ELSEVIER

Carbohydrate Polymers **24** (1994) 139–148
© 1994 Elsevier Science Limited.
Printed in Great Britain. All rights reserved
0144-8617/94/$07.00

Structure and properties of the polysaccharides from pea hulls. Part 1: Chemical extraction and fractionation of the polysaccharides

R.M. Weightman,† C.M.G.C. Renard* & J.-F. Thibault

Institut National de la Recherche Agronomique, Laboratoire de Biochimie et Technologie des Glucides, rue de la Géraudière, BP 527, 44026 Nantes Cedex 03, France

(Received 9 December 1993; accepted 28 January 1994)

Pectins and hemicelluloses were extracted sequentially from the alcohol insoluble residue (AIR) of pea hulls using chemical agents. The AIR was rich in carbohydrates (942 mg g^{-1}), notably cellulosic glucose, and was poorly lignified (6 mg g^{-1}). Approximately 4 and 6% of the AIR could be sequentially extracted using CDTA and HCl (CSP and HSP respectively). After such depectination treatments, 4, 6 and 4% of the AIR were extracted by increasing concentrations of KOH (0·05 M (OHSP-I), 1 M (1OH-I) & 4 M (4OH-I), respectively). By contrast, alkali treatments alone (KOH 0·05 M (OHSP-II), 1 M (1OH-II) & 4 M (4OH-II)) extracted 2, 4 and 5% of the AIR, respectively. Hemicelluloses extracted by 1 M and 4 M KOH in both series were further fractionated through precipitation, upon neutralization of the extracts. Precipitates accounted for 82, 60, 74 and 60% of total hemicelluloses in the 1OH-I, 4OH-I, 1OH-II & 4OH-II extracts respectively, and appeared to be essentially pure, acidic xylans. More variation was seen in the composition of the soluble polymers, which were rich in xylose, glucose and uronic acids. Extracts were fractionated on Sephacryl S500 and DEAE Sepharose CL-6B. In the 'pectic' fractions, high molecular weight arabinans were found in HSP as well as xylose-rich polymers. Concentrated alkalis extracted small amounts of low molecular weight pectic material, but only after prior depectination had been carried out. The final residue accounted for around 61% of the AIR after depectination and alkali treatment, and contained 91% cellulose; after alkali treatment alone, it accounted for about 84% of the AIR and was rich in glucose (~70%) and uronic acids (~16%).

INTRODUCTION

The testa of the pea seed is an interesting plant organ to study. Early in the life of the developing seed it plays an important role in processing the nutrients which enter the cotyledons for storage (Rochat & Boutin, 1992). By contrast, in the mature seed, the cells of the testa form a protective barrier against fungal pathogens. Its properties as a protective organ, which are also vital to seed growth, are due to the presence of a number of different cell types and a high concentration of polysaccharide components.

The testae can be separated easily from the cotyledons and are commonly described as pea hulls. Recently, pea hulls have received interest as a fibre-rich product of commercial value which is richer in complex carbohydrates than wheat bran, is virtually tasteless, and has useful physicochemical properties when incorporated as a food ingredient. Little work has been carried out in order to characterise these polysaccharides, with the exception of the arabinoxylan described by Banerji and Rao (1963), or more recently the polysaccharides solubilised after extrusion cooking (Ralet *et al.*, 1993*a*,*b*). Such information would be essential in explaining the physiological effects of pea hulls in the diet, their poor digestibility (Longstaff & McNab, 1989), and their physicochemical properties when used as 'fibre' additives or as bulking agents. The aim of this study was to characterise the polysaccharides

*To whom correspondence should be addressed.
†Present address: ADAS, Soil & Water Research Centre, Anstey Hall, Maris Lane, Trumpington, Cambridge, UK, CB2 2LF.

of commercial pea hulls, in order to perform further structural investigations on the important fractions and to have a structural basis for explaining some of their physico-chemical properties.

EXPERIMENTAL

Materials

Pea hulls of approximately 11% moisture content were provided by Sofalia (Ennezat, France) and stored at ambient temperature until required.

Preparation of alcohol insoluble residue (AIR)
Low molecular weight compounds were extracted from pea hulls in boiling 70% (v/v) ethanol for 20 min. The hulls were filtered on sintered glass, and subsequently washed (6×) with 70% (v/v) ethanol at room temperature. Washing was repeated until extracts were free of sugars, as monitored by addition of phenol/sulphuric acid reagent (Dubois *et al.*, 1956). AIR was dried by solvent exchange (ethanol 96% and acetone), oven dried at 40°C, then milled in an IKA Universal Mill (particle size distribution after treatment: 88% in range 0·16–0·5 mm).

Methods

Sequential extraction of AIR
Two extraction schemes were employed and these are summarised in Table 1; the first employed five successive extraction steps, and began with five samples of the AIR. At each extraction step, one residue was recovered on sintered glass, washed repeatedly with water, dried by solvent exchange and its yield recorded. This procedure gave replicate extracts for all but the final extract. Extractions were as follows; AIR (5 g) was extracted in sequence with: (1) 50 mM cyclohexane-*trans*-1,2-diamine-*NNN'N'*-tetra acetate (CDTA pH 4·8, 20°C, 150 ml), 16 h, 3 h, 3 h; (2) HCl (50 mM, pH 1·6, 85°C, 3 × 0·5 h, 150 ml) and washed (3×) with water; (3) 0·05 M KOH, (4) 1 M KOH and (5) 4 M KOH, each 16 h, 3 h, 3 h, (4°C, 150 ml, containing sodium borohydride 12·6 mM). The second extraction scheme utilised only the three alkaline extraction steps, and consequently started with three (5 g) residues ((6) 0·05 M KOH, (7) 1 KOH, (8) 4 M KOH, conditions as above). The residues were centrifuged after each extraction, and the extracts and washings recovered. CDTA and HCl extracts were brought to pH 5·5 with sodium acetate. Alkaline extracts were acidified to pH 5 with acetic acid. All extracts were concentrated by rotary evaporation and extensively dialysed against distilled water prior to freeze-drying. For the 1 M and 4 M KOH extracts in both extraction schemes, a white precipitate formed on neutralisation and was recovered by centrifugation, rinsed with distilled water and freeze-dried.

Table 1. Extraction schemes and conditions used during the sequential chemical extraction of commercial pea hulls

	Solvent	Concentration	Duration (h)	Temperature (°C)
1st extraction series				
CSP	CDTA	(50 mM)	16 h, 3 h, 3 h	20
HSP	HCl	(50 mM)	3 × 0·5 h	85
OHSP-I	KOH	(50 mM)	16 h, 3 h, 3 h	4
1OH-I	KOH	(1·0 M)	16 h, 3 h, 3 h	4
4OH-I	KOH	(4·0 M)	16 h, 3 h, 3 h	4
2nd extraction series				
OHSP-II	KOH	(50 mM)	16 h, 3 h, 3 h	4
1OH-II	KOH	(1·0 M)	16 h, 3 h, 3 h	4
4OH-II	KOH	(4·0 M)	16 h, 3 h, 3 h	4

Analyses
All values are presented on a dry weight basis, dry matter being determined by drying at 120°C for 2 h. Ash was determined as the residue after incineration overnight at 550°C then 1 h at 900°C. Lignin was measured as the dry weight of organic matter in the residue remaining after refluxing AIR with CTAB (*N*-cetyl-*NNN* trimethyl ammonium bromide solution) 20 g/litre in 1 N sulphuric acid for 1 h, prior to dispersion in 72% sulphuric acid (3 h, 20°C), and finally washing extensively with distilled water (Klason, 1931). Starch was estimated as 0·9 × glucose released after sequential digestion of AIR with Termamyl and amyloglucosidase (Batey, 1982). Protein content of extracted polysaccharides was determined using the method of Lowry *et al.* (1951) with a bovine serum albumin standard. Uronic acids were determined by the automated *m*-phenylphenol method (Thibault, 1979) using a galacturonic acid standard. Total neutral sugars were determined by the automated orcinol method (Tollier & Robin, 1979). All fractions were hydrolysed in 2 N sulphuric acid (3 h, 100°C) for measurement of individual neutral sugars, with an additional pre-treatment of 72% sulphuric acid (1 h, 20°C) for AIR and its chemically-extracted residues (Saeman *et al.*, 1954). The individual sugars were reduced, acetylated and analysed as their alditol acetates by GLC (Englyst & Cummings, 1984). Differentiation of galacturonic and glucuronic acid was carried out on a portion of the acid hydrolysates, using a Dionex Carbopac PA1 HPLC column, with a PAD detector (Dionex Corporation, USA). Sugars were eluted from the HPLC column using a linear acetate gradient over 15 min, of 0–300 mM sodium acetate in 150 mM NaOH (flow rate of 1 ml min^{-1}) followed by isocratic elution (300 mM sodium acetate/150 mM NaOH) for 5 min prior to re-equilibration of the column (10 min) in 150 mM NaOH. Methanol and acetic acid were determined on an Aminex HPX 87 H, HPLC column (Voragen *et al.*, 1986), after saponification with isopropanol/NaOH.

Chromatography

Solutions (5 ml) of polysaccharides (10 mg) in 0·05 M sodium acetate buffer pH 4·8, were loaded onto a DEAE-Sepharose CL-6B column (20 cm × 2 cm) for anion exchange chromatography. The column was eluted with 60 ml of 0·05 M sodium acetate buffer pH 4·8 at 45 ml h^{-1}. Bound material was then eluted using a linear gradient (240 ml, 0·05 M–1 M acetate buffer pH 4·8) followed by 45 ml of 1 M sodium acetate buffer. Fractions (4·5 ml) were collected and analysed as above for neutral and acidic sugars. Samples were dissolved overnight at room temperature in 0·1 M sodium acetate buffer, pH 5, centrifuged, and the supernatant (0·6 ml, approximately 10 mg polysaccharide ml^{-1}) loaded onto a Sephacryl S-500 column (65 cm × 2 cm) for gel filtration chromatography. Elution was carried out ascendingly with 0·1 M sodium acetate buffer, pH 5 at 60 ml h^{-1} and fractions (2 ml) were collected and analysed for their galacturonic and neutral sugar contents. Fractions were pooled, reduced if necessary by rotary evaporation, dialysed against distilled water, and recovered by freeze-drying. Fractionated polysaccharides were hydrolysed and analysed for neutral and acidic sugars as described above.

RESULTS AND DISCUSSION

Alcohol insoluble residue (AIR)

Preparation of AIR mainly solubilises the sucrose and galactosyl oligosaccharides which are found in pea seeds (Holl & Vose, 1980). Most of the oligosaccharides are found stored in the cotyledons of the mature seed, and so the loss after preparation of AIR was only 3% of the weight of the starting material. In the commercial product (from de-hulling whole pea seeds) starch may be present as a contaminating material originating from the cotyledons, but in the present study only accounted for 5 mg g^{-1} of AIR. Ash concentration was 22 mg g^{-1}, slightly lower than the ash concentration found in whole peas. The concentration of glucose (Table 2) was quite high compared with the range of 43–46% reported by Longstaff and McNab (1989), Englyst *et al.* (1988) and Ralet *et al.* (1993*a*). Comparable values have been reported by Selvendran (1984) who measured non-starch polysaccharide (NSP) glucose concentrations of 57% in pea hulls, and Longstaff and McNab (1991) who measured NSP-glucose concentrations of 60% in faba bean (*Vicia faba*) hulls. The AIR was also rich in

Table 2. Composition (% of DM) and yields (% of AIR) of residues and polysaccharides extracted from pea hulls sequentially with CDTA (CSP), HCl (HSP), 0·05 M KOH (OHSPI), 1 M KOH & 4 M KOH (1OHI & 4OHI respectively), or extracted sequentially with 0·05 M KOH, 1 M & 4 M KOH (OHSP-II, 1OH-II & 4OH-II respectively)

	Yield (%) (+/− SD[a])	Composition (mg g^{-1})											
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	AUA	%GalA/ %GlcA[b]	Methanol (DM)[c]	Acetic acid (DA)[c]	Protein
Pea hulls (AIR)		16	tr.	39	122	6	28	581	150	97/3	4(13)	17	nd
1st extraction series													
CSP	4·2(0·83)	19	3	58	83	tr.	26	7	640	100/0	28(24)	16(7)	42
HSP	5·8(0·48)	53	14	249	144	tr.	70	23	393	96/4	37(51)	16(12)	90
OHSP-I	4·3(0·24)	32	10	17	89	9	37	7	536	100/0			112
1OH-I													
1OH-IA[d]	4·9(0·63)	tr.	tr.	7	773	tr.	tr.	23	47	38/62			54
1OH-IB	1·1(0·02)	24	19	97	240	tr.	56	138	218	93/7			109
4OH													
4OH-IA[d]	2·4	tr.	tr.	79	883	tr.	tr.	tr.	43	15/85			79
4OH-IB	1·6	20	19	50	187	4	58	178	211	95/5			221
4OH-IR Residue	61·1	10	tr.	9	27	tr.	tr.	906	44	100/0			nd
2nd extraction series													
OHSP-II	2·2(0·05)	31	7	144	90	tr.	52	19	342	96/4			204
1OH-II													
1OH-IIA[d]	3·2	tr.	tr.	22	624	tr.	tr.	37	62	28/72			144
1OH-IIB	1·1	31	11	148	130	8	60	89	186	91/9			261
4OH-II													
4OH-IIA[d]	3·1	tr.	tr.	13	843	tr.	tr.	30	57	48/52			68
4OH-IIB	2·1	23	13	112	132	4	53	108	334	96/4			118
4OH-IIR Residue	83·8	13	tr.	35	66	tr.	10	679	157	98/2			nd

[a] SD, standard deviation; $n = 4$ for CSP & HSP; $n = 3$ for OHSP-I & OHSP-II; $n = 2$ for 1OH-I & 1OH-II.
[b] GalA, galacturonic acid; GlcA, glucuronic acid.
[c] DM, degree of methylation; DA, degree of acetylation.
[d] A: precipitated fractions (1OH-IA, 4OH-IA, 1OH-IIA & 4OH-IIA) and B: fractions soluble after neutralisation (1OH-IB, 4OH-IB, 1OH-IIB & 4OH-IIB).
tr.: traces.
nd: not determined.

xylose and uronic acids, broadly in agreement with the concentrations of sugars measured in pea hulls by Longstaff and McNab (1989). The uronic acids were poorly methylated, in agreement with data of Ralet *et al.* (1993*a*) for pea hulls and contrasting with the highly methylated pectins commonly found in fruits and in vegetable tissues. Acetylation degree was not calculated for the AIR from pea hulls, as it has been shown (Ralet *et al.*, 1993*b*) to contain acetylated xylans.

The lower polysaccharide concentrations reported previously for pea hulls (74–80%) have been explained in terms of high concentrations of lignin. However in this study, 'Klason' lignin only accounted for $6\,mg\,g^{-1}$ of AIR, similar to the concentration reported for sweet lupin hulls which also contain approximately 50% cellulose and only 0·4–1·7% lignin (Bailey *et al.*, 1974; Brillouet & Riochet, 1983). Thus, pea hulls cannot be considered a source of 'typical' lignified secondary cell walls as classified by Selvendran (1985); a more representative source would be the mature stem of peas (18% 'Klason' lignin, Åman & Graham, 1987). It seems likely that the differences arise from incomplete solubilisation of the cellulose with short prehydrolysis and hydrolysis times.

Chemical extraction of AIR

Yields of extracted polysaccharides were quite variable, and yield data are presented with their relevant standard deviations (SD) in Table 2. Variation in composition between replicates was much less than variation in yields and thus compositional data are presented without SD for clarity. Extracts were subsequently pooled for further chemical analysis. Eight extracts were recovered in total (Table 2), five after successive extraction with chelating agent (CSP), hot acid (HSP), 0·05 M alkali (OHSP-I), 1 M alkali (1OH-I) and 4 M alkali (4OH-I), and three after successive extraction with 0·05 M alkali (OHSP-II), 1 M alkali (1OH-II) and 4 M alkali (4OH-II). Within each 1 and 4 alkali treatments, polysaccharides were further fractionated into either precipitates 'A' (1OH-IA, 1OH-IIA, 4OH-IA, 4OH-IIA), or soluble polysaccharides 'B' (1OH-IB, 1OH-IIB, 4OH-IB, 4OH-IIB) which precipitated or remained soluble, respectively, on neutralisation of the alkaline extract.

The final residue after extraction with 4 M alkali in series I (4OH-IR) gave a yield of 61% of AIR and contained 91% glucose, confirming the high concentration of cellulose found in the pea hulls. The first extraction series released the major part, but not all of the pectic substances in the AIR. The three successive depectination treatments had similar efficiencies, with yields in uronic acid of 18% (CSP) and 15% (HSP and OHSP-I), whereas only small amounts of galacturonic acid were solubilised by the concentrated alkalis. Uronic acid content of the 4OH-IR residue was 4·4%, and still represented a significant proportion (approximately 18%) of the uronic acid originally present in the AIR (Table 2). The global recovery of this extraction series was only 85%, with more marked losses for arabinose (recovery: 66%), uronic acids (72%) and rhamnose (75%). These losses are probably due to formation of oligomers, as the recovery for glucose was 97%.

Individual yields from the second extraction series were lower (range 1·2–3·6% of AIR) than from the first series (range 1·2–6·4% of AIR) and consequently, total yield from the second (approximately 13% of AIR) was also lower than that of the first extraction series (27% of AIR). By contrast with the first extraction series, the final residue after 4 M KOH extraction (4OH-IIR residue; Table 2) contained 15·7% uronic acid, representing 88% of the original uronic acid. Dilute alkali, when used as a first treatment, only extracted 5% of the uronic acid, a third of the amount extracted after CDTA and acid treatment. The global recovery of this extraction series was 96%, with marked losses only for xylose (recovery: 88%). This time, all of the arabinose (96%) and the uronic acids (101%) could be accounted for in the extracts and residue.

Pectins were mostly extracted by the three first treatments of the first extraction series. In both series, the major part of the concentrated alkali extract precipitated ('A' fractions), with much lower yields of the soluble 'B' fractions.

Precipitate ('A' fractions)

In both extraction series, because of their poor solubility, 'A' fractions (1OH-IA, 1OH-IIA, 4OH-IA and 4OH-IIA) were not purified further by chromatographic methods. These major fractions formed 82, 60, 74 and 60% of the total extract in 1OHI, 4OHI, 1OH-II and 4OH-II respectively. They were rich in xylose (>60%), and contained minor quantities of uronic acid (4–6%), arabinose and glucose (see Table 2). Slightly less arabinose was present in the polysaccharides from extraction series I, which may reflect loss of some arabinose during the HCl depectination treatment. Since the major constituent of the uronic acids was glucuronic acid, these polysaccharides probably represent relatively pure acidic xylans, such as the heteroxylan isolated by Ralet *et al.* (1993*b*) from the water-soluble fraction of extruded pea hulls. Analysis of yields and composition of the 1OHI and 4OHI fractions showed that these polysaccharides together contained 48% (series I), or 38% (series II) of the total xylose originally present in the AIR. It remains to be seen from structural analysis how the xylans isolated in the present study relate to the neutral and acidic xylans solubilised after extrusion cooking of pea hulls, described by Ralet *et al.* (1993*b*). Ryden and Selvendran (1990) report that the 'hemicelluloses' from parenchymatous tissues of runner beans could not be fractionated in this way, whereas Selvendran and King (1989) isolated a range of acidic xylans

from the parchment layers of mature runner bean pods, the major polysaccharides being heterogeneous of d.p. 90–200. Bailey *et al.* (1974) carried out chemical extraction of lupin hulls, and reported that the 'hemicellulose A' fraction which they isolated, formed 70% of the hemicellulose. However Aspinall *et al.* (1966) recovered a 'hemicellulose-A' fraction from soya bean hulls, from which they purified a glucurono-xylan through removal of arabinan by copper complexing, and more recently Swamy and Salimath (1990) purified, by removal of arabinan, acidic xylans from red gram. Thus these glucurono-xylans appear typical of secondary cell walls of the legume seed hull.

Pectic and soluble 'B' fractions

First extraction series

In addition to large quantities of uronic acids, the major non-cellulosic neutral sugars, arabinose and xylose were extracted and could be found in each major fraction (CSP, HSP and OHSP-I), as well as in the 'B' fractions. Extraction of relatively high amounts of pectin (18% of the uronic acids, versus only about 2% with water (Ralet *et al.*, 1993*a*)) by CDTA from this tissue is probably related to the low methylation degree of the pectins in the pea hulls. The pectins extracted by CDTA do have a low methylation degree, in contrast to what has been found previously, e.g. for apples (Renard *et al.*, 1990). The fraction solubilised by hot acid has a methylation degree of about 50, implying the presence in pea hulls of at least two populations of pectins. The ratio of uronic acid to rhamnose was 28:1, 7:1 and 17:1 in CSP, HSP and OHSP-I respectively. These data show that less branched pectic substances with low methylation degrees were extracted by chelating agent, and that a second population of pectic polymers originating in the cell wall matrix was more highly branched, had higher methylation degrees and was held in the wall by acid-labile linkages. Arabinose was found at a higher concentration than xylose only in the HSP extract, where it probably originated from arabinose-rich side chains of pectic polymers. In addition to uronic acid (essentially galacturonic acid), xylose and arabinose, the 'B' fractions contained relatively high amounts of glucose and some galactose and fucose.

Second extraction series

The yield of OHSP-II was approximately one half of that of OHSP-I and it contained a higher concentration of protein than did OHSP-I. Dilute alkali alone extracted only about 5% of the uronic acids, so that pectin extraction was very low in this series. Alkali-labile linkages thus seem to play a secondary part in the pea hulls cell walls. Jarvis *et al.* (1981) suggested that mild alkali might cleave bonds which attach uronosyl residues to other cell wall components and therefore that the branched pectic substances which are extracted by such a treatment are strongly held in the wall matrix by ester linkages. While these results generally support their view, they also show that mild alkali treatment yields significantly more polysaccharides when carried out after acid extraction (yields; 15 and 5% of the uronic acids of AIR for OHSP-I and OHSP-II, respectively) despite the possible loss of some material through degradation during prior acid extraction. This observation suggests that both acid- and alkali-labile linkages are implicated in retaining these acidic complexes in the matrix. The uronic acid to rhamnose ratio of 11:1 for the OHSP-II extract again suggests that branched pectins were extracted by this treatment, and that here the less branched pectins remain in the wall residue. Relatively more arabinose with respect to xylose was extracted at each step in the second series, compared with the alkaline extracts in the first series, the difference being particularly striking in OHSP-II. This was probably due to hydrolysis of arabinose moieties to dialysable oligomers by the acid treatment used in the first series. While the overall yields of the 'B' polymers and their neutral sugars composition were similar between extraction series, in the second series, relatively more protein and uronic acids were extracted with respect to neutral sugars (Table 1). Although quantitatively the 'B' fractions are relatively minor components of the cell wall, these may be important cross-linking compounds, as the major part can only be extracted with strong alkali.

Further fractionation was carried out, by anion-exchange and gel filtration chromatography, to attempt purification of the major polysaccharides.

Anion-exchange chromatography

Composition and chromatograms of polysaccharides fractionated on DEAE Sepharose CL-6B are shown in Figs 1 and 2. All extracts contained material both free and bound to the ion-exchange column, with the exception of the mild alkali treatment in the first series (Fig. 1(c); OHSP-I) which contained almost no free fraction. In all cases some material was strongly retained on the column and could not be removed by 1 M sodium acetate buffer. Particularly low uronic acid recoveries were obtained for CSP (35%), OHSP-I (16%), 4OH-IA (28%) and OHSP-II (46%). In the case of CSP and OHSP-I, total recovery of the neutral sugars suggested presence of an almost pure galacturonan. High concentrations of xylose in every fraction contrast with the composition of the polysaccharides isolated from the parenchymatous tissues of runner beans by Ryden and Selvendran (1990), which only contained significant quantities of xylose in neutral fractions. HSP gave good yields of both uronic acid (68%) and neutral sugars (86%), with the free fraction being quantitatively important and rich in arabinose (63 mol%). By contrast with the profiles of the polysaccharides from OHSP-I, the OHSP-II extract (Fig. 2(a)) contained a significant

neutral fraction rich in arabinose (A; 63 mol%), as well as a range of polymers rich in uronic acids, eluted by increasing concentration of buffer. The high arabinose concentration in the neutral fraction of OHSP-II strongly suggests that an arabinan was extracted by the alkali alone, although obviously in a much lower yield than that in HSP (Fig. 1(b)).

Polysaccharides in the 'B' fractions (1OH-IB, 1OH-IIB, 4OH-IB and 4OH-IIB) all gave similar elution profiles on DEAE Sepharose CL-6B, although the relative proportions of bound to free polymers and the composition of the fractions varied. The 'B' polymers were each further separated into three fractions, a major non-retained fraction rich in arabinose, xylose and glucose, and two retained fractions, the fraction eluting last being richer in uronic acids and rhamnose. The non-

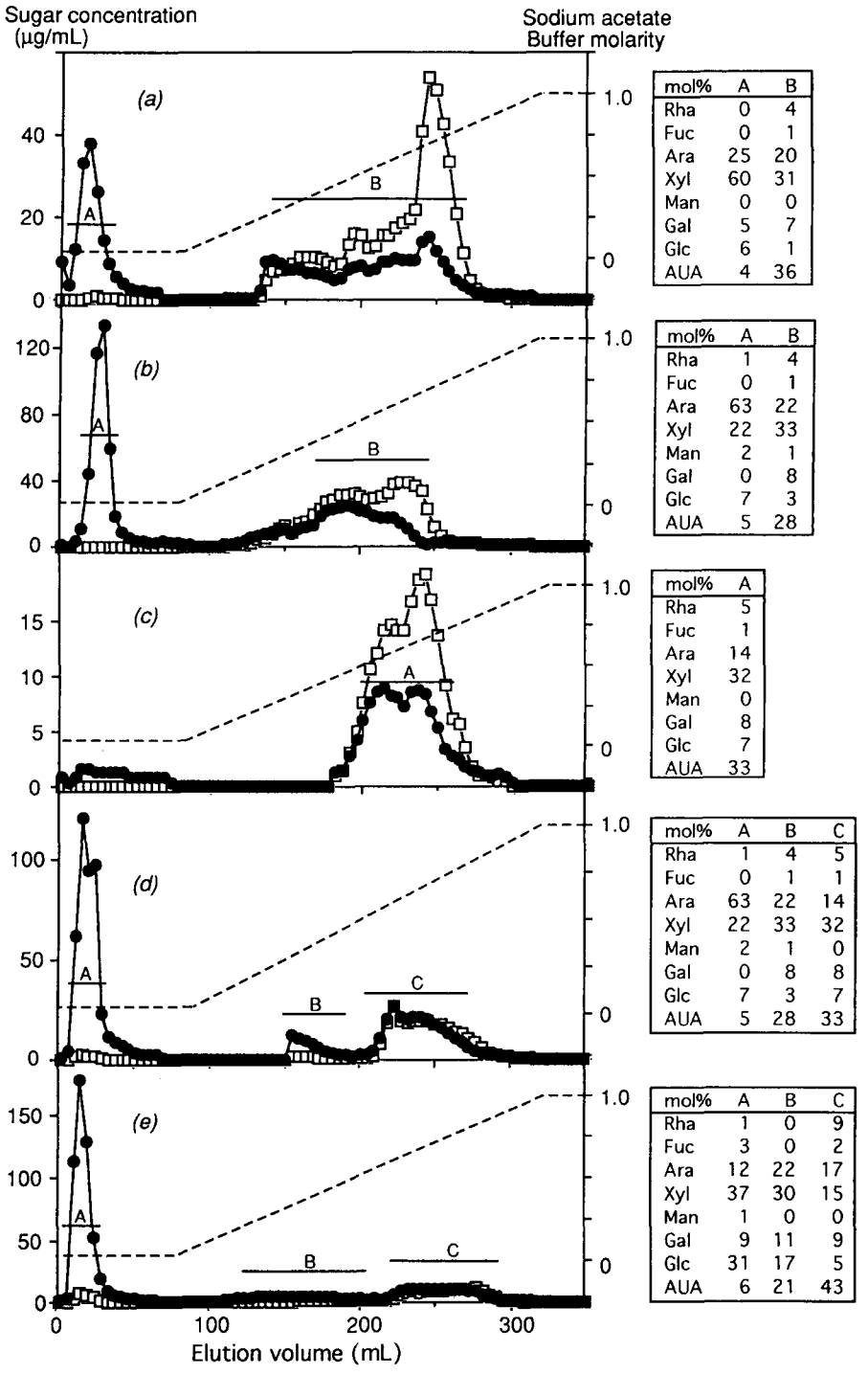
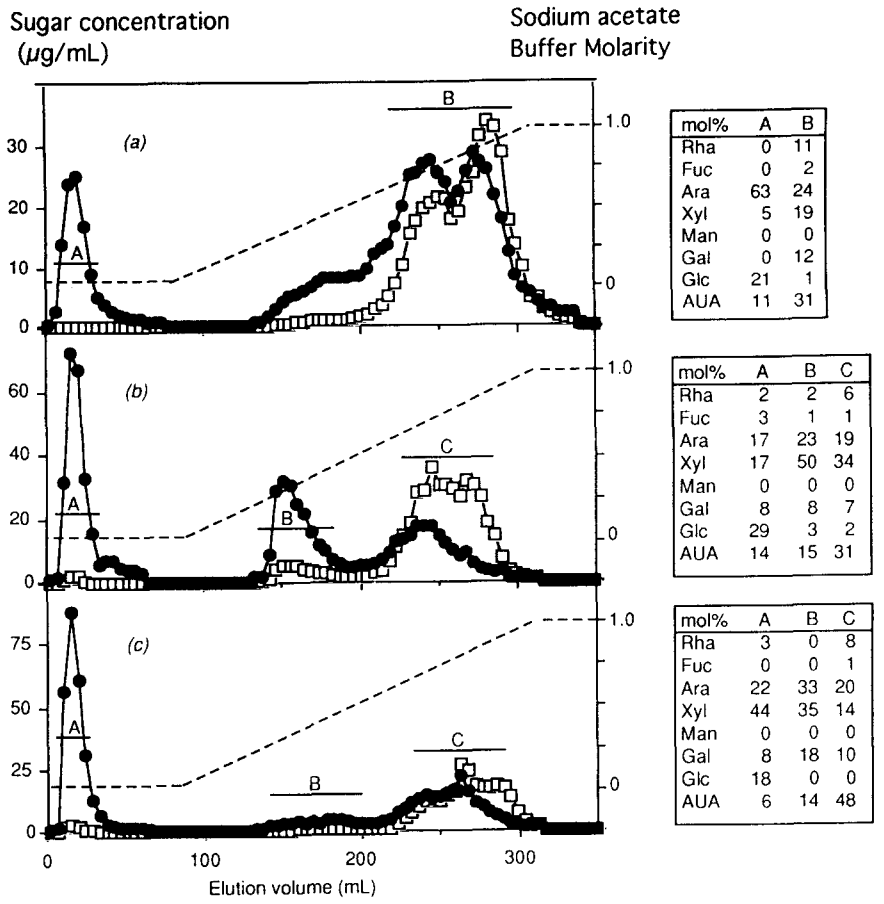


Fig. 1. Elution profiles of pectic and hemicellulose-B fractions on DEAE Sepharose CL-6B, after extractions with CDTA (a), HCl (b), 0·05 M KOH (c), 1 M KOH (d) and 4 M KOH (e). Galacturonic acid (□), neutral sugars (●), acetate buffer molarity (----).



mol%	A	B
Rha	0	11
Fuc	0	2
Ara	63	24
Xyl	5	19
Man	0	0
Gal	0	12
Glc	21	1
AUA	11	31

mol%	A	B	C
Rha	2	2	6
Fuc	3	1	1
Ara	17	23	19
Xyl	17	50	34
Man	0	0	0
Gal	8	8	7
Glc	29	3	2
AUA	14	15	31

mol%	A	B	C
Rha	3	0	8
Fuc	0	0	1
Ara	22	33	20
Xyl	44	35	14
Man	0	0	0
Gal	8	18	10
Glc	18	0	0
AUA	6	14	48

Fig. 2. Elution profiles of pectic and hemicellulose-B fractions on DEAE Sepharose CL-6B, after extractions with 0·05 M KOH (a), 1 M KOH (b) and 4 M KOH (c). Galacturonic acid (□), neutral sugars (●), acetate buffer molarity (----).

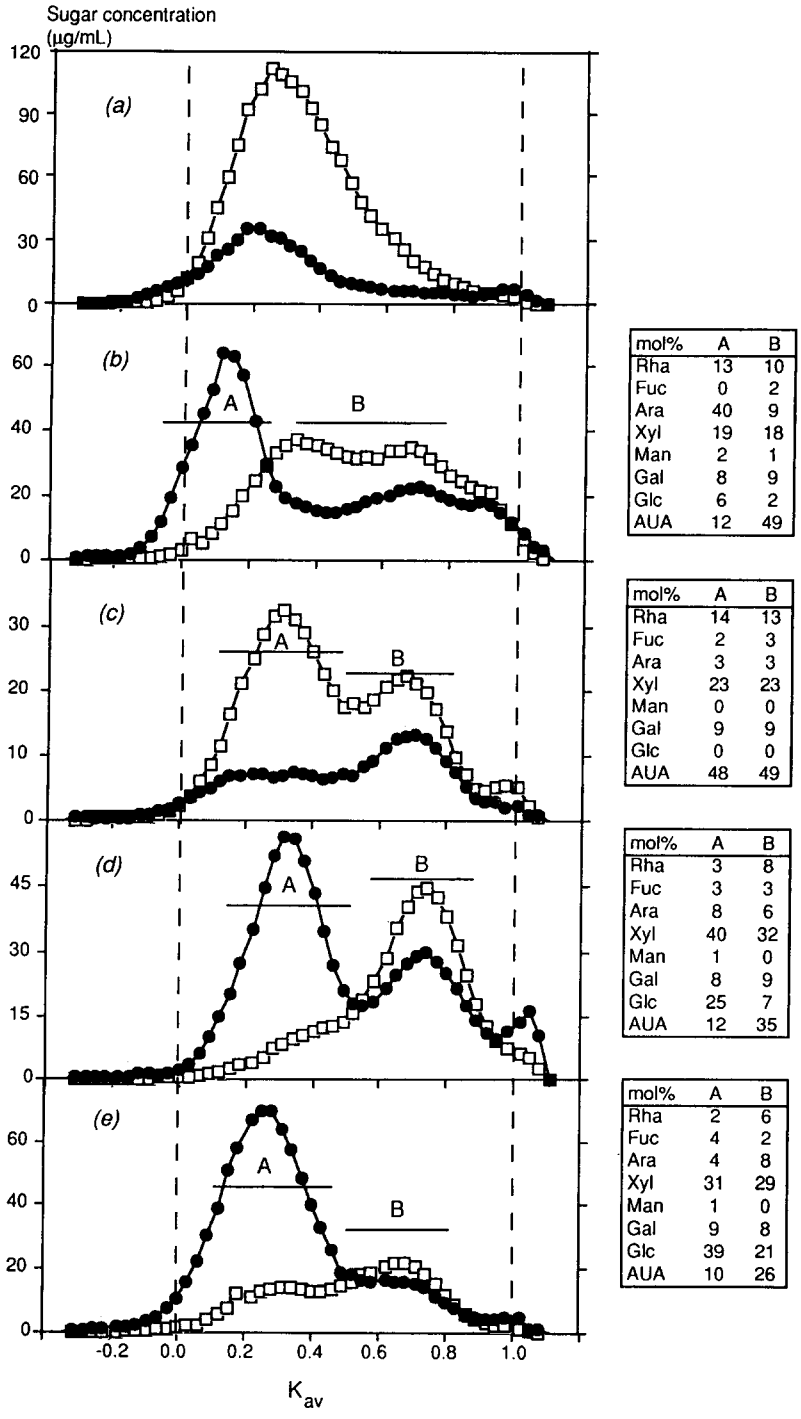
retained fraction of 1OH-IB was particularly rich in arabinose. The non-retained fractions of 1OH-IIB, 4OH-IB and 4OH-IIB contained more xylose and glucose. In 1OH-IIB and 4OH-IB the presence of fucose in addition to galactose, glucose and xylose suggests the existence of a xyloglucan as found in the cotyledons of peas (Brillouet & Carré, 1983) or the parenchymatous tissue of runner beans (Ryden & Selvendran, 1990). The high molar proportions of arabinose and xylose in the acidic fractions of the 1OH & 4OH extracts (Fig. 1(d) and (e); Fig. 2(b) and (c)) suggest a range of hemicellulose-pectic polymers, some of them having complex structures.

Gel filtration chromatography

The elution profiles of the polysaccharides chromatographed on Sephacryl S500 are shown in Figs 3 and 4. In both extraction procedures, recoveries of approximately 100% were obtained from the column. The polysaccharides from the first series showed a broad range of molecular weight (Fig. 3), whereas those of the second extraction series were predominantly of high hydrodynamic volumes (above $K_{av} \sim 0{\cdot}5$).

Neutral sugars and galacturonic acid were not distributed similarly in each polysaccharide fraction. In CSP, neutral and acidic sugars eluted mostly as one peak at about K_{av} 0·2. In HSP (Fig. 3(b)), two clear peaks were seen, a neutral one eluting between K_{av} 0·2 and 0·4 and rich in arabinose (63 mol%), and a second, acidic peak, between K_{av} 0·4 and 0·9. An arabinan of high molecular weight was obtained in spite of the degradative conditions used in this extraction. In the alkali extracts of the first series (Figs 3(c)–(e)) two peaks were seen at K_{av} 0·4 and 0·7. In OHSP-I both peaks were essentially acidic, whereas in the polysaccharides extracted by progressively stronger alkali, the first peak eluted at $K_{av} = 0{\cdot}4$ was neutral and quantitatively the most important of the two. In the second extraction series, the polysaccharides eluted as a single broad peak at $K_{av} = 0{\cdot}4$. Again the polysaccharides became progressively more neutral as the concentration of alkali increased. The contrast between the elution profiles of the KOH extracts from the first and second series was quite marked; the acidic population eluted at $K_{av} = 0{\cdot}7$ in the first series (OHSP-I, 1OH-IB & 4OH-IB) was not present in the latter (OHSP-II, 1OH-IIB & 4OH-IIB) on which previous depectination had not been carried out. Elution profiles from gel chromatography thus gave large contrasts between extraction schemes which were not readily apparent during anion exchange chromato-

graphy. The polymers extracted only by strong alkali after acid (series 1; fraction 'B' in each of Figs 3(c)–(e)) and eluted at $K_{av} = 0{\cdot}7$ are of a smaller molecular size than those extracted by alkali alone, and are probably artefacts of CDTA or HCl extractions. HCl tends to remove the large arabinose side chains of the pectic molecules, and the mild alkali treatment (OHSP) might then cleave ester linkages as suggested above, extracting small pectic fragments associated with the hemicellulose. These small molecular size polymers bear a striking resemblance in sugar composition to the xylogalacturonans isolated by rhamnogalacturonase from apple modified hairy regions by Voragen *et al.* (1992) and which eluted at the void volume on Sephacryl S200 in their study. These polymers could, however, also be analogous to the pectic–xylan complexes of low degree of polymerization isolated from asparagus tissues by Waldron and Selvendran (1992).



mol%	A	B
Rha	13	10
Fuc	0	2
Ara	40	9
Xyl	19	18
Man	2	1
Gal	8	9
Glc	6	2
AUA	12	49

mol%	A	B
Rha	14	13
Fuc	2	3
Ara	3	3
Xyl	23	23
Man	0	0
Gal	9	9
Glc	0	0
AUA	48	49

mol%	A	B
Rha	3	8
Fuc	3	3
Ara	8	6
Xyl	40	32
Man	1	0
Gal	8	9
Glc	25	7
AUA	12	35

mol%	A	B
Rha	2	6
Fuc	4	2
Ara	4	8
Xyl	31	29
Man	1	0
Gal	9	8
Glc	39	21
AUA	10	26

Fig. 3. Elution profiles of pectic and hemicellulose-B fractions on Sephacryl S500, after extractions with CDTA (a), HCl (b), 0·05 M KOH (c), 1 M KOH (d) and 4 M KOH (e). Galacturonic acid (□), neutral sugars (●).

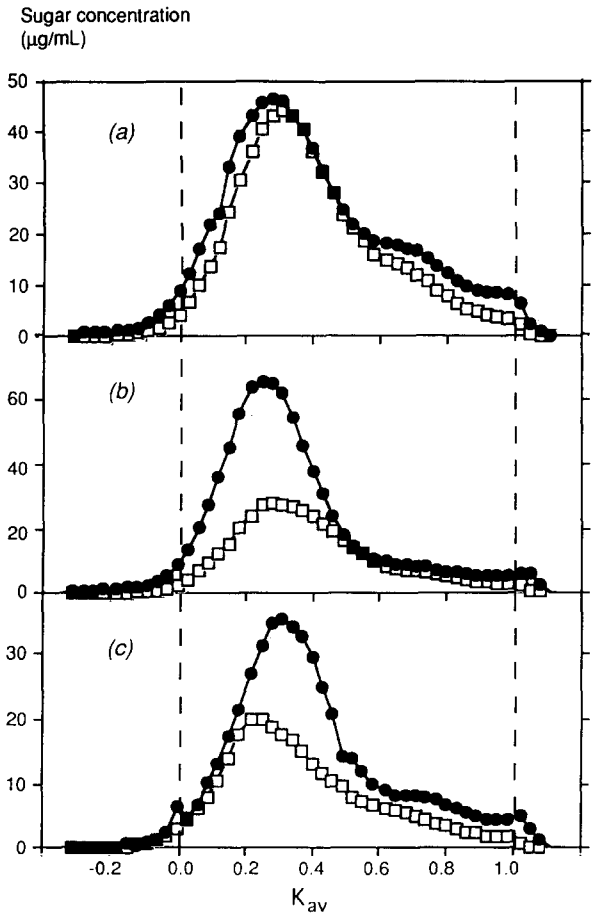


Fig. 4. Elution profiles of pectic and hemicellulose-B fractions on Sephacryl S500, after extractions with 0·05 M KOH (a), 1 M KOH (b), and 4 M KOH (c). Galacturonic acid (□), neutral sugars (●).

CONCLUSIONS

The pea testa contains a parenchymatous layer, approximately ten cells deep, but quantitatively the majority of the total cell wall material may originate in the palisade layer, only one cell deep but with apparently thickened cell walls (Gassner, 1973). The pea hulls contain pectins, which are largely extractable by CDTA. An arabinan and significant quantities of xylose-containing polymers are present associated with the pectic substances. Xylans that precipitate upon neutralisation form the major part of the 'hemicellulose' fraction, similar to those reported previously for legume seed hulls, and the xylans extracted from the parchment-layer cells of runner beans. These xylans could largely be extracted without previous depectination.

Thus the range of polysaccharides isolated probably represents the heterogenous nature of the material, in terms of the different cell types present; cuticle, palisade, and parenchyma all being important fractions. It is generally assumed that secondary cell types have heavily lignified cell walls, however this was not the case in the present study. Recently Waldron and Selvendran (1990 and 1992) have suggested that xylan complexes are the precursors to lignification in asparagus parenchymatous tissues and it is possible that these initials form in the pea hull tissues, but that lignification does not progress further.

These data suggest that extrusion cooking reported by Ralet *et al.* (1993*a*,*b*) solubilised preferentially highly branched pectic polymers, as well as acidic xylans. The pectins of legume seeds have been studied only briefly previously, and although Aspinall *et al.* (1967) suggested the acidic polysaccharides of the soy bean hull had similar structural features to those of the cotyledon of that species, information is scarce on the pectins of seed hulls of the *Papilionoideae*. Work is required to further characterise the xylose-rich pectic substances from pea hulls and this will be presented in a following paper.

ACKNOWLEDGEMENT

This research was supported through the award of a fellowship to one of the authors (R.M.W.) by the European Community.

REFERENCES

Åman, P. & Graham, H. (1987). Whole crop peas. 1. Changes in botanical and chemical composition and rumen *in vitro* degradability during maturation. *Anim. Feed Sci. Technol.*, **17**, 15–31.

Aspinall, G.O., Hunt, K., Morrison, I.M. (1966). Polysaccharides of soybeans. Part II. Fractionation of hull cell wall polysaccharides and the structure of a xylan. *J. Chem. Soc.*, **C**, 1945–9.

Aspinall, G.O., Hunt, K. & Morrison, I.M. (1967). Polysaccharides of soy-beans. Part V. Acidic polysaccharides from the hulls. *J. Chem. Soc.*, **C**, 1080–6.

Bailey, R.W., Mills, S.E. & Hove, E.L. (1974). Composition of sweet and bitter lupin seed hulls with observations on the apparent digestibility of sweet lupin seed hulls by young rats. *J. Sci. Food Agric.*, **25**, 955–61.

Batey, I.L. (1982). Starch analysis using thermostable alpha-amylases. *Starch/Stärke*, **34**, 125–8.

Banerji, N. & Rao, C.V.N. (1963). Structural studies on an arabinoxylan from pea skin (*Pisum sativum*). Part 1. Methylation studies. *Can. J. Chem.*, **41**, 2844–8.

Brillouet, J.-M. & Carré, B. (1983). Composition of cell walls from cotyledons of *Pisum sativum*, *Vicia faba* and *Glycine max*. *Phytochemistry*, **22**(4), 841–7.

Brillouet, J.-M. & Riochet, D. (1983). Cell wall polysaccharides and lignin in cotyledons and hulls of seeds from various lupin (*Lupinus* L.) species. *J. Sci. Food Agric.*, **34**, 861–8.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, **28**(3), 350–66.

Englyst, H.N. & Cummings, J.H. (1984). Simplified method for the measurement of total non-starch polysaccharides by gas liquid chromatography of constituent sugars as alditol acetates. *Analyst*, **109**, 937–42.

Englyst, H.N., Bingham, S.A., Runswick, S.A., Collinson, E. & Cummings, J.H. (1988). Dietary non starch polysaccharides in fruit, vegetables and nuts. *J. Human Nutr. Diet.*, **1**, 247–86.

Gassner, G. (1973). *Mikroskopische Untersuchung Pflanzlicher Lebensmittel*. Gustav Fischer Verlag., Stuttgart, pp. 66–9.

Holl, F.B. & Vose, J.R. (1980). Carbohydrate and protein accumulation in the developing field pea seed. *Can. J. Plant Sci.*, **60**, 1109–14.

Jarvis, M.C., Hall, M.A., Threlfall, D.R. & Friend, J. (1981). The polysaccharide structure of potato cell walls: Chemical fractionation. *Planta*, **152**, 93–100.

Klason, P. (1931). Bericht der vereins der papier und zellstaftchemisker. In *La Chimie du Bois*, eds. Barry, Hawley & Wise. Dunod, Paris, pp. 174–85.

Longstaff, M. & McNab, J.M. (1989). Digestion of fibre polysaccharides of pea (*Pisum sativum*) hulls, carrot and cabbage by adult cockerels. *Brit. J. Nutr.*, **62**, 563–77.

Longstaff, M. & McNab, J.M. (1991). The inhibitory effects of hull polysaccharides and tannins of field beans (*Vicia faba* L.) on the digestion of amino acids, starch and lipid and on digestive enzyme activities in young chicks. *Brit. J. Nutr.*, **65**, 199–216.

Lowry, G.H., Rosebrough, N.J., Lewsfarr, A. & Randall, R.J. (1951). Protein measurement with the folin reagent. *J. Biol. Chem.*, **193**, 265–75.

Ralet, M.-C., Della Valle, G. & Thibault, J.-F. (1993*a*). Raw and extruded fibre from pea hulls. Part 1. Composition and physico-chemical properties. *Carbohydr. Polym.*, **20**, 17–23.

Ralet, M.C., Saulnier, L. & Thibault, J.-F. (1993*b*). Raw and extruded fibre from pea hulls. Part 2. Structural study of the water-soluble polysaccharides. *Carbohydr. Polym.*, **20**, 25–34.

Renard, C.M.G.C., Voragen, A.G.J., Thibault, J.F. & Pilnik, W. (1990). Studies on apple protopectin. 1. Extraction of insoluble pectin by chemical means. *Carbohydr. Polym.*, **12**, 9–25.

Rochat, C. & Boutin, J.-P. (1992). Role of the seed coat in pea embryo nutrition. In *Proc. of the First European Conf. on Grain Legumes*, AEP, Paris, pp. 147–8.

Ryden, P. & Selvendran, R.R. (1990). Cell wall polysaccharides and glycoproteins of parenchymatous tissues of runner bean (*Phaseolus coccineus*). *Biochem. J.*, **269**, 393–402.

Saeman, J.F., Moore, W.E., Mitchell, R.L. & Millet, M.A. (1954). Techniques for the determination of pulp constituents by quantitative paper chromatography. *Tappi*, **37**, 336–43.

Selvendran, R.R. (1984). The plant cell wall as a source of dietary fibre: Chemistry and structure. *Am. J. Clin. Nutr.*, **39**, 320–37.

Selvendran, R.R. (1985). Developments in the chemistry and biochemistry of pectic and hemicellulosic polymers. *J. Cell. Sci. Suppl.*, **2**, 51–88.

Selvendran, R.R. & King, S.E. (1989). Structural features of the cell wall polysaccharides of the parchment layers of the pods of mature runner beans. *Carbohydr. Res.*, **195**, 87–99.

Swamy, N.R. & Salimath, P.V. (1990). Structural features of acidic xylans isolated from red gram (*Cajanus cajan*) husk. *Carbohydr. Res.*, **197**, 327–37.

Thibault, J.-F. (1979). Automatisation du dosage des substances pectiques par la méthode au méta-hydroxydiphényl. *Lebensm.-Wiss. u.-Technol.*, **12**, 247–51.

Tollier, M.T. & Robin, J.P. (1979). Adaptation de la méthode à l'orcinol sulfurique au dosage automatique des oses neutres et totaux. *Ann. Technol. Agric.*, **28**, 1–15.

Voragen, A.G.J., Schols, H.A. & Gruppen, H. (1992). Structural studies of plant cell wall polysaccharides using enzymes. In *Proc. of the Inter. Symp. Plant Polymeric Carbohydrates*. 1992.

Voragen, A.G.J., Schols, H.A. & Pilnik, W. (1986). Determination of the degree of methylation and acetylation of pectins by HPLC. *Food Hydrocolloids*, **1**, 65–70.

Waldron, K.W. & Selvendran, R.R. (1992). Cell wall changes in immature *asparagus* stem tissue after excision. *Phytochemistry*, **31**, 1931–92.