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Enzymatic degradation of cell wall polysaccharides from soybean meal

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Abstract

Soybean meal, soybean water unextractable solids (WUS) and extracts thereof, which contain particular cell wall polysaccharides, were incubated with a number of cell wall degrading enzymes. The intact cell wall polysaccharides in the meal and WUS were hardly degradable, while the extracts from WUS were well degraded. The arabinogalactan side chains in the pectin-rich ChSS fraction (Chelating agent Soluble Solids) could to a large extent be removed from the pectins by the combined action of endo-galactanase, exo-galactanase, endo-arabinanase and arabinofuranosidase B. The remaining polymer was isolated and represented 30% of the polysaccharides in the ChSS fraction. Determination of the sugar composition showed these polymers to be very highly substituted pectic structures. It still contained 5 mol% of arabinose and 12 mol% of galactose, representing 7% and 12%, respectively, of the arabinose and galactose present in the ChSS fraction before degradation. Further, the presence of uronic acid (50 mol%) and of xylose (18 mol%) indicated the presence of a xylogalacturonan.

Keywords: Enzymatic; Polysaccharides; Soybean; Cell wall

1. Introduction

The structure of the cell wall polysaccharides from soybean was the subject of a number of investigations in the sixties. A first determination of the constituent sugars of the fractionated soybean polysaccharides is described by Kawamura and Narasaki (1961). Morita (1965a); Morita (1965b) and Morita et al. (1967) determined the structure of an arabinogalactan in the ''hot-water-extract'' from defatted soybean flour. They concluded that the backbone of the polysaccharide is a β-1,4-linked polygalactopyranose chain with little branching. The arabinofuranosyl residues are attached as 1,5-linked side chains, with an average length of two sugar units, to galactose residues in the 1,4-linked main chain by 1,3-linkages. Aspinall et al. (1967a) describe the same structure to be present in an extract from soybean meal, obtained with a 2% ethylene diaminetetra-acetic acid disodium salt solution.

A different picture is provided by Labavitch et al. (1976). A soybean fraction containing mainly arabinose and galactose was degraded by a purified endo-galactanase and their results indicate that the arabinose in this soybean fraction is organized primarily in rather large oligo or polyarabinosides.

The soybean arabinan-galactan described by Labavitch et al. (1976) was obtained by alkaline extraction, by which neutral sugar containing side chains might be detached from branched pectic polysaccharides. Bacic et al. (1988) and O'Neill et al. (1990) already reported neutral polysaccharides like arabinans, galactans and arabinogalactans to be attached to rhamnogalacturonan in pectic polysaccharides from other sources. In all the other previously mentioned investigations, the defatted soybean meal had also been in contact with a sodium hydroxide solution before extraction of polysaccharides. Aspinall et al. (1967a) themselves already raise the possibility that the neutral polysaccharide might have arisen as a degradation product from the acidic polysaccharide, which is also present in the extract, by inadvertent cleavage of glycosidic bonds of uronyl residues by base-catalyzed β-elimination.

To confirm these findings, we deproteinated soybean meal under milder conditions, to prevent the occurrence of β-elimination, by using SDS (sodium dodecylsulphate) and DTT (1,4-dithiothreitol). A CDTA (1,2-diaminocyclohexane-*N*,*N*,*N*',*N*'-tetraacetic acid) extract of soybean meal pretreated this way contained 25 mol% of arabinose and 37 mol% of galactose. This extract showed only one symmetrical peak on size-exclusion chromatography and no neutral fraction was obtained by anion-exchange chromatography (Huisman et al., 1998). This indicated that the arabinose and galactose residues present in this extract are part of the pectins.

Information about the structure of polysaccharides can

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Table 1
Sugar composition of soybean polysaccharide fractions expressed as mol%

Fraction	Sugar composition								
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Uronic acids	Carbohydrate content[a]
WUS	2	3	19	8	2	29	21	17	89.3
ChSS	2	3	25	6	1	37	1	26	52.7
Polymeric residue (P)	6	7	4	18	2	12	1	50	69.7

[a] Expressed as % w/w

be obtained by both chemical and enzymatic degradation of polysaccharide fractions, followed by the identification of the formed degradation products. However, chemical hydrolysis with dilute acid cleaves the glycosidic bonds in a rather unspecific way, which prevents the conversion of the obtained knowledge into a hypothetical structure of the polymer. Pure enzymes have a high substrate specificity and form characteristic oligomers, therefore they are a valuable tool in structure elucidation (Voragen et al., 1993). We now report on the enzymatic degradation of soybean cell wall polysaccharides and the first steps in the structural elucidation of a pectin-rich extract from soybean cell wall polysaccharides.

2. Materials and Methods

2.1. Materials

Water unextractable solids (WUS) were isolated from solvent-extracted, untoasted soybean meal and sequentially extracted as described by Huisman et al., (1998).

2.2. Enzymatic degradation of soybean polysaccharides

Solutions [0.25% (w/w)] in 0.05 M sodium acetate buffer (pH 5.0) containing 0.01% NaN_3 were incubated with a number of enzymes or enzyme combinations at 30°C rotating 'head over tail'. Soybean meal, WUS, ChSS, DASS (Dilute Alkali Soluble Solids) and 1 M ASS (1 M Alkali Soluble Solids) were used as substrates in the degradation studies with cloned enzymes. Incubations with purified enzymes were performed with the ChSS extract. Between two subsequent enzyme incubations and at the end of all incubations the enzymes were inactivated by heating at 100°C for 10 min. Polysaccharides degrading activities were determined by HPSEC and HPAEC analyses of the digests.

Endo-galactanase, endo-arabinanase, rhamnogalacturonan hydrolase (RG hydrolase), rhamnogalacturonan acetyl esterase (RGAE) and Polygalacturonase-1 (PG-1), an endo-polygalacturonase, were cloned from *Aspergillus aculeatus* (Kofod et al., 1995) and were kindly provided by Novo Nordisk A/S (Bagsvaerd, Denmark). The cloned micro-organisms produce low amounts of their own enzymes in addition to the genetically introduced enzyme, so the cloned enzymes are not completely pure. Pectin methyl esterase

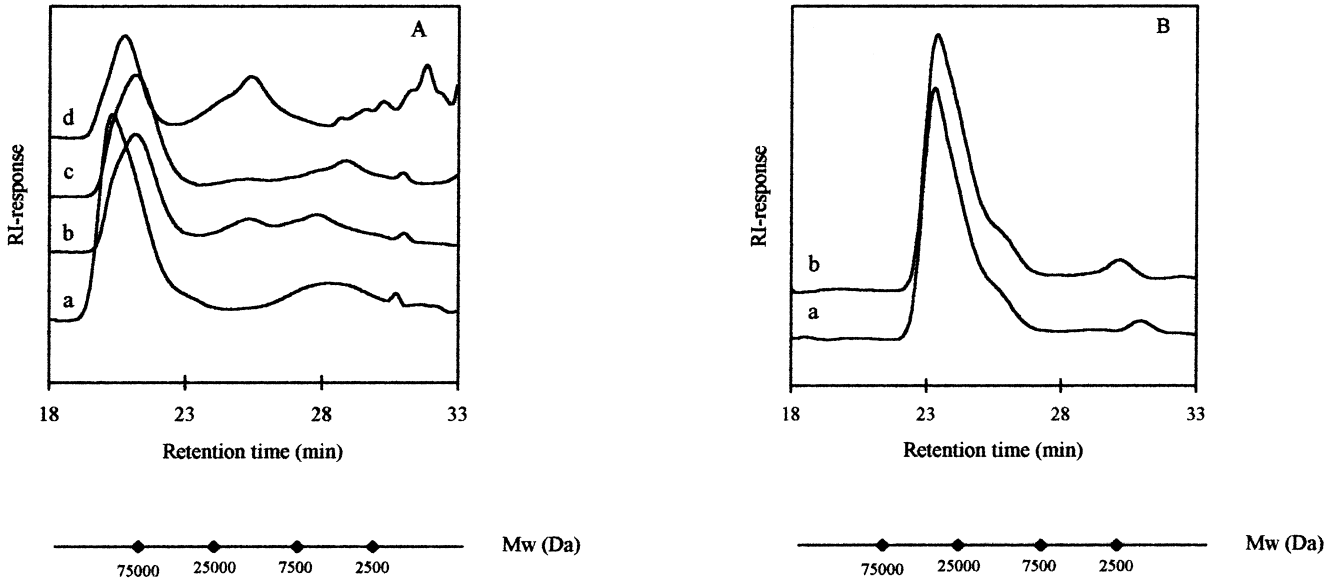

Fig. 1. HPSEC elution patterns of the soybean ChSS fraction after incubation with cloned enzymes for 24 h. A: (a) before; and (b) after incubation with endo-galactanase; (c) endo-arabinanase; (d) RG hydrolase and RGAE. B: (a) before; and (b) after incubation with PG-1 and PE.

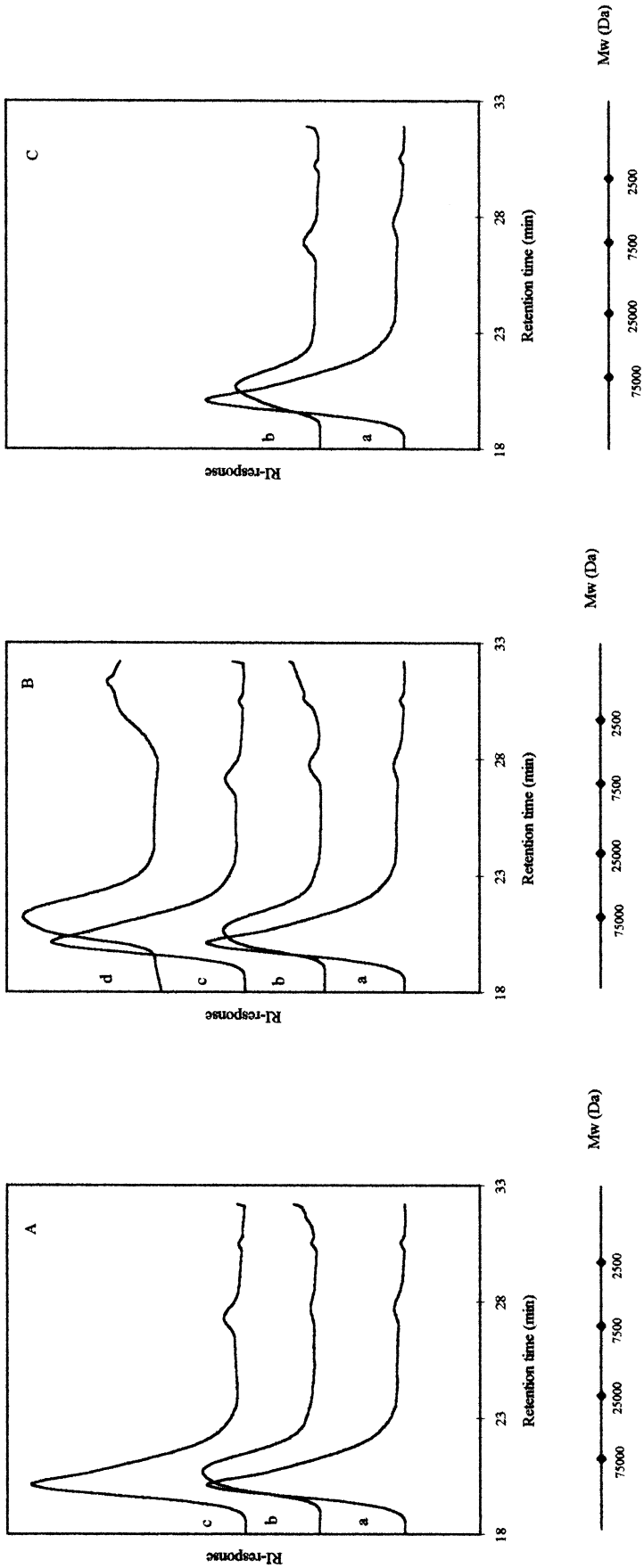

Fig. 2. HPSEC elution patterns of the soybean ChSS fraction, A: (a) before; and (b) after incubation with endo-galactanase; (c) endo-arabinanase. B: (a) before; and (b) after incubation with endo-galactanase and exo-galactanase; (c) endo-arabinanase and arabinofuranosidase B; and (d) endo-galactanase and arabinofuranosidase B. C: (a) before; and (b) after incubation with a combination of endo-galactanase, exo-galactanase, endo-arabinanase and arabinofuranosidase B. All digest were incubated with the previously mentioned enzyme(s) for 24 h.

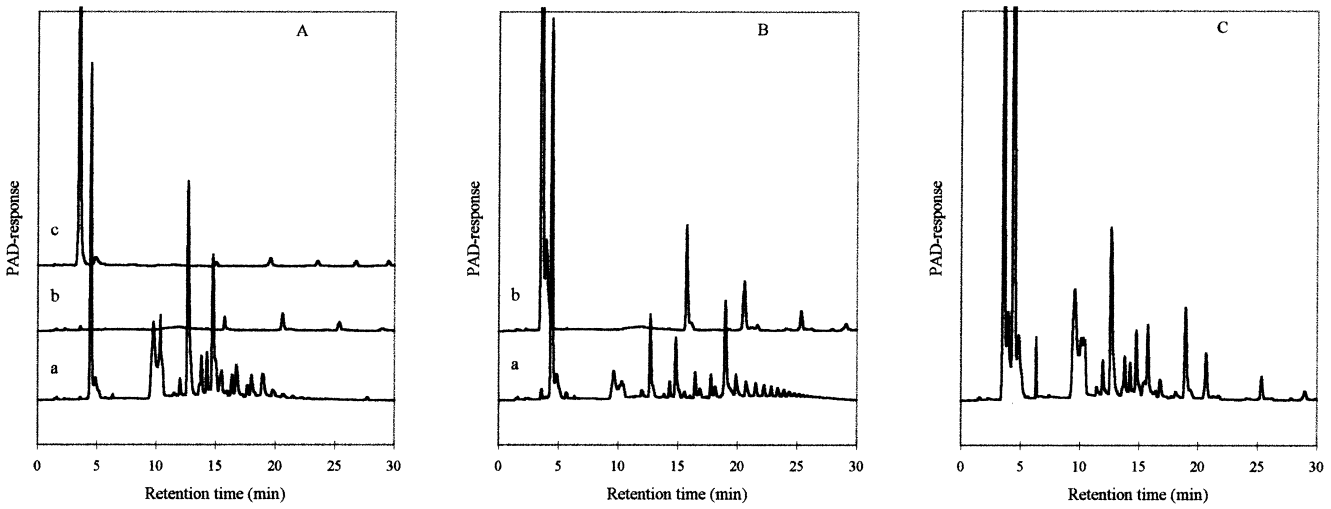

Fig. 3. HPAEC elution patterns of the soybean ChSS fraction after incubation with, A: (a) endo-galactanase; (b) endo-arabinanase; (c) arabinofuranosidase B. B: (a) endo-galactanase and exo-galactanase; (b) endo-arabinanase and arabinofuranosidase B. C: combination of endo-galactanase, exo-galactanase, endo-arabinanase and arabinofuranosidase B. All digest were incubated with the previously mentioned enzyme(s) for 24 h.

(PE) was purified from a culture medium of *Aspergillus niger* (Baron et al., 1980).

The arabinogalactan degrading enzymes used to degrade the ChSS extracts were purified from culture filtrates of fungi. Endo-arabinanase originated from *Aspergillus aculeatus* and arabinofuranosidase B from *Aspergillus niger* (Rombouts et al. 1988). Exo-galactanase was purified from a fraction obtained during the arabinofuranosidase A purification from *Aspergillus niger* (Rombouts et al., 1988). The endo-galactanase is purified from Pectinex Ultra-SP-L, a technical enzyme preparation derived from *Aspergillus aculeatus* [Novo-Nordisk Ferment (Switzerland) Ltd., Dittingen, Switzerland] as described by van de Vis et al. (1991). The amount of enzymes used was 0.13 μg protein/ml substrate solution for endo-arabinanase and arabinofuranosidase B, 0.5 μg protein/ml substrate solution for exo-galactanase and 0.05 μg protein/ml substrate solution for endo-galactanase.

2.3. Isolation of the polymeric residue remaining after enzymatic degradation of the arabinogalactan side chains from soybean pectins

A 1% ChSS solution (250 mg/25 ml) in 0.05 M sodium acetate buffer (pH 5.0) containing 0.01% NaN_3 was incubated with endo-galactanase, exo-galactanase, endo-arabinanase and arabinofuranosidase B at 30°C for 48 h, rotating 'head over tail'. After incubation the enzymes were inactivated (10 min, 100°C) and the supernatant was applied onto a Sephacryl S-100 HR column, which was initially equilibrated in 0.05 M sodium acetate buffer pH 5.0, using a Hiload System (Amersham Pharmacia Biotech AB, Uppsala, Sweden). Elution was carried out using the same buffer and the elution rate was 2.5 ml/min. Fractions (10 ml) were collected and assayed by automated methods (Tollier and Robin, 1979 and Thibault, 1979) for neutral sugar content and uronic acid content. The appropriate fractions were pooled, concentrated, dialyzed, freeze dried and analyzed for neutral sugar composition and uronic acid content.

2.4. Analytical methods

Neutral sugar composition was determined by gas chromatography according to Englyst and Cummings (1984), using inositol as an internal standard. The samples were pretreated with 72% (w/w) H_2SO_4 (1h, 30°C) followed by hydrolysis with 1 M H_2SO_4 for 3 h at 100°C and the constituent sugars were analyzed as their alditol acetates.

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

High-Performance Size-Exclusion Chromatography (HPSEC) was performed on a SP8800 HPLC (Spectra Physics, San José, CA, USA) equipped with three columns (each 300 × 7.5 mm) of Bio-Gel TSK in series (60XL, 40XL and 30XL; Bio-Rad Labs., Richmond, CA, USA) in combination with a TSK guard column (40 × 6 mm) and elution at 30°C with 0.4 M sodium acetate buffer pH 3.0 at 0.8 ml/min. Calibration was performed using dextrans. The eluate was monitored using a Shodex SE-61 refractive index detector (Showa Denko K.K., Tokyo, Japan).

High-Performance Anion-Exchange Chromatography (HPAEC) was performed on a Dionex Bio-LC system (Sunnyvale, CA, USA) as described by Schols et al. (1994). The gradient was obtained by mixing solutions of 0.1 M NaOH and 1 M sodium acetate in 0.1 M NaOH.

For the determination of arabinogalactan oligomers, the

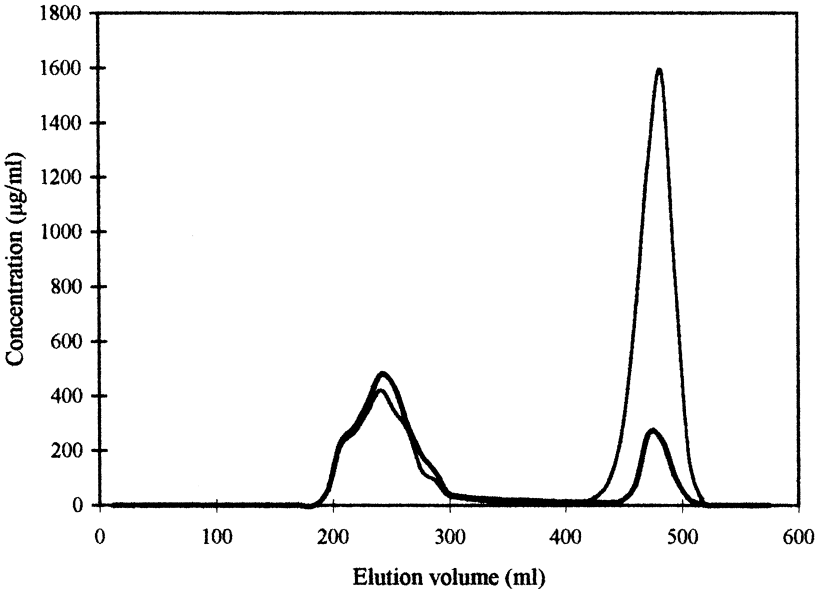

Fig. 4. Elution profile of the digest of soybean ChSS, after incubation with endo-galactanase, exo-galactanase, endo-arabinanase and arabinofuranosidase B, on Sephacryl S-100 HR. Uronic acid concentration (—), neutral sugar concentration (–).

(4 × 250 mm) CarboPac PA100 column (Dionex) was equilibrated with 0.1 M NaOH. Twenty µl of the sample was injected and a linear gradient to 0.4 M sodium acetate in 0.1 M NaOH in 40 min was applied. The column was washed for 5 min with 1 M sodium acetate in 0.1 M NaOH and equilibrated again for 15 min with 0.1 M NaOH. Calibration was performed with standard solutions of arabinose, galactose and a series of arabinan oligomers.

3. Results and discussion

3.1. Degradation of soybean polysaccharides with cloned enzymes

A first screening of the degradability of soybean meal, WUS and extracts thereof was performed with cloned enzymes, because purified enzymes are available in only very small amounts. Incubation is performed with endo-galactanase, endo-arabinanase, RG hydrolase, a combination of RG hydrolase and RGAE or a combination of PG-1 and PE. Since RG hydrolase is hindered by the presence of *O*-acetyl groups (Schols et al., 1990) RGAE was added to remove these groups. Similar reasons underlie the addition of PE to PG-1, to remove methylester groups. Hydrolysis by PG-1 preferably takes place next to a free carboxyl group and PE is able to remove the methoxyl groups from methylated galacturonic acid residues.

The digests obtained from soybean meal and WUS contained some arabinose and galactose, probably because of side-activities in the enzyme preparations. Endo-arabinanase was able to solubilize an amount of arabinan oligomers, which was relatively small considering the high arabinose content (Table 1). Although the sugar composition of soybean WUS indicates the presence of rhamnogalacturonan regions, RG hydrolase — whether combined with RGAE or not — did not release the characteristic RGase oligomers described by Schols et al. (1994). The combination of PG-1 and PE was able to release some galacturonic acid monomers in addition to the neutral sugar residues, but did not release galacturonan oligomers in spite of the high galacturonic acid content of the WUS. Analysis with HPSEC showed that none of these enzyme preparations were able to solubilize polysaccharides from both the soybean meal and WUS (elution patterns are not shown). So none of these enzymes were able to degrade the cell wall as present in soybean meal and soybean WUS, only some small neutral degradation products and some galacturonic acid residues were released. The network of the cell wall polysaccharides present in soybean appears to be too complex or too dense to be penetrated by the applied enzymes. Removal of the protein, which accounts for about 60% of the soybean meal, from the cell wall material did not increase the susceptibility of the polysaccharides for enzymatic degradation, as the degradability of the WUS is not improved compared to the degradability of the soybean meal.

Disruption of this cell wall polysaccharide network by sequential extraction can possibly increase the degradability by enzymes. Therefore, the degradability of the pectin-rich extracts — ChSS, DASS and 1 M ASS — from soybean WUS was determined by incubation of the extracts with the cloned enzymes mentioned previously.

Analyses of the digests with HPSEC (only the digests of ChSS are shown in Fig. 1) exhibit large decreases of the molecular weight of the polysaccharides in these extracts, except for the PG-1 and PE digests of ChSS and DASS. HPAEC analyses of the digests show the release of

galactose and arabinose monomers in addition to small amounts of arabinan, galactan and arabinogalactan oligomers. RG hydrolase and PG-1 are also able to release galacturonic acid residues. The amounts and diversity of oligomers formed with the various enzyme preparations and from the three substrates are different.

Extraction of the pectic polysaccharides did indeed increase their degradability, although this mainly concerned the degradability of the (arabino)galactan side chains. All the enzymes used seem only to be able to degrade part of the (arabino)galactan side chains present in the pectin-rich extracts from soybean WUS. RG hydrolase and PG-1 preparations, which are theoretically able to degrade the pectin backbone, do not release their typical degradation products. The only degradation detected is that of the (arabino)galactan side chains because of side activities of the preparation. Even after enzymatic removal of *O*-acetyl and methoxyl groups from the uronic acid residues, degradation of the pectin backbone could not be determined.

These results indicate that soybean pectic polysaccharides differ from pectic polysaccharides from other sources in that they are not degraded by the enzymes as used in this experiment. The fact that the backbone could not be degraded indicates that the galacturonic acid residues are presumably highly substituted or that they are not present in extended galacturonan chains.

Screening of the degradability of soybean polysaccharides as described previously was performed with cloned enzymes. However, these enzyme preparations still contain some side-activities. Therefore, we continued our studies on the characterization of the cell wall polysaccharides with highly purified enzymes. They allow better conclusions about which parts of the polysaccharides are degraded. The next step in the characterization of the polysaccharides will use these purified enzymes to investigate which parts of the polysaccharides in the pectin-rich ChSS fraction are degraded, to determine which degradation products are formed and to obtain the part of the polymers which is not degraded.

3.2. Degradation of the ChSS fraction with (combinations of) arabinan/galactan degrading enzymes

The galacturonic acid-rich ChSS fraction is rich in arabinose and galactose residues (Table 1). This indicates the presence of pectins with a considerable amount of arabinan, galactan or arabinogalactan side chains. Incubation with enzymes able to (partially) degrade these side chains, can provide information about their structure. Therefore, incubations with endo-galactanase, exo-galactanase, endo-arabinanase and arabinofuranosidase B are performed.

First, the ChSS fraction was incubated with all enzymes separately. The elution patterns of the endo-galactanase digest and of the endo-arabinanase digest (24 h incubation) after HPSEC and HPAEC are shown in Figs. 2A and 3A. In addition, incubations with combinations of the enzymes were performed, both simultaneously and subsequently after inactivation of the first enzyme. Figs. 2(B),(C) and 3(B),(C), show only the elution patterns of the most interesting digests, because a number of digests are (almost) identical.

The molecular weight distributions shown in Fig. 2(A) demonstrate that endo-galactanase is the only tested enzyme which is able to reduce the hydrodynamic volume of the polymers. Endo-galactanase causes a small shift of the molecular weight and a simultaneous decrease of the amount of polymeric material (70% of the original area of the high molecular weight peak remains after 90 min and 65% after 24 h). A small peak of intermediate molecular weight with a retention time of 26 min is formed after 90 min of incubation (not shown). These rather large degradation products are further degraded after prolonged incubation [Fig. 2(Ab)]. Since endo-galactanase is only able to degrade the (arabino)galactan side chains of the pectins in the ChSS fraction, the release of the intermediate products indicates that these side chains can be of considerable length. Endo-arabinanase is not able to cause any change in the molecular weight distribution of the ChSS fraction. Van de Vis et al. (1994) also found that endo-arabinanases showed very little activity on soybean arabinogalactan. Exo-galactanase and arabinofuranosidase B (not shown), do not influence the HPSEC elution pattern. Although the exo-enzymes do release monomeric sugar residues from the polysaccharide, the effect of these enzymes on the hydrodynamic volume of heterogeneous polysaccharides is usually negligible.

To determine whether the presence of branches on the galactan chain influence the degradation by endo-galactanase, the ChSS fraction was incubated with combinations of endo and exo-galactanase and of endo-galactanase and arabinofuranosidase B. Combined action of endo-galactanase and exo-galactanase, both simultaneously [Fig. 2(Bb)] and subsequently (not shown), brings about a shift in the molecular weight distribution which is identical to the action of endo-galactanase alone. The amount of remaining polymeric material decreased further to 60% of the polymers in the ChSS blank, suggesting that exo-galactanase is able to release galactose from the polymers, and enhances endo-galactanase action. This additional degradation does not effect the hydrodynamic volume of the remaining polymers. Incubation of the ChSS fraction with the combination of endo-galactanase and arabinofuranosidase B causes a shift to lower molecular weight, which is slightly larger than the shift caused by endo-galactanase alone (Fig. 2(Bd)]. The amount of the remaining polymer in the digest is also a little smaller, approximately 60% of the polymers in the blank. The resulting elution pattern is quite similar to the profile of the digest obtained by endo and exo-galactanase. The sequence in which the enzymes are added to the ChSS fraction does not influence the HPSEC elution pattern, suggesting that arabinofuranosidase B is able to remove arabinofuranosyl groups from arabinogalactan side chains,

but this does not increase the susceptibility of these chains for endo-galactanase.

Labavitch et al. (1976) demonstrated that rather large arabinan chains are present in a fraction from soybean meal, which are expected to be degraded by endoarabinanase. The absence of a shift in the molecular weight distribution in Fig. 2(Ac) and the absence of large amounts of arabinan oligomers in Fig. 3(Ab) shows that endo-arabinanase alone is not able to degrade the arabinan chains of the ChSS fraction. A linear (1,5)-α-L-arabinan is a better substrate for endo-arabinanase than a branched arabinan, and since the degree of branching of pectic arabinans from various plant tissues is found to be relatively high (Beldman et al., 1997), it might be necessary to linearize the arabinan chain before degradation by endo-arabinanase can take place. To rule out the hindrance of arabinofuranosyl side chains, the ChSS fraction was incubated with endo-arabinanase and arabinofuranosidase B, simultaneously. However, this also did not result in a change of the molecular weight distribution of ChSS [Fig. 2(Bc)].

The combination of endo-galactanase, exo-galactanase, endo-arabinanase and arabinofuranosidase B is very effective in degrading the ChSS fraction. This digest shows the largest decrease of the hydrodynamic volume and the smallest peak of remaining polymeric material [Fig. 2(Cb)]. About 45% of polymeric material remained. Arabinose and galactose represent over 60 mol% of the polysaccharides in the ChSS fraction (Table 1). This indicates that this combination of enzymes can remove almost all arabinogalactan side chains and that the enzymes act in synergism, because the total effect of the combination of enzymes is greater than the sum of the individual effects.

The HPAEC elution patterns of the digests obtained after incubation of the ChSS fraction with endo-galactanase, endo-arabinanase and arabinofuranosidase B is shown in Fig. 3(A). Degradation with endo-galactanase releases relatively large (arabino)galactan oligomers, and after incubation for 90 min, the main oligomer seems to be a tetraose (not shown). After prolonged incubation (24 h), this series of oligomers is further degraded by the endo-galactanase to mainly mono, di and trimers [Fig. 3(Aa)]. Endo-arabinanase was not able to degrade the ChSS fraction [Fig. 3(Ab)], the digest contains very few oligomeric degradation products. Exo-galactanase releases galactose monomers, as expected (not shown), although it could not be concluded whether the galactose residues were released from the non-reducing end of galactan chains or from galactose branches. Arabinofuranosidase B releases mainly arabinose monomers and some arabinan oligomers from the ChSS fraction [Fig. 3(Ac)].

When endogalactanase is combined with exogalactanase [Fig. 3(Ba)] the amount of galactose increases compared with the amount of galactose in the endo-galactanase digest. The elution patterns show a decrease of the amount of galactan and/or arabinogalactan oligomers, although some larger oligomers seem still to be present in the digest. The oligomers formed by endo-galactanase are probably further degraded by the exo-galactanase activity. The endo-galactanase and arabinofuranosidase B digest of the ChSS fraction (not shown) contains the same oligomers as the endo-galactanase digest. The amount of arabinose monomers is much higher than in the endo-galactanase digest and identical to the amount of arabinose monomers in the arabinofuranosidase B digest. Thus, arabinofuranosidase B appears to be unable to further degrade the oligomers formed by endo-galactanase; the enzyme is only able to release arabinose residues from the arabinans present in the polymeric material.

Combined action of endo-arabinanase and arabinofuranosidase B releases a considerable amount of arabinose residues and small amounts of arabinose oligomers, oligomers with a degree of polymerization up to 5 can be detected in Fig. 3(Bb). Rombouts et al. (1988) and Beldman et al. (1997) showed that arabinofuranosidase B releases arabinose as the sole product, resulting in linearized arabinan chains which are better substrates for the endo-enzyme. The release of larger arabinan oligomers may have taken place, but the arabinofuranosidase B present in the incubation mixture is able to degrade these oligomers. This synergism between endo-arabinanase and arabinofuranosidase B on soybean polysaccharides was also found by van de Vis et al. (1994).

The combination of endo-galactanase, exo-galactanase, endo-arabinanase and arabinofuranosidase B, which showed the largest decrease of the molecular weight and the smallest peak of remaining polymeric material in the HPSEC elution pattern, shows the release of high amounts of arabinose and galactose residues and a large number of different oligosaccharides: arabinan oligomers, galactan oligomers as well as arabinogalactan oligomers [Fig. 3(C)].

Enzymatic degradation of the neutral side chains of soybean pectin shows that these side chains can be of considerable length. Combined action of endo-galactanase, endo-arabinanase, exo-galactanase and arabinofuranosidase B is required to remove the larger part of these side chains and this indicates that arabinogalactan side chains are present in addition to arabinan and galactan side chains.

3.3. Characterization of the residual polysaccharide after removal of the arabinogalactan side chains from soybean ChSS

The incubation of the ChSS fraction with endo-galactanase, exo-galactanase, endo-arabinanase and arabinofuranosidase B is performed on a larger scale to enable isolation and further characterization of the remaining polymeric residue. The elution pattern of the Sephacryl S-100 HR column shows two peaks; the first peak originates from the remaining polymeric part of the pectin (P) and the second from the oligomeric degradation products (Fig. 4). This polymeric residue yields 30% of the polysaccharides present in the ChSS fraction, representing 12% of the polysaccharides present in soybean WUS.

Table 1 shows the sugar composition of the polymeric residue (P) and allows comparison with the sugar compositions of the WUS and the ChSS fraction from which it originates. The combined activities of the enzymes used were able to remove almost all arabinose, only 7% of the arabinose present in the ChSS fraction remained. The amount of remaining galactose residues was higher and represents 12% of the galactose present in the ChSS fraction before degradation. Additional incubation of a small sample of residue (P) with the same set of enzymes was performed to determine if more arabinose and galactose residues could be removed with an excess of enzymes. Both, HPSEC and HPAEC analysis of the digest, did not show any change of the molecular weight distribution or the release of any degradation product, so further removal of the arabinogalactan side chains was not possible using these enzymes.

The main constituent sugar of the polymeric residue P was uronic acid (50 mol%). Striking is the presence of 18 mol% xylose, which may indicate the presence of a xylogalacturonan. Preliminary results obtained in enzymatic degradation of this polymeric residue after acid hydrolysis with an exo-galacturonase (Beldman et al., 1996) showed the release of the characteristic dimer of xylose and galacturonic acid, thus, confirming the presence of a xylogalacturonan. In addition, the polymer contains 6 mol% rhamnose, which is very likely to be present in rhamnogalacturonan regions in the remaining pectin. In the ChSS fraction, on average 36% of the uronic acid residues carry an acetic acid group and 35% carries a methoxyl group. The degree of methylation and acetylation in the polymeric residue P could not be determined, because the available amount of this polymer was too small. However, the acetyl and methoxyl substituents are probably still present in the remaining polymer, because the incubation and isolation conditions are not likely to have removed any of these groups. Thus, the polymeric residue (P) of ChSS appears to be a highly substituted pectic structure.

4. Conclusions

It appears that rather pure cloned enzymes, having only one major activity, can not or hardly degrade the polysaccharides in the intact soybean cell wall. Previous research showed purified enzymes also to be rather limited in the degradation of soybean cell wall material (Schols et al., 1993). Even incubation with very powerful commercial enzyme mixtures did not lead to the degradation of the intact cell wall polysaccharides. However, polysaccharides extracted from soybean WUS can be degraded by enzymes more easily. Extraction of part of the polysaccharides from the cell wall network might enlarge the pores present in this network and enable enzymes to penetrate and reach the available hydrolysis sites. This indicates that it is necessary to disrupt the network of the cell wall polysaccharides to enable the enzymes to degrade them. Still, it is not yet clear whether the proper enzymes are missing or that the inaccessibility of the substrate is the main reason.

The combination of endo-galactanase, exo-galactanase, endo-arabinanase and arabinofuranosidase B is very effective in degrading the arabinogalactan side chains in the ChSS fraction. Analysis of the degradation products and the observed synergistic action of some enzyme combinations indicate that rather large branched arabinogalactan side chains are present in soybean pectic structures. About 30% of the sugars present in the ChSS fraction are recovered as the undegradable remaining polymer (P) in the digest after this incubation. This polymer still contains 4 mol% of arabinose and 12 mol% of galactose, which could not be removed by these enzymes. This polymer appears to be a very highly substituted pectic structure, containing rhamnogalacturonan regions and presumably xylogalacturonan. From an unfractionated soybean polysaccharide preparation, various acidic xylose-containing oligosaccharides were obtained by partial hydrolysis, by Aspinall et al. (1967b). However, this is the first time that indications for the presence of *polymeric* xylogalacturonan regions in a pectic polysaccharide fraction from soybean meal were obtained. The presence of xylogalacturonan regions was indicated in pectins extracted from pea hulls (Weightman, et al., 1994), apple (Schols et al., 1995), watermelon and cotton (Yu and Mort, 1996).

Further research will focus on the elucidation of the structure of both the remaining polymer (P) and the (arabino)galactan oligomers which are formed after degradation of the soybean ChSS fraction with endo-galactanase, exo-galactanase, endo-arabinanase and arabinofuranosidase B. This will include chromatography techniques, degradation with specific enzymes, and MS and NMR analyses. Knowledge of the structures of these oligosaccharides and of the mode of action of the enzymes can lead to the elucidation of the structure of the (arabino)galactan side chains from soybean cell wall pectin. The structure of the other cell wall polysaccharides of soy bean will be another topic of investigation.

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