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Characterisation of cell wall polysaccharides from rapeseed (*Brassica napus*) meal

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ABSTRACT

To enable structural characteristics of individual cell wall polysaccharides from rapeseed (*Brassica napus*) meal (RSM) to be studied, polysaccharide fractions were sequentially extracted. Fractions were analysed for their carbohydrate (linkage) composition and polysaccharide structures were also studied by enzymatic fingerprinting.

The RSM fractions analysed contained pectic polysaccharides: homogalacturonan in which 60% of the galacturonic acid residues are methyl-esterified, arabinan branched at the O-2 position and arabinogalactan mainly type II. This differs from characteristics previously reported for *Brassica campestris* meal, another rapeseed cultivar. Also, in the alkali extracts hemicelluloses were analysed as xyloglucan both of the XXGG- and XXXG-type decorated with galactosyl, fucosyl and arabinosyl residues, and as xylan with O-methyl-uronic acid attached. The final residue after extraction still contained xyloglucan and remaining (pectic) polysaccharides next to cellulose, showing that the cell wall matrix of RSM is very strongly interconnected.

1. Introduction

Rapeseed (*Brassica napus*) meal (RSM) is a by-product from the production of rapeseed oil and is used as animal feed. The high demand for energy has led to increased production of biodiesel and as a consequence more RSM has become available for the animal feed industry. Nowadays in Europe, 24% of oilseed meal used in feed originates from rapeseed compared to 59% from soybean and 12% from sunflower (Krautgartner et al., 2012). A drawback of RSM is that, compared to soybean meal, it is high in cell wall polysaccharides, which cannot be degraded by endogenous enzymes of monogastric animals and improvement of digestibility by pre-treatment of RSM is needed (Pustjens et al., 2012). However, knowledge on cell wall polysaccharide structure in RSM is currently limiting.

Oil seeds are dicotyledonous plants. They are rich in polysaccharides, like pectins, hemicelluloses and cellulose, which together form a complex network within the plant cell wall. Pectins are a very diverse group of polysaccharides consisting of the structural elements homogalacturonan (HG), rhamnogalacturonan I (RG-I) and II (RG-II), and xylogalacturonan. HG is a linear polymer of α-1,4-linked galacturonic acid units, which can be methyl-esterified on the O-6 position. Also, they can carry acetyl-groups on the O-2 and/or O-3 position (Schols & Voragen, 2002). RG-I consists of a backbone of alternating rhamnosyl and galacturonyl residues, to which neutral side chains, such as arabinans and arabinogalactans, are proposed to be attached (Schols & Voragen, 2002). The hemicelluloses in oil seeds are mainly xyloglucans, which are polysaccharides with a β-1,4-linked glucosyl backbone with 1,6-linked xylosyl units, to which galactosyl and fucosyl residues can be attached.

Rapeseed derives from several species belonging to the genus Brassicaceae. In this paper *B. napus* is studied, of which the cell wall polysaccharides have not been characterised so far. *B. napus* is a crossbreed between *Brassica campestris* and *Brassica oleracea*. From three cultivars rapeseed is grown and used in industry (Sheidai, Noormohamadi, Mirabdolbaghi-Kashani, & Ahmadi, 2003). Characterisation of polysaccharides from *B. campestris* meal has been published (Ghosh et al., 2004; Siddiqui & Wood, 1971, 1972, 1974, 1977a, 1977b). From the *B. oleracea* cultivar, only the cell wall polysaccharides from its cabbages have been analysed (Westereng et al., 2009), and not from the meal of its seeds. ×The aim of this study was to characterise cell wall polysaccharides from *B. napus* meal, which will help to understand and improve the limited digestibility of these polysaccharides in this abundant by-product for monogastric animals.

2. Materials and methods

2.1. Plant material

Rapeseed meal (ADM, Hamburg, Germany, 2009) was provided by Nutreco Nederland B.V. (Boxmeer, The Netherlands).

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2.2. Isolation of water unextractable solids (WUS) and sequential extraction

Rapeseed meal (RSM) was ground using a Retsch mill (Retsch, Haan, Germany) to pass a 0.5 mm sieve. This meal (40 g) was extracted three times with 1 L of demineralized water during 1 h at 70 °C. After each extraction, solubles were separated from the insoluble residue by filtration on a G2-glass filter. Water soluble solid (WSS) fractions were combined and (without previous dialysis) freeze-dried.

Water unextractable solids (WUS) were sequentially extracted (25 mL extractant per gram material) starting with 0.05 M 1,2-diaminocyclohexane-N,N,N′,N′-tetraacetic acid (CDTA) and 0.05 M ammonium-oxalate in 0.05 M NaOAc (pH 5.2) at 70 °C (Chelating Agent Soluble Solids, ChSS). Chelating agent unextractable solids were further extracted with 0.05 M NaOH containing 20 mM $NaBH_4$ at 4 °C (Dilute Alkali Soluble Solids, DASS). Again, the unextracted solids were further extracted with 4 M NaOH containing 20 mM $NaBH_4$ at 4 °C (4 M Alkali Soluble Solids, 4MASS). Similarly, extraction with 6 M KOH containing 20 mM $NaBH_4$ was performed at 4 °C (6 M Alkali Soluble Solids, 6MASS). The 6 molar alkali extraction was performed using KOH instead of NaOH, because at this concentration KOH was found to improve extraction of xyloglucan, more than NaOH (Nishitani & Masuda, 1983). The final unextracted material is called the residue (RES). After each extraction, solubilised polymers were separated from the insoluble residue by filtration on G2-glass filters. ChSS were dialyzed against 0.1 M NH_4OAc buffer at pH 5.2 prior to dialysis against demineralized water at 4 °C. DASS, 4MASS, 6MASS and RES were neutralised with 1 M HCl, dialysed against demineralized water at 4 °C and freeze-dried.

2.3. Enzymatic fingerprinting

RSM fractions (in 10 mM NaOAc buffer pH 5.0, 5 mg/mL) were incubated with pure and well-characterised enzymes. The choice for specific enzymes to demonstrate the presence of specific polysaccharides was made based on the carbohydrate composition of the RSM fractions. The enzymes used were polygalacturonase (*Aspergillus aculeatus*; 190 μg protein/mL (Kofod, Mathiasen, Heldt-Hansen, & Dalbøge, 1995), beta-galactosidase (*Aspergillus niger*; 15 μg protein/mL (Laboratory of Food Chemistry, unpublished data)), endo-galactanase (*A. niger*; 26 μg protein/mL, (Vis, Searle-van Leeuwen, Siliha, Kormelink, & Voragen, 1991), endo-arabinanase (*A. aculeatus*; 7.3 mg protein/mL, (Rombouts et al., 1988), exo-arabinanase (*Chrysosporium lucknowense* C1 (Kühnel et al., 2010)), endo-mannanase (*A. niger*; 542 μg protein/mL (Düsterhöft, Bonte, & Voragen, 1993)), a xyloglucan specific endo-glucanase (*A. aculeatus* (Pauly et al., 1999)) and endo-xylanase I (*Aspergillus awamori*; 22 μg protein/mL (Kormelink, Searle-Van Leeuwen, Wood, & Voragen, 1993)). Besides these pure enzymes, a commercial cellulase preparation (CellicCTec, Novozymes, Bagsvaerd, Denmark) was used. Enzymes were dosed at 0.5 μg enzyme-protein per 5 mg substrate.

The incubations were performed in 10 mM NaOAc buffer (pH 5.0) at 40 °C rotating 'head-over-tail' for 24 h. All enzymes were inactivated by heating at 100 °C for 10 min. Digests were analysed by HPSEC, HPAEC and MALDI-TOF MS, as described in Section 2.4.

2.4. Analytical methods

Dry matter content was determined in duplicate by drying overnight in an oven at 103 °C.

Protein content was determined in duplicate by the Dumas method (AOAC, 1995) on a Thermo Quest NA 2100 Nitrogen and Protein Analyser (Interscience, Troy, NY, USA). Sample (~10 mg) was weighed into a cup and directly analysed. D-methionine was used for calibration (Nx5.3 ((Mosse, 1990)).

Lignin content was determined gravimetrically according to NREL in duplicate. After pre-hydrolysis with 72% (w/w) H_2SO_4 for 1 h at 30 °C, samples were hydrolysed with 1 M H_2SO_4 at 100 °C for 3 h. Samples were filtered over G4 glass filters. The filtrate was measured for acid soluble lignin (ASL) spectrophotometrically at 205 nm. ASL was calculated according to the formula: ASL = (A * B * C)/(D * E), with A = absorption relative to 1 M H_2SO_4, B = dilution factor, C = filtrate volume, D = extinction coefficient of lignin (110 g L^{-1} cm^{-1}), and E = weight of substrate (g). The washed residue was dried (105 °C, 18 h), and weighed as acid insoluble lignin. Total lignin was calculated as the sum of acid soluble and acid insoluble lignin (Sluiter et al., 2011).

Neutral sugar composition was determined in duplicate according to Englyst and Cummings (1984). After a pre-hydrolysis with 72% (w/w) H_2SO_4 for 1 h at 30 °C, the samples were hydrolysed with 1 M H_2SO_4 at 100 °C for 3 h. The monosaccharides were derivatised to their alditol acetates and analysed by gas chromatography (Focus-GC, Thermo Scientific, Waltham, MA, USA). Inositol was used as internal standard.

Uronic acid content was determined in duplicate according to the automated colorimetric m-hydroxydiphenyl assay (Thibault, 1979), using an auto-analyser (Skalar Analytical B.V., Breda, The Netherlands). Galacturonic acid was used for calibration.

Acetyl- and methyl-ester content was determined in duplicate by High Performance Liquid Chromatography (HPLC) after treatment of the sample (20 mg/mL) with 0.4 M NaOH in isopropanol/water (1:1 (v/v)) on an Ultimate 3000 System (Dionex, Sunnyvale, CA, USA) equipped with an Aminex HPX-87H ion exclusion column (7.8 mm × 300 mm; BioRad Laboratories, Hercules, CA, USA). The samples were eluted with 5 mM sulphuric acid at 40 °C and at a flow rate of 0.6 mL/min. Elution was followed by refractive index detection (Shodex RI 101; Showa Denko K.K., Kawasaki, Japan). Quantification was performed using HPLC-grade acetic acid and methanol standards. Also, samples were analysed without alkali addition in order to correct for presence of free acetic acid and methanol.

High Performance Size Exclusion Chromatography (*HPSEC*) was performed on an Ultimate 3000 System (Dionex) equipped with a set of four TSK-Gel superAW columns (Tosoh Bioscience, Tokyo, Japan) in series: guard column (6 mm ID × 40 mm) and separation columns 4000, 3000 and 2500 (6 mm ID × 150 mm). Samples (25 μL) were eluted with filtered aqueous 0.2 M sodium nitrate for 25 min at 40 °C and at a flow rate of 0.6 mL/min followed by refractive index detection (Shodex RI 101; Showa Denko K.K.). Calibration was performed using pullulan standards of 180, 738 Da and 6, 12, 23, 47, and 112 kDa (Sigma, St. Louis, MO, USA).

High Performance Anion Exchange Chromatography (*HPAEC*) was performed on an ICS-5000 System (Dionex) equipped with a CarboPac PA 1 column (2 mm × 250 mm), and Pulsed Amperiometric Detection. Elution was performed with a flow rate of 0.3 mL/min and a temperature of 40 °C. The elution profile used for quantification of di- and oligosaccharides was: 0–5 min isocratic 0.1 M NaOH, 5–15 min linear 0–0.1 M NaOAc in 0.1 M NaOH, 15–25 min linear 0.1–0.3 M NaOAc in 0.1 M NaOH, isocratic for 5 min at 1 M NaOAc in 0.1 M NaOH, followed by 20 min isocratic at 0.1 M NaOH. WSS was ten times diluted before analysis The gradient used for analysis of enzyme digests was: 0–45 min linear from 0 to 0.7 M NaOAc in 0.1 M NaOH, isocratic for 5 min at 1 M NaOAc in 0.1 M NaOH, followed by 15 min isocratic at 0.1 M NaOH. Enzyme digests were ten times diluted before analysis.

Matrix Assisted Laser-induced Desorption/Ionisation Time-of-Flight Mass Spectrometry (*MALDI-TOF-MS*) was performed using an UltraFlextreme workstation (Bruker Daltronics, Bremen, Germany) equipped with a Smartbeam II laser of 355 nm and operated in the

Table 1
Composition of rapeseed meal (RSM) and its fractions (%w/w).

	RSM	WSS	WUS	ChSS	DASS	4 MASS	6 MASS	RES
Yield[a]	100 (*100*)	26 (*27*)	64 (*59*)	6 (*3*)	4 (*2*)	9 (*5*)	10 (*18*)	31 (*39*)
Total sugar[b]	36	37	33	16	14	17	60	41
Protein (Nx5.3)[b]	30	14	34	41	44	33	18	23
Lignin (acid soluble)[b]	24 (10)	n.a[c]	22 (9)	n.a.	n.a.	n.a.	n.a.	23 (6)
Acetyl esters[b]	0.26	0.26	0.55	0.11	0	n.a.	n.a.	n.a.
Methyl esters[b]	0.15	0.49	18	0.50	0.35	n.a.	n.a.	n.a.

RSM: rapeseed meal; WSS: water soluble solids; WUS: water unextractable solids; ChSS: Chelating Agent Soluble Solids; DASS: Dilute Alkali Soluble Solids; 4MASS: 4 molar alkali soluble solids; 6MASS: 6 molar alkali soluble solids; RES: residue.
The numbers is brackets are expressed as the carbohydrate yield in g carbohydrate in the fraction/100 g total carbohydrate.
[a] Expressed as g/100 g RSM, as g carbohydrate/100 g total carbohydrate between brackets.
[b] Expressed as %w/w.
[c] Not analysed.

positive mode. After a delayed extraction time of 70 ns, the ions were accelerated to a kinetic energy of 25 kV and detected using a FlashDetector. The data were collected from averaging 200 laser shots, with the lowest laser energy needed to obtain sufficient spectra. External calibration was performed using maltodextrin (Paselli MD-6, AVEBE, Veendam, The Netherlands). Samples were desalted prior to analysis using AG 50W-X8 Resin (BioRad Laboratories, Hercules, CA, USA). 1 μL desalted sample was mixed with 1 μL matrix solution of 10 mg/mL 2,5-dihydroxy-benzoic acid (Bruker Daltronics) in 50% (v/v) acetonitrile and dried under a stream of air.

Glycosidic linkage analysis was performed as described elsewhere in Oosterveld, Beldman, Schols, & Voragen (1996). Polysaccharides were methylated, followed by hydrolysis with 2 M TFA for 1 h at 121 °C and permethylated monomers were converted into their alditol acetates. Treatment with methyliodide was performed twice in order to obtain sufficient permethylation. The partially methylated alditol acetates were identified by GC–MS using a Trace GC coupled to a DSQ-II (both Thermo Scientific) equipped with a Restek RTX-35MS column (Restek Corporation, Bellefonte, PA, USA). A temperature gradient was applied from 120 °C to 250 °C in 52 min, proceeded by a hold time of 5 min at 250 °C. MS detection of masses 50–450 *m/z* was performed.

3. Results and discussion

3.1. General composition of RSM

Rapeseed meal (RSM) was analysed for its carbohydrate, protein and lignin contents (Table 1). Protein (Nx5.3; 30% w/w) and lignin contents (24% w/w) of our *B. napus* meal, were similar to *B. campestris* meal, another rapeseed cultivar published previously (Bell, 1993; Slominski & Campbell, 1990). The carbohydrate content (36% w/w) was higher compared to the 28% (w/w) found for *B. campestris* meal (Ghosh et al., 2004).

The carbohydrate composition of RSM is presented in Table 2. RSM was high in glucosyl (40 mol%), arabinosyl (19 mol%) and uronyl residues (15 mol%). Compared to *B. campestris* meal (Ghosh et al., 2004), in *B. napus* meal only about half of the arabinosyl and galactosyl residues were present, but twice as much glucosyl residues were found. The content of uronyl residues was the same.

3.2. Water soluble fraction of RSM

When extracted with hot water (70 °C), 26% of the dry matter and 27% of the total carbohydrate content of RSM was solubilised (Table 1). The water soluble fraction (WSS) mainly contained glucosyl (64 mol%) and some galactosyl residues (17 mol%). HPAEC analysis showed that this WSS fraction mainly contained small saccharides, like fructose, sucrose, raffinose and stachyose, which contributed to the total carbohydrate content of the WSS for 4, 50, 3 and 18%, respectively. These values are in agreement with previously published data for *B. campestris* (Knudsen & Li, 1991; Siddiqui & Wood, 1977a).

For *B. campestris* meal, besides small saccharides also a water-soluble arabinan (Eriksson, Andersson, Westerlund, & Åman, 1996) and an acidic arabinogalactan (Siddiqui & Wood, 1972) have been reported. In our *B. napus* meal 6% (w/w) of total carbohydrate in WSS was arabinosyl and 5% (w/w) uronyl residues, but their polymeric structure was not further investigated.

3.3. Sequential extraction of WUS

The water unextractable solids (WUS) fraction was composed of 33% carbohydrates, mainly glucosyl (32 mol%), arabinosyl (25 mol%), uronyl (18 mol%) and xylosyl residues (12 mol%) (Table 2). Of the uronic acids residues 35% was substituted with methanol groups and 36% with acetic acid groups. In order to investigate the polysaccharide structures in detail, the water

Table 2
Molar sugar composition and sugar yields of rapeseed meal (RSM) and its fractions (RSM: rapeseed meal; WSS: water soluble solids; WUS: water unextractable solids; ChSS: Chelating Agent Soluble Solids; DASS: Dilute Alkali Soluble Solids; 4 MASS: 4 molar alkali soluble solids; 6 MASS: 6 molar alkali soluble solids; RES: residue).

	Molar composition[a]							Yield[b]						
	Rha	Ara	Xyl	Man	Gal	Glc	UA	Rha	Ara	Xyl	Man	Gal	Glc	UA
RSM	2	19	8	6	10	40	15	100	100	100	100	100	100	100
WSS	tr[c]	7	1	7	17	64	5	0	9	4	31	43	42	8
WUS	1	25	12	4	8	32	18							
ChSS	2	15	4	2	4	3	71	3	2	1	1	1	0	12
DASS	tr	44	6	3	10	9	29	0	4	1	1	2	0	3
4 MASS	tr	17	20	15	13	30	5	0	4	11	12	6	3	2
6 MASS	1	29	22	2	13	23	11	13	26	48	6	22	10	12
RES	4	20	6	4	6	40	20	81	36	28	27	24	36	46
Recovery								97	81	93	78	98	91	83

[a] Expressed as anhydro-units: Ara: arabinose; Xyl: xylose; Man: mannose; Gal: galactose; Glc: glucose; UA: uronic acids.
[b] Expressed as g/100 g monosaccharide unit.
[c] Trace.

unextractable solids (WUS) were sequentially extracted with chelating agent (ChSS) to release calcium-bound pectins, dilute alkali (DASS) to release pectins tightly bound to hemicellulose, and 4 and 6 molar alkali (4 MASS and 6 MASS) to release hemicelluloses. Cellulose will remain in the residue (RES).

The yields and general composition of the fractions are shown in Table 1, while the detailed carbohydrate composition of the fractions is presented in Table 2. Fructose and fructose-containing oligosaccharides influence the carbohydrate composition and yields, since they are not correctly analysed using the alditol acetate method. ChSS and DASS yields were relatively low, which was unexpected. Proteins were gradually extracted, which resulted in fractions having a quite high protein content (14–44% w/w). It has to be noted that the protein content of the ChSS fraction is also influenced by the presence of residual CDTA in this fraction. Based on dry matter, the residue after extraction of RSM was the largest fraction, which is mainly due to the recovery of lignin in the residue (23% w/w). This is 30% of the lignin present in the RSM, meaning the rest of the lignin is co-extracted in the alkali fractions.

The mass balance of the extraction (Tables 1 and 2), showed a recovery of 86% of the dry matter of RSM. Based on the total carbohydrate content, 94% could be recovered, of which recovery of each individual constituent monosaccharides was in the range of 78–98% (Table 2). From *B. campestris* meal less material could be recovered during the extraction procedures reported (Ghosh et al., 2004; Siddiqui & Wood, 1977a), which expectedly resulted in less representative fractions.

Molecular weight distribution of the polysaccharides in extracted RSM fractions was analysed by HPSEC (Supplemental Figure 1). Different populations of polysaccharides were distinguished. The DASS fraction contained polysaccharides with a molecular mass of around 14 kDa, as based on pullulan standards, and some smaller saccharides of 1.6 and 4 kDa. The 4 MASS fraction contained polysaccharides of around 25 and 350 kDa and smaller saccharides of 2.3 kDa. The 6 MASS fraction contained even larger polysaccharides of around 440 kDa and also (poly)saccharides of 2.3 and 25 kDa as also found in the 4 MASS fraction. ChSS is not shown, since CDTA is difficult to remove from pectins (Mort, Moerschbacher, Pierce, & Maness, 1991), thereby interfering with the HPSEC-analysis.

3.4. Sugar composition of RSM fractions

The ChSS fraction, representing 3% of total carbohydrate of RSM, was high in uronyl residues (71 mol%), most likely present as homogalacturonan (Table 2). Of these uronic acid residues 60% was substituted with methanol esters and 9% with acetic acid esters. Next to uronyl residues, arabinosyl residues were abundant (14 mol%), which is expected to be present as arabinan side chains, as often reported for pectin structures (Schols & Voragen, 2002).

The DASS fraction, representing 2% of total carbohydrate content of RSM, was high in arabinosyl (44 mol%) residues, probably originating from arabinan side chains of pectin present (Table 2). The latter was indicated by the presence of 29 mol% uronyl residues.

The 4 MASS fraction, representing 4% of total carbohydrate content of RSM, contained quite some xylosyl (20 mol%) and glucosyl (30 mol%) residues, which is expected to be present as xyloglucan (Vierhuis, Schols, Beldman, & Voragen, 2001). Also arabinosyl (17 mol%), mannosyl (15 mol%) and galactosyl (13 mol%) residues were present. This could indicate the presence of arabinan, (galacto-)mannan and (arabino-)galactan.

The 6 MASS fraction, representing 17% of total carbohydrate content of RSM, consisted of arabinosyl (29 mol%), xylosyl (22 mol%) and glucosyl residues (23 mol%), which could indicate the presence of arabinan, arabinoxylan and/or xyloglucan. Also, galactosyl (12 mol%) and uronyl residues (11 mol%) were present.

Table 3
Sugar linkage composition of rapeseed meal (RSM) fractions; expressed as anhydro-units: Ara: arabinose; Xyl: xylose; Man: mannose; Gal: galactose; Glc: glucose; UA: uronic acids (mol%).

	WUS	DASS	4 MASS	6 MASS
t-Ara[a]	18	41	16	15
1,2-Ara		1	1	
1,5-Ara	10	9	2	11
1,2,5-Ara	6	29	10	4
Total Ara	34	80	29	30
t-Xyl	9	3	7	12
1,2-Xyl	2	1	10	3
1,4-Xyl	3	1	3	7
Total Xyl	14	5	20	22
1,4,6-Man			3	1
Total Man			3	1
t-Fuc	2	1	2	5
1,2,4-Fuc	7	3		8
Total Fuc	9	4	2	13
t-Gal	3		4	6
1,2-Gal			5	7
1,3-Gal			7	
1,3,6-Gal		7	4	
Total Gal	3	7	20	13
1,4-Glc	25	5	17	6
1,4,6-Glc	16		11	15
Total Glc	41	5	28	21
T/B[b]	1.10	1.15	1.04	1.36

[a] t: terminal.
[b] T/B: ratio terminally linked residues: branching points.

The residue, representing 39% of total carbohydrate content of RSM, contained mainly (40 mol%) glucosyl residues and also quite some arabinosyl and uronyl residues (each 20 mol%). The latter indicates that are still (pectic) polysaccharides tightly associated with cellulose microfibrils (Pauly et al., 1999). This lead to the assumption that the cell wall polysaccharide matrix of rapeseed meal is very strongly associated. A similar conclusion has been proposed for *B. campestris* meal (Ghosh et al., 2004).

More detailed analysis is necessary to analyse the polymeric structure of the extracted cell wall polysaccharides from RSM. This is studied using linkage type analysis, which will give information on the type of carbohydrate linkages present between the constituent monosaccharides (Section 3.5) and using pure enzymes, which will be helpful in elucidating the presence of specific polysaccharides (Section 3.6).

3.5. Glycosidic linkage type of RSM fractions

Data obtained from linkage type analysis of the RSM fractions (Table 3) can only be used in a qualitative way instead of quantitative due to the poor DMSO solubility and the high amount of uronic acids, which are not detected in this method, present of some samples (Hilz, Bakx, Schols, & Voragen, 2005).

Arabinosyl residues in the DASS and 4MASS fraction were mainly present as terminal residues (51% of the arabinosyl residues present) or linked via 1,2,5-bonds (36% of the arabinosyl residues) (Table 3). This indicates the presence of an arabinan with branches at the O-2 position. This is different from *B. campestris* meal, for which a highly branched arabinan was reported with branching at both the O-2 and O-3 position. The arabinosyl residues in the 6MASS fraction were mainly present as terminal units and linear 1,5-linked-arabinosyl residues. The arabinan present in this fraction is much more linear than the arabinan present in the DASS

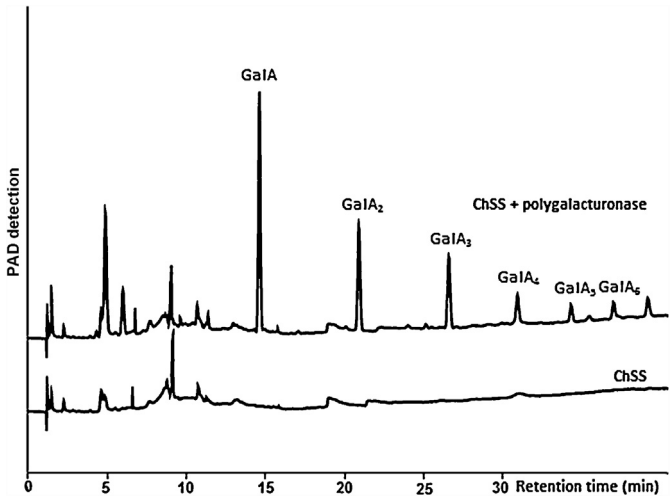

Fig. 1. HPAEC chromatogram of RSM ChSS before and after incubation with polygalacturonase. GalA: galacturonic acid; $GalA_n$: linear galacturonic acid-oligosaccharides of DP_n (n = 2–6).

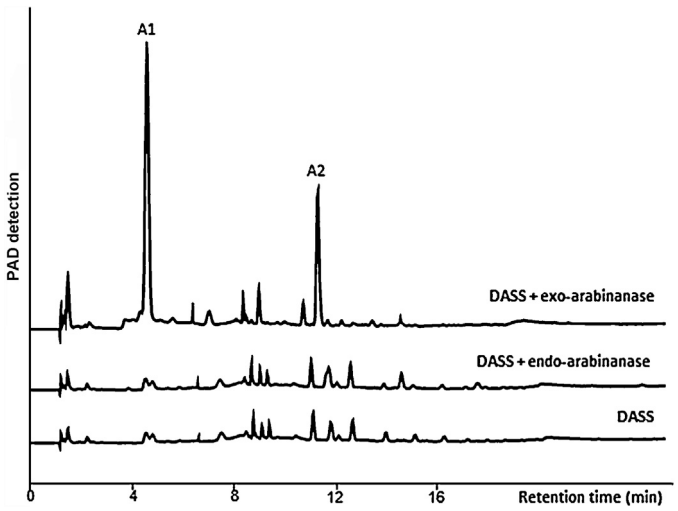

Fig. 2. HPAEC chromatogram of RSM DASS before and after incubation with endo- and exo-arabinanase (A1 = arabinose, A2 = arabinobiose).

and 4MASS fraction. The linearity of the arabinan could be responsible for the fact that these arabinan structures were more difficult to extract (Peña & Carpita, 2004).

Besides arabinosyl residues, the 4MASS fraction contains galactosyl residues, which were mainly 1,3-linked. Most likely, our *B. napus* meal contained arabinogalactan type II, since also some terminal and 1,3,6-linked galactosyl residues were found. Type II arabinogalactan was also found in *B. campestris* meal (Siddiqui & Wood, 1972).

Glucosyl residues in the 4MASS fraction were mainly 1,4- and 1,4,6-linked, and xylosyl was present as terminal residues and 1,2-linked, as was also described for the xyloglucan found in *B. campestris* meal (Siddiqui & Wood, 1971). The xyloglucan in *B. napus* meal is also fucosylated, similar to *B. campestris* meal (Siddiqui & Wood, 1977b). Around 15% of the xylosyl residues were 1,4-linked, which means that besides xyloglucan, a xylan is present.

3.6. Enzymatic fingerprinting of RSM fractions

The ChSS fraction could not be analysed by linkage type analysis, due to the high content of uronyl residues, which are not detected is this in this method. However, enzymatic fingerprinting was successful in characterising the polysaccharides present. After incubation of the ChSS fraction with polygalacturonase, a range of galacturonic acid-oligosaccharides of DP 1–8 were formed (Fig. 1), originating from homogalacturonan structural elements. In addition, after incubation of ChSS fraction with endo-arabinanase, arabinose oligomers of DP 2–4 were produced (data not shown), indicating the presence of an arabinan. This is in line with results from linkage type analysis (Section 3.5).

Incubation of the DASS fraction with an endo-arabinanase did not show any degradation products, but when an exo-arabinanase was added arabinose and arabinobiose were formed (Fig. 2). This confirms the result from linkage type analysis that DASS contained a branched arabinan. Next, a combination of beta-galactosidase and endo-galactanase showed formation of galactose and galactobiose. This could indicate that there is arabinogalactan type I, having β-1,4-linkages, or galactomannan present as also described for *B. campestris* meal previously (Siddiqui & Wood, 1972). However, in the linkage type analysis, only 1,3,6-linked galactosyl residues were analysed in the DASS fraction, no 1,4 linkage was found.

Simultaneous incubation of the 4MASS fraction with β-galactosidase and endo-galactanase resulted in hexose-oligomers up to DP 6, analysed with MALDI-TOF-MS. After mannanase incubation, hexose-oligosaccharides up to DP 7 were formed. Due to the known specificity of the enzymes used, it can be said that these hexose oligomers formed were built from galactosyl and mannosyl residues. This indicates the presence of galactomannan. When the 4 MASS fraction was incubated with a xyloglucan-specific glucanase, typical xyloglucan oligomers were formed (Fig. 3A). It is known that only part of the polysaccharide fraction can be degraded by this enzyme (Pauly et al., 1999). From the part that is degraded, XSGG, LSGG (both XXGG-type), XXFG and XLFG (both XXXG-type) were formed (based on nomenclature described by Fry et al. (1993)). This indicated the presence of galactosyl and fucosyl decorations on the xylosyl residues. The XXFG and XLFG moieties have also been reported for the 4MASS fraction of *B. campestris* meal (Ghosh et al., 2004). Siddiqui and Wood (1977b) reported the presence of 1,4,6-linked glucosyl and 1,4-linked glucosyl in a molar ratio of 3:1. In our view, this can be attributed to the presence of XXXG-type xyloglucan. From a phylogenetic point of view the XXXG-type structures are expected, while the XXGG-type are not. The fact that in *B. napus* meal both the XXGG- and XXXG-type xyloglucan are found is not common, although it has been described before in the Poaceae family for rice (Vierhuis et al., 2001b).

When the 4 MASS fraction was incubated with an endo-xylanase, pentose oligomers up to DP 5 with an O-methylated uronic acid attached were formed. Such glucuronoxylan oligomeric structures have been reported before for *B. campestris* meal (Ghosh et al., 2004).

Incubation of the 6MASS fraction with endo-xylanase revealed the formation of pentose-oligomers up to DP 6 with O-methylated uronic acid attached (data not shown), comparable to the 4MASS fraction. Besides xylosyl and uronyl residues, also arabinosyl, galactosyl and glucosyl residues were found (Table 2). Therefore this fraction was also incubated with xyloglucan-specific glucanase, endo-arabinanase, and the combination of endo-galactanase and beta-galactosidase. However, all incubations did not show release of oligosaccharides. This indicates that the structure of carbohydrates extracted in the 6MASS fraction was too complex to be degraded by the enzymes used.

Degradation of the RES fraction with a commercial cellulase preparation confirmed the presence of hexose oligomers (from cellulose) next to xyloglucan-oligomers (Fig. 3B). The xyloglucan-oligosaccharides confirmed that the xyloglucan in the residue consisted of building blocks of the XXGG-type and XXXG-type decorated with galactosyl, arabinosyl and fucosyl residues. When

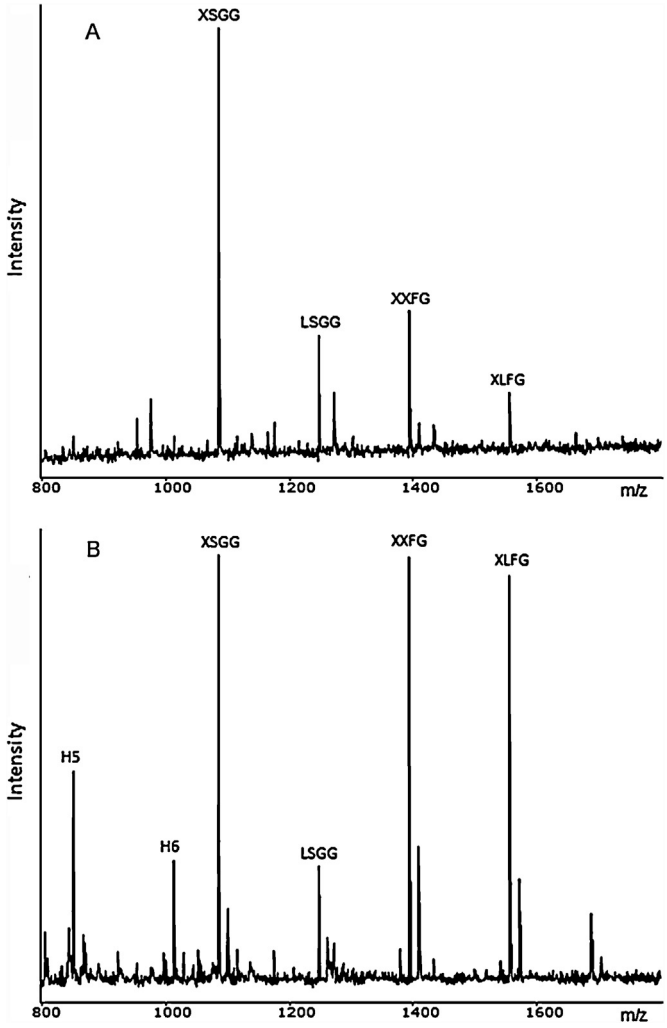

Fig. 3. MALDI-TOF-MS spectrum of (A) RSM 4MASS incubated with a xyloglucan-specific glucanase and (B) RSM RES incubated with a commercial cellulase preparation. H = hexose; nomenclature of xyloglucan-oligomers according to Fry et al. (1993).

Table 4
Summary of polysaccharides present in *B. napus* meal (this paper) and *B. campestris* meal (Siddiqui & Wood, 1977a).

	B. napus meal	*B. campestris* meal
1,5-linked arabinan branched at O-2	+	+
1,5-linked arabinan branched at O-3		+
Galactomannan	+	
Homogalacturonan	+	+
Rhamnogalacturonan I	+	
Type I arabinogalactan		
Type II arabinogalactan	+	+
Glucuronoxylan	+	+
XXGG-type xyloglucan	+	
XXXG-type xyloglucan	+	+

the residue was incubated with a xyloglucan-specific glucanase, no degradation was observed (data not shown), as was expected because xyloglucan and cellulose can be tightly bound by hydrogen bonding (Pauly et al., 1999).

Next to cellulose and xyloglucan, the residue also contained quite some rhamnosyl, arabinosyl and uronyl residues, probably originating from pectic polysaccharides. A cellulose-arabinan complex (Zykwinska, Thibault, & Ralet, 2008) and a cellulose-rhamnogalacturonan (Oechslin, Lutz, & Amadò, 2003) have both been described before.

4. Conclusions

The comparison of polysaccharides present in *B. napus* and *B. campestris* meal is presented in Table 4. Although both meals belong to the same phylogenetic family of Brassicaceae and similar polysaccharides were found, also distinct differences in cell wall polysaccharide structures are present. *B. napus* contained arabinan with only O-2 branches (instead of branching at O-2 and O-3) and XXGG-type xyloglucan (besides XXXG-type xyloglucan), which were not found in *B. campestris*. Homogalacturonan, arabinogalactan and glucuronoxylan are similar in structure.

In the residue after sequential extraction, besides cellulose, still pectic polysaccharides were found. This implies that RSM has a rigid matrix and therefore different pre-treatments have to be explored to improve accessibility of the cell wall polysaccharides and to increase digestibility for monogastric animals.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.07.059.

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