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Lebensm.-Wiss. u.-Technol., 21, 198–205 (1988)

Further Characterization of Acid- and Alkali-soluble Pectins from Sugar Beet Pulp

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(*Received January 25, 1988; Accepted February 23, 1988*)

Pectins are solubilized from an alcohol insoluble residue by sequential extraction with hot dilute acid (HP) and cold dilute alkali (OHP). They have a fairly low molecular weight, a relatively large amount of associated neutral sugars and contain small but significant amounts of feruloyl groups and hydroxyproline-rich protein. They differ by their degrees of methylation and acetylation as OHP are partly deesterified during the extraction. Arabinose, galactose and rhamnose are the major neutral sugars and are located in the hairy fragments. All the feruloyl groups are recovered in the hairy fragments whereas only part of the proteic material is found in these fragments.

Introduction

Sugar beet pulp constitutes the by-product of sugar refining industries and is available in large amounts. After pressing and drying, it is mainly used as cattle feed. However, some new interests for sugar beet pulp have appeared. Structurally, sugar beet pectins consist, as other pectins (1–6), in large homogalacturonic or smooth regions and small rhamnogalacturonic regions carrying neutral sugar side chains or hairy fragments but they have an original feature in containing (1,2) some feruloyl groups ester-linked to the hairy fragments. This substitution which has also been reported (7) for spinach pectins, leads to a new way of gelation for sugar beet pectins since they can be crosslinked through their feruloyl groups in presence of a mixture of peroxidase/hydrogen peroxide (8) or of ammonium persulfate (9). Water-soluble products of an increased molecular weight or gels are obtained depending on the concentration of the pectins. This coupling reaction could therefore increase the usefulness of sugar beet pectins.
The sugar beet pulp pectins (1,2) have been previously studied by chemical and enzymic methods after sequential extraction from an alcohol insoluble residue. Our aim is now to have new insights in their structure by a further characterization of the hairy fragments. Special attention is paid to their protein content, amino-acid composition as well as their phenolic acid content. In this work, the extractions with water and oxalate are omitted as they solubilize less than 10% of the pectins (1).

Material and methods

Material

Pressed sugar beet pulps come from the factory in Artenay. The alcohol insoluble residue (AIR) is obtained as described by Barbier and Thibault (10).

Methods

Extraction and purification of the pectic substances. The pectic substances are sequentially extracted from AIR by hot dilute acid (0.05 M HCl, 30 min at 85°C), and cold dilute alkali (0.05 M NaOH, 30 min at 4°C). Each extraction is carried out three times (1). The alkali residue is dried by solvent exchange and then air dried. The pectins are obtained after precipitation with four volumes of 96% ethanol and then purified by precipitation with cupric ions as described by Michel *et al.* (11).

Chromatography. Ion-exchange chromatography is performed on a column (6 × 1.6 cm) of DEAE Sephacel equilibrated with 0.05 M sodium acetate buffer (pH 4.8). Pectic substances (4 mg) are loaded onto the column and the gel is washed with 50 ml of 0.05 M sodium acetate buffer (pH 4.8). The bound material is eluted by a linear sodium acetate buffer gradient at pH 4.8 (ionic strength 0.05–0.08 M, 90 ml). Fractions of 5 ml are collected.
The initial pectins are chromatographied on a Sepharose CL-2B column (86 × 2.0 cm) and degraded pectins are chromatographied on a Sephacryl S200 column (79 × 2.0 cm) or on a Sepharose CL-6B column (50 × 1.6 cm) and on a Biogel P2 column (203 × 1.6 cm). Elution on Sepharose CL-2B and CL-6B is carried out in ascending direction with sodium acetate buffer (ionic strength = 0.1 M, pH 4) at a flow rate of 20 ml/h. Samples (6 mg) dissolved in the same buffer are applied onto the column and fraction of 4 ml are collected. Elution on Sephacryl S200 is carried out in a descending direction with sodium acetate buffer (ionic strength = 0.05 M, pH 4) at a flow rate of 20 ml/h. Samples (up to 100 mg) are loaded onto the column and fractions of 3 ml are collected.
Results are expressed as a function of $K_{av} = (V_e - V_0)/(V_t - V_0)$ where V_e, V_0, V_t are the elution volume of the fraction, the void volume of the column and the total volume of the column, respectively. V_0 and V_t are determined as the elution volumes of carboxyl-methyl cellulose and galacturonic acid, respectively.
Chromatography on Biogel P2 is performed as described elsewhere (12). About 1 mg of material is injected onto the column and aliquot of the effluent (6 ml/h) is continuously analyzed by the m-hydroxydiphenyl and the orcinol methods. Elution profiles represent the absorbancy at 520 nm (m-hydroxydiphenyl detection) and at 420 nm (orcinol detection) versus the elution volume without correction for the interference of galacturonic acid during the orcinol detection.

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Viscosity measurement. Intrinsic viscosity values ([m], ml/g) are obtained at 25°C with an automatic Fica viscosimeter. Solutions of pectins (3 mg/ml) in 0.155 M sodium chloride are used and diluted with 0.155 M NaCl. The viscosity average molecular weights ($\overline{M}_v$) are calculated according to Owens *et al.* (13).

Hydrolysis by an endopolygalacturonase. A purified endopolygalacturonase (14) (endo-PG, E.C.3.2.1.15.) is used for the degradation of the pectins. HP are first demethylated and deacetylated by alkali as described elsewhere (5). Reaction mixtures contain 2 mg/ml of pectin in 0.05 M acetate buffer, pH 4.1 and enzyme (0.2 nKat/ml). Hydrolysis extents are calculated from the increase in reducing power as determined by the method of Nelson (15) using galacturonic acid as standard.

Analytical methods. All the values are calculated on a moisture free basis. Proteins (N × 6.25) are determined by the Kjeldahl procedure and by the Folin–Ciocalteu reagent as described by Potty (16). Ash is measured after incineration overnight at 550°C, then 1 h at 800°C. Methanol and acetic acid are determined by HPLC (17) (Aminex HPX 87H), methanol is also determined by the method of Wood and Siddiqui (18) and acetic acid by GLC (19).
Phenolic acids are liberated by treatment of pectins with 2 M NaOH at 35°C for 2 hours under argon. After acidification (pH 2) and extraction using diethyl ether, they are analyzed by HPLC (20) on Rsil C18 5 nm with water/methanol/acetic acid (73/26/1, v/v) as eluent at a flow rate of 0.9 ml/min. Total phenols and proteins are measured by colorimetry (16). Feruloyl groups are determined spectrophotometrically at 375 nm on freshly prepared solution of pectin in 0.05 M glycine/NaOH (pH 10) buffer using a molar extinction coefficient of 31,600 (7). Feruloyl groups in column eluates are monitored by spectrophotometry at 375 nm, the pH of the fractions being adjusted to 10 by NaOH 1 N. When the concentration of the fractions is too low, the content in ferulic acid is measured at 325 nm; in these conditions, the extinction coefficient is determined and is equal to 22,900.
The amino acid composition is determined after acid hydrolysis by 6 M HCl as their ninhydrine derivatives (480 nm for hydroxyproline) on a Kontron Liquimat III autoanalyzer (21) (Duorum DC 6A) or as their phenylthiocarbamyl derivatives by HPLC (22) (Nucleosil C18) using UV detection.
Uronic acids (as galacturonic acid) are determined by the automated m-hydroxydiphenyl method (23) in the solutions and by the method of Ahmed and Labavitch (24) in the AIR and the residue after pectin extraction.
Neutral sugars were estimated by the automated orcinol method (25) using arabinose as a standard. Corrections were made for interference from galacturonic acid. Neutral sugars are analyzed by GLC as their alditol acetates after sulfuric acid hydrolysis of the polysaccharides (26).

Results

Alcohol insoluble residue (AIR)
The yield of AIR is 88 g/100 g of dry sugar beet pulp. The AIR composition is shown in **Table 1**. Polysaccharides account for 60.4% of the AIR. The high content in galacturonic acid (20.3%) suggests the presence of an important amount of pectic substances. Arabinose (19.1%), galactose (4.5%) are the major non cellulosic neutral sugars. Glucose (12.5%) is present in relatively important amount and originates mainly from cellulose. Xylose and mannose occur in very small amount (1–2%). Ash represents 10.1% and the protein content (N × 6.25) is 6.7%.

Extraction and characterization of the pectins
Pectins are removed from the AIR by a sequential extraction with hot dilute acid (HP) and cold dilute alkali (OHP). Yields and composition are reported in **Table 1**. The total recovery including the residue is 68.2%. More than one fourth of the AIR is recovered in the two pectic fractions. HP account for 18.7% (w/w) and OHP for 9.5% (w/w). Sequential extraction releases the bulk of galacturonic acid and only 12% of the initial galacturonic acid is still present in the final residue. Rhamnose, arabinose and galactose are mostly removed while glucose, xylose and mannose remain in the residue. 94% of the arabinosyl residues are released but only 14.8 and 9.2% are recovered in HP and OHP, respectively. Most of them must be lost during the precipitation of the pectins in alcohol. In the final residue, by comparison with the AIR, ash content (9.5%) does not significantly change in contrast to protein content (11.2%) which strongly increases.
The content in galacturonic acid of crude HP and OHP is low, 44.4 and 40.6%, respectively. Total neutral sugars are 22.1 and 29.9% for the HP and OHP, respectively. Pectic fractions are composed of the same neutral sugars, mainly arabinose, galactose and rhamnose. Mannose and glucose are not detected. Feruloyl groups, as measured by spectrophotometry, appear in similar low amounts in HP and OHP (<1%). These pectins are submitted to ion exchange chromatography on DEAE Sephacel in order to assess their purity (**Fig. 1**). Recoveries in galacturonic acid are 93.6 and 40.5% for HP and OHP, respectively. OHP which are dimethylated and partly deacetylated during the extraction, are largely irreversibly bound to the gel. HP elute as a single peak at an ionic strength of 0.48 M, indicating that they are homogeneous in degree of methylation. The feruloyl groups are eluted together with the pectins, indicating that they are probably linked to them. Some neutral sugars (3.6 and 5.6% of initial HP and OHP, respectively), are not covalently bound to the gel. They represent the neutral polysaccharides which are not covalently linked to the pectic backbone.
HP and OHP are purified by precipitation with cupric ions and then rechromatographied on the same gel (**Fig. 1**). The

Table 1 Sugar composition (% of dry wt) of AIR, of the crude, purified pectins and their hairy fragments, and of the final residue

		Before purification		After purification		Hairy fragments		
Fraction	AIR	HP	OHP	HP	OHP	HP	OHP	Final residue
Yields (%)		18.7[a]	9.5[a]	72[b]	84.8[b]	29.0[c]	35.0[c]	40.0[a]
Galacturonic acid	20.3	44.4	40.6	55.8	52.7	17.6	19.6	6.1
Rhamnose	1.1[d]	1.6	2.9	2.8	2.8	7.2	6.7	0.7
Arabinose	19.1	15.1	18.5	12.4	14.8	45.8	37.5	2.8
Xylose	1.8	tr[e]	tr	0.3	0.8	0.8	0.6	3.3
Mannose	1.1	—	—	—	—	—	—	2.1
Galactose	4.5	5.3	8.5	4.7	7.7	17.9	29.1	3.7
Glucose	12.5	tr	tr	tr	tr	0.6	tr	21.3

[a] Yields from AIR. [b] Yields from crude pectins. [c] Yields from purified pectins. [d] Neutral sugars determined by GLC as alditol acetates. [e] traces

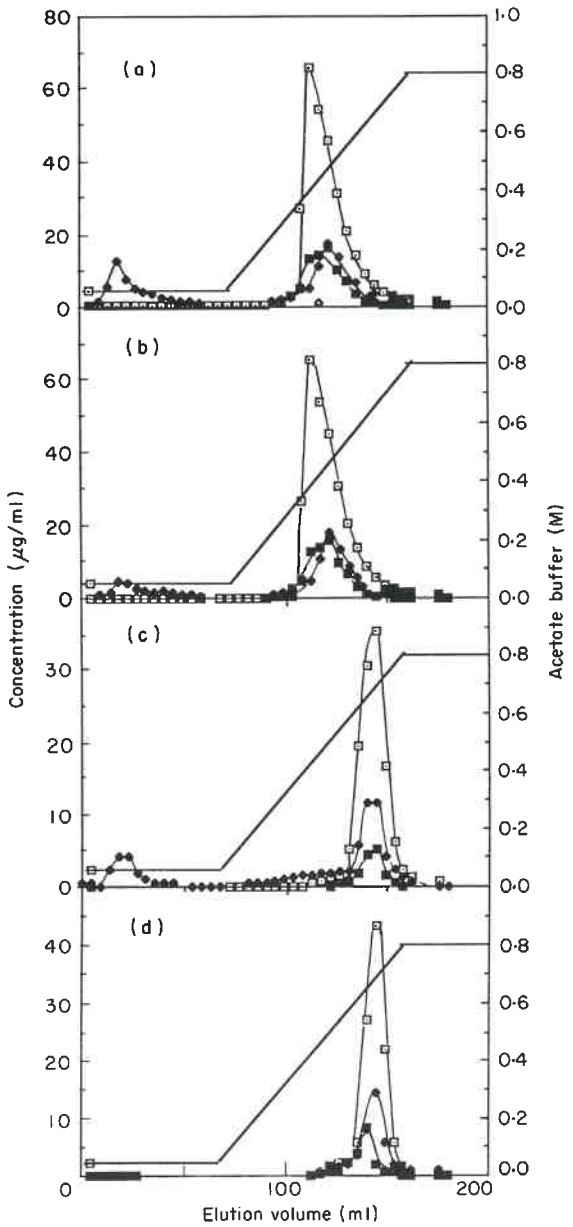

Fig. 1 Ion exchange chromatography on DEAE-Sephacel of the crude and purified pectins from sugar beet pulp. a, crude HP; b, purified HP; c, crude OHP; d, purified OHP. □, galacturonic acid; ◆, neutral sugars; ■, ferulic acid × 10

unbound material represents less than 1% after purification. The bound materials behave similarly on the DEAE Sephacel showing that the purification step did not change their structure.

The purification procedure increases the galacturonic acid content (**Table 1**). HP and OHP contain 55.8 and 52.7% of galacturonic acid, respectively. The amount of total neutral sugars is 20.4 and 26.3% for HP and OHP, respectively. In both, arabinose is the major neutral sugar followed by galactose and rhamnose. Xylose is present in minor amount. Mannose is not detected. By comparison of the neutral sugar composition of crude and purified pectins, arabinose and galactose are mainly affected by the purification. In HP, the proportion of arabinose removed is higher than that of galactose; in OHP, equal amounts of arabinose and galactose are removed.

The degree of methylation (**Table 2**) is ~64% for HP and ~5% for OHP due to demethylations occurring during the alkali extraction. The degree of acetylation is ~23% for HP and ~10.7% for OHP. Some acetyl groups remain in OHP indicating that acetyl ester linkages are more alkali-resistant than the methyl ester ones. Even after purification, HP and OHP contain significant amounts of proteins (**Table 2**) respectively 3.5 and 3.6% for HP and OHP by the Potty's method and 3.8 and 5.4% by the method of Kjeldahl.

This proteic fraction is analyzed for amino-acid composition (**Table 2**). Hydroxyproline, aspartic acid, glutamic acid, lysine and valine are the major amino-acids. Serine and threonine are present but in minor amounts. HP contain a high proportion of hydroxyproline compared to that of OHP (33.6% of total amino-acids in HP and 8.1% of total amino-acids in OHP). OHP show a relatively high quantity of tyrosine (8.5% of total amino-acids).

The total amount of phenols measured by colorimetry (1.2 and 1.1% for HP and OHP, respectively) is higher than the total amount of phenolic acids measured by HPLC. Therefore, the purified pectins contain some phenols which react with the Folin-Ciocalteu reagent and which are not phenolic acids. The major phenolic acid detected by HPLC is ferulic acid (92.0 and 95.4% of the phenolic acids of HP and OHP, respectively) while a smaller proportion of p-coumaric acid is found (7.3 and 4.4% of the phenolic acids of HP and OHP, respectively) (**Fig. 2**). Acceptable agreement is found in feruloyl content determined by spectrophotometry (0.7 and 0.6% of HP and OHP, respectively) and HPLC (0.6 and 0.5% of HP and OHP, respectively). The degrees of feruloylation (moles of feruloyl groups per 100 moles of arabinose and galactose) are 1.8 and 1.7 for HP and OHP, respectively.

Table 2 Non sugar composition (% of dry wt) of the purified pectins and their hairy fragments

	Purified pectins		Hairy fragments	
	HP	OHP	HP	OHP
Methanol[a]	6.3 (64.1)	0.6 (6.7)	nd	nd
Methanol[b]	6.2 (63.1)	0.4 (5.0)	nd	nd
Acetic acid[b]	4.3 (23.0)	1.8 (10.2)	nd	nd
Acetic acid[c]	4.4 (23.5)	1.9 (10.7)	nd	nd
Total phenols[d]	1.1	1.2	1.9	1.3
Total phenolic acids[e]	0.7	0.6	1.7	1.2
Ferulic acid[e]	0.7	0.6	1.8	1.3
Ferulic acid[f]	0.6 (1.8)	0.5 (1.7)	1.7 (1.7)	1.2 (1.3)
Proteins[d]	3.8	5.4	5.1	4.9
Proteins[g]	3.5	3.6	nd	nd
Total amino acids	3.9	3.6	3.9	3.48
Aspartic acid	0.23	0.31	0.12	0.12
Threonine	0.18	0.21	0.2	0.21
Serine	0.20	0.25	0.25	0.21
Glutamic acid	0.26	0.37	0.44	0.35
Proline	0.15	0.19	0.36	0.3
Glycine	0.2	0.16	0.1	0.07
Alanine	0.2	0.17	0.02	0.14
Valine	0.26	0.36	0.42	0.32
Isoleucine	0.18	0.11	0.07	0.08
Leucine	0.11	0.22	0.07	0.03
Tyrosine	0.11	0.26	0.36	0.3
Phenylalanine	0.08	0.11	0.04	0.02
Lysine	0.25	0.3	0.02	0.22
Histidine	0.08	0.16	0.28	0.22
Arginine	0.1	0.13	0.08	0.01
Hydroxyproline	1.31	0.29	1.0	0.8
Cysteine	nd	nd	0.02	0.08
Methionine	nd	nd	0.04	0.03

[a] Measured by the method of Wood and Siddiqui. [b] Measured by HPLC. [c] Measured by GLC. Values in parentheses are degree of methylation or degree of acetylation. [d] Measured by colorimetry. [e] Measured by HPLC. [f] Measured by spectrophotometry. Values in parentheses are degree of feruloylation. [g] Measured by the Kjeldahl method (N × 6.25). nd, not determined

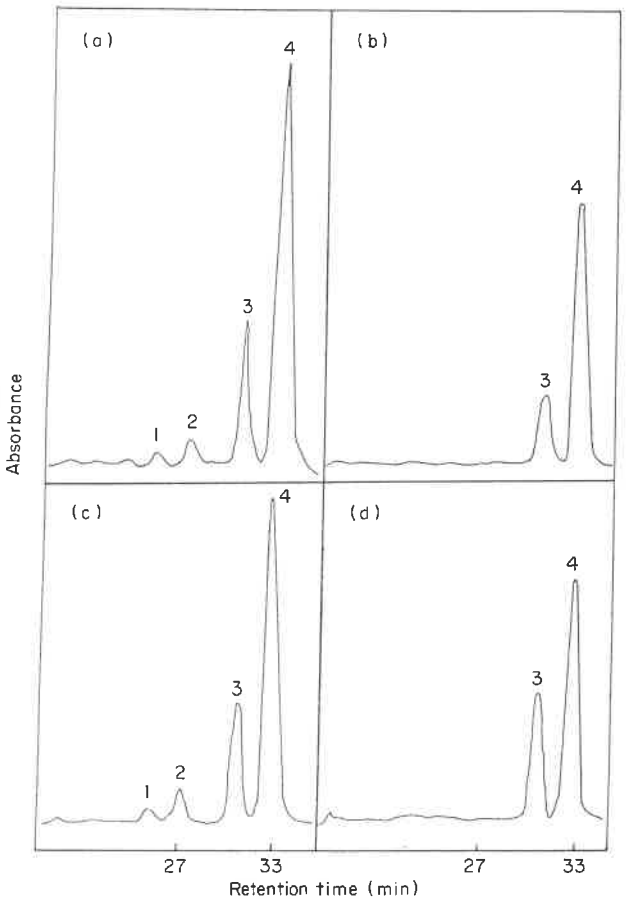

Fig. 2 HPLC chromatograms of phenolic acids from the purified pectins and their hairy fragments. a, purified HP; b, HP hairy fragments; c, purified OHP; d, OHP hairy fragments. 1, cis p-coumaric acid; 2, trans p-coumaric acid; 3, cis ferulic acid; 4, trans ferulic acid

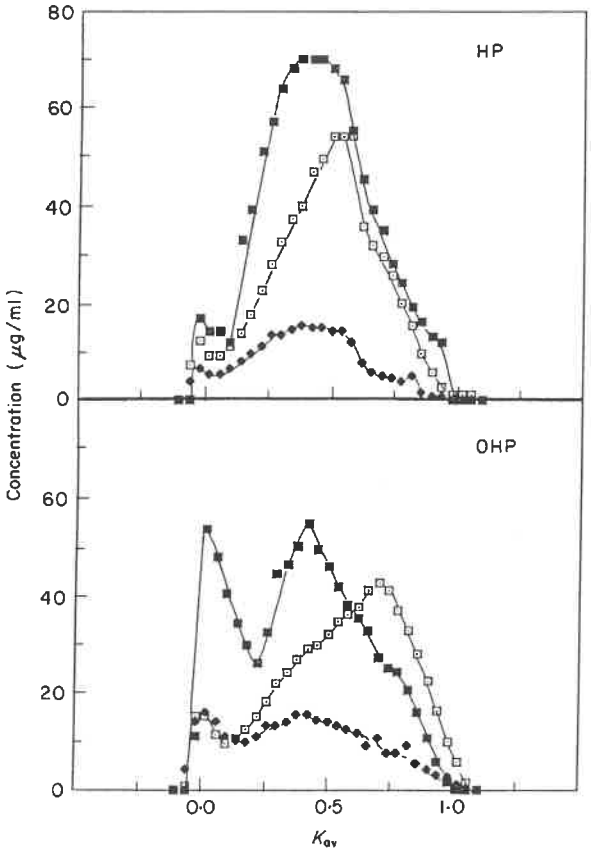

Fig. 3 Gel filtration chromatography on Sepharose CL-2B of the sugar beet pectins. □, galacturonic acid; ◆, neutral sugars; ■, ferulic acid × 10^2

The purified pectins are chromatographied on Sepharose CL-2B (**Fig. 3**). HP show a fairly continuous variation of molecular weight and are chemically heterogeneous as indicated by the variation of neutral sugars to galacturonic acid ratio over the fractionation range. OHP apparently consist of two populations, one at a $K_{av} = 0.37$, rich in neutral sugars and one at a $K_{av} = 0.7$, poor in neutral sugars. The feruloyl groups apparently elute together with the neutral sugars for HP as well as for OHP.

The intrinsic viscosities are 247 ml/g and 200 ml/g for HP and OHP, respectively and the viscosity average molecular weights are 45,800 and 39,200 for HP and OHP, respectively. Huggins coefficients are close to 0.5 in every case. In contrast to what is expected from gel filtration, OHP have a lower $\overline{M}_v$ than HP.

Isolation and characterization of the hairy fragments

The pectic molecules are hydrolysed by endo-PG in order to remove most of the smooth regions and to isolate the neutral sugar side chains and the rhamnose kinks. Hydrolysis by endo-PG requires demethylated and deacetylated galacturonic acid residues; in the conditions used, the feruloyl groups are proved to be not removed. The extent of hydrolysis by endo-PG is higher (33%) for HP than for OHP (26.2%). This results from the residual acetyl groups present in OHP, as shown by the fact that preliminary deacetylation increases the percentage of hydrolysis to 36.3%. The products of hydrolysis are chromatographied on Sephacryl S200 (**Fig. 4**) and are separated in two large peaks: one, near or at the void volume of the column, poor in galacturonic acid (19 and 13.9% of the initial galacturonic acid present in HP and OHP, respectively)

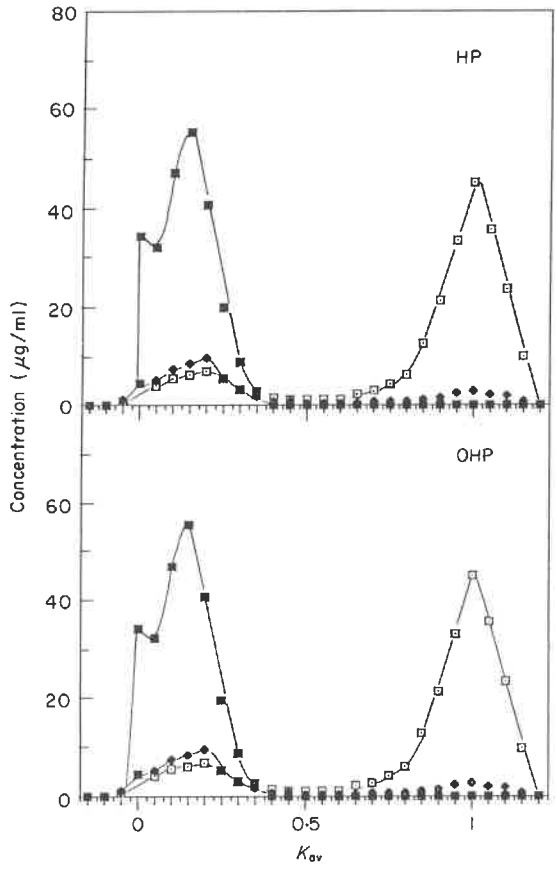

Fig. 4 Gel filtration chromatography on Sephacryl S200 of the endo-PG-degraded HP and OHP. □, galacturonic acid; ◆, neutral sugars; ■, ferulic acid × 10^2

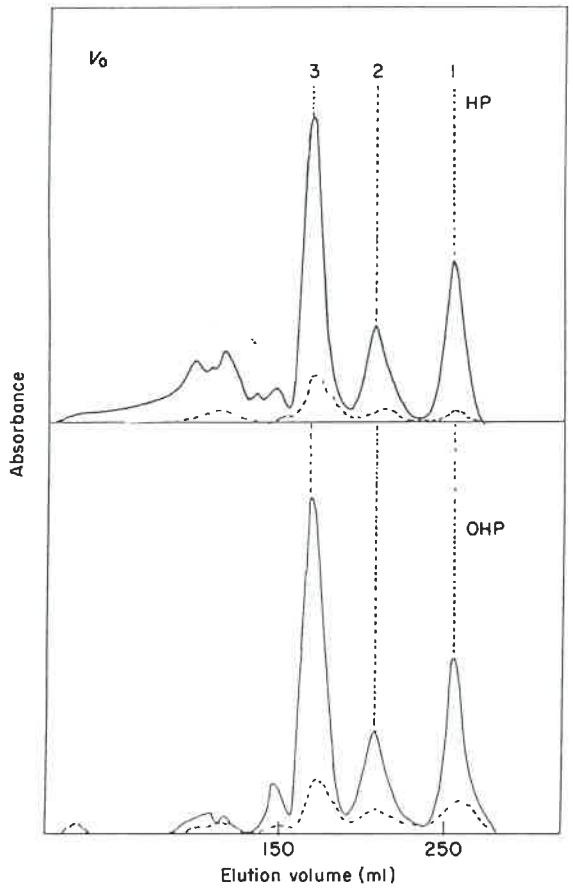

Fig. 5 Gel filtration chromatography on Biogel P2 of the material eluted at the Vt from the Sephacryl S200 column. 3, 2, and 1 peaks corresponding to tri-, di- and galacturonic acid, respectively. (—), orcinol response; (....), m-hydroxydiphenyl

and rich in neutral sugars (83.8 and 82.3% of the initial neutral sugars in HP and OHP, respectively) and in feruloyl groups (90 and 96% of the initial feruloyl groups in HP and OHP, respectively) which represents the hairy fragments; the second one, near or at the total volume, with almost all the galacturonic acids (91 and 86.5% of the galacturonic acids in HP and OHP, respectively) and small amounts of the neutral sugars (16.2 and 17.6% of the initial neutral sugars in HP and OHP, respectively) and of the feruloyl groups (10 and 3.7% of the initial feruloyl groups in HP and OHP, respectively). Chromatography on Biogel P2 of the material eluted at the total volume reveals the presence of oligogalacturonides, tetra, tri, di, and galacturonic acids (11.7, 45.89, 21.1, 17.2% of the galacturonic acid found in peaks for HP, respectively and 6.1, 45.0, 24.9, 24.0% of the galacturonic acid found in the peaks for OHP, respectively) (**Fig. 5**). The action of the endo-PG is nearly complete and trigalacturonic acid is the major final product. This material corresponds to the smooth regions of the pectic molecules. The material eluted at the void volume of the column is dialyzed against distilled water to remove acetate buffer and oligogalacturonides associated with neutral sugar side chains of the hairy fragments under the conditions of chromatography (2). This last point is confirmed by the fact that the neutral sugars to galacturonic acid ratios increase (3.4–4.1% for HP hairy fragments and 3.1–3.7% for OHP hairy fragments) after dialysis.

Table 1 shows the composition of the pectins in comparison to that of the hairy fragments. Neutral sugars account for 72.3 and 73.9% of the HP and OHP hairy fragments, respectively and the molar neutral sugar to galacturonic acid ratios are about 5 for HP and 4.4 for OHP. The molar rhamnosyl residue to galacturonic acid ratios are 0.42 and 0.5 for HP and OHP, respectively indicating that the backbone in the hairy fragments have a very kinked structure.

The neutral sugar composition of the hairy fragments to that of the HP are close: 94% of the rhamnose, 86.8% of the arabinose, 84% of the galactose and 77.3% of the xylose are recovered in the HP hairy fragments. In the OHP hairy fragments, 80.7% of the rhamnose, 81.4% of the arabinose, almost all the galactose but only 26% of the xylose are present. Xylose may exist as short side chains or single units attached to galacturonic acid in small fragments eluted at the total volume of the Sephacryl S200.

The contents in feruloyl groups of the two hairy fragments measured either by spectrophotometry or by HPLC are higher than those of the initial pectins (**Table 2**) and phenolic compounds other than ferulic acid have not been detected by HPLC (**Fig. 2**). The recovery and the calculation of the degree of feruloylation show that almost all the feruloyl groups are located in the hairy fragments.

The phenols measured by colorimetry (1.9 and 1.3% for HP and OHP hairy fragments, respectively) is close to the phenolic acids measured by HPLC (1.7 and 1.2% for HP and OHP hairy fragments, respectively). Phenol compounds other than ferulic acid are probably eliminated with oligolacturonides on gel filtration chromatography.

The hairy fragments compared to HP and OHP are not enriched in proteic material. Part may be removed with oligogalacturonides during gel filtration chromatography. These results show that all the proteic material is not covalently linked to the hairy fragments. Amino-acid composition of the hairy fragment proteic material is different from that of initial pectins. Hydroxyproline, proline, glutamic acid, valine and tyrosine are the major amino-acids in HP and OHP hairy fragments. HP hairy fragments compared to HP are richer in tyrosine and OHP hairy fragments to OHP in hydroxyproline.

The hairy fragments are chromatographied on Sepharose CL-6B (**Fig. 6**). The hairy fragments appear more homogeneous than the initial pectins as attested by a narrow molecular weight distribution and the relative constancy along the fractionation range of the column of the weight ratio neutral sugars to galacturonic acid. This is true for HP as well as for OHP; however, the latter contained still some material which is eluted at the void volume of the column.

The intrinsic viscosities are 42.3 and 39.02 ml/g for HP and OHP hairy fragments, respectively and the $\overline{M}_v$ are 12,300 and 11,600 assuming that the Owens *et al.* relation can be applied to those molecules. The HP hairy fragments are characterized by a high value (1.3) of the Huggins coefficient. The $\overline{M}_v$ values are smaller than those expected from gel filtration and the OHP hairy fragments which contain some material eluted at the V_0 show the lowest $\overline{M}_v$ values. In the conditions of chromatography, some aggregations could explain the disagreement in the molecular weight values of the hairy fragments between the two methods.

Discussion

Before pectin extraction, the preliminary step which consists in removing residual sucrose and inactivating enzymes is performed by boiling in ethanol. An acceptable quantity of material can thus be prepared but this procedure and particularly the drying may induce some artefacts such as coprecipitation of proteins, of phenolic compounds and of salts, or may modify the solubility of polysaccharides (27,28).

AIR is mainly composed of polysaccharides and its composition is close to that previously published (29,30). Pectins and cellulose are the main polysaccharides; xylose and mannose-containing polysaccharides are present in minor amounts. Some proteins are found.

From the AIR, pectic substances are isolated by a sequential

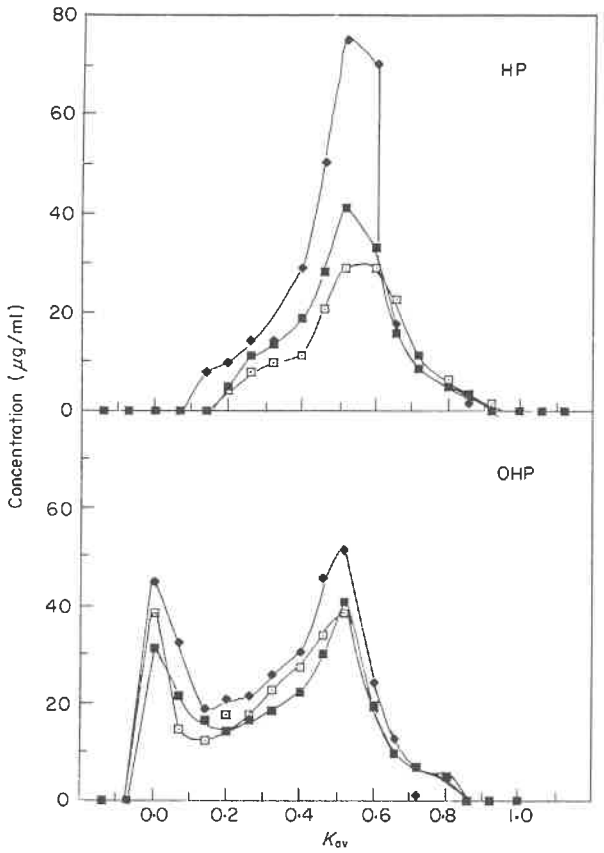

Fig. 6 Gel filtration chromatography on Sepharose CL-6B of the HP and OHP hairy fragments. □, galacturonic acid; ◆, neutral sugars; ■, ferulic acid × 10^2

extraction with hot dilute acid and with cold dilute alkali. The amount of extracted pectins is very similar to that obtained by Rombouts and Thibault (1) and the bulk is extracted with 0.05 M HCl at 85°C probably by splitting some glycosidic bonds, mainly those involving (31) arabinose and rhamnose. Few ester linkages and few bonds between galacturonic acid residues may also be split (31). Some pectins are extracted by subsequent treatment with cold 0.05 M NaOH which could cleave some ester linkages as those involving (32) serine residues. The β-elimination reactions are limited by concomitant demethylation (33).

The residue left after the extraction is enriched in cellulose, in xylose and mannose-containing polysaccharides and in proteins. All the pectic substances are not removed and the residue still contains galacturonic acid. The remaining pectic substances may be tightly bound to the other cell wall components (34) but it cannot be ruled out that some of the pectic molecules became insoluble after the processing and drying of the AIR (28,29).

HP and OHP have fairly low molecular weight and relatively high content in neutral sugars. Arabinose, galactose and rhamnose are the preponderant neutral sugars in agreement with previous work (1,2); xylose is found in minor amounts. The results show that neutral sugars are located in the hairy fragments with the exception of xylose which may exist as short chains attached to the galacturonic backbone as in apple pectins (3) and may be removed on gel filtration with oligogalacturonides. The rhamnose is involved in the pectic backbone and may constitute the point of attachment of the neutral sugar side chains (35–38). The other neutral sugars may occur in short side chains but are also found in more or less branched multiple units (35–38). The isolation of an arabinan (39) from sugar beet pectins and the structural studies by ^{13}C-n.m.r. (40) suggest the presence of a highly branched arabinan as side chains in sugar beet pectins.

An important characteristic of the sugar beet pectins is the presence of acetyl groups. Acetylation has been already reported only for some other pectins (41,42). In sugar beet pectins, Rombouts and Thibault (43) have shown that most of them are linked to the homogalacturonic regions. The presence of these substituents hinders the hydrolysis of the sugar beet pectins by the endo-PG through blocking the binding sites and this fact explains why the OHP which contain some residual acetyl groups are degraded by the enzyme to a lower extent than the demethylated and deacetylated HP.

Another special feature of sugar beet pectins is that they contain ferulic acid and p-coumaric acid. These hydroxycinnamic acids account for all the phenolic acids in the purified pectins and are ester-linked to the hairy fragments. The presence of such phenolic acids has been reported in the monocotyledons (44) and in the Chenopodiacees (1,2,7,38). In the latter to which sugar beet and spinach belong, phenolic acids are linked to the pectins (1,2,7,38). Our studies confirm that they are exclusively linked to the hairy fragments of the pectins. This is in agreement with studies (7) showing that the principal feruloylation sites are the arabinose and galactose termini of the side chains of the pectic substances in the spinach cell wall. Therefore feruloyl groups are accessible and may cross-link the polymers to which they are bound within the wall through coupling reactions leading to diferulate residues. In monocotyledons, there are several reports of natural occurrence of diferulic acid (37,45–47) and its presence in spinach is mentioned by Fry (7). Such coupling reactions which may be catalysed by an extracellular peroxidase in the walls, may play an important role in controlling the cell expansion. It may explain the fact that pectin extraction by cold dilute alkali (1,2,38,48) which could split some feruloyl ester linkages.

In the purified pectins, the presence of other phenols is suggested by the colorimetric measurements. These unidentified compounds are not recovered in the hairy fragments because they are probably eliminated with the oligogalacturonides during gel filtration. Their presence in the purified pectins may result from an artefact of the isolation procedure of pectic substances. This fact has been yet underlined by Rombouts and Thibault (2).

The presence of some proteins has been previously reported in highly purified pectins (10,49–52). In sugar beet pectins, proteins are rich in hydroxy-amino-acid, acid amino-acids, serine and threonine. This amino-acid composition is comparable to that of some glycoproteins which have been isolated from different plant tissues (53–55) and in which the presence of uronic acid and rhamnose arising probably from pectic material is often mentioned. The detection of significant amount of such glycoproteins in a range of plant tissues suggests that they may be part of the walls and that they do not come from artefacts during the extraction conditions. They may be linked to the pectic backbone through their carbohydrate parts which are mainly composed of arabinose and of galactose. Only part (about 30%) of the proteic material is recovered in the hairy fragments and the amino-acid composition has changed. The remainder is probably removed with oligogalacturonides during gel filtration. Therefore, the hairy fragments are not the only site of the association between cell wall proteins and pectic substances. The occurrence of some crosslinks between pectins and proteins through oxidative coupling of their phenolic compounds (feruloyl groups and tyrosine) may be plausible as there is apparently a relative enrichment in tyrosine of the proteic fraction associated to HP hairy fragments. More work is required to clarify the association of proteins and polysaccharides in the cell wall.

Conclusion

The aim of this paper was to describe the isolation and the characterization of the sugar beet pectins and their hairy fragments. The sugar beet pectins consist of large smooth regions and small hairy fragments. The ferulic acid is the main phenolic compound; it is ester linked to the hairy fragments. Some proteic material remains after purification in pectins and their hairy fragments. Their origin has not not yet been established. We shall further investigate the structure of the neutral sugar side chains and the location of the feruloyl groups.

Acknowledgements

The authors thank Mrs M. J. Crepeau for excellent technical assistance.

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