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Xyloglucan from soybean (*Glycine max*) meal is composed of XXXG-type building units

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Abstract

Soybean cell wall material was depectinated by extraction with a hot chelating agent and a cold dilute alkali. The hemicelluloses were solubilised from the residue with 1 and 4 M KOH solutions, resulting in 1 M Alkali Soluble Solids (1 MASS) and 4 M Alkali Soluble Solids (4 MASS) fractions. The polysaccharides extracted with 1 M KOH were fractionated by ion-exchange chromatography, yielding a neutral and a pectic population. The sugar composition of the neutral population indicated the presence of xyloglucans and possibly xylans. Enzymatic degradation with endo-xylanases and endo-glucanases showed the presence of xyloglucans only. Analysis of the digest formed after incubation of the neutral population with endo-glucanase V using both HPAEC and MALDI-TOF MS showed the formation of the characteristic poly-XXXG xyloglucan oligomers (XXG, XXXG, XXFG, XLXG, and XLFG).

Keywords: Xyloglucan; Soybean meal; XXXG-type building unit

1. Introduction

Soybeans (*Glycine max*) belongs to the pea family of the Leguminosae. Their primary cell wall is built up of skeletal cellulose microfibrils and the so-called matrix polymers, which include xyloglucans, xylans, pectins and proteins. The cell walls of dicots consist of two main interpenetrating networks, one of cellulose and hemicellulose and one of pectin (McCann & Roberts, 1991).

Hemicelluloses are non-cellulosic wall polysaccharides other than pectins (McCann & Roberts, 1991), which can be extracted from the walls with alkaline solutions, typically 1–4 M. The requirement for relatively strong alkali for their extraction from the wall is due to strong hydrogen bonding between the hemicellulose and cellulose microfibrils. The hemicelluloses vary greatly in different cell types and in different species. In most cell types, one hemicellulose predominates, with others present in smaller amounts (Brett & Waldron, 1990).

In the cell walls of most Dicotyledonae, the principal hemicelluloses are xyloglucans. Other hemicelluloses, such as gluco- and galactoglucomannans, galactomannans, (1 → 3)-β-D-glucans, and glucuronoarabinoxylans are found in much lower amounts (Carpita & Gibeaut, 1993). Xyloglucans are linear chains of (1 → 4)-β-D-glucan with xylosyl residues added at regular sites to the O-6 position of the glucosyl units. Additional sugar residues, like galactose, fucose and arabinose, are added to the O-2 of some xylosyl residues (Carpita & Gibeaut, 1993; Hayashi, 1989; Vincken, York, Beldman & Voragen, 1997a; York, van Halbeek, Darvill & Albersheim, 1990). The galactose residues can be O-acetylated (Kiefer, York, Darvill & Albersheim, 1989).

In previous publications, the structure of the pectic substances in the pectin network of the cell wall has been described extensively (Huisman, Schols & Voragen, 1998; Huisman, Schols & Voragen, 1999; Huisman, Fransen, Kamerling, Vliegenthart, Schols & Voragen, submitted). In the current study we will describe the structural elucidation of the most prominent hemicellulose in soybean cell wall material.

2. Materials and methods

2.1. Material

Polysaccharides have been sequentially extracted from water unextractable solids (WUS) isolated from solvent-extracted, untoasted soybean meal (Huisman et al., 1998). The 1 M Alkali Soluble Solids (1 MASS) and 4 MASS

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Table 1
Sugar composition of soybean meal fractions as mol%

Fraction	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Uronic acids
WUS	2	3	19	8	2	29	21	17
1 MASS	2	3	23	11	1	35	5	20
1 MASS neutral	2	4	8	24	1	19	40	3
1 MASS charged	2	3	22	10	Trace amount	34	6	25

fractions were obtained by extraction with 1 and 4 M KOH, respectively.

2.2. Ion-exchange chromatography

Approximately 250 mg of 1 MASS was fractionated on a column (100 × 2.6 cm, id) of DEAE Sepharose Fast Flow, which was initially equilibrated in 0.005 M NaAc-buffer pH 5.0, using a Hiload System (Amersham Pharmacia Biotech AB, Uppsala, Sweden).

Elution was carried out sequentially with 530 ml of 0.005 M NaAc-buffer pH 5.0, a linear gradient from 0.005 to 1 M NaAc buffer pH 5.0 (1060 ml), a linear gradient 1–2 M NaAc-buffer pH 5.0 (530 ml) and 265 ml 2 M NaAc-buffer pH 5.0. Residual bound polysaccharides were washed from the column with 530 ml of 0.5 M NaOH. The elution rate was 10 ml/min except for the first step, in which the sample was applied onto the column and the elution rate was 5 ml/min. Fractions (20 ml) were collected and assayed by automated methods for neutral sugar content (Thibault, 1979) and uronic acid content (Tollier & Robin, 1979). The appropriate fractions were pooled, concentrated, dialysed, freeze dried and analysed for neutral sugar composition and uronic acid content.

2.3. Enzymatic degradation

The unbound, neutral population from anion-exchange chromatography (1 MASS neutral) was treated with pure and well-defined enzymes, endo-xylananse I (Kormelink, Searle-van Leeuwen, Wood & Voragen, 1993a; Kormelink, Gruppen, Viëtor & Voragen, 1993b) and endo-glucanase I and V (Beldman, Searle-van Leeuwen, Rombouts & Voragen, 1985). Solutions (0.25% (w/w)) of 1 MASS neutral in 50 mM NaAc buffer (pH 5.0) containing 0.01% NaN_3 were incubated at 30°C rotating 'head over tail', during 24 h. The enzyme concentration used in these experiments was 1 μg/ml. The enzymes were inactivated by heating at 100°C for 10 min. The digests were analysed by High-performance size-exclusion chromatography (HPSEC) and High-performance anion-exchange chromatography (HPAEC).

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 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.

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

HPSEC was performed on a SP8800 HPLC (Spectra Physics) equipped with three columns (each 300 × 7.5 mm, id) of Bio-Gel TSK in series (40XL, 30XL and 20XL; Bio-Rad Labs.) in combination with a TSK guard column (40 × 6 mm, id) 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.

HPAEC was performed on a Dionex Bio-LC system (Schols, Voragen & Colquhoun, 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 xylan oligomers the gradient described by Verbruggen et al. (1998) was used. Calibration was performed with a standard xylan digest. For the determination of xyloglucan oligomers the gradient described by Vincken, Beldman, Niessen and Voragen (1996a) for the CarboPac PA-100 column was used. Xyloglucan oligomers prepared from apple cell wall polysaccharides (Vincken et al., 1996a) were used as standards.

MALDI-TOF MS. The matrix solution was prepared by dissolving 9 mg 2,5-dihydroxybenzoic acid and 3 mg 1-hydroxy isoquinoline in 700 μl distilled water and 300 μl acetonitril. A 1 μl volume of this solution was placed on the sample plate together with 1 μl of the sample solution and allowed to dry at room temperature. The sample plate was then placed in the instrument.

MALDI-TOF mass spectra were recorded with a Voyager-DE RP Biospectrometry Workstation (PerSeptive Biosystems, Inc., Framingham, MA, USA) equipped with a nitrogen laser operating at 337 nm (3-ns pulse duration), a single stage reflector, and delayed extraction. The accelerating voltage used was 12 kV and the delay time setting was 200 ns. Each spectrum was produced by accumulating data from 100–256 laser shots. Mass spectra were calibrated with an external standard containing galacturonic acid oligomers (degree of polymerisation 2–9).

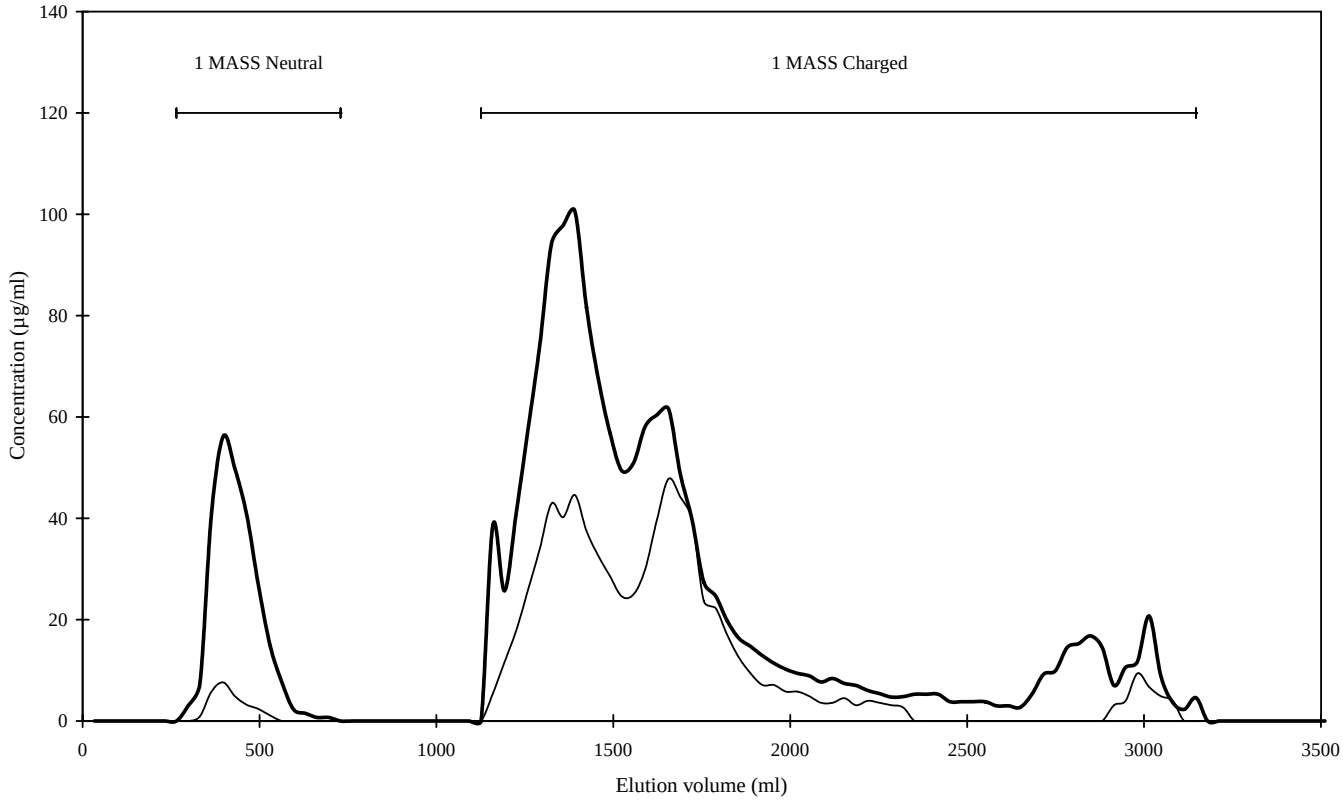

Fig. 1. Elution profile of soybean 1 MASS on anion-exchange chromatography. Uronic acid concentration (—), neutral sugar concentration (**—**).

3. Results and discussion

3.1. Fractionation of the 1 MASS extract

In a previous paper we described the sequential extraction of soybean meal WUS (Huisman et al., 1998). The WUS were first extracted with CDTA and 0.05 M NaOH, to remove the pectic substances. The residue was extracted with 1 M KOH to yield the 1 MASS extract. This extract contains 16% of the polysaccharides in the WUS. The sugar composition (Table 1) indicates the presence of both pectic substances and hemicelluloses, and these hemicelluloses might be xyloglucans and xylans.

The 1 MASS extract was fractionated using anion-exchange chromatography. The elution profile (Fig. 1) shows that the 1 MASS extract contains both a neutral (1 MASS neutral) and a charged (1 MASS charged) population. The sugar compositions of both populations are shown in Table 1. The recovery per individual sugar residue is satisfactory, varying between 90 and 114%. An aberrant observation is the high recovery of glucose (190%). An unexpected amount of glucose was washed from the column with 0.5 M NaOH. The origin of this glucose-rich material could not be established. It is of no concern during this research, because this late-eluting material was not further studied.

The neutral population yields 10.2% of the recovered polysaccharide material. The high contents of xylose and glucose are an indication for the presence of xyloglucans, and the presence of fucose suggests that the xyloglucans are fucosylated. The major part of the galactose can also be accounted for by xyloglucans. Assuming that the xyloglucan oligomers shown in Table 2 occur in equal amounts, xyloglucan would account for 10 mol% of galactose. Both, the arabinose and the remaining galactose residues can also occur in the side chains of the small amount of rhamnogalacturonan eluting with the neutral polysaccharides. The neutral sugar containing side chains apparently prevented the interaction of galacturonic acid residues with the column material, and these pectic substances consequently eluted in the void. This was also seen for a part of the soybean pectic substances extracted with chelating agent and dilute alkali (Huisman et al., 1998).

3.2. Enzymatic degradation of the hemicelluloses from 1 MASS

The specificity of enzymes can be used to show the presence of particular polysaccharides (Voragen, Schols & Gruppen, 1993). In this study endo-glucanase I and V, and endo-xylanase I are used to demonstrate the presence of (xylo)glucans and arabinoxylans, respectively. Endo-glucanase I is known to have a high activity towards glucans and a much lower activity towards xyloglucan, and endo-glucanase V has a high activity towards xyloglucan (Vincken, Beldman & Voragen, 1997b).

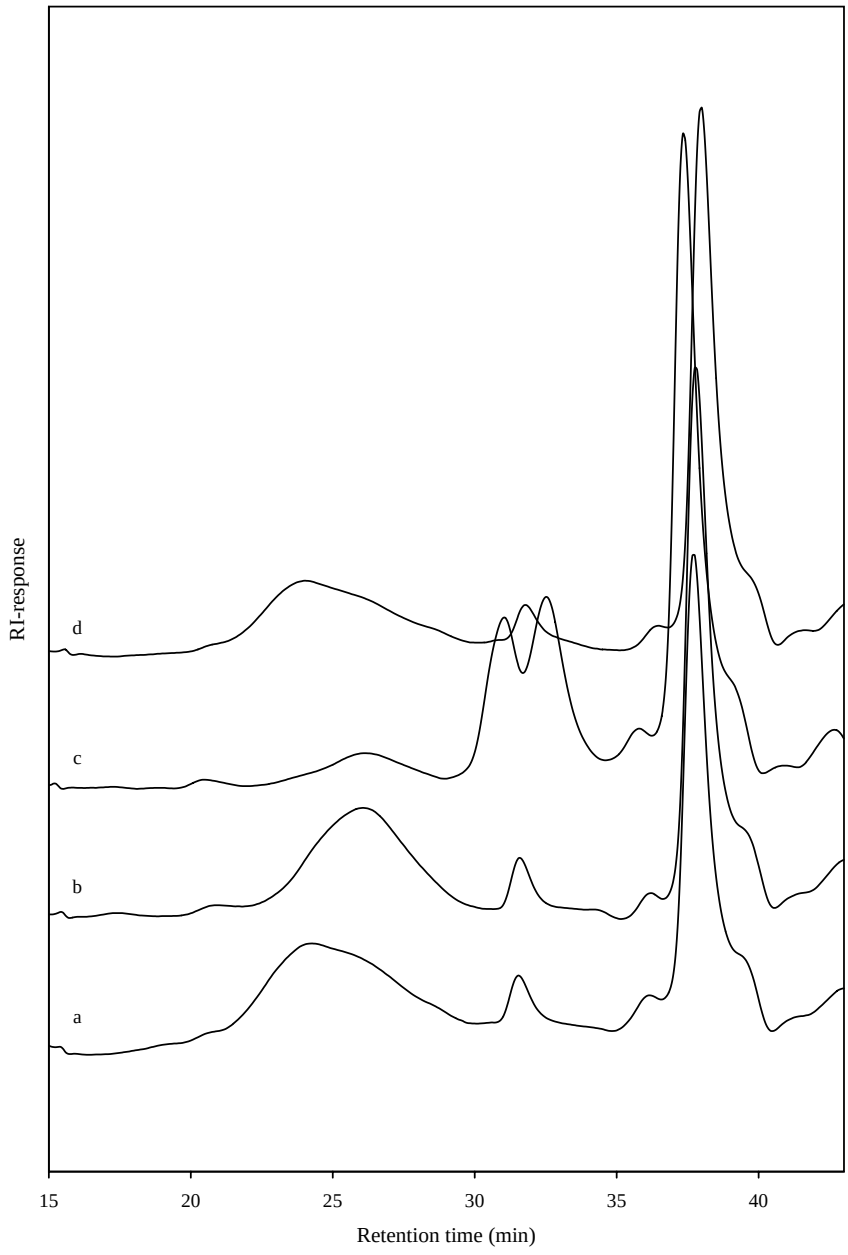

Fig. 2. HPSEC elution profiles of: (a) 1 MASS neutral; (b) endo-glucanase I digest; (c) endo-glucanase V digest; and (d) endo-xylanase I digest.

The elution profile of the endo-xylanase I digest on HPSEC is similar to the elution pattern of the blank 1 MASS neutral (Fig. 2, lines d and a). Also, the elution profile on HPAEC does not show the release of any (arabino)xylan oligomers (Fig. 3A). So, endo-xylanase I appears to be unable to degrade any polysaccharides in this fraction, demonstrating that the presence of arabinoxylans in the 1 MASS neutral fraction is not very likely. Although the presence of a heavily branched arabinoxylan, which is resistant to degradation with endo-xylanase I, cannot be ruled out completely.

Endo-glucanases (I and V), on the other hand, are able to degrade the neutral polymers. Incubation of the 1 MASS neutral fraction with endo-glucanase I results in a small decrease of the molecular masses of the polysaccharides. Endo-glucanase V causes almost complete degradation of the polymers in the 1 MASS neutral fraction (Fig. 2, line c) to products that elute at 31 and 32.5 min. So the neutral population of the 1 MASS fraction most probably contains xyloglucans, and no linear glucans.

The HPAEC profiles of these two digests (Fig. 3B) confirm these results. Endo-glucanase I is not able to release oligomeric degradation products (line b). Endo-glucanase V, on the other hand, releases large amounts of oligomeric degradation products (line c). The retention times of the oligomers in HPAEC analysis are identical to those formed from apple (Vincken et al., 1996a) and sugar beet xyloglucan (Oosterveld, Beldman, Schols & Voragen, 1999) by

Table 2
The structure of oligomers obtained after incubation of 1 MASS neutral by endo-glucanase V

Code	Structure	$[M + Na]^+$
XXXG	Glc-Glc-Glc-Glc | | | | Xyl Xyl Xyl	1085
XXFG	Glc-Glc-Glc-Glc | | | | Xyl Xyl Xyl | Gal | Fuc	1393
XLFG	Glc-Glc-Glc-Glc | | | | Xyl Xyl Xyl | | Gal Gal | Fuc	1555
XLXG	Glc-Glc-Glc-Glc | | | | Xyl Xyl Xyl | Gal	1247

endo-glucanase V. The structures of the oligomers in the apple xyloglucan endo-glucanase V digest are known, and it can be assumed in all probability that the structure of the oligomers in the digest from soybean xyloglucan are identical. Small changes in the substitution pattern of the xyloglucan oligomers result in considerable changes in their retention time (Vincken, Wijsman, Beldman, Nissen & Voragen, 1996b), and their occurrence in this digest could consequently be ruled out.

The digest is also analysed by MALDI-TOF MS (Fig. 4). The signal with mass 1085 is caused by a sodium-cationised oligomer composed of four hexoses and three pentoses. The signal with mass 1101 is caused by the potassium-cationised oligomer composed of the same residues. Signals 1247 and 1263 correspond to the sodium- and potassium-cationised oligomer with an additional hexose, $hexose_5pentose_3$. The signal 1393 (and 1409) is indicative for $hexose_5pentose_3$-deoxyhexose. The last two signals, 1555 and 1571, correspond to a decamer containing an additional hexose.

From both HPAEC and MALDI-TOF MS it can be concluded that the oligomers obtained after incubation with endo-glucanase V were; XXG, XXXG, XXFG, XLXG, and XLFG, named according to the nomenclature of Fry et al. (1993), and are shown in Table 2.

The presence of these (already known) xyloglucan oligomers in the digest shows that three out of four glucose residues carry a side chain; it is composed of XXXG-type building units (Vincken et al., 1997a). This was also seen for xyloglucans from many species such as apple, sycamore, tamarind and sugar beet (Oosterveld et al., 1999; Vincken et al., 1996a; York et al., 1990). This is in contrast with xyloglucans from potato and tomato, both belonging to the Solanaceae, for which the presence of two adjacent unbranched glucose residues is characteristic (Vincken et al., 1996b). The presence of XXFG and XLFG also indicates that the xyloglucans from soybean are fucosylated, similar to xyloglucans from other sources (Hisamatsu, Impallomeni, York, Albersheim & Darvill, 1991; York et al., 1990). The fucose residue is substituted to the O-2 of a galactosyl unit, resulting

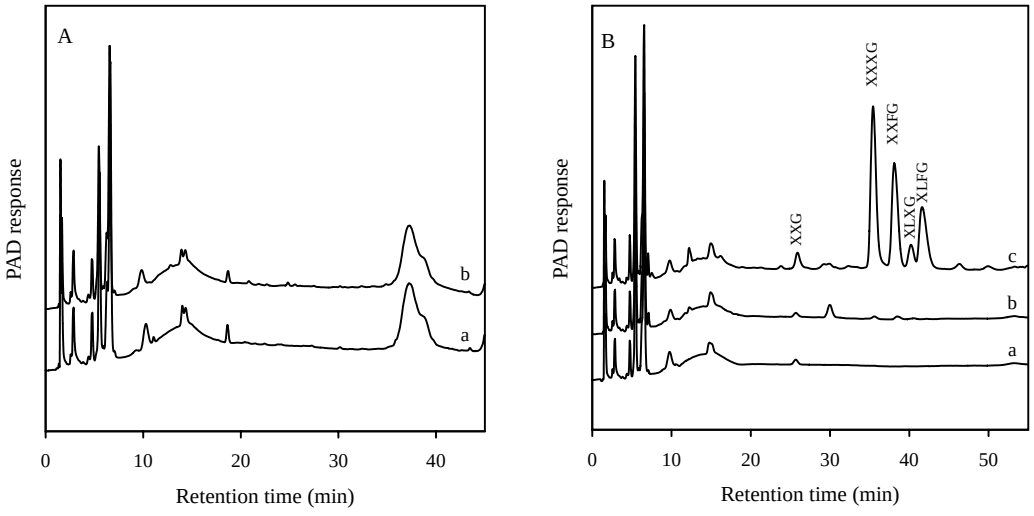

Fig. 3. HPAEC elution profiles of (Aa) 1 MASS neutral; and (Ab) endo-xylanase I digest, obtained with a gradient for xylan oligomers (Verbruggen et al., 1998), and of (Ba) 1 MASS neutral; (Bb) endo-glucanase I digest; and (Bc) endo-glucanase V digest obtained with a gradient for xyloglucan oligomers (Vincken et al., 1996a).

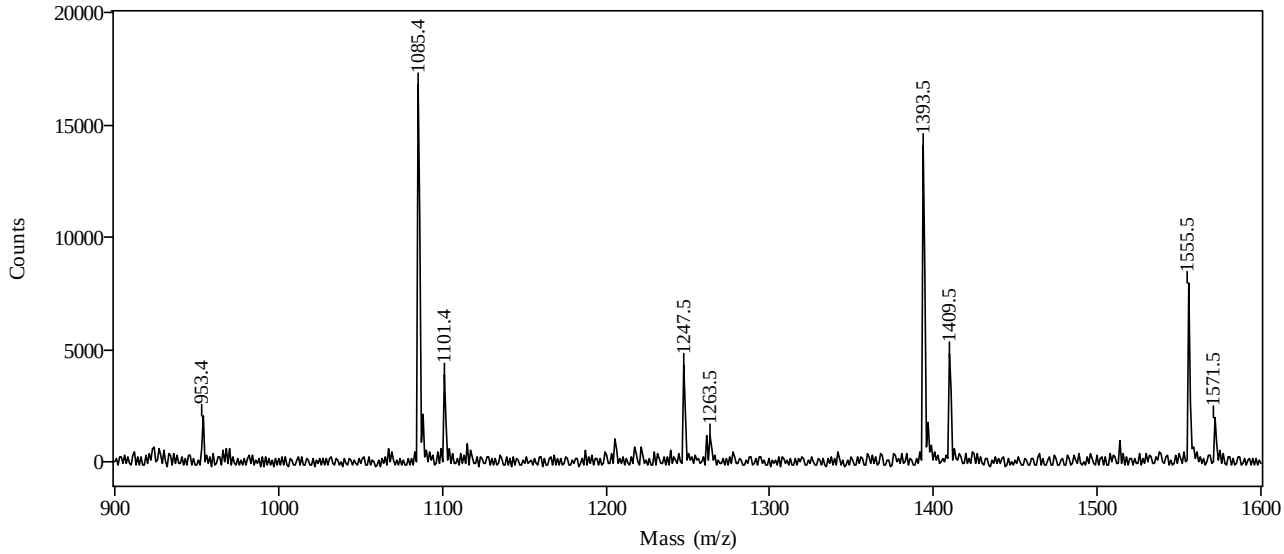

Fig. 4. MALDI-TOF mass spectrum of the endo-glucanase V digest of 1 MASS neutral.

in a trisaccharide side chain attached to the glucan backbone.

Information about the structure of the xyloglucan from soybean *seeds* was not published before, unlike that of the hypocotyl. The xyloglucan from suspension-cultured soybean cells, started from callus tissue derived from a hypocotyl of a seedling of soybean, was mainly constructed of two kinds of oligosaccharide repeating units, a heptasaccharide (XXXG) and a nonasaccharide (XXFG) (Hayashi, Kato & Matsuda, 1980). These structures also correspond with a xyloglucan of the poly-XXXG type.

The sugar composition of the 4 MASS fraction from soybean WUS also indicates the presence of xyloglucan. Degradation of the 4 MASS fraction with endo-glucanase I and V showed that this fraction contains a small amount of glucan and that the major constituent is xyloglucan. HPAEC and MALDI-TOF MS of the digests showed that these xyloglucans are also composed of XXXG-type building units, resembling the structure of the xyloglucans extracted by the 1 M KOH solution. They distinguish themselves from the xyloglucans in the 1 MASS fraction only in the effort needed to extract them from the cell wall material, which is expressed in the higher concentration of alkali used. The xyloglucans in the 4 MASS fraction are probably more tightly hydrogen-bonded to the cellulosic microfibrils or enclosed in these microfibrils and were released due to the swelling of cellulose caused by the high concentration of alkali (Edelmann & Fry, 1992).

4. Conclusions

The polysaccharides in the 1 MASS extract from soybean WUS contain both pectic substances and neutral polysaccharides, which can be separated by ion-exchange chromatography. The neutral population represents 1.6% of the polysaccharides of soybean WUS. The sugar composition and the formation of specific xyloglucan oligomers after degradation of the neutral polysaccharides in the 1 MASS extract with endo-glucanase V proves that this fraction consists of xyloglucans. The structure of the xyloglucan oligomers was determined by comparison of their elution behaviour on HPAEC with that of well-known references, and by molecular mass analysis by MALDI-TOF MS. It can be concluded that the xyloglucans are fucosylated and are composed of XXXG-type building units, similar to xyloglucans from many other plants. The structure of xyloglucan from soybean seeds has not been described before.

Combination of the specificity of enzymatic degradation with HPAEC and mass spectrometry proves to be very efficient in characterising polysaccharides isolated from any plant material. This applies among others to xyloglucans, as was shown in this research.

The sugar composition of the 1 MASS charged fraction obtained after anion-exchange chromatography indicates that it is rich in pectic substances. Further research will focus on the structure of these pectins, and comparison of their structure with the pectic substances in the ChSS and DASS fractions from soybean meal.

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References

Beldman, G., Searle-van Leeuwen, M. J. F., Rombouts, F. M., & Voragen, A. G. F. (1985). *European Journal of Biochemistry, 146*, 301–308.

Blumenkrantz, N., & Asboe-Hansen, G. (1973). *Analytical Biochemistry*, *54*, 484–489.

Brett, C., & Waldron, K. (1990). chap. 2. *Physiology and biochemistry of plant cell walls*, Boston: Hyman.

Carpita, N. C., & Gibeaut, D. M. (1993). *Plant Journal*, *3*, 1–30.

Edelmann, H. G., & Fry, S. C. (1992). *Carbohydrate Research*, *228*, 423–431.

Englyst, H. N., & Cummings, J. H. (1984). *Analyst*, *109*, 937–942.

Fry, S. C., York, W. S., Albersheim, P., Darvill, A., Hayashi, T., Joseleau, J.-P., Kato, Y., Lorences, E. P., Maclachlan, G. A., McNeil, M., Mort, A. J., Reid, J. S. G., Seitz, H. U., Selvendran, R. R., Voragen, A. G. J., & White, A. R. (1993). *Physiology of Plant*, *89*, 1–3.

Hayashi, T. (1989). *Annual Review of Plant Physiology and Plant Molecular Biology*, *40*, 139–168.

Hayashi, T., Kato, Y., & Matsuda, K. (1980). *Plant and Cell Physiology*, *21*, 1405–1418.

Hisamatsu, M., Impallomeni, G., York, W. S., Albersheim, P., & Darvill, A. G. (1991). *Carbohydrate Research*, *211*, 117–129.

Huisman, M. M. H., Schols, H. A., & Voragen, A. G. J. (1998). *Carbohydrate Polymers*, *37*, 87–95.

Huisman, M. M. H., Schols, H. A., & Voragen, A. G. J. (1999). *Carbohydrate Polymers*, *38*, 299–307.

Huisman, M. M. H., Fransen, C. T. M., Kamerling, J. P., Vliegenthart, J. F. G., Schols, H. A. & Voragen, A. G. J. Submitted for publication.

Kiefer, L. L., York, W. S., Darvill, A. G., & Albersheim, P. (1989). *Phytochemistry*, *28*, 2105–2107.

Kormelink, F. J. M., Searle-van Leeuwen, M. J. F., Wood, T. M., & Voragen, A. G. J. (1993a). *Journal of Biotechnology*, *27*, 249–265.

Kormelink, F. J. M., Gruppen, H., Viëtor, R. J., & Voragen, A. G. J. (1993b). *Carbohydrate Research*, *249*, 355–367.

McCann, M. C., & Roberts, K. (1991). In C. W. Lloyd (Ed.), *The cytoskeletal basis of plant growth and form*, (pp. 109–129). London: Academic Press.

Oosterveld, A., Beldman, G., Schols, H. A., & Voragen, A. G. J. (1999). *Carbohydrate Research*, *00*, 00 in press.

Schols, H. A., Voragen, A. G. J., & Colquhoun, I. J. (1994). *Carbohydrate Research*, *256*, 97–111.

Thibault, J.-F. (1979). *Lebensm Wissenschaftliche Technology*, *12*, 247–251.

Tollier, M., & Robin, J. (1979). *Annals of Technology in Agriculture*, *28*, 1–15.

Verbruggen, M. A., Spronk, B. A., Schols, H. A., Beldman, G., Voragen, A. G. J., Thomas, J. R., Kamerling, J. P., & Vliegenthart, J. F. G. (1998). *Carbohydrate Research*, *306*, 265–274.

Vincken, J.-P., Beldman, G., Niessen, W. M. A., & Voragen, A. G. J. (1996a). *Carbohydrate Polymers*, *29*, 77–85.

Vincken, J.-P., Wijsman, A. J. M., Beldman, G., Niessen, W. M. A., & Voragen, A. (1996b). *Carbohydrate Research*, *288*, 219–232.

Vincken, J.-P., York, W. S., Beldman, G., & Voragen, A. G. J. (1997a). *Plant Physiology*, *114*, 9–13.

Vincken, J.-P., Beldman, G., & Voragen, A. G. J. (1997b). *Carbohydrate Research*, *298*, 299–310.

Voragen, A. G. J., Schols, H. A., & Gruppen, H. (1993). In F. Meuser & D. J. Manners & W. Seibel (Eds.), *Plant polymeric carbohydrates*, (pp. 3–15). Cambridge: Royal Society of Chemistry.

York, W. S., van Halbeek, H., Darvill, A. G., & Albersheim, P. (1990). *Carbohydrate Research*, *200*, 9–31.