Cell Wall Metabolism in Ripening Fruit

IV. Characterization of the Pectic Polysaccharides Solubilized during Softening of ‘Bartlett’ Pear Fruit

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ABSTRACT

Fractionation of pectic polysaccharides from the juice of ripening ‘Bartlett’ pears (Pyrus communis) gave two general types of polyuronides. The major type was a homogalacturonan (HGA) whose molecular weight decreased upon ripening. The other type comprised heteropolymers composed of various amounts of arabinose, rhamnose, and galactose. Treatment of the major arabinose-containing heteropolymeric fraction with high molecular weight (400,000) with a pear exo-polygalacturonase to degrade contaminating HGA gave a polyuronide which was inert to tomato endopolygalacturonase. Glycosyl-linkage analysis of this arabinose-rich fraction revealed results expected from a rhamnogalacturonan I-like polysaccharide with large, highly branched arabinose side chains (RG-I). A linkage between HGA and RG-I was not found. RG-I, in ripening pears, appeared to be degraded with the initial loss of much of its arabinose.

The loss of flesh texture experienced by pome fruits upon ripening is attributable, in part, to dissolution of the ordered arrangement of cell-wall and middle lamella polysaccharides (12). Cell-wall preparations derived from progressively ripe fruit show a decrease in the content of pectic polysaccharides especially. Accompanying the preferential loss of galacturonic acid and of certain neutral sugars, notably D-galactose from apple (11), and L-arabinose from pear (9), cell walls are corresponding increases in the ripening fruit of water-soluble pectic polysaccharides containing these sugars (1, 16). These are of interest because they accumulate and are readily isolated in the form in which they existed at the time of extraction. They were not obtained with the use of extraction procedures (chemical or enzymic) that could release from isolated cell wall preparations polymers that could be altered from those present in vivo. The structures of the water-soluble pectic polysaccharides result from native biosynthetic and degradative processes and the analysis of these structures will give clues to the structure of pectin in native undegraded cell walls and to the enzymes involved in ripening-associated pectin breakdown. Their accumulation raises several questions concerning their origin, biological function, and apparent metabolic stability. The latter may be due to a lack of suitable hydrolytic enzymes in the ripening fruit or to a peculiar structure which protects the pectic polysaccharides from further degradation. Potent glycoside hydrolyzing activities of pear fruit, namely PG2 (16), and α-L-arabinofuranosidase (2) might be expected to rapidly degrade the water-soluble pectins which appear to accumulate in ripening pears (1).

The present paper describes the change in quantity of the major pectic polysaccharide fractions obtained from the juice of progressively more ripe ‘Bartlett’ pear fruit. The characterization of these pectins in terms of composition, mol wt, linkage, and susceptibility to the isolated enzymes named above shows that the two major pectin types in pears, HGA, and a rhamnogalacturonan I-like polymer with a high arabinose content are not linked in pear juice and are degraded by very different mechanisms as pear fruit ripen.

MATERIALS AND METHODS

Source of Fruit

Mature green Bartlett pears (Pyrus communis) were obtained from a commercial fruit storage cooperative at Sacramento, CA, and stored at −1°C until needed (less than 1 month). Fruits were selected for uniformity of size and freedom from blemishes. Fruits were ripened at 20°C. Changes in flesh firmness were determined using a UC fruit firmness tester (10) fitted with a 0.79 cm tip. Firmness data are reported as Newtons (N).

Preparation of Pectin Fractions from Pear Juice

Fruit at designated stages of ripeness (based on flesh firmness) were peeled, cored, and sliced. Fruit flesh slices were pressed through several layers of cheesecloth. The clear juice was added directly to 4 volumes of boiling ethanol and the mixture boiled for a further 10 min. After storage at 2°C, precipitated material was collected by centrifugation (8000g, 5 min) in a Sorvall model RC2-B refrigerated centrifuge. The pellet was suspended in 50 mM Na-acetate (pH 5.0) until solution appeared complete (generally 30 min) and the mix-

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2 Abbreviations: PG, polygalacturonase; PE, pectin esterase; PGA, polygalacturonic acid; HGA, homogalacturonan; RG-I, rhamnogalacturonan I; endo, endoglycosidase.

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ture centrifuged as above. The supernatant solution was added to 4 volumes of ethanol and held at 2°C overnight. The precipitated material was again collected by centrifugation, dissolved in pH 5.0 acetate, and applied to a column of DEAE-agarose (Bio-Rad) that had been equilibrated with the same buffer. The column was eluted with portions of pH 5.0 acetate containing 0, 100, and 500 mM NaCl as indicated in Figure 2. Column fractions were assayed colorimetrically for uronic acids according to Blumenkrantz and Asboe-Hansen (4) and for pentose using the orcinol reaction (6). The fractions containing the most prominent peak eluted by each buffer were pooled and added to 4 volumes of ethanol. The precipitates were collected by centrifugation, dissolved in distilled water, and lyophilized. The lyophilized material was dissolved in 50 mM Na acetate (pH 5.0) applied to a column of Bio-Gel A-5m (Bio-Rad), and eluted with the same buffer. Column fractions were assayed as above and fractions containing the prominent peaks pooled as indicated in Figures 3 and 4, precipitated with 4 volumes of ethanol, and the precipitated material dissolved in distilled water and lyophilized. The carbohydrate composition of each pooled fraction was analyzed by GLC.

Carbohydrate Analysis

Neutral sugar compositions of samples of all pectin fractions were determined by GLC of the derived alditol acetates according to the technique of Albersheim et al. (3). Glycoside linkage compositions were determined by methylation analysis according to the method of Hakamori (7) as modified by Sandford and Conrad (19) but using K+ DMSO− (8). The methylated polysaccharides were carboxyl reduced with Na borodeuteride according to the Waeghe et al. (23) modification of the procedure of Taylor and Conrad (21) and subsequently was hydrolyzed, reduced (NaBD4), and acetylated (23) prior to GLC analysis. GLC was performed with a Sigma 3 instrument (Perkin Elmer) fitted with a DB225 column operated at an H2 pressure of 15 psi and at 210°C for alditol acetates and from 150 to 210°C (1° min−1 oven programming) for partially methylated alditol acetates. The integration of peak areas and calculation of sugar concentrations was carried out with a Sigma 10 data system (Perkin Elmer). The identity of chromatographic peaks was determined by cochromatography against standards and combined GC-MS performed at the Facility for Advanced Instrumentation (University of California, Davis). Response factors for determination of concentrations of partially methylated alditol acetates from GLC peak areas were those of Sweet et al. (20).

Methyl Ester Determination

The method of Wood and Siddiqui (24) was used for analysis of the methyl ester content of pectin fractions.

Enzyme Preparations

α-L-Arabinofuranosidase was prepared from ripe Bartlett pear fruit (2). Pear acetone powder, 0.4 g, prepared by the method of Dick et al. (5) was suspended in 20 mL of cold 5 mM Na phosphate (pH 6.5) and the mixture centrifuged at 10,000g for 10 min. The pelleted material was washed twice by resuspension in 5 mM phosphate and centrifugation. The supernatant solutions were discarded. The pellet material was resuspended in 5 mM phosphate containing 1.0 mM NaCl (10 mL), centrifuged, and the supernatant used as a source of α-L-arabinofuranosidase activity. The activity was assayed using p-nitrophenyl-α-L-arabinofuranoside (0.1% w/v, Sigma Chemical Co.) as substrate in 0.1 mM Na acetate (pH 4.5) at 30°C. Incubation was terminated by addition of 1 mM NaOH. Free p-nitrophenol was measured by reading absorbance at 405 nm. The velocity was 0.03 μmol p-nitrophenol per min per mL of enzyme preparation. The activity of this preparation against polysaccharides was measured in pH 4.5 acetate at 30°C using 1 mM of enzyme preparation and 2 mL of 1.0% solutions of PGA, pear pectin fractions, arabin (beet, Koch-Light Ltd.), and an arabinogalactan (Larch, Sigma Chemical Co.). Hydrolytic activity toward polysaccharides was determined by the reducing test of McFeeters (14).

exo-PG

The purification of an exo-PG from Bartlett pears was patterned after that of Pressley and Avants (18) for ‘Anjou’ pears. The ultra-filtered (mol wt 10,000 cut-off) high salt extract of ripe pear fruit was subjected to gel filtration on ACA 34 (LKB) (1.5 x 40 cm gel bed) developed with 0.15 mM NaCl, 5 mM Na acetate (pH 5.5) at 4°C. Two major peaks of PG activity were obtained. Fractions from each peak were pooled and the protein precipitated by addition of ammonium sulfate to 80% saturation. The precipitated material from the first eluted peak was dissolved and chromatographed on a column of Agarose 5m (1.5 x 120 cm gel bed) developed with the same buffer. One peak of PG activity was obtained which was devoid of activity toward p-nitrophenyl-α-L-arabinofuranoside but did contain some PE. The PG active peak fraction was concentrated by addition of ammonium sulfate to 80% saturation, and the precipitated material was collected by centrifugation. The pellet was dissolved in 0.2 mM Na acetate (pH 5.0) and syringe-filtered. PG activity was assayed using 0.1% PGA (Sigma), in 0.1 mM Na acetate (pH 4.5), 5 mM CaCl2 at 30°C. Quantitation of hydrolysis was by the reducing test of McFeeters (14). One unit of PG activity was the amount that released 1 μmol of galacturonic acid per hour.

Polylacturonase II of tomato (17), was a gift of Dr. Alan Bennett of the Department of Vegetable Crops, University of California, Davis. The activity was assayed as for the pear PG above. The activity of the pear and tomato PGs toward pear pectin fractions II, III, and IV (0.5% w/v solutions) was tested in 0.1 mM Na acetate (pH 4.5) at 30°C. Hydrolysis was quantified by measuring generation of reducing sugar (14).

RESULTS

The change of the sugar content of the water soluble, 80% ethanol precipitable polysaccharide fraction of freshly squeezed pear juice which accompanies softening of Bartlett pear fruit during ripening is shown in Figure 1. The increase in uronic acid and arabinose content of this fraction is comparable to the analogous fraction of pear fruit homogenate reported earlier (1).
Anion-exchange chromatography of each of the pectic polysaccharide-containing fractions from pear juice on DEAE-agarose by stepwise elution gave four well-defined fractions. Data for fruits of 8N firmness are presented in Figure 2. The void-volume fraction (fraction I), which had only a trace of uronic acid, was not examined further. Fraction II of Figure 2, was eluted with a larger volume of the initial column buffer. The isolated (i.e. reprecipitated) fraction comprised less than 10% of the uronic acid content of the applied sample from the juice of pears of any firmness examined. It was composed primarily (98%) of polymeric uronic acid with trace amounts (<1% each) of arabinose, galactose, and xylose. Approximately 60% of the GaIA residues were methyl esterified. Upon gel permeation chromatography of fraction II on Bio-Gel A 5m (data not shown), the larger portion (80%) of the uronic acid eluted at the total volume of the column and was devoid of neutral sugar residues. A smaller portion (about 20%) of the uronic acid of the applied sample was quite heterodisperse with a peak corresponding to a mol wt of 40,000 (based on comparison with dextran standards) and contained all of the neutral sugar of the applied sample. Most of the uronic acid was eluted from the DEAE-agarose column (Fig. 2) with 0.1 m NaCl (fraction III) and with 0.5 m NaCl (fraction IV).

Variations, relative to fruit firmness, in the amounts and relative proportions of the major fractions (III and IV) from the DEAE column (Fig. 2) may be seen in Table I. The composition of the isolated fraction III remains essentially constant as the pears ripen. A significant change in fraction IV with ripening is the decrease in uronic acid content and accompanying increase in rhamnose content. There is an appreciable loss of fraction IV in the overripe (8 N firmness) fruit. To test whether this loss is due to a preferential vulnerability of a class of pectin molecules in fraction IV to degradation by soluble pear fruit enzymes, replicate pears of firm-

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**Table I. Carbohydrate Composition of DEAE-Agarose Fractions of the 80% Ethanol Precipitable Fraction of Pear Fruit Juice from Pears at Different Stages of Ripeness**

<table>
<thead>
<tr>
<th>Firmness</th>
<th>Fraction</th>
<th>GalA/100 mL juice</th>
<th>GalA</th>
<th>Ara</th>
<th>Rha</th>
<th>Xyl</th>
<th>Gal</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>III</td>
<td>64</td>
<td>85</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>14</td>
<td>III</td>
<td>120</td>
<td>81</td>
<td>14</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>14</td>
<td>IV</td>
<td>40</td>
<td>34</td>
<td>56</td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>III</td>
<td>150</td>
<td>84</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>IV</td>
<td>18</td>
<td>30</td>
<td>53</td>
<td>10</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

The neutral sugar values were determined on duplicate aliquots by gas chromatography of the alditol acetate derivatives (3). Agreement of values was within 5%. The uronic acid values were determined by the method of Blumenkrantz and Asboe-Hansen (4) performed on replicate samples. (Data from a different lot of pears, at different firmness values but within the range indicated here, gave data consistent with those presented here.)
ness 14 N were juiced, and the juice held at 20°C for 1 h before the pectin was precipitated with boiling ethanol. While the overall yield of polymeric uronic acid from such juice was little changed from juice treated with hot ethanol immediately upon juicing, the composition of the derived fraction IV (but not fraction III) changed substantially: the relative uronic acid content increased to 53% and the arabinose decreased to 36% (cf. Table I).

The order of elution of fractions III and IV from the ion exchange column is explainable in terms of their methyl esterification: 60% for fraction III and 51% (leading half) and 41% (trailing half) for fraction IV.

DEAE fractions III and IV contained unique pectin molecular species as indicated by their further fractionation on BioGel A 5 m (Figs. 3 and 4). Fraction III (Fig. 3) gave three distinct classes of polymers in terms of composition (Table II). Fraction IIIA, with a peak centered at mol wt 400,000, contained virtually all of the neutral sugar of the applied sample, had a broad range of molecular sizes, and was heterogeneous in composition across the peak (data not shown) with the ratio of uronic acid to neutral sugar increasing with increasing elution volumes. The void volume fraction was probably aggregated material from fraction A since it had a similar composition and invariably reappeared upon rechromatography of fraction IIIA. The small neutral sugar content of fraction IIIB was probably contamination by fraction A. Fraction C, which eluted near the total volume of the column, was heterodisperse since its peak was three times as broad as the total volume marker (galacturonic acid). The relative amounts of fractions IIIB and IIC (both composed only of uronic acid) varied considerably in juice of pears of different ripeness, C predominating in softer pears; e.g., a definite peak of uronic acid at mol wt 40,000 (fraction B) was seen from pears of firmness 14 N, in which fraction C comprised 35% of the uronic acid. In replicate pears of firmness 9.8 N (shown in Fig. 3), fraction C comprised 70% of the uronic acid of the applied sample.

Agarose chromatography revealed that fraction IV was composed of two classes of polymers varying in terms of mol wt distribution (Fig. 4) and composition (Table II). Fraction D was a broad peak centered at mol wt 400,000. It was similar to fraction IIIA in size distribution but very different in composition. At least two-thirds of the sugar residues were arabinose and about one-fourth or less were uronic acid. The void volume fraction was probably an aggregate of fraction D since it had the same composition and reappeared upon rechromatography of isolated fraction D on the same column.

The relative uronic acid content of fraction D decreased upon ripening (from 25 to 19 mol% for pears of firmness 14 N to 8 N, respectively) and the rhamnose content increased significantly (4–7.5 mol%). Although the breadth of fraction D clearly indicated that its component polymers were heterodisperse, the composition of successive cuts across the peak was constant. However, fraction D was nevertheless composed of a variety of polymer molecules, different in composition, since chromatography of an analytical sample of fraction D on DEAE-agarose with gradient salt elution gave a single asymmetrical peak whose relative uronic acid content varied twofold across the peak (data not shown).

Fraction IV E (mol wt 40,000, Fig. 4) was quite unique in composition (Table II). The uronic acid content decreased somewhat with ripening and the neutral sugar content increased (data not shown). The relative amounts of fractions D and E also changed with ripening; the proportion (in mg%) of uronic acid in the two fractions (D/E) decreased from 39/61 to 29/71 while the arabinose D/E ratio went from 98/2 to 92/8 for preparations from pears of firmness 14 N (ripe) and

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**Table II. Carbohydrate Composition of Fractions from Agarose 5m Column Chromatography of DEAE-Agarose Fractions III and IV of the 80% Ethanol Precipitable Fraction of Pear Fruit Juice from Pears of Firmness 14 N**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>GalA</th>
<th>Rha</th>
<th>Ara</th>
<th>Gal</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>62</td>
<td>2</td>
<td>33</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>96</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>25</td>
<td>4</td>
<td>67</td>
<td>3</td>
</tr>
<tr>
<td>E</td>
<td>89</td>
<td>4</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>
toward PGA and Ill. (Data shown are from pears of firmness 9.8 N). The column was calibrated with dextran polysaccharide and galacturonic acid.

**Table III. Relative Activities of Pear and Tomato Polygalacturonase toward PGA and Pear Pectin Fractions**

Substrate concentration was 0.5%. The velocity for PGA was 0.027 μmol of reducing groups per h per 10 μL of the pear PG and 0.17 μmol of reducing groups per h per 1.25 μg of the tomato PG. Values are expressed relative to PGA.

<table>
<thead>
<tr>
<th>Pectin Substrate</th>
<th>Relative Activity of Pear Enzyme</th>
<th>Relative Activity of Tomato Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction III</td>
<td>2.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Fraction III (saponified)</td>
<td>2.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Fraction IV</td>
<td>1.9</td>
<td>0.43</td>
</tr>
<tr>
<td>Fraction IV (saponified)</td>
<td>1.7</td>
<td>0.71</td>
</tr>
<tr>
<td>PGA</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

of firmness 8 N (overripe); i.e. fraction E increased and fraction D decreased when pears became overripe.

**Enzyme Treatment**

Treatment of 1% solutions of fractions II, III, IV, citrus PGA, beet araban, and larch arabinogalactan each with 0.03 units of an α-L-arabinofuranosidase (extracted from pear fruit) resulted in an insignificant release of reducing sugar in 2 h.

Pear pectin fractions III and IV were readily attacked by an exo-PG (18) purified from Bartlett pears and by tomato PGIII, an endo enzyme (17). Table III shows that the pear enzyme was equally active toward unsaponified and saponified pectins while the tomato enzyme hydrolyzed saponified pectins more rapidly. Reducing sugar was released from isolated fraction IVD by the pear exo-enzyme at a rate one-third that from PGA under comparable conditions. After incomplete (one-eighth of the uronic acid glycosidic linkages were cleaved)
digestion of fraction IVD by the exo-enzyme, undigested material was collected by 80% ethanol precipitation and subjected to agarose chromatography (Fig. 5). The major peak had the same elution characteristics as undigested fraction IVD but with a composition of a significantly higher arabinose/uronic acid ratio, i.e. GalA (13%), Ara (72%), Rha (8%), Gal (6%) (cf. Table II). The other peak in Figure 5, at the total volume of the column, contained uronic acid only (i.e. no detectable neutral sugars).

Treatment of the major peak of pear exo-PG-treated fraction IVD with 12.5 μg of tomato PGII, an amount sufficient to degrade PGA at a rate of 1.7 μmol/h under comparable conditions, released no reducing sugars during 6 h. Ethanol precipitation and rechromatography on agarose gave only one peak with the shape, position, and sugar composition as before treatment (data not shown).

The results of linkage analysis of the PG-treated fraction IVD, performed by methylation, carboxyl reduction, hydrolysis, reduction, and acetylation and GC-MS analysis, are shown in Table IV.

DISCUSSION

The increased content of polymer uronic acid and arabinose in Bartlett pear juice in a water-soluble, 80% ethanol-precipitable form (Fig. 1) which accompanies ripening of the fruit is a well-documented phenomenon (1). The heterogeneity of this polyuronide fraction of pear fruit in terms of monomer composition and molecular size of its constituent polysaccharides is extreme but does not appear to be continuous. Two compositional types of polyuronides are the major constituents: HGA and heteropolymers containing, in addition to galacturonic acid, varying proportions of the neutral sugars, arabinose, rhamnose, and galactose. Relatively low mol wt (<10,000) HGAs account for the greatest portion of uronic acid material in ripe and overripe pears (Fig. 3; Tables I and II). The increase in the low mol wt HGA as pears become overripe, apparently at the expense of higher mol wt HGA, indicates that the endogenous substrate is attacked by the endogenous PGs (18) but possibly is not degraded by these enzymes to the monomer or smaller oligomer (80% ethanol-soluble) form at a rapid rate. The level of methyl esterification (60%) may protect the HGA from extensive hydrolysis by the PGs (in spite of the presence of endogenous PE [2]) so that the 80% ethanol-insoluble forms accumulate in the ripening fruit.

Three neutral sugar-containing polyuronide fractions which differed from each other primarily in their arabinose content were partially resolved by anion exchange chromatography and gel filtration (Figs. 2–4). It is likely that each of these fractions contained containing HGA unlinked to the neutral sugar-containing polyuronide. This conclusion arises from the action of PGs on the fraction with the highest arabinose content (fraction IVD, Fig. 4). Both an exo-PG from pears (18) and PGII from tomatoes (17), an endo-enzyme, are active toward this fraction. However, the initial partial hydrolysis of it by the exo-PG gave an arabinose-pectin of undiminished mol wt (but increased arabinose proportion) plus a low mol wt GalA-containing fraction. The latter fraction could have been produced by exo-PG action on a separate HGA or on an HGA tail at the nonreducing side of the Ara-substituted portion of the RG-I backbone. The absence of a change in behavior upon gel filtration while the Ara/GalA ratio doubled suggests that the former suggestion is the more likely. The absence of endo PG action on the exo-PG-treated arabinopolymers of fraction IVD are distinct molecules. This is not in agreement with the model structure proposed for apple pectic substances (22).

The linkage analysis (Table IV) of the PG treated arabinopolymers with a backbone containing D-galacturonosyl and 1-rhamnosyl residues, linked 1→4 and 1→2, respectively, with short side chains of D-galactose, linked 1→4, and large, highly branched arabinan side chains both linked to the 4-position of every second rhamnose residue, on average, of the backbone. The linkage data in Table IV indicate a degree of under-methylation of arabinosyl residues. It is therefore not possible to estimate the exact nature of the interarabinose linkages. Nonetheless, assuming that the arabinan chains are linked only to the 4-position of rhamnose residues of the rhamnogalacturonan backbone, the chain length of the araban (average of 30 residues, based on total Ara residues in Table IV) and the percentage of terminal arabinose (16%) clearly indicate that the araban is highly branched. The undermethylation of arabinosyl residues is probably due to a peculiar steric hindrance of certain internal arabinose residues since there was no indication of undermethylation of galacturonic acid residues.

This arabinorhamnogalacturonan was not attacked by the PGs from pears or tomatoes. Therefore, evidence has not been found for a linkage between an RG-I and HGA in the pectin of pear juice as suggested for the pectins of apples (22). It is conceivable that such a linkage also does not exist among the water insoluble pectic polysaccharides associated with the cell wall. The solubilization of both HGA and RG-I-like polymers upon treatment of cell-wall preparations with PG-containing enzyme preparations may occur by a different mechanism than cleavage of a PG-susceptible glycosidic linkage between them.

The accumulation of the arabinorhamnogalacturonan (fraction IVD, Fig. 4) in the juice of ripening pears may be attributed to its resistance to hydrolysis by enzymes which have been described in pear fruit. The terminal arabinofuranosyl groups were not attacked by an α-1-arabinofuranosidase (see above), the most active glycosidase in pear fruit extracts (2). The rhamnogalacturonan backbone was not hydrolyzed by an endo PG from tomato (17) and would presumably also not be attacked by the analogous endo PG activity in pears (18). The decrease in this fraction in overripe pears must be due to an activity other than those mentioned above. (Glycosidases active on cell wall substrates but not on artificial substrates have recently been described [15]). It is possible that it is degraded initially to the RG of fraction IVE (Fig. 4) since the latter fraction increased in concert with the decrease in fraction IVD and the difference in composition was largely arabinose (Table II). An activity cleaving arabinosyl residues from side chains was evident in pear juice since there was a
loss of arabinose from fraction IVD upon incubation of the juice prior to hot ethanol precipitation of the pectin fraction (see above).

LITERATURE CITED