Carbohydrate Polymers **20** (1993) 25–34

Raw and extruded fibre from pea hulls. Part II: Structural study of the water-soluble polysaccharides

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(Received 1 March 1992; revised version received 26 May 1992; accepted 28 May 1992)

The soluble fraction (9%) obtained by aqueous extraction of an extruded pea hull fibre gave 3 peaks on DEAE-Sepharose-6B. The first population (25%) was not bound to the gel and contained xylose, arabinose and glucose (15:6:1), the second one (12%), eluted for an ionic strength of 0·08 M, contained xylose, arabinose and glucuronic acid (18:1:1), and the third one (63%), eluted for an ionic strength between 0·3 M and 0·8 M, contained galacturonic acid, arabinose, xylose, rhamnose and galactose (5·3:3:1·8:1·7:1). The structure of these fractions was studied through enzymic degradation, methylation, ^{1}H-NMR and ^{13}C-NMR. The first one was composed mainly of a heteroxylan having a (1 → 4)-β-D-xylan backbone (average d.p. 103) with only very few branching points at O-2, and of a slightly branched arabinan. The second one was an acidic xylan. Both heteroxylans were acetylated. The third one was pectic in nature with a main chain consisting of (1 → 4) linked galacturonic acid, partly substituted at the O-2 and O-3 positions, and (1 → 2) linked rhamnose residues which were mainly substituted at the O-4 position. This acidic fraction was appreciably hydrolyzed by an endopolygalacturonase, indicating the presence of homogalacturonan regions in the molecules. Arabinose, galactose and xylose were present as side-chains.

INTRODUCTION

Pea hull cell walls are lignified tissues by opposition to non-lignified tissues which are found in the cotyledons (Selvendran, 1985). Many studies have been carried out on the non-lignified tissues of seeds (Brillouet & Carré, 1983; Crawshaw & Reid, 1984; Carré *et al.*, 1985), but few reports of the isolation of polysaccharides from seed hulls are available. The water-soluble polysaccharides of field-bean (*Dolichos lablab*) hulls are composed mainly of highly branched β-(1 → 3)(1 → 6) arabinogalactans (Salimath & Tharanathan, 1982*a*). Ammonium oxalate extracts some pectic substances rich in arabinose and xylose (Salimath & Tharanathan, 1982*b*). Successive extractions of soy-bean hulls with aqueous reagents give several xylose-rich pectic substances in addition to galactomannans and xylans (Aspinall *et al.*, 1967*b*).

In the preceding paper (Ralet *et al.*, 1992), we have chemically and physico-chemically characterized pea hull polysaccharides and we have shown that extrusion-cooking of pea hull fibre increased the water-soluble fraction by increasing the water-solubility of arabinose, galactose and rhamnose, and, to a more limited extent, of galacturonic acid and xylose. In this work we have studied and characterized the water-soluble polysaccharides of an extruded sample (GF3) showing an increased water-solubility (9%) compared to the initial product (3·6%). Structural features of two hemicellulosic and one pectic fractions from pea hulls are reported in this paper.

EXPERIMENTAL

Materials

Yellow pea hulls (*Pisum sativum*) from Sofalia (Chappes, France) were extruded with a Clextral B-45 twin-screw

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extruder. GF3 was chosen as it was extruded under mild conditions (temperature 100°C; screw speed 243 rpm; water added 60% of dry matter; feed rate 15 kg/h) with a specific mechanical energy of 231 kWh/t. Aqueous extraction was carried out at 25°C as reported in the previous paper (Ralet *et al.*, 1992) and water-soluble fractions were concentrated and freeze-dried.

Exo-arabinanase (E.C. 3.2.1.55) was purified from a commercial preparation of PECTINEX AR (Novo Industri, Bagsvaerd, Denmark) (Lahaye, 1992).

Endo-xylanase (E.C. 3.2.1.8.) was obtained from a preparation of *Clostridium thermolacticum* (Pellerin *et al.*, 1991).

Endo-polygalacturonase (E.C. 3.2.1.15) was isolated from a preparation of *Kluyveromyces fragilis* as described by Versteeg (1979).

Methods

Analytical methods

Uronic acids (as galacturonic acids) were determined by the automated *m*-phenylphenol method (Thibault, 1979). Total neutral sugars were quantified by the orcinol method (Tollier & Robin, 1979) using arabinose as a standard. Reducing sugars were determined according to the method of Nelson (1944). The individual sugars were reduced, acetylated and analysed by GLC (Blakeney *et al.*, 1983) after hydrolysis by 2N trifluoroacetic acid (2 h, 121°C). Inositol was used as an internal standard. The differentiation between galacturonic and glucuronic acid was performed by HPLC (Aminex HPX-87H; 50°C; 10^{-3} N H_2SO_4; 0·6 ml/h) after acid hydrolysis (2N trifluoroacetic acid; 2 h; 121°C).

De-esterification

Aqueous solutions (0·2% w/v) were maintained at 2°C in an ice/salt bath and the pH was adjusted to 13 with 1M NaOH at 2°C. After 1 h, the solution was brought to pH 4·5 with 1 M HCl. The solution was then dialysed (cut-off 8000 D) against distilled water and freeze-dried.

Copper precipitation

To a solution of polysaccharides (100 mg) in distilled water (10 ml) was added aqueous 7% copper sulphate (750 µl). The precipitate was separated from the supernatant by centrifugation, extensively washed with distilled water and solubilized in 2·5% ethylene diamine tetra-acetic acid disodium salt (Na_2EDTA, 10 ml). The solution was extensively dialysed (cut-off 8000 D) against distilled water and freeze-dried.

Enzymic degradation

Degradations with *exo*-arabinanase, *endo*-xylanase and *endo*-polygalacturonase were carried out in 0·05 M sodium acetate buffer (pH 5·0). Polysaccharides solutions were incubated for 24 h at 40°C or 30°C (1 nkat of enzyme/mg of sugar to be released in the polysaccharide). After 24 h hydrolysis, the same amount of enzyme was added and the reaction was continued for a further 8 h.

The enzymic degradations were followed by the analysis of the reducing sugars using arabinose, xylose or galacturonic acid as standards.

Chromatography

Ion-exchange chromatography was performed on a column (50 cm × 1·6 cm) of DEAE-Sepharose CL-6B equilibrated with 0·01 M sodium acetate buffer (pH 4·8). Sample (150 ml of a solution at 3·4 mg/ml) was loaded onto the column and the gel was washed with 500 ml of 0·01 M sodium acetate buffer (pH 4·8). The bound material was eluted with 0·08 M acetate buffer and then with a linear sodium acetate buffer gradient at pH 4·8 (0·08 to 1 M; 400 ml). Fractions (9 ml) were collected and colorimetrically assayed for their content in neutral sugars (Tollier & Robin, 1979) and galacturonic acid (Thibault, 1979).

Chromatographies on a column (70 cm × 2·1 cm) of Sephacryl S-200 were carried out at a flow rate of 25 ml/h with 0·1 M sodium acetate buffer (pH 4). Fractions (5 ml) were collected and assayed as previously described.

All fractions were extensively dialysed (cut-off 8000 D) against distilled water until the conductivity of the washing was <2 µS.

Reduction of the uronic acids

1-cyclohexyl-3-(2-morpholinoethyl)-carbodi-imide metho-*p*-toluenesulphonate (Fluka; 10 mol per mol carboxylic acid) was added to a solution of de-esterified polysaccharide (6 ml, 1 mg/ml) in 8 M urea. The pH was kept constant at 4·75 with 0·02 M HCl for 2 h. A 1 M $NaBH_4$ solution (250 mol $NaBH_4$ per carboxylic acid equivalent) was then added during 30 min, maintaining the pH at 7 with 1 M HCl, using 1-octanol as anti-foaming agent (York *et al.*, 1985). After 90 min, the solution was extensively dialysed against distilled water and freeze-dried. This reduction was carried out twice.

Methylation analysis

Fractions were methylated by the Hakomori (1964) method. A time of contact with the dimethylsulfinyl anion of 1 h was used. Acidic fractions were converted into their H^+ form by percolating the solutions through an Amberlite IR-20 resin to ensure dissolution in dimethyl-sulphoxide. Methylated polysaccharides were extracted with $CHCl_3$-CH_3OH (2:1, v:v), washed three times with distilled water, air-dried at 40°C and then hydrolysed with 90% formic acid (1 h, 100°C) and 2 M trifluoroacetic acid (90 min, 120°C), converted into their alditol acetates using perchloric acid as a catalyst

(Harris *et al.*, 1984) and analysed by GLC on (i) a fused-silica capillary column (30 m × 0·32 mm) bonded with OV-1; 150°C for 10 min, then 2°C/min to 190°C; injector temperature 210°C; detector temperature 240°C; split 60–80 ml/min; hydrogen as carrier gas at 0·7 bars; and (ii) a fused-silica capillary column (30 m × 0·32 mm) bonded with OV-225; 175°C for 15 min, then 5°C/min to 220°C; injector temperature 210°C; detector temperature 240°C; split 60–80 ml/min; hydrogen as carrier gas at 0·7 bars. Peak identification was based on retention times using inositol as internal standard. Peak areas were corrected by response factors as described by Sweet *et al.* (1975).

^{1}H- and ^{13}C-NMR spectroscopy

^{13}C and ^{1}H-NMR spectra of ~0·8% solutions of polysaccharides in D_2O were recordered at 80°C with a Bruker AM 500 spectrometer operating at 125·76 and 500·14 MHz, respectively. Spectral width of 25 KHz and recycling time of 1·05 s were used for ^{13}C-NMR and chemical shifts were measured in ppm from the signal of external DMSO and converted to values related to tetra-methyl-silane. Spectral width of 12 KHz and recycling time of 1·4 s were used for ^{1}H-NMR and chemical shifts were measured in ppm from the signal of external sodium 2, 2-dimethyl-2-silapentane-5-sulphonate.

RESULTS

Fractionation

The elution pattern of the water-soluble polysaccharides from extruded pea hulls on DEAE-Sepharose CL-6B is shown in Fig. 1. The recovery was close to 100%.

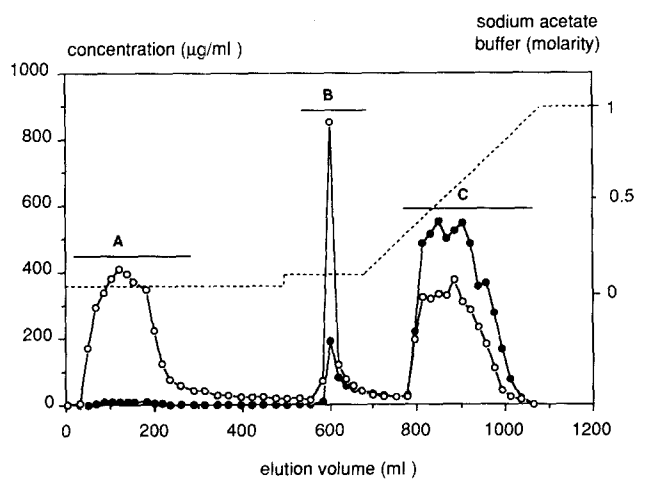

Fig. 1. Ion-exchange chromatography of the water-soluble fraction of extruded pea hulls on DEAE-Sepharose CL-6B: (○) neutral sugars; (●) galacturonic acid; (---) sodium acetate buffer molarity.

Three fractions were obtained;

(A) a fraction not bound to the gel accounting for 25% of the injected total sugars;
(B) a weakly acidic fraction (12% of the injected total sugar) eluted for an ionic strength of 0·08 M; and
(C) a strongly acidic fraction (63% of the injected total sugars) eluted for an ionic strength between 0·3 M and 0·8 M.

Fraction (A) contained a high amount of xylose (67·4 mol%), appreciable amounts of arabinose (26·5 mol%) and traces of glucose, mannose and galactose but was devoid of uronic acids. Fraction (B) had little arabinose (4·9 mol%), glucuronic acid (4·6 mol%) and was very rich in xylose (85·8 mol%); traces of glucose, mannose and galactose were also detected. Fraction (C) was rich in galacturonic acid (41 mol%) with only traces of glucuronic acid, but with arabinose (23·4 mol%), xylose (13·9 mol%), rhamnose (13·4 mol%) and galactose (7·7 mol%).

Structure of fraction (A)

Composition and structure of the whole fraction

Fraction (A) gave 12 partially methylated sugars (Table 1). The ratio (1·0) between terminal residues and branched units and the good correlation with direct analysis, suggested that no under-methylation had occurred. Xylose was essentially present as (1 → 4) linked units, ~92% being unsubstituted, ~6% carrying a single substitution at O-2 and ~2% consisting of non-reducing end-groups. No substitution at O-3 or double substitution at O-2 and O-3 were detected. Arabinose was mainly present as (1 → 5) linked units (~81%), as terminal residues (~16%) and as (1 → 3, 5) linked units (5%). Minor amounts (6%) of glucose were detected, 80% being (1 → 4) linked. Terminal, (1 → 6) and (1 → 4, 6) linked units were also detected. It is likely that these glucose units correspond to soluble fragmented starch contaminating the fraction. The large amount of (1 → 4) linked xylose units and of (1 → 5) linked arabinose units indicated that fraction (A) contains either a single polymeric material, e.g. an heteroxylan slightly substituted at O-2 with arabinofuranose residues present as relatively long side-chains (average d.p. = 5), or two independent polymers such as a (1 → 4) linked xylan backbone with the predominent single arabinofuranosyl substituants at O-2, and a (1 → 5) linked arabinan.

^{1}H-NMR analysis (Fig. 2(a)) revealed signals for anomeric protons of α-D-arabinofuranosyl residues at 5·05 ppm and 5·15 ppm and of β-D-xylopyranosyl residues at 4·25–4·7 ppm (Capon & Thacker, 1964; De Silva *et al.*, 1986; Bengtsson & Åman, 1990). On the basis of methylation results and by comparing their relative intensities, the signal at 5·15 ppm was assigned

Table 1. Methylation analysis of native, *exo*-arabinanase-degraded and *endo*-xylanase-degraded fraction (A)

	Methyl ether	A	A II	A II'	A II_1	A III	A III'
Arabinose	235 Ara	4·1	3·4	0·4	13·3	6·5	1·9
	234 Ara	—	—	—	0·5	—	—
	25 Ara	—	—	—	0·7	—	—
	23 Ara	21·2	13·6	1·0	72·9	37·6	40·4
	2 Ara	0·9	0·3	—	5·7	1·7	2·1
	Ara	—	—	—	1·5	—	—
		26·2 (26·5)[a]	17·3 (19·3)	1·4 (2·2)	94·6 (92·9)	45·8 (48·3)	44·4 (43·2)
Xylose	234 Xyl	0·9	0·8	1·0	0·5	1·8	1·6
	23 Xyl	62·1	78·5	95·8	2·4	18·0	24·8
	3 Xyl	4·2	2·9	0·5	—	5·4	1·6
		67·2 (67·4)	82·2 (79·6)	97·3 (96·2)	2·9 (3·4)	25·2 (23·8)	27·9 (27·1)
Galactose	246 Gal	—	—	—	—	2·6	1·4
	234 Gal	0·3	—	—	—	—	—
	236 Gal	1·0	—	—	0·8	1·6	3·3
	26 Gal	—	—	—	0·9	—	1·2
	23 Gal	—	—	—	0·3	—	1·1
		1·3 (0·5)	—	—	2·5 (3·5)	4·2 (2·0)	6·9 (8·0)
Glucose	2346 Glc	0·6	—	—	—	3·1	3·8
	236 Glc	4·2	—	0·4	—	17·9	9·2
	234 Glc	0·8	—	—	—	1·6	1·3
	23 Glc	0·4	—	—	—	2·3	3·2
	Glc	—	0·5	—	—	—	—
		6·0 (4·5)	0·5 (1·2)	0·4 (1·7)	—	24·9 (22·9)	17·5 (15·7)
Mannose	236 Man	—	—	—	—	—	3·3
		0 (1·1)	—	—	—	—	3·3 (6·0)

[a]Values in parenthesis are relative mole percent of constituent sugars obtained by direct sugar analysis.

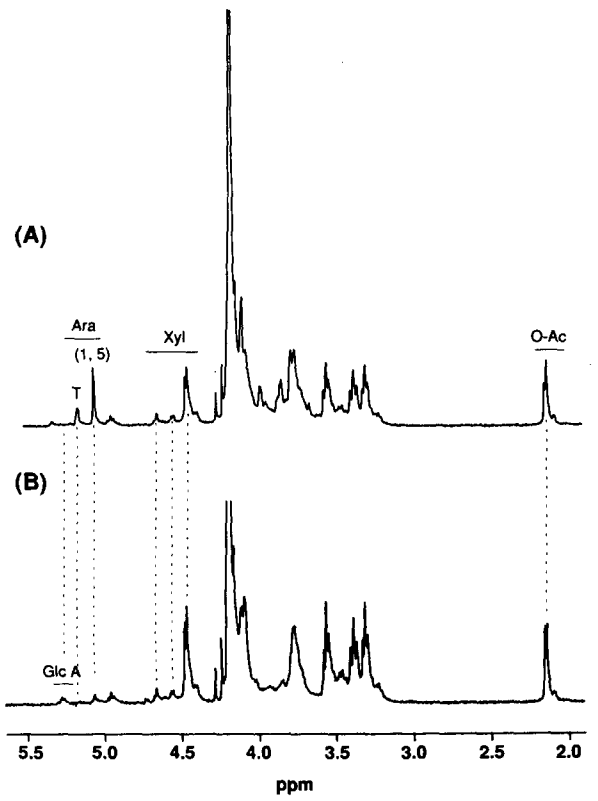

Fig. 2. ^{1}H-NMR spectra of fraction (a) and (b) from the DEAE Sepharose CL-6B column.

to the terminal arabinofuranosyl residues and the signal at 5·05 ppm to the (1 → 5) linked arabinofuranosyl residues. No signal at 5·4 ppm, relevant to terminal arabinofuranosyl residues linked to O-3 of the branched xylopyranosyl residues (Bengtsson & Åman, 1990) could be detected. The sample was highly acetylated as shown by the signals around 2·2 ppm. It can be calculated by integrations of the signals assigned to anomeric xylose residues and of the signals assigned to acetyl groups that 7 out of 10 xylose residues are acetylated; a value close to that measured by Selvendran & King (1989) for xylans of the parchment layer of pods of mature runner beans. Integrations of the signals in the anomeric region showed a ratio 0·36 for arabinose to xylose residues, in agreement with the sugar analysis (0·39).

^{13}C-NMR spectrum is shown in Fig. 3(a). This spectrum was interpreted on the basis of methylation and literature data (Brillouet *et al.*, 1982; Brillouet & Joseleau, 1987; Bengtsson & Åman, 1990). The most important signals were assigned to internal (1 → 4) linked β-D-xylosyl residues. The resonances around 101·9 ppm, 76·5 ppm, 73·8 ppm, 72·9 ppm, and 63·2 ppm correspond to C_1, C_4, C_3, C_2, and C_5, respectively, of the internal units of the xylan main chain. Intense resonances at 107·7 ppm, 82·5 ppm, 81·0 ppm, 76·9 ppm, and 67·1 ppm were ascribed, respectively, to C_1, C_4, C_2,

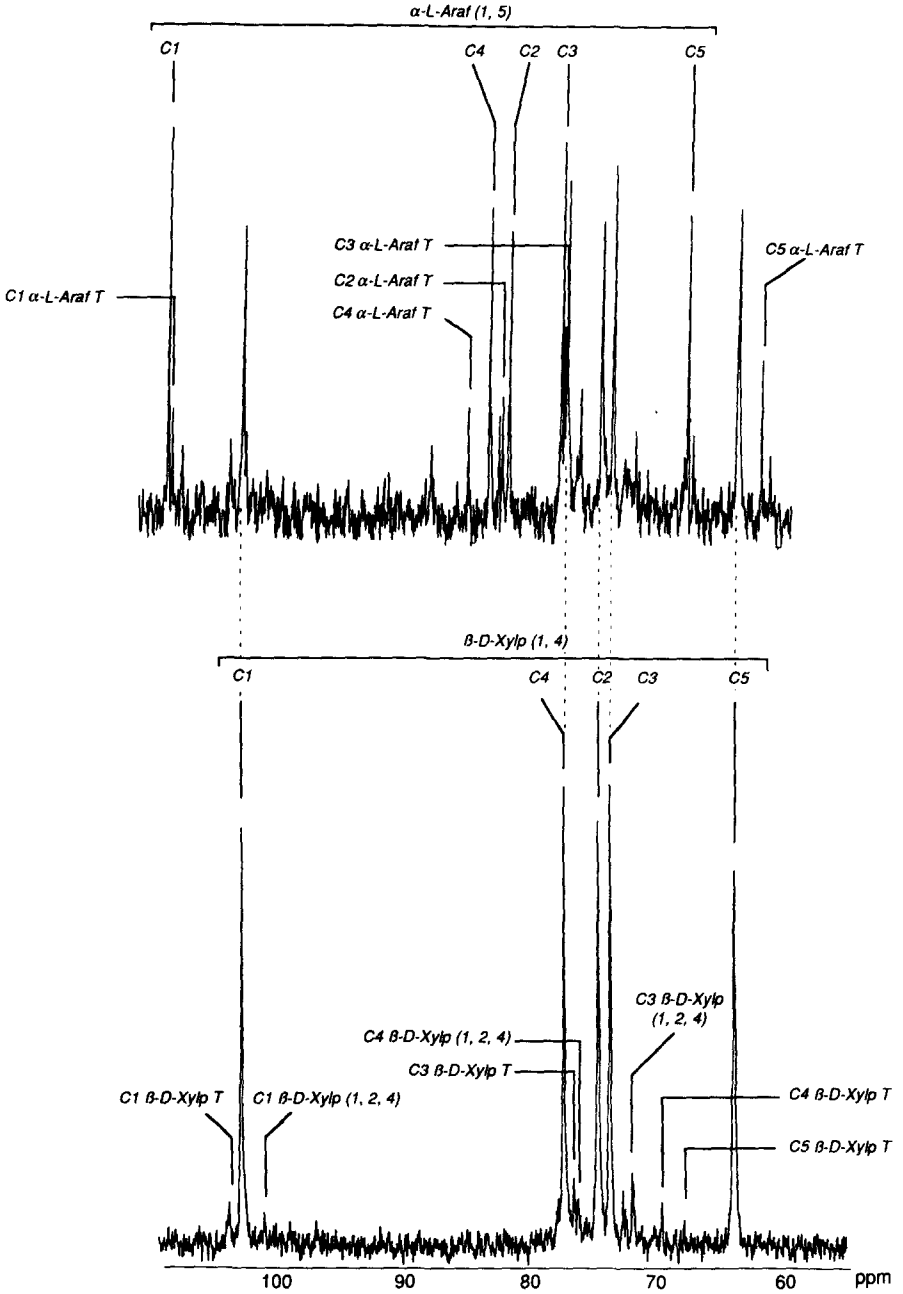

Fig. 3. ^{13}C-NMR spectra of fraction (a) and (b) from the DEAE Sepharose CL-6B column.

C_3 and C_5 of (1 → 5) linked arabinofuranosyl residues. Signals at 107·4 ppm, 84·2 ppm, 81·5 ppm, 76·7 ppm and 61·3 ppm were assigned, respectively, to C_1, C_4, C_2, C_3 and C_5 of terminal α-L-arabinofuranosyl residues. Signal for the carboxyl groups of glucuronic acid was absent in fraction (A), confirming the neutral character of the heteroxylan. Signals were observed at low field (20·3–21·1 ppm) for acetyl groups.

Gel-filtration chromatography

When chromatographed on Sephacryl S-200 (Fig. 4(a)), fraction (A) revealed a main population (A II; 75% of total sugars injected) eluted as a narrow peak at K_{av} ~0·25. A minor population (A I; 5%) was eluted at the void volume. A third population (A III) was eluted as a large peak between K_{av} 0·5 and K_{av} 0·9 (20%). Sugar analysis revealed that (A I) contained xylose (54·3%), arabinose (29·4%), glucose (8·9%) and mannose (7·4%). Fraction (A II) was composed of xylose (79·6%) and arabinose (19·3%) and contained traces of glucose. Fraction (A III) contained xylose (23·8%), larger amounts of arabinose (48·3%), residual glucose (22·9%), and traces of galactose and mannose. Fraction (A II), eluting at K_{av} 0·25 on Sephacryl S-200 (Fig. 4(a)), gave 6 partially methylated sugars, among which (1 → 4) linked xylose and (1 → 5) linked arabinose were predominant (Table 1). An approximate d.p. of 103 for the xylan backbone was obtained from the ratio of

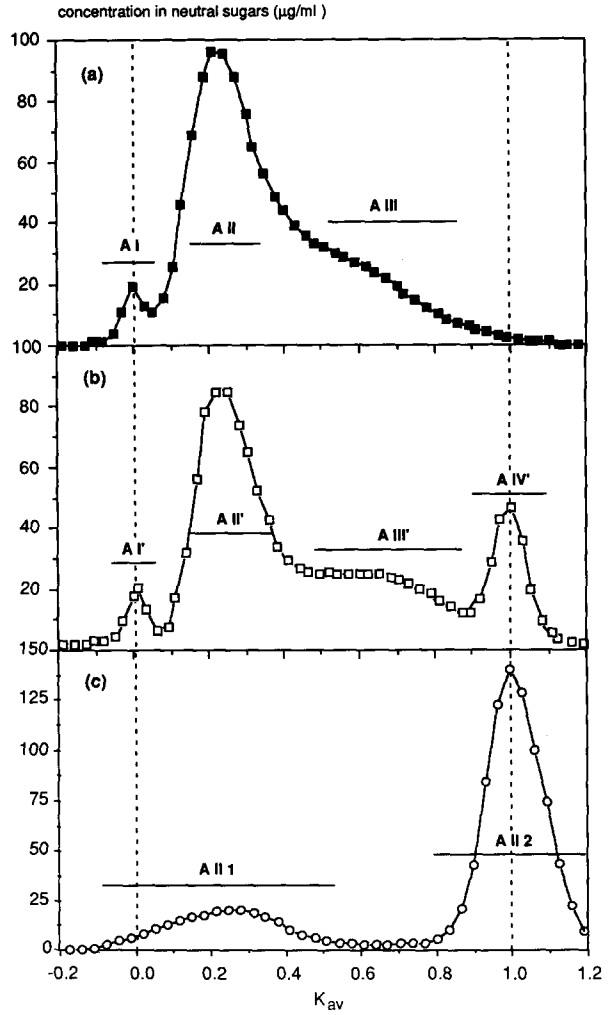

Fig. 4. Gel permeation chromatography on Sephacryl S-200 of fraction (A). (a) native, (b) *exo*-arabinanase degraded, and (c) *endo*-xylanase degraded.

(1 → 4) linked + (1 → 2, 4) linked xylose residues to terminal xylose residues. Fraction (A III), eluting at K_{av} 0·65 on Sephacryl S-200 (Fig. 4(a)), was composed of numerous fragmented polysaccharidic fractions since it gave 12 partially methylated sugars (Table 1). Xylose was essentially present as (1 → 4) linked units, arabinose as (1 → 5) linked units and glucose as (1 → 4) linked units. Traces of (1 → 3) and (1 → 4) linked galactose were also detected.

As fractionation by gel-permeation chromatography (Fig. 4(a)) did not lead to clear-cut separation, degradation by an *exo*-arabinanase and by an *endo*-xylanase were applied in order to check the existence of single or multiple populations.

Hydrolysis by an exo-*arabinanase*

About 50% of the total arabinose was removed after 24 h and additional enzyme did not increase this yield. The elution pattern of the hydrolysate on Sephacryl S-200 (Fig. 4(b)) showed that A I and A III were not affected and that the amount of A II decreased without changes in the K_{av}. A further peak (A IV'; 14% of the total sugars) appeared at the total volume where arabinose units accumulated (Fig. 4(b)). Fraction (A II'), eluting at K_{av} 0·25 on Sephacryl S-200 (Fig. 4(b)), was composed of xylose and arabinose in a molar ratio (44:1) and contained traces of glucose. Fraction (A III'), eluting at K_{av} 0·65 on Sephacryl S-200 (Fig. 4(b)) contained larger amounts of arabinose (molar ratio xylose:arabinose 0·6:1) and 14·6% of residual glucose.

In fraction A II', terminal non-reducing as well as (1 → 5) linked arabinosyl units were affected by the enzymic treatment (Table 1). The decrease of the proportion of (1 → 2, 4) linked xylose indicated the presence of arabinofuranosidase-like activity and showed that at least part of the arabinose residues are linked to the xylan backbone.

Hydrolysis by an endo-*xylanase*

To get unambiguous chromatographic results, hydrolysis by the *endo*-xylanase was carried out on the major fraction (A II) isolated by gel filtration chromatography on Sephacryl S-200. The *endo*-xylanase released 48% of the xylose. The reducing power of the medium reached a plateau at 24 h and did not increase when additional enzyme was added. When chromatographed on Sephacryl S-200, *endo*-xylanase degraded fraction (A II) revealed 2 peaks (Fig. 4(c)). The peak A II_1 at K_{av} 0·25 (Fig. 4(c)), representing 16% of the total sugars, was composed mainly of arabinose with traces of xylose, and galactose. Methylation analysis (Table 1) showed that arabinose was essentially present as (1 → 5) linked and terminal units (77·1% and 14·1% of total arabinose, respectively). This arabinan was slightly substituted at O-3 (6%) and some double substitutions were also present (1·6%). Traces of (1 → 3) linked arabinofuranosyl units were detected (Table 1). The peak A II_2 at the total volume contained xylose and traces of arabinose and glucose. These results are consistent with the presence of a slightly branched neutral xylan and a free arabinan.

Structure of fraction (B)

Composition and structure of the fraction

Fraction (B) gave 12 partially methylated sugars. A ratio of 0·8 between terminal residues and branched units was found. Glucuronic acid units, known to be linked to the O-3 atoms of the xylan backbone as single-unit side chains (Aspinall *et al.*, 1954), could explain this rather low ratio. Xylose was predominantly (1 → 4) (88·2%) linked but there were also significant amounts of (1 → 2, 4), (1 → 3, 4), and (1 → 2, 3, 4) linked xylopyranosyl residues (1·9%, 1·0% and 1·2%, respectively). Terminal residues were present in relatively large amounts (2·2%), part of them being probably carried as side chains (Wilkie & Woo, 1977; Bacic & Stone, 1981). Arabinose was predominantly terminally-

linked (1·7%) but (1 → 5) linked arabinofuranosyl residues were also present (1·5%). Traces of galactose, essentially terminally-linked, and of glucose were also detected.

^{1}H-NMR analysis (Fig. 2(b)) revealed signals for β-D-xylopyranosyl residues at 4·25–4·7 ppm (Capon & Thacker, 1964; De Silva *et al.*, 1986; Bengtsson & Åman, 1990). The small signal present around 5·3 ppm could be assigned to α-glucuronic acid by comparison to literature data (Di Fabio *et al.*, 1984). It can be calculated by integrations of the signals assigned to anomeric xylose residues and of the signals assigned to acetyl groups at 2·2 ppm, that 7 out of 10 xylose residues are acetylated, as in fraction (A). Integrations of the signals in the anomeric region showed a ratio 0·05 for arabinose to xylose residues, in agreement with the sugar analysis (0·06).

^{13}C-NMR spectrum of fractions B is shown in Fig. 3(b). The most prominent signals were assigned to internal (1 → 4) linked β-D-xylosyl residues. The resonances around 101·9 ppm, 76·5 ppm, 73·8 ppm, 72·9 ppm, and 63·2 ppm correspond to C_1, C_4, C_3, C_2, and C_5, respectively, of the internal units of the xylan main chain. The signals at 102·8 ppm, 75·7 ppm, 72·3 ppm, 68·8 ppm, and 65·5 ppm were assigned to C_1, C_3, C_2, C_4, and C_5, respectively, of the terminal xylosyl groups by comparing with previously published data (Brillouet *et al.*, 1982; Brillouet & Joseleau, 1987). Finally, resonances around 100·1 ppm, 76·5 ppm, 75·4 ppm, 71·1 ppm, and 63·2 ppm were assigned to C_1, C_2, C_4, C_3, and C_5, of the branched residues by considering changes due to the 2-substitution and relative intensities of the signals. The absence of signal around 78–80 ppm, generally ascribed to C_3 of the O_3-branched xylosyl residues (Brillouet *et al.*, 1982; Bengtsson & Åman, 1990), confirms the low appearance of that type of linkage as suggested by the methylation analysis. Typical signals were also observed at high fields (173·9 ppm) for the carboxyl groups of glucuronic acid and at low field (20·3–21·1 ppm) for acetyl groups.

These results indicate the presence of an arabino-glucurono-xylan built on a central core of (1 → 4) linked β-D-xylopyranosyl residues, ~93% being unsubstituted, ~3% carrying a single substitution at O-2, ~1% carrying a single substitution at O-3, ~1% being doubly branched at O-2 and O-3 and ~2% consisting of non-reducing end-groups.

Structure of fraction (C)

Composition and structure of the whole fraction

Fraction (C) gave 18 partially methylated alditol acetates (Table 2) and a terminal/substituted ratio of 0·9 was found.

Fraction (C) contained mainly (1 → 5) linked arabinosyl residues (74% of total arabinose) and terminal arabinosyl residues accounted only for 15% of total arabinose. The low proportion (11%) of (1 → 3, 5) linked arabinosyl residues indicated a relatively low degree of branching in this fraction. Arabinose residues are likely involved in a structure with a backbone of (1 → 5) linked arabinofuranosyl residues with few branching points on position 3. Rhamnose occurred in (1 → 2) linked, (1 → 2, 4) linked and terminal forms (14·2%, 5·8% and 0·8%, respectively). The (1 → 2) and (1 → 2, 4) linked rhamnoses are typical constituents of rhamnogalacturonans (Darvill *et al.*, 1980; Aspinall, 1980). Xylose was predominantly (1 → 4) linked (44%) but large amounts of terminal (31%) and (1 → 3, 4) linked (25%) xylosyl residues were also present. Galactose was present as seven different derivatives. (1 → 3) linked galactose was the dominant feature (44% of total galactose) followed by (1 → 4) linked (22%), (1 → 6) linked (12%), terminal (11%) and (1 → 3, 6) linked (6%). Type I galactans, consisting of a main chain of (1 → 4) linked galactosyl residues, and type II galactans, consisting of a main chain of (1 → 3) linked galactosyl residues substituted by short chains of (1 → 6) linked galactosyl residues, seem to be present in fraction (C). The low amount of (1 → 3, 6) linked galactosyl residues indicated that the galactosyl units are only slightly branched.

After de-esterification with cold dilute sodium hydroxide, 35% reduction of fraction (C) was achieved in one step. A second reduction was then applied and 82% reduction was achieved. After carboxyl-reduction, (1 → 4) linked galactose became the preponderant glycosidic linkage after (1 → 5) linked arabinose (Table 2). The (1 → 4) linked galacturonic acid and the (1 → 2) linked rhamnose residues can be considered to form the pectic backbone of fraction (C). Comparison with the unreduced material showed an increase in (1 → 3, 4) linked and (1 → 2, 4) linked galactose, indicating possible branching at position 2 and 3 of galacturonic acid. Terminal and (1 → 4) linked glucose increased after carboxyl-reduction, arising from glucuronic acid as end group or linked through O-4, possibly by terminal rhamnose (Aspinall & Rosell, 1977; Saulnier *et al.*, 1988). Glucuronic acid was reported to be linked to fucose and galactose residues in the pectic polysaccharides of soy-beans (Aspinall *et al.*, 1967*b*) and lemon-peel (Aspinall *et al.*, 1968).

Gel-filtration chromatography

When chromatographed on Sephacryl S-200 (Fig. 5(a)), fraction (C) revealed two populations; one, of high molecular weight (C I) was rich in neutral sugars (70·9 mol%) and the other, of a lower molecular weight (C II), rich in galacturonic acid (69·8 mol%). Arabinose was the main neutral sugar in fraction (C I), representing 54 mol% of the neutral sugars. Xylose (20 mol%), galactose (14·2 mol%) and rhamnose (11·7 mol%) were also present in this fraction. Fraction (C II) contained lower proportions of arabinose (19 mol%) and larger

Table 2. Methylation analysis of native, carboxyl-reduced, copper-precipitated and *endo*-polygalacturonase-degraded fraction (C)

	Methyl ether	C	C carboxyl-reduced	C copper precipitated	C *endo*PG fraction 1
Rhamnose	234 Rha	0·8	0·4	0·9	0·8
	34 Rha	14·2	9·3	16·3	12·8
	3 Rha	5·8	4·0	4·5	6·5
	Rha	—	—	—	0·7
		20·9 (22·8)[a]	13·7 (13·4)	21·7 (21·6)	20·8 (19·8)
Arabinose	235 Ara	6·3	4·3	9·5	6·9
	23 Ara	31·5	21·5	22·9	41·3
	2 Ara	4·5	4·4	2·5	0·8
		42·3 (39·7)	30·2 (23·4)	34·8 (33·7)	49·0 (45·9)
Xylose	234 Xyl	6·2	4·1	14·4	6·0
	23 Xyl	8·7	6·9	11·2	5·3
	2 Xyl	—	—	—	—
	3 Xyl	4·9	3·0	3·0	4·6
	Xyl	—	—	1·8	—
		19·8 (23·5)	14·0 (13·9)	30·4 (28·2)	15·8 (16·1)
Galactose	2346 Gal	1·7	2·7	1·6	3·8
	246 Gal	6·9	7·2	3·1	6·1
	234 Gal	1·9	1·7	1·5	1·3
	236 Gal	3·5	19·2	3·4	0·9
	26 Gal	0·3	3·1	—	—
	23 Gal	0·4	4·2	—	—
	24 Gal	1·0	1·0	1·0	1·2
		15·7 (13·0)	39·1 (47·4)	10·7 (10·6)	13·4 (15·2)
Glucose	2346 Glc	0·5	1·4	—	—
	236 Glc	0·9	1·6	2·4	0·9
	234 Glc	—	—	—	—
	23 Glc	—	—	—	—
		1·4 (1·0)	3·0 (0·6)	2·4 (3·5)	0·9 (0·7)

[a]Values in parenthesis are relative mole percent of constituent sugars obtained by direct sugar analysis.

proportions of xylose (33·8 mol%) and rhamnose (29 mol%).

Fraction (C) was not degraded by *endo*-xylanase, indicating that xylose units were linked as single or oligomeric units to the rhamnogalacturonic backbone. Shibuya & Nakane (1984) attributed the presence of (1 → 4) and (1 → 3, 4) linked xylopyranosyl residues in pectic polysaccharides of rice endosperm cell walls to arabinoxylan contamination.

This fraction was tentatively separated by copper precipitation.

Copper precipitation

Sixty percent of fraction (C) was precipitated by copper. The precipitate was enriched in galacturonic acid (60 mol%) and impoverished in arabinose (13·5 mol%), galactose (4·3 mol%) and rhamnose (8·6 mol%). Xylose content remained constant. It can be calculated that 60% of the rhamnose, 50% of the arabinose, 80% of the xylose, 45% of the galactose and 88% of the galacturonic acid initially present were recovered after precipitation.

Methylation analysis revealed some discrepancies between fraction (C) and the copper precipitate (Table 2). Terminal/substituted ratios of 1·8 were found, suggesting that galacturonic acid residues in this fraction also carry some side-chains. Arabinose and galactose contents were lower while xylose content was increased in the precipitate. A lower proportion of (1 → 2, 4) linked rhamnose (21% of the total rhamnose) was detected. Although (1 → 5) linked arabinose was still the major glycosidic linkage, its proportion was lower (66% of the total arabinose) than in the native fraction (75% of the total arabinose), suggesting a lower d.p. of the arabinan side-chains. Higher proportions of (1 → 4) linked galactose and lower proportions of (1 → 3) linked galactose were observed. Xylose was present in higher quantities essentially because of the increased content of terminal xylose.

When chromatographed on Sephacryl S-200 (Fig. 5(b)), the copper precipitate revealed the same peaks as the parent fraction (C), both of them being enriched in galacturonic acid. The proportions of the

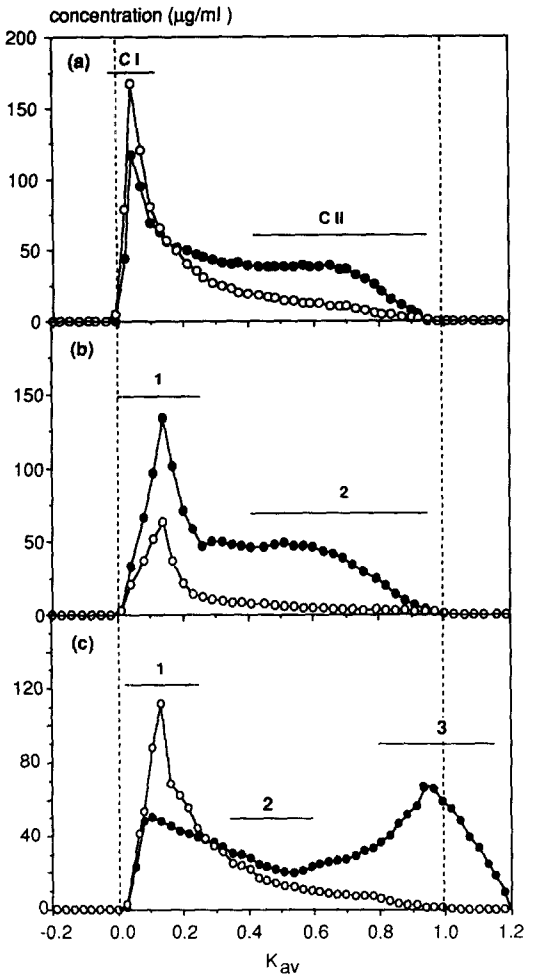

Fig. 5. Gel permeation chromatography on Sephacryl S-200 of fraction (C). (a) native, (b) copper precipitated, and (c) *endo*-polygalacturonase degraded: (○) neutral sugars; (●) galacturonic acid.

different neutral sugars were unchanged in the second peak eluting at K_{av} 0·6 while a decrease in the proportion of arabinose and a simultaneous increase in the proportion of xylose and rhamnose were noticed in the peak at K_{av} 0·15.

Hydrolysis by an endo-*polygalacturonase*

De-esterified fraction (C) was degraded by an *endo*-polygalacturonase and 27% of the galacturonic acid was released. The reducing power of the medium reached a plateau at 16 h and the reaction was complete as additional enzyme did not increase it.

Endo-polygalacturonase-degraded polysaccharides were chromatographed on Sephacryl S-200 (Fig. 5(c)). The peak eluting at K_{av} 0·15 and representing 36% of the injected sugars, was composed of arabinose, galacturonic acid, rhamnose, xylose and galactose (3:2·7:1·3:1·1:1). This fraction, corresponding to hairy fragments of the pectic molecules,was methylated and the results are shown in Table 2. The structures were very similar to those of the parent fraction. However, arabinose residues were present in larger amounts and xylose residues in lower amounts in this fraction.

A second fraction was collected between K_{av} 0·35 and K_{av} 0·6. Finally, a large peak, mainly composed of galacturonic acid with traces of xylose, rhamnose, arabinose and galactose (59:2·5:2·3:1·1:1) was eluted near the total volume. It can be calculated that ~50% of the uronic acid injected were recovered in the third peak. Although the extent of the degradation of fraction (C) by pure *endo*-polygalacturonase was quite moderate (~50% of the total galacturonic acid), the results indicate that there are homogalacturonan regions in the molecules.

DISCUSSION

The water-soluble fraction from an extruded pea hull fibre contained both hemicellulosic and pectic fractions. The major hemicellulosic fraction consisted of a neutral heteroxylan having a (1 → 4)-β-D-xylan backbone (average d.p. 103) with only very few branching points at O-2. This structure was very close to that of the esparto grass arabinoxylan found by Aspinall & Ferrier (1958). Xylans from dicotyledonous plants such as wood xylans, are usually characterized by side-chains of 4-*O*-methyl-D-glucuronic acid or D-glucuronic acid (Radhakrishnamurthy & Srinivasan, 1957). An associated arabinan was also present in the neutral fraction. This arabinan was built on a central core of (1 → 5) linked units slightly substituted at O-3 (6%). The fair proportion of (1, 3 → 5) linked arabinose indicate a relatively low degree of branching compared to isolated arabinan from white lupin (*Lupinus albus* L.) cotyledons (Carré *et al.*, 1985).

An acidic xylan was also isolated from the water-soluble fraction of extruded pea hulls. This xylan was typical of dicotyledonous plants and revealed the same essential structure as that of the parchment layer of mature runner bean pods xylans extracted with hot water (Selvendran & King, 1989). Besides terminal arabinofuranosyl substituents, longer chains terminated with galactopyranosyl or xylopyranosyl residues similar to those found by Wilkie & Woo (1977) on bamboo heteroxylans and by Bacic & Stone (1981) on wheat aleurone layer heteroxylans may be present.

Pectic substances are the major components of the water-soluble fraction of extruded pea hulls. These pectic substances are particularly rich in neutral sugars, especially arabinose and surprisingly xylose. Aspinall has reported that some pectins carry terminal xylopyranose residues on O-3 of (1 → 4) linked galacturonic acid residues (Aspinall, 1980). In tragacanthic acid, pollen and rapeseed galacturonan (Aspinall & Baillie, 1963; Bouveng, 1965; Siddiqui & Wood, 1976), most of the xylose is attached as single units to the galacturonan chain. Recently, Ryden &

Selvendran (1990*a*) have suggested that these branch points could carry oligosaccharidic substituents and (1 → 4) linked xylopyranosyl residues were detected in potato pectic polysaccharides and in the middle lamella of suspension-cultured bean cells (Northcote *et al.*, 1989; Ryden & Selvendran, 1990*b*). Analytical and literature data suggest that mono and oligosaccharidic xylose side-chains could be preferentially linked to the galacturonic acid residues.

Hemicellulosic as well as pectic substances, thought to help the seed coats to imbibe water during germination (Selvendran, 1985), have been solubilized after extrusion-cooking of a pea-hull sample. Hemicellulosic substances were composed of neutral and acidic xylans and of a slightly branched arabinan. The pectic substances extracted seem to have very complex structures with numerous and very diverse side-chains among which (1 → 5) linked arabinans were the major feature, associated with type I and II (arabino)-galactans. The occurrence of large amounts of xylose residues in the pectic fraction was of particular interest and further studies are needed to elucidate the mode of linkage of these xylose monomers and oligomers to the rhamnogalacturonan.

ACKNOWLEDGEMENT

We thank Sofalia SA (Chappes, France) for financial support and Dr M. Lahaye and Dr. P. Debeire for the gift of purified enzymes. We gratefully acknowledge the help of Dr. M. Lahaye for NMR analysis and of Dr. C.M.G.C. Renard for fruitful discussion.

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