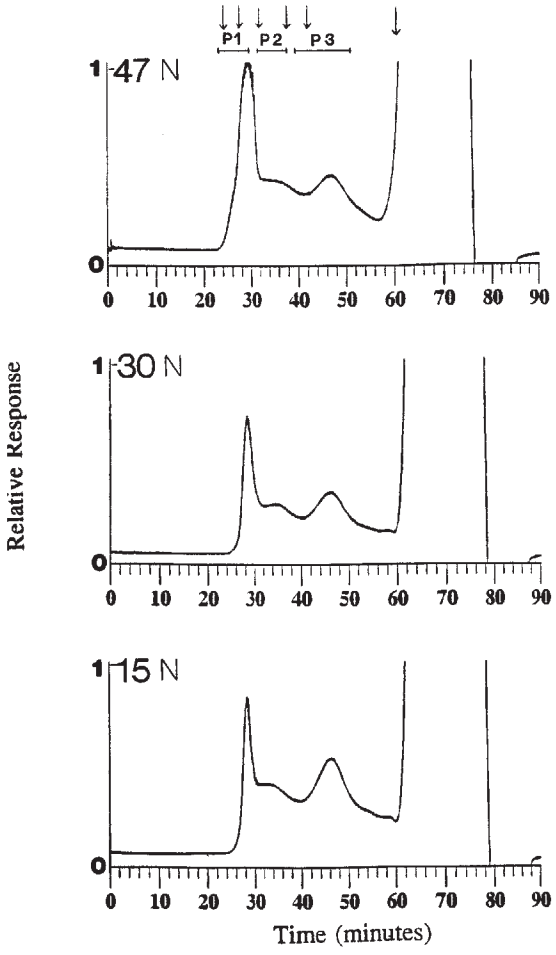

Fig. 9. Toyopearl HW55S apparent molecular size profiles of 4 mol·L^{-1} KOH plus boric acid extract for cell walls of peaches differing in firmness. P1, P2, and P3 represent peak 1, peak 2, and peak 3. See Fig. 1 for conditions. Sugar composition of P1, P2, and P3 are shown in Table 9.

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