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Carbohydrate Polymers 37 (1998) 87–95

Carbohydrate Polymers

Cell wall polysaccharides from soybean (*Glycine max*.) meal. Isolation and characterisation

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Received 20 January 1997; revised 8 July 1997; accepted 22 July 1997

Abstract

Cell wall material was isolated as water unextractable solids (WUS) from soybean meal. The isolation of WUS yields a fraction that contains 92% of the polysaccharides present in soybean meal and only a few other components. Arabinose, galactose, uronic acids and glucose (cellulose) were the major constituent sugars. WUS was sequentially extracted with chelating agent (chelating agent soluble solids, ChSS), dilute alkali (dilute alkali soluble solids, DASS), 1 M alkali (1 M alkali soluble solids, 1 M ASS) and 4 M alkali (4 M alkali soluble solids, 4 M ASS) to leave a cellulose-rich residue (RES). ChSS was the major extract, yielding 38% of the polysaccharides present in the WUS. All extracts and the residue were characterised by their sugar composition and their molecular-weight distribution. The extracts ChSS and DASS were fractionated by anion exchange chromatography. They showed identical elution patterns: an unbound fraction, five bound fractions of which one fraction eluted only with alkali. Anion exchange chromatography was also performed after saponification of both pectin-rich extracts, again resulting in identical elution patterns.

Keywords: Polysaccharides; Soybean meal; Isolation; Characterisation

1. Introduction

One of the basic products of the soybean is oil. The by-product of the industrial oil extraction, soybean meal, is enriched in proteins and cell wall polysaccharides. Soybean meal is used in livestock feeds and as a raw material for the production of soy protein isolate. The polysaccharides in soybean meal are badly utilised by monogastric animals. Partial degradation of these polysaccharides with enzymes could improve the utilisation by animals. Knowledge of the polysaccharide structure is needed to select enzymes which are able to degrade the polysaccharides in a way that is optimal for the uptake by and well-being of the animals. In the production of soy protein isolate from dehulled and defatted soybean meal, the purity of the protein product is desired to be above 90%. Enzymes able to remove polysaccharides from the protein fraction could be of importance in protein isolation. Identification and selection of such enzymes requires knowledge of the polysaccharide structure in the soybean cell walls.

Some structures of soybean cell wall polysaccharides have already been partly elucidated during the sixties. Within this group of polysaccharides, the arabinogalactans have been studied most intensively (Morita, 1965a, b; Aspinall et al., 1967a; Labavitch et al., 1976), and shown to contain chains of 1,4-linked β-D-galactopyranose residues in which some residues carry through C-3 a side chain of (1,5-linked) L-arabinofuranose residue(s). However, a large variation is present in the degree of branching and in the distribution of the substituents over the main chain. Aspinall and Cottrell (1971) also isolated a highly branched arabinan, containing 1,3- and 1,5-linked arabinofuranose residues.

Within the group of acid polysaccharides, the sugar composition has been determined and a number of hydrolysis products have been characterised. The results indicate a main chain consisting of D-galacturonic acid and L-rhamnose residues and side chains containing mainly galactose and arabinose residues. Two noteworthy observations were firstly the presence of oligosaccharides containing contiguous rhamnose residues, and secondly the presence of xylosyl-galacturonic acid dimers, indicative of the presence of xylogalacturonan (Aspinall et al., 1967b). The structure of the pectin molecules as a whole has not yet been investigated.

Until now, no survey has been published in which fractions from soybean meal are characterised with respect to their (polysaccharide) composition. Moreover, isolated

0144–8617/98/$ - see front matter
PII: S0144-8617(97)00111-2

soybean polysaccharides have also not been investigated in detail, except for chelating agent extracted pectic substances by Brillouet and Carré (1983). The purpose of the present investigation was to isolate the intact cell wall polysaccharides from soybean meal and to characterise them in order to perform further structural investigations of the important fractions.

2. Materials and methods

2.1. Plant material

Solvent-extracted, untoasted soybean meal was obtained from Cargill BV (Amsterdam, The Netherlands).

2.2. Isolation of water-unextractable solids (WUS)

Dehulled, defatted, untoasted soybean meal was ground to pass through a 0.5-mm sieve. This meal (800 g) was extracted with 3 l distilled water containing 0.05% NaN_3 during 2 h at room temperature. The suspension was centrifuged (11 000 ***g***; 30 min). The pellet was resuspended, and this procedure was repeated four times. The combined supernatants were subjected to ultrafiltration using a tubular system (Cobe Nephross BV, Boxtel, The Netherlands), resulting in a filtrate (UFF) containing material smaller than 5000 Da and a retentate (UFR) containing polymers larger than 5000 Da.

Subsequently, the protein was extracted from the residue with 3 l 1.5% (w/v) sodium dodecylsulphate solution containing 10 mM 1,4-dithiothreitol, during 3 h at room temperature. After centrifugation (11 000 ***g***; 30 min), this extraction was repeated three times. The final pellet was washed twice with distilled water. The combined supernatants were dialysed, concentrated and freeze-dried (SDSS).

The residue was then suspended in 1 l of distilled water (pH 5.0) at 85°C, and starch gelatinisation was allowed to proceed for 1 h. The residue obtained after centrifugation (11 000 ***g***; 30 min) was suspended in 1 l buffer solution (pH 6.5) containing 10 mM maleic acid, 10 mM NaCl, 1 mM $CaCl_2$ and 0.05% NaN_3. Porcine pancreatic α-amylase (2 mg; Merck art. 16312) was added and the mixture incubated at 30°C for 19 h. After centrifugation (11 000 ***g***; 30 min), the residue was washed with 1 l of hot distilled water (65°C) and centrifuged again. The α-amylase digestion and hot water washing were repeated once. The combined supernatants were dialysed, concentrated and freeze-dried (HWS), and the remaining unextractable residue was resuspended in distilled water and freeze-dried (WUS).

2.3. Sequential extraction of WUS

Soybean WUS (20 g) was sequentially extracted, based on the procedure described by Redgwell and Selvendran (1986), with 0.05 M 1,2-diaminocyclohexane-*N,N,N′,N′*-tetraacetic acid (CDTA) and 0.05 M NH_4-oxalate in 0.05 M NaAc-buffer, pH 5.2 (eight times 600 ml) at 70°C for 1 h (chelating agent soluble solids, ChSS); washed with distilled water (two times 600 ml) and these extracts added to the ChSS fraction; extracted with 0.05 M NaOH (three times 600 ml) at 2°C for 1 h (dilute alkali soluble solids, DASS); 1.0 M KOH + 20 mM $NaBH_4$ (5 times 600 ml) at room temperature for 2 h (1 M alkali soluble solids, 1 M ASS); and 4 M KOH + 20 mM $NaBH_4$ (three times 600 ml) at room temperature for 2 h (4 M alkali soluble solids, 4 M ASS). After each extraction, solubilised polymers were separated from the insoluble residue by centrifugation (19 000 ***g***; 30 min). All extracts were acidified to pH 5.2 (if necessary) by glacial acetic acid, concentrated, dialysed and freeze-dried. ChSS (including the two supernatants obtained after washing the residue from this extraction step) were dialysed against 0.1 M NH_4Ac buffer (pH 5.2) before dialysing against distilled water. The final residue (RES) was suspended in water, acidified to pH 5.2, dialysed and freeze-dried.

2.4. Ion-exchange chromatography

Approximately 500 mg of ChSS, saponified ChSS (sChSS), DASS and saponified DASS (sDASS) were fractionated on a column (550 mm × 15 mm) of DEAE Sepharose Fast Flow, which was initially equilibrated in 0.005 M NaAc-buffer pH 5.0, using a Hiload System (Pharmacia).

The ChSS and DASS fractions were suspended in water, the insoluble residues were removed by centrifugation (5400 ***g***; 5 min), and the supernatants were applied on to the column. Saponification of ChSS and DASS was performed by dissolving them in 0.1 M NaOH (0°C, 16 h) followed by neutralisation with 0.1 M HAc. These saponified samples were also centrifuged and the supernatants applied on to the column.

Elution was carried out sequentially with 400 ml of 0.005 M NaAc-buffer pH 5.0, a linear gradient from 0.005 to 0.5 M NaAc buffer pH 5.0 (1200 ml), a linear gradient 0.5–2 M NaAc-buffer pH 5.0 (1000 ml) and 400 ml 0.005 M NaAc-buffer pH 5.0. Residual bound polysaccharides were washed from the column with 400 ml of 0.5 M NaOH. The elution rate was 10 ml/min, except for the first step, in which the sample was applied on to the column and the elution rate was 2.5 ml/min. Fractions (23 ml) were collected and assayed by automated methods (Tollier and Robin, 1979; Thibault, 1979) for neutral sugar content and uronic acid content. The appropriate fractions were pooled, concentrated, dialysed, freeze-dried and analysed for neutral sugar composition and uronic acid content.

2.5. Analytical methods

Moisture content was determined by drying at 105°C until no further decrease in weight was observed.

Table 1
Yield and composition of soybean meal and fractions thereof (percentage dry weight)

	Soybean meal	UFF	UFR	SDSS	HWS	WUS	*Recovery*
Yield	100	19.5	39.6	18.5	0.4	15.7	*93.7*
Protein content	57.3	21.1	87.8	84.2	15.4	2.1	*95.7*
Starch content	1.0	0	0	0	8.5	0	*3.4*
NSP content	15.4[a]	50.2	13.7	3.0	43.3[a]	95.8	*102.4*[b]
Acetic acid groups	1.1	1.2	0.7	0.3	1.4	2.8	*92*
Methanol groups	0.3	0.1	0.2	t	1.1	1.1	*92*
Fructose	0.6	6.6	3.5	0	0	0	*446*
Sucrose	5.4	0	0	0	0	0	*0*
Raffinose	0.8	0.5	0.1	0	0	0	*17*
Stachyose	4.9	8.4	1.2	0	0	0	*43*

t = trace amount.
[a]After enzymatical removal of starch.
[b]In this calculation, UFF and UFR are omitted.

Starch content was determined enzymatically using a test kit (Boehringer, Mannheim, Germany).

Non-starch polysaccharide content of soybean meal and HWS was determined according to Englyst and Cummings (1984). The starch was enzymatically hydrolysed, the residue was dried and the sugar composition was determined.

Neutral sugar composition was determined by gas chromatography according to Englyst and Cummings (1984), using inositol as an internal standard. The samples were pre-treated with 72% w/w H_2SO_4 (1 h, 30°C) followed by hydrolysis with 1 M H_2SO_4 for 3 h at 100°C, and the constituent sugars were analysed as their alditol acetates. Cellulosic glucose was calculated as the difference between the content of glucose with and without prehydrolysis.

Uronic acid content was determined by the automated colorimetric *m*-hydroxydiphenyl assay (Blumenkrantz and Asboe-Hansen, 1973; Thibault, 1979; Tollier and Robin, 1979) using an auto-analyser (Skalar Analytical BV, Breda, The Netherlands). Corrections were made for interference by neutral sugars present in the sample.

Protein content was determined by a semi-automated micro-Kjeldahl method (Roozen and van Boxtel, 1979). The conversion factor used was 6.25.

Degree of acetylation and methylation was determined by HPLC after saponification with 0.4 M NaOH (Voragen et al., 1986). Quantification was performed using acetic acid and methanol standards.

High-performance size-exclusion chromatography (HPSEC) was performed on an SP8800 HPLC (Spectra Physics) equipped with three columns (each 300 × 7.5 mm) of Bio-Gel TSK in series (60XL, 40XL and 30XL; Bio-Rad Labs) in combination with a TSK guard column (40 × 6 mm) and elution at 30°C with 0.4 M NaAc buffer pH 3.0 at 0.8 ml/min. Calibration was performed using dextrans, ranging from 500 to 4 kDa. The eluate was monitored using a Shodex SE-61 Refractive Index detector.

High-performance anion-exchange chromatography (HPAEC) was performed on a Dionex Bio-LC system as described by Schols et al. (1994). The gradient was obtained by mixing solutions of 0.1 M NaOH and 1 M NaAc in 0.1 M NaOH.

For the determination of small neutral oligomers, fructose, sucrose, raffinose and stachyose, the (4 × 250 mm) CarboPac PA1 column was equilibrated with 0.016 M NaOH. Twenty microlitres of the sample were injected, and a linear gradient to 0.1 M NaOH in 33 min was applied, followed by a linear gradient from 0 to 0.04 M NaAc in 0.1 M NaOH in 12 min. The column was washed for 5 min with 1 M NaAc in 0.1 M NaOH, then 5 min with 0.1 M NaOH and then equilibrated again for 12 min with 0.016 M NaOH. Calibration was performed with standard solutions of fructose, sucrose, raffinose and stachyose.

3. Results and discussion

3.1. Yield and composition of the WUS

The yield and composition of the fractions of soybean meal obtained during the isolation of WUS are shown in Table 1. The recovery of this fractionation is 94%. The major part of the material is water-soluble (UFF and UFR), namely 59%. The yield of the WUS fraction is 16%.

The protein content of the soybean meal is very high, as expected (57%). The recovery of protein in this procedure is 96%. The major part of the protein present in soybean meal is recovered in the UFR fraction (61%); these are the water-soluble proteins. The SDS/DTT solution is able to extract another 27% of the proteins in the material. The amount of protein recovered in the WUS fraction is 0.6% of the protein present in the soybean meal and represents only 2.1% of the WUS.

The soybean meal contains 1% of starch. Starch molecules are degraded by the use of α-amylase in the extraction of HWS, and degradation products are removed during dialysis. Only 3.4% of the starch resists degedation with α-amylase, these are limit dextrins from amylopectin.

The polysaccharide content of the soybean meal (15.4%) and the HWS fraction (43.3%) is determined after removal

Table 2
Sugar composition of soybean meal and fractions thereof (mol%)

Fraction	Sugar composition								Carbohydrate content[a]
	rha	fuc	ara	xyl	man	gal	glc	Uronic acids	
Soybean meal[b]	2	3	19	8	3	28	21	18	14.5
UFF	0	0	2	1	6	29	58	3	40.7
UFR	1	t	4	1	13	26	49	6	12.4
SDSS	7	1	11	4	19	14	18	25	2.9
HWS[b]	1	1	23	4	2	33	10	26	40.7
WUS	2	3	19	8	2	29	21	17	89.3

t = trace amount.
[a]Expressed as % w/w.
[b]After enzymatical removal of starch.

of starch and oligomeric sugars. In the determination of the polysaccharide content of the other fractions (UFF, UFR, SDSS and WUS), this step was omitted, because none of these fractions contained starch anymore. Thus, the polysaccharide content of the soybean meal does not include the neutral oligomeric sugars, whereas the 'polysaccharide content' of the UFF and UFR fractions includes these small sugars. Therefore, the calculation of the recovery of polysaccharides (102%) only includes the polysaccharide contents of the SDSS, HWS and WUS fractions. Of the total polysaccharides in these fractions, 95% is recovered in the WUS fraction. The acetic acid and methanol groups are believed to be present as substituents of the uronic acid residues in the polysaccharides. They are expected to be recovered in the WUS fraction, which is confirmed by the results in Table 1, showing that 40% of the acetic acid groups and 58% of the methanol groups are recovered in the WUS fraction. The water-soluble polysaccharides (in the UFF and UFR fractions) contain 45% of the acetic acid groups and 33% of the methanol groups.

The small sugars—fructose, sucrose, raffinose and stachyose—are water-soluble and thus recovered in the UFF and UFR fraction. The soybean meal contains 0.6% fructose, 5.4% sucrose, 0.8% raffinose and 4.9% stachyose, which is in agreement with the figures of Sosulski et al. (1982) who found 6.35% sucrose, 1.15% raffinose and 2.85% stachyose. The recoveries of sucrose (0%), raffinose (17%) and stachyose (43%) detected in the fractions are very low. For fructose, however, the recovery is unrealistically high (446%). A possible explanation for this observation is that the di-, tri-, and tetrasaccharide are degraded by endogenous enzymes, which would lead to the formation of glucose, fructose and galactose containing oligomers and monomers. If all oligomers that had not been recovered had been degraded, the recovery of fructose would have been 600%. The lacking 150% can be explained by the new unidentified oligomers appearing in the HPAEC patterns of the UFF and UFR fractions. This enzymatic degradation of oligosaccharides takes place during ultrafiltration of the cold water-soluble fraction, because so far, no heat treatment has been given to inactivate enzymes present in the soybean meal. Enzyme activities that might be present in the residue are inactivated by SDS and DTT during the next extraction step and will not degrade the polysaccharides present in the residue. Since 95% of the polysaccharides were recovered in the WUS fraction and no indications for polysaccharide degrading activities were found, it is believed that degradation of polysaccharides did not occur, and research directed towards the WUS was continued.

The sugar composition of all fractions from soybean meal was determined and is shown in Table 2. The polysaccharides in the meal and in the WUS fraction consist mainly of galactose, glucose (mainly cellulose), arabinose and uronic acids. The kind of uronic acid was not determined, but Aspinall et al. (1967a, b) has shown that the uronic acids present in soybean cotyledon meal are primarily galacturonic acids. The cellulose content of the soybean meal and the WUS fraction is 2.7 and 17.7% w/w, respectively.

In the determination of the polysaccharide content of the UFF and UFR fractions, starch and oligomeric sugars were not removed prior to hydrolysis. Fructose, raffinose (gal–glc–fru) and stachyose (gal–gal–glc–fru) interfere with the analysis of the sugar composition of these fractions. In this analysis, fructose is partly determined as mannose and glucose. The major sugars in both the UFF and the UFR fraction are glucose, galactose and mannose. The amount of mannose residues in the UFR fraction (13%) is higher than the mannose content of the UFF fraction (6%), whereas the amount of fructose (present as monomer and in raffinose and stachyose) in the UFR fraction is slightly lower than in the UFF fraction. This is an indication of the presence of mannose-containing polysaccharides. This can be a result of incomplete removal of the hulls from the soybean meal, since soybean hulls consist, to a large extent, of galactomannans, which are isolated by extraction with cold water (Whistler and Saarnio, 1957; Aspinall and Whyte, 1964).

The SDSS and HWS fraction are not of importance in our study, because the amounts of polysaccharides extracted in these two steps are very low. The WUS fraction, however, is really enriched in polysaccharides. The most important constituent sugars are galactose, glucose (cellulose), arabinose

Table 3
Yield on sugar basis (%) and sugar composition of extracts from WUS expressed as mol%, and as a percentage of that particular sugar in the WUS (in parentheses)

		Sugar composition								Carbo-hydrate content[a]	DA[b]	DM[c]
Extract	Yield	rha	fuc	ara	xyl	man	gal	glc	Uronic acids			
WUS	100	2	3	19	8	2	29	21	17	89.3	49	36
ChSS	38	2 (40)	3 (42)	25 (48)	6 (30)	1 (15)	37 (49)	1 (1)	26 (53)	52.7	36	35
DASS	7	2 (8)	3 (8)	24 (9)	6 (6)	1 (3)	38 (10)	1 (t)	25 (10)	73.9	22	29
1 MASS	16	2 (15)	3 (22)	23 (20)	11 (23)	1 (7)	35 (21)	5 (4)	20 (18)	74.2		
4 MASS	7	1 (4)	3 (9)	12 (4)	28 (24)	2 (8)	18 (5)	26 (9)	10 (4)	84.5		
RES	18	1 (8)	0 (0)	3 (3)	3 (6)	4 (41)	2 (1)	76 (65)	11 (11)	75.9		
Recovery	*86%*	*75%*	*81%*	*84%*	*88%*	*74%*	*85%*	*80%*	*96%*	*86%*	*30%*	*41%*

t = trace amount.
[a]Expressed as % w/w.
[b]Expressed as mol acetic acid/100 mol uronic acids.
[c]Expressed as mol methanol/100 mol uronic acids.

and uronic acids. This is an indication of the presence of a considerable amount of pectins in the WUS. These results are in agreement with Brillouet and Carré (1983), who also found galactose to be the major sugar constituent, followed by galacturonic acid, arabinose and glucose in soybean cotyledon cell walls.

From the data in Table 2, it can be concluded that this isolation procedure yields a WUS fraction in which almost all cell wall polysaccharides are recovered and which is almost free of other components. The sugar composition of the WUS fraction is very similar to that of the soybean meal, which indicates that no sugar residues were specifically removed during the isolation procedure. Surprisingly, over 90% of the uronic acids present in the soybean meal were recovered in the WUS fraction. In the isolation procedure of cell wall material from, for instance, onions (Redgwell and Selvendran, 1986), apples (Schols et al., 1995) and olives (Huisman et al., 1996), a significant amount of the pectins is water- or buffer-soluble. This could be an indication of more complex pectic molecules or greater diversity within the architecture of the soybean cell walls compared with other plant cell walls.

3.2. Sequential extraction of the WUS

CDTA and NH_4-oxalate are most generally used to abstract Ca^{2+} from the cell walls, and most of the pectic polysaccharides held in the walls by ionic cross-links will be solubilised. The ChSS fraction, which is the main fraction (38%), is rich in arabinose, galactose and uronic acids. The remaining pectic polysaccharides are probably ester cross-linked within the wall matrix and are (partially) extracted with dilute alkali and recovered in the DASS fraction. The sugar composition of this extract is identical with the composition found for the ChSS fraction. The galactose: arabinose ratio found in both the ChSS and DASS fraction is 1.5:1, and the uronic acid:rhamnose ratio is 14:1. The sugar composition of the ChSS and DASS fractions is quite similar to that of the EDTA-soluble pectic substances extracted by Brillouet and Carré (1983); their extract also contains pectic substances rich in galactose and arabinose with a molar ratio of 1.5:1 and has a uronic acid:rhamnose ratio of 13:1.

Further extraction of the residue with stronger alkali (1 and 4 M KOH) solubilises small amounts of additional pectic material along with the hemicelluloses. Besides arabinose, galactose and uronic acids, the 1 M ASS fraction contains also 11 mol% of xylose. Xylose and glucose are the predominant sugars in the 4 M ASS fraction, which may indicate the presence of xyloglucan in this extract. The final α-cellulose residue (RES) still contains a small amount of uronic acids, representing 11% of the uronic acids present in the WUS. These uronic acids can be galacturonic acids as well as glucuronic acids. The galacturonic acids might be present as pectic molecules tightly bound to or firmly entangled in the cellulose/hemicellulose network. The glucuronic acids will probably be present as hemicellulose substituents. The yields and sugar compositions of the extracts from WUS are shown in Table 3.

The degree of acetylation and methylation in the WUS are, respectively, 49 and 36%. The ChSS fraction has a degree of methylation of 35% and a remarkably high degree of acetylation of 36%. The low recoveries of the acetyl groups and methyl esters after sequential extraction (30% and 41%, respectively) are caused by the fact that part of these groups are saponified during extraction with alkali, and as a result of that cannot be determined. Although extraction of the DASS fraction was performed using NaOH, part of the methyl and acetyl groups were still present (DA = 22% and DM = 29%). This phenomenon was also found by Ros et al. (1996) extracting a diluted sodium hydroxide soluble pectin fraction from the albedo of lemons. Assuming that these substituents will be partially saponified during the extraction of DASS, the degree of

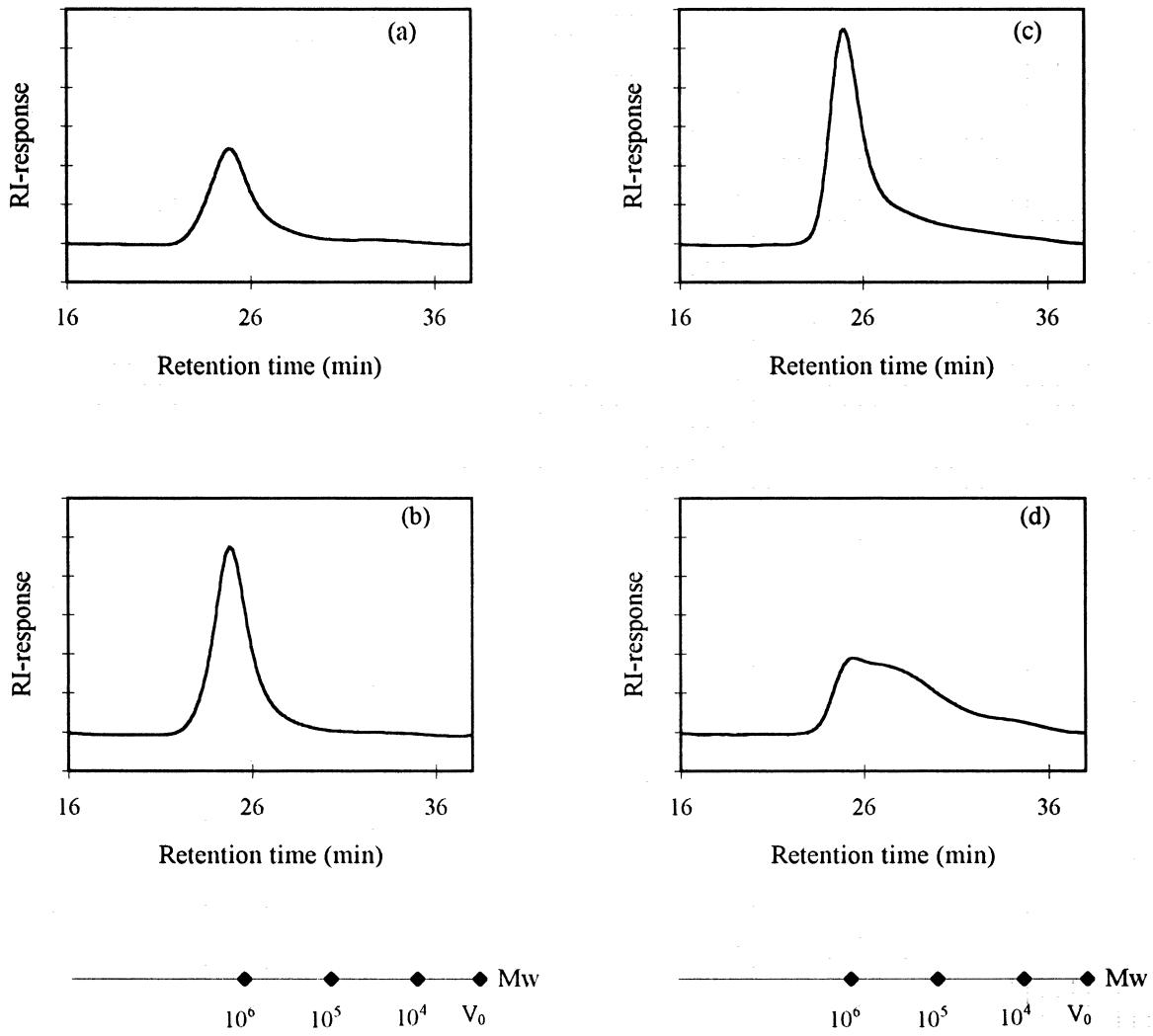

Fig. 1. HPSEC elution patterns of (a) ChSS, (b) DASS, (c) 1 M ASS and (d) 4 M ASS extracted from soybean WUS.

acetylation and methylation of this extract will be underestimated.

The extracts were further characterised by the determination of their molecular weight distributions, which are shown in Fig. 1. The ChSS and DASS fractions show almost identical symmetrical peaks, with an average molecular weight of about 10^6 Da based on calibration with dextrans. Since uronide-containing polymers have a larger hydrodynamic volume than dextrans, due to intramolecular electrostatic repulsion by charge effects and therefore elute faster than expected on the basis of their molecular weight (Schols et al., 1991), the molecular weight of these two fractions containing pectin-rich polysaccharides, will be smaller than mentioned above. The average molecular weight of the 1 M ASS is similar to that of the first two fractions, but the distribution tails to lower molecular weights. The molecular weight distribution of the 4 M ASS fraction is much broader, and the average molecular weight is lower than the preceding three extracts. For each extract, a standardised amount was solubilised as much as possible. The differences in the areas under the peaks are partly caused by differences in the sugar contents of the extracts, which depends on the amount of residual salt and water in the fractions. The ChSS fraction has the lowest sugar content (52.7%), due to the fact that the CDTA is difficult to dialyse away from pectins (Mort et al., 1991). The differences in the solubility of the extracts are also partly responsible for the differences in the areas under the peaks. This mainly concerns the elution pattern of the 4 M ASS fraction, because this fraction is not completely soluble, whereas the others are.

3.3. Anion-exchange chromatography of ChSS and DASS

The polysaccharides present in the pectin-rich extracts ChSS and DASS are very similar with respect to their sugar compositions and molecular weight distributions. For further characterisation, these polysaccharides were fractionated, based on their charge density. The soluble parts of these extracts, representing over 90% of the polysaccharides of the extracts, are applied onto the column. The elution pattern of ChSS and saponified ChSS (sChSS) are

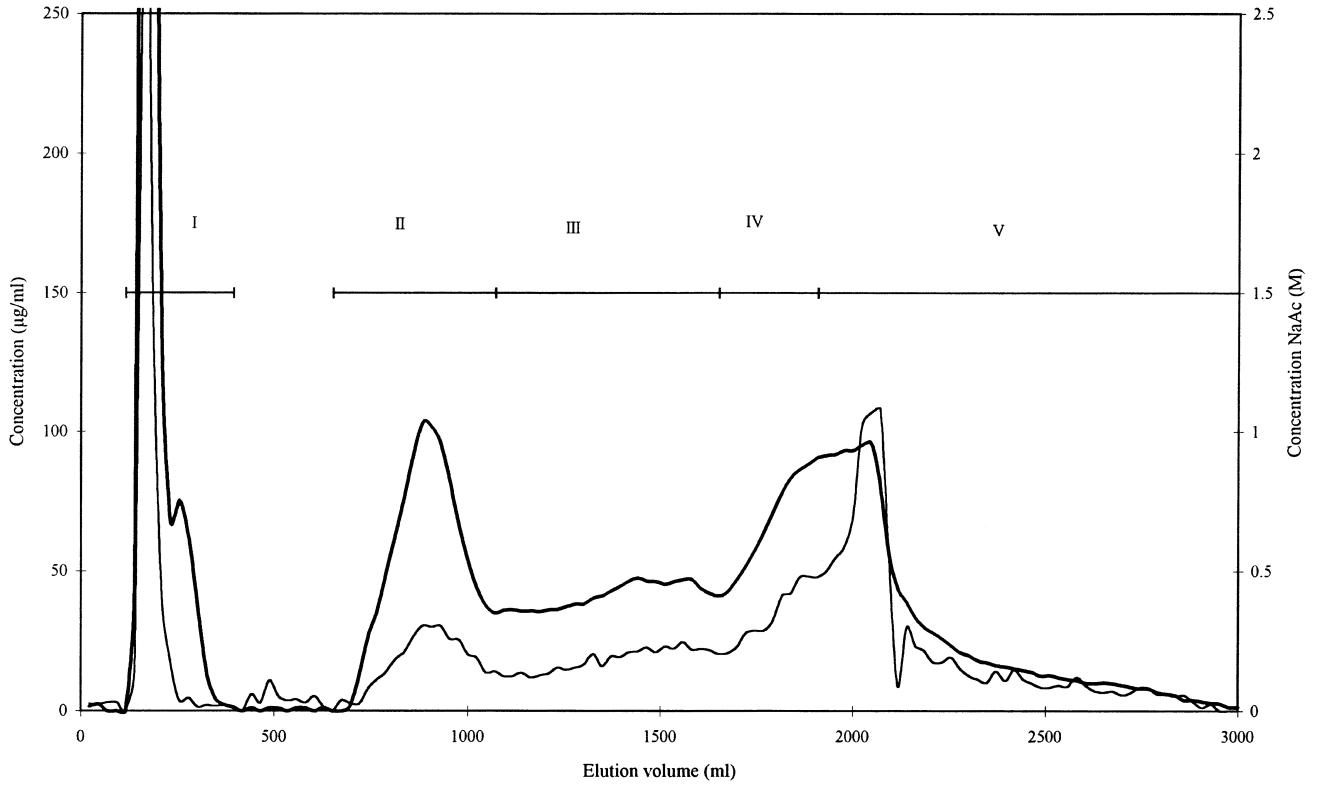

Fig. 2. Elution profile of soybean ChSS on anion-exchange chromatography. Uronic acid concentration (————), neutral sugar concentration (————).

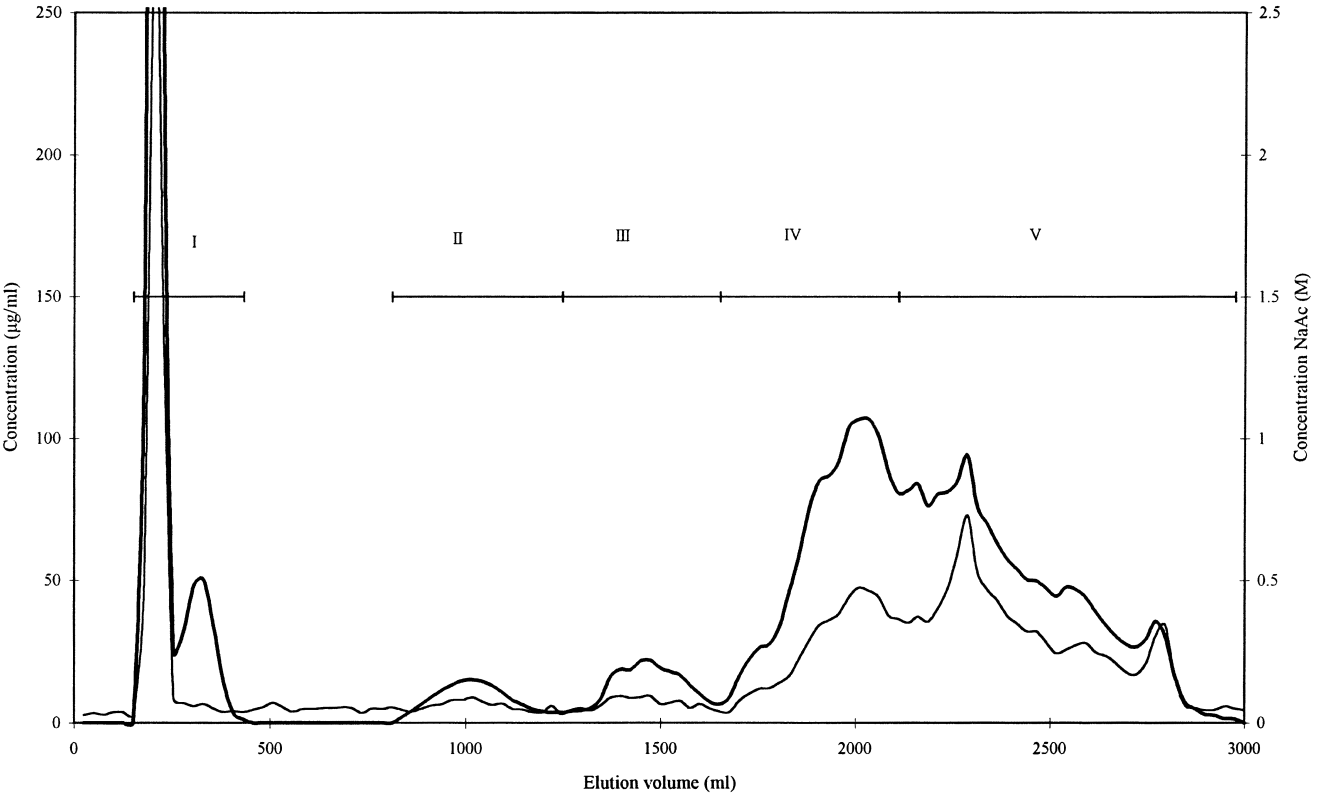

Fig. 3. Elution profile of saponified soybean ChSS on anion-exchange chromatography. Uronic acid concentration (————), neutral sugar concentration (———).

Table 4
Yield on sugar basis (%) and sugar composition (mol%) of fractions obtained after anion-exchange chromatography of ChSS and saponified ChSS

		Sugar composition							
	Yield	rha	fuc	ara	xyl	man	gal	glc	Uronic acids
ChSS		2	3	25	6	1	37	1	26
Residue	8.2	2	2	21	8	t	34	9	24
Pool I	24.8	2	3	29	6	1	41	1	19
Pool II	14.7	2	3	24	6	t	45	1	19
Pool III	13.2	2	4	22	8	1	39	1	24
Pool IV	11.2	2	3	23	8	1	35	2	27
Pool V	19.0	2	2	21	7	1	29	1	37
Alkali wash	7.4	2	2	21	7	2	32	2	32
Recovery	98.4	104	97	98	114	100	103	263	88
					sChSS				
Residue	4.9	2	2	21	8	2	33	13	21
Pool I	27.8	2	3	27	6	1	44	1	18
Pool II	6.1	2	2	26	4	1	48	3	13
Pool III	5.0	1	2	24	5	1	47	4	16
Pool IV	21.2	2	3	24	6	1	44	1	19
Pool V	22.8	2	4	22	9	1	31	1	30
Alkali wash	8.0	2	2	21	7	2	25	3	40
Recovery	95.7	97	96	98	107	128	105	292	77

t = trace amount.

shown in Figs. 2 and 3, respectively. The residues contain less than 10% of the polysaccharides present in the different extracts, and are enriched in glucose.

The elution patterns of DASS and saponified DASS (sDASS) are identical with those of ChSS and sChSS, and therefore not shown here. The unbound fractions are pooled as pool I and the bound fractions are pooled to give pools II–V as indicated in Figs. 2 and 3. The sugar composition and the uronic acid content of these pools, the residue remaining after centrifugation of the suspensions (residue) and the strongly bound polysaccharides washed from the column with 0.5 M NaOH (alkali wash), are presented in Table 4. The data obtained for DASS and sDASS are again analogous and are not shown.

The recovery of the fractionations is high. All the pools are rich in arabinose, galactose and uronic acids. As can be seen from Figs. 2 and 3, the relative uronic acid content of the fractions increased with increasing salt concentration of the eluent. This is confirmed by the ratio neutral sugars to uronic acids, which decreases with increasing retention time of the fraction. At the same time, a shift from galactose to arabinose takes place, the arabinose:galactose ratio increased from 0.52:1 (pool II) to 0.70:1 (pool V) for ChSS, from 0.55 to 0.72 for sChSS, from 0.53 to 0.75 for DASS and from 0.53 to 0.76 for sDASS.

Pool I consists of the unbound polysaccharides, and is therefore expected to have a low uronic acid content. However, this pool contains about 20 mol% uronic acids. A possible explanation for not binding is that the neutral sugar containing side chains prevent the interaction of the uronic acids with the column material, and the polysaccharides will be eluted in the void. Alternatively, these unbound uronic acid containing polysaccharides could represent methyl esterified (neutral) pectic material. This later possibility is in contradiction with the fact that this unbound fraction is also found in the elution pattern of the saponified extracts.

The bound polysaccharides from the saponified extract are slightly lower in their uronic acid content than those from the unsaponified extract, except for the alkali wash. Furthermore, a shift in the amount of polysaccharides from pool II and III to pool IV and V can be detected. By saponification of the methanol groups, the pectins will possess more charged groups and will therefore need a higher ionic strength of the buffer to be eluted.

4. Conclusions

Isolation of the cell wall polysaccharides from soybean meal, which contains 57% of proteins, yields a fraction containing almost all polysaccharides present in the meal and few other components. A complete mass balance for both proteins and polysaccharides of the recovered fractions during the isolation of the WUS is given. The sugar compositions of the soybean meal and the isolated WUS fraction are quite similar, indicating that no polysaccharides were specifically removed during the isolation procedure.

Sequential extraction of the isolated cell walls with solutions, which selectively solubilise particular polysaccharides, results in two pectin-rich extracts (ChSS and DASS), an extract that contains pectins as well as hemicelluloses (1 M ASS), an extract mainly containing hemicelluloses (4 M ASS) and a cellulose-rich residue. The pectin-rich extracts have identical sugar compositions and contain predominantly galactose, arabinose and uronic acids. The 1 M

ASS fraction contains xylose in addition to the former three sugars, and the hemicellulose-rich fraction contains mainly xylose and glucose.

Besides having identical sugar compositions, ChSS and DASS also exhibit similar molecular weight distributions and similar behaviour in anion exchange chromatography. The sugar composition of the pools obtained by ion exchange chromatography of ChSS and DASS were also the same. So far, no indications have been found to state that ChSS and DASS are structurally different, although it is obvious that their arrangement in the cell wall was not identical because they were obtained with different extractants.

Further research will be directed towards a more detailed characterisation of the extracts by methylation analysis and degradation with specific enzymes. Another item of interest is to obtain information about physical properties of the isolated polysaccharides. From the sugar composition of the ChSS and DASS extracts, and the pools thereof obtained by anion exchange chromatography, the presence of xylogalacturonan in these extracts is expected. Isolation and characterisation of a xylogalacturonan containing fraction will also be one of the objects of further research.

Acknowledgements

This research is supported by the Dutch Technology Foundation (STW), Gist-brocades and the Product Board for Feeding Stuffs (VVR).

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