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Polysaccharides from *Sesamum indicum* meal: Isolation and structural features

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Abstract

Defatted *Sesamum indicum* seed cake was extracted, following two separate sequences, and the effects of extraction medium on yield and composition of the extracts were compared. Polysaccharides extracted sequentially with dilute acid and alkali represented 250 mg/g of defatted meal. The isolated polymers contained arabinan, rhamnogalacturonan I (RG I) and arabinogalactan proteins. Polysaccharides extracted during chlorite treatment and with dilute alkali had a higher proportion of rhamnose, suggesting a more branched variety of polymer. Three extracts, which were further characterized by size exclusion chromatography, gave two overlapping peaks. Structural characterisation of hemicellulosic polysaccharides, isolated with KOH, using specific enzyme hydrolysis, ion exchange chromatography (HPAEC) and matrix assisted laser desorption ionisation-time-of-flight (MALDI-TOF) mass spectroscopy, showed that sesame meal xyloglucan (XG) contained XXXG, XXFG and XXLG, and XLLG (named according to Fry et al., 1993) as the major building sub-units in the ratio of 1:0.9:0.3. Hydrolysis with endo-β-(1 → 4)-D-xylanase and analysis of the xylan derived oligosaccharides showed the presence of monomeric xylose (40%), xylobiose (46%) and acidic xylan oligosaccharides containing 4-*O*-methyl-D-glucuronic acid residues (14%).

Keywords: *Sesamum indicum*; Seed cake; Arabinan; RGI; Arabinogalactan protein; Xyloglucan; Xylan

1. Introduction

Sesamum indicum L. syn *S. orientale* L. is an erect, branched or unbranched annual and one of the most ancient of cultivated crops in India. The average yield of mustard seed is about 157 kg/ha (Wealth of India, 1972). The agronomic value of the plant lies in the seed. Most of the sesame seed produced in India is utilised for production of oil, with seed cake as by-product. Sesame meal is the residue obtained from seed after extraction of oil. However, due to the limited degradation of these plant cell wall polysaccharides in the digestive tract of monogastric animals, sesame meal is poorly utilised in animal feed. This is likely caused by the complex structure of the walls, which makes them not easily accessible to enzymic degradation. One interesting feature of sesame meal is its richness in polysaccharides and protein, which can be used for its functional/or biological properties in the making of suitable functionalised derivatives (Zaghloul & Prakash, 2002), for the preparation of modified protein (Bandyopadhyay & Ghosh, 2002) or hypoglycaemic activity (Takeuchi, Mooi, Inagaki, & He, 2001). Understanding the composition, structure and location of cell wall polysaccharides is therefore, essential for the development of a better use of this agricultural by-product.

To date, most studies on *Sesamum* have concerned glycosides (Suzuki, Miyase, & Ueno, 1993), quinones

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(Hasan, Furumoto, Begum, & Fukui, 2001), flavonoids (Anila & Vijayalakshmi, 2000), sugars (Wealth of India, 1972) and proteins (Tzen, Cao, Laurent, Ratnayake, & Huang, 1993). Less attention has been paid, however, to the structural features of polysaccharides present in sesame cultivated in India. To the best of our knowledge, only one study dealing only with the sugar composition (Wankhede & Tharanathan, 1976) has been reported, and detailed structural information is missing. Unfortunately, partial structural characterization is not sufficient for interpretation in terms of functional properties and behaviour, and further knowledge is required. This report is part of a larger study aimed at characterizing different polysaccharide families of sesame meal. The structural analysis was carried out using enzymatic degradation of the hemicellulosic fractions with specific enzymes and analysis of the resulting fragments by combination of GC, GC–MS, HPAE–PAD chromatography and MALDI-TOF mass spectrometry. In addition, as sesame meal is an important source of water soluble polysaccharides, we have used mild acidic treatment, a process used for the industrial extraction of pectin from apple pomace and citrus peels (Aravantinos-Zafiris & Oreopoulou, 1992) for the extraction of polysaccharides. The yields and compositions of the dilute alkali- and water-extracted polysaccharides are also presented and discussed.

2. Materials and methods

2.1. General analyses

The analyses were made at least in duplicate and the results presented are their mean values. All evaporations were carried out under reduced pressure temperature below $\leqslant 50$ °C. Klason lignin was determined as previously described (Adams, 1965).

2.2. Material and preliminary treatments

The seed cake was obtained from the local market and was treated sequentially with hexane (48 h) and acetone (24 h) in a Soxhlet apparatus to remove lipids. The defatted sesame meal (DM) was then air dried and ground.

2.3. Isolation of polysaccharides

2.3.1. Extraction with water

The defatted seed meal (DM, 5 g) was extracted sequentially with: (i) water (300 ml, pH 5.5) for 16 h at 4 °C and then for 4 h at 35 °C (CWE, 360 mg), followed by (ii) hot water (300 ml, pH 5.5) at 80 °C for 30 min (HWE, 480 mg).

2.3.2. Dilute HCl extraction

In a separate experiment DM (4 g) was thrice extracted with 0.05 M HCl (200 ml, pH 1.8) solution at 80 °C for 30 min (designated as 'A', yield 520 mg).

2.3.3. Delignification

Lignins were removed from the acid-extracted residue using sodium chlorite in acidic solutions (pH 4.7) at 80 °C for 15 min (twice) and the material recovered was dialyzed and referred to as sodium chlorite soluble material (SC, yield 400 mg).

2.3.4. Alkali extraction

Polymers were extracted from the delignified material using the following extraction conditions (Fig. 1): (i) 0.05 M KOH + 0.4% $NaBH_4$ for 16 h at 4–6 °C, followed by 4 h at 30–35 °C, (OH, 480 mg), (ii) 1 M KOH + 0.4% $NaBH_4$ for 4 h at 30–35 °C, followed by 16 h at 4–6 °C (1OH, 160 mg) and (iii) 4 M KOH + 0.4% $NaBH_4$ for 4 h at 30–35 °C followed by 16 h at 4–6 °C (4OH, 150 mg).

All alkaline extracts were acidified to pH 5 over an ice-bath, dialyzed exhaustively and finally lyophilized. The resulting 4 M KOH-insoluble residue was washed thoroughly with water containing acetic acid, and then with deionized water, and finally dried by solvent exchange to yield the INS residue (1200 mg).

2.3.5. Isolation of arabinogalactan proteins with β-glucosyl Yariv reagent

AGPs were isolated according to Schultz, Johnson, Currie, and Bacic (2000). Briefly, to a solution of A in 1% NaCl (w/w) an equal volume of Yariv reagent, also in 1% NaCl was added. The mixture was kept at 4–6 °C for 16 h and then centrifuged. The pellet was washed with 1% NaCl, followed by pure methanol (3 times each). The pellet was then dried and treated with sodium metabisulphite (10%). The resulting solution was then dialyzed and freeze dried to yield the sesame arabinogalactan proteins (AGPs).

2.4. Preparation of xyloglucan oligosaccharides

The two hemicellulosic fractions 1OH or 4OH (15 mg) were separately dissolved in 6 ml of 50 mM NaOAc (pH 5.0) and the mixtures incubated with 30 units of endoglucanase (Megazyme International, Ireland) for 24 h at 37 °C with constant shaking. The enzyme resistant polymers were then precipitated in 80% ethanol (v/v) and removed by centrifugation. The soluble fractions containing the xyloglucan oligosaccharides (1XGose and 4XGose) were concentrated under reduced pressure and finally lyophilised.

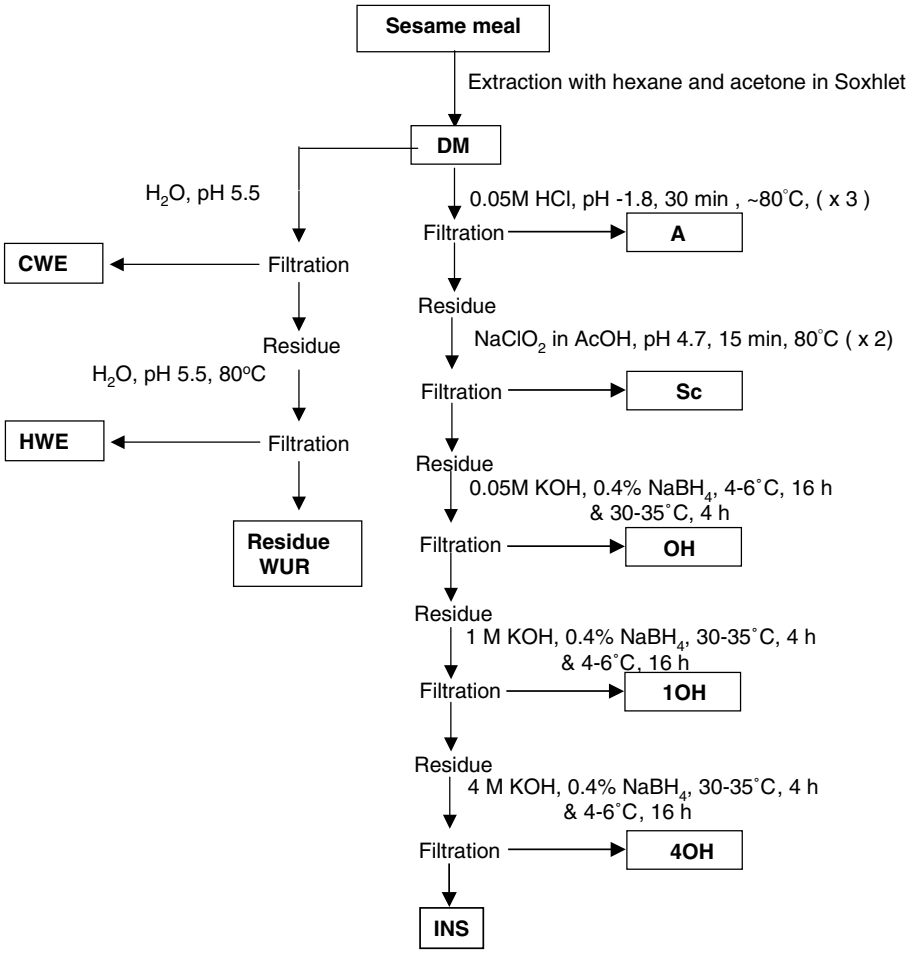

Fig. 1. Scheme for the isolation of polysaccharides from *S. indicum* meal by sequential extraction with inorganic solvents.

2.5. Preparation of xylan oligosaccharides

Hydrolysis of the 10 mg of xylan rich fraction (1OH) was performed in 5 ml of 10 mM NaOAc (pH 5.0) using 40 units of endo-xylanase (Megazyme International, Ireland) at 37 °C for 24 h with constant stirring. To remove enzyme resistant polymeric material, the digest was treated with 4 volumes of cold ethanol, the suspension was stored overnight at 4 °C and then centrifuged. Xylan oligomers (1Xose) were recovered by concentrating the supernatant under a stream of nitrogen at 40 °C and lyophilising the concentrated solution.

2.6. Sugar analysis

Total sugars were determined by the phenol–sulfuric acid assay using galactose as standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Total uronic acids were assayed colorimetrically by the *m*-hydroxydiphenyl assay according to the procedure outlined by Ahmed and Labavitch (1977). Uronic acids in the sulfuric acid hydrolysate were identified by TLC as previously described (Mondal, Ray, Thakur, & Ghosal, 2003). The neutral sugar compositions of fractions were determined after hydrolysis with sulfuric acid (2 M, 100 °C, 2 h), reduction and acetylation (Blakeney, Harris, Henry, & Bruce, 1983).

2.7. Protein and amino acid analysis

Amino acids were released by hydrolysis with 6 M HCl at 110 °C for 22 h in a sealed tube and were analyzed as described (Mazumder et al., 2002).

2.8. IR spectroscopy

All samples were dried at 35–44 °C in a vacuum over P_2O_5 for 72 h prior to analysis. Infrared spectra were recorded on a JASCO FTIR 420 spectrophotometer using a KBr disc.

2.9. HPAE–PAD chromatography

The 80% ethanol soluble oligo- and monosaccharides were analysed on a Dionex DX 500 system equipped with a GP 50 gradient pump, an eluent degas module, a

CarboPac PA-1 column and a pulse amperometric detector (PAD). Samples (10–100 μl) were injected and eluted (1 ml/min) with the following NaOAc gradient in 100 mM NaOH as described (Ray et al., 2004).

2.10. MALDI TOF mass spectrometry

Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS), in reflectron mode was performed using a Micromass (Manchester, UK) Tof spec E MALDI-TOF mass spectrometer. 2,5-Dihydroxybenzoïc acid (10 mg/ml) was used as matrix.

3. Results and discussion

3.1. Composition of sesame meal

The defatted meal (DM) of *S. indicum* contained 19% 2 M sulfuric acid hydrolysable sugars residues of which about one fifth were uronic acids (Table 1). The main neutral sugars were glucose, arabinose, xylose, galactose and mannose, together with smaller quantities of rhamnose and a trace amount of fucose. When hydrolysed by Saeman's method, DM released mainly glucose (data not shown). It also contained 35% of lignin and 21% of protein.

3.2. Extraction of polysaccharides

The scheme for the sequential extraction of polysaccharides from DM is given in Fig. 1. The yields of the extracted polymers are given in Table 1. Dilute acid and alkali extracted 13% (A) and 12% (OH) of the material present in the defatted seed cake (DM). Moreover, 10% of DM was also recovered from the solution produced during sodium chlorite treatment, a process known to remove lignin. Pectin is reported to be most stable at pH 4, and both below and above this value, depolymerisation and deesterification of the pectin chain may occur (Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). During extraction with dilute-acid and alkali, some degradation of the pectin chain may occur, which could be responsible for the higher yields obtained under these conditions. Therefore, extraction of polysaccharides was also carried out using milder condition (water, pH 5.5) as shown in Fig. 1. The yields of water extracted polymer isolated at room temp. (CWE) and high temp (HWE) were 7% and 10%, respectively, of DM dry weight.

3.3. Sugar composition of extracted polymers

The neutral sugar contents of fractions obtained under neutral (CWE and HWE), mildly acidic (A and SC) or alkaline (OH) conditions varied from 18% to 24%, whereas, their uronic acid content were from 2% to 11% (Table 1). Sugar compositional analysis, which revealed arabinose as the major neutral sugar in most of these fractions, suggests the presence of arabinan. The presence of galacturonic acid, the identity of which was confirmed by TLC, together with rhamnose, galactose and arabinose indicates the probable presence of RG I in all of these fractions. Interestingly, polysaccharides extracted during chlorite treatment or with dilute alkali had higher proportion of rhamnose, suggesting a more branched variety of polymer. The presence of glucose, xylose, galactose and fucose residues in HWE fraction indicates the probable presence of xyloglucan (XG) (Table 1).

3.4. Arabinogalactanproteins

Considering that the yield of fraction 'A' is relatively high, it contained galactose and arabinose and its pro-

Table 1
Yields and sugar composition of the defatted seed cake of *S. indicum* and fractions isolated from it by sequential extraction with inorganic solvents (see text for identification of fractions)

	DM	A	AGP	SC	OH	1OH	4OH	INS	CWE	HWE
Yield[a]	100	13	Nd	10	12	4	4	30	7	10
NS[b]	15	24	35	23	20	33	34	7	18	20
UA[b]	4	4	1	11	6	8	6	3	2	3
Rha[c]	1	Tr	1	2	2	–	–	Tr	1	1
Ara[c]	26	66	19	25	31	4	1	2	51	27
Xyl[c]	16	10	10	41	38	82	26	15	10	20
Glc[c]	39	5	15	18	13	11	28	80	13	24
Fuc[c]	–	–	Tr	–	–	Tr	Tr	–	–	1
Man[c]	8	6	8	Tr	5	1	37	2	8	8
Gal[c]	11	12	47	15	12	2	8	1	16	19

–, not detected; Nd, not determined; Tr, trace; NS, neutral sugar; UA, uronic acid.
[a] Percentage by weight of the DM dry weight.
[b] Percentage by weight of fraction dry weight.
[c] Mol%.

Table 2
Amino acid composition of the dilute HCl-extracted polymer (A) isolated from *S. indicum* seed cake

Amino acids	mol%
Aspartic acid/asparagine	8
Threonine	0.4
Serine	8.1
Glutamic acid/glutamine	28.4
Proline	5.7
Glycine	19.5
Alanine	11.2
Cysteine	0
Valine	2.5
Methionine	0
Isoleucine	1.0
Leucine	3.9
Tyrosine	1.6
Phenyl alanine	2.6
Histidine	1.5
Lysine	2.1
Arginine	3.5

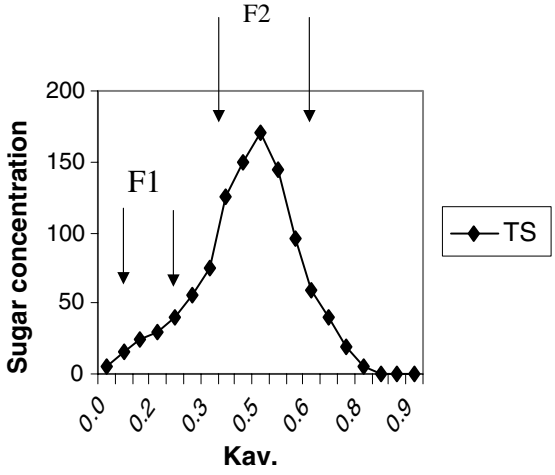

Fig. 2. SEC elution profile of acid soluble polysaccharide fraction (A) obtained from defatted seed cake of *S. indicum*.

tein content is high (24%), we have tested its reactivity with β-glucosyl Yariv reagent. 11% of fraction 'A' dry weight was precipitable with this reagent. Sugar compositional analysis of this material shows that it was enriched in galactose residues and, to a lesser extent, arabinose, confirming the presence of AGP (Table 1). It also contained mannose and xylose residues probably originating from *N*-glycans and polymers containing xylose. The amino acid composition of proteins associated with fraction 'A' showed that glutamic acid/glutamine, glycine, alanine and serine were the major constituents (Table 2).

3.5. Extraction and composition of hemicellulosic polysaccharides

As shown in Table 1, 8% of the DM dry weight were recovered from 1 and 4 M KOH-soluble fractions. Sugar compositional analysis shows that xylose alone accounted for >80% of the total carbohydrate of the 1 M KOH soluble fraction (1OH), indicative of the presence of xylan. The 4 M KOH soluble material (4OH) on the other hand, contained glucose and xylose, together with smaller quantities of galactose and trace amounts of fucose indicating the presence of XG, another important hemicellulosic polysaccharide. The high amount of mannose present in the 4OH fraction probably arises from mannose containing polymers. Finally, after Seaman hydrolysis, INS showed a very high glucose content (80%), demonstrating the abundance of cellulose in this fraction.

3.6. Size exclusion chromatography

To check if some degradation had occurred during extraction with dilute acid and alkali, selected fractions were analysed by gel permeation chromatography, using Sephacryl S-1000. As seen in Fig. 2, the water-extracted polymers gave two overlapping peaks, within the Kav. ranges from 0.1 to 0.8. The first peak (F1), which accounted for 26% of the total sugar eluted, had Kav. from 0.1 to 0.3, whereas, the second peak (F2) eluted between 0.3 and 0.8.

3.7. Structure of xyloglucan

To gain information on the structure of XG, the 4OH fraction was treated with a specific endo-(1 → 4)-β-D-glucanase. Sugar compositional analysis of the enzyme-generated oligomers (4XGose fraction) indicated the presence of glucose and xylose residue as the major sugar together with smaller quantities of galactose and trace amounts of fucose residues (Table 3).

MALDI-TOF mass spectrum (Fig. 3(a)) of 4XGose showed the presence of two XG oligosaccharides having $[M + Na]^+$ at 1085 and 1247 of higher abundance, one having $[M + Na]^+$ at 1410 of medium abundance and three having $[M + Na]^+$ at 953, 1393 and 1555 of smaller abundance. Taking into consideration the specificity and mode of action of the endo-(1 → 4)-β-D-glucanase, sugar

Table 3
Sugar composition (mol%) of the fractions derived from the hemicellulosic fractions of *S. indicum* seed cake by enzyme hydrolysis (see text for identification of fractions)

	1Xose	1XGose	4XGose
Ara	2	15	9
Xyl	91	37	19
Glc	4	39	45
Fuc	–	4	2
Man	Tr	5	22
Gal	3	–	4

–, not detected; Tr, trace.

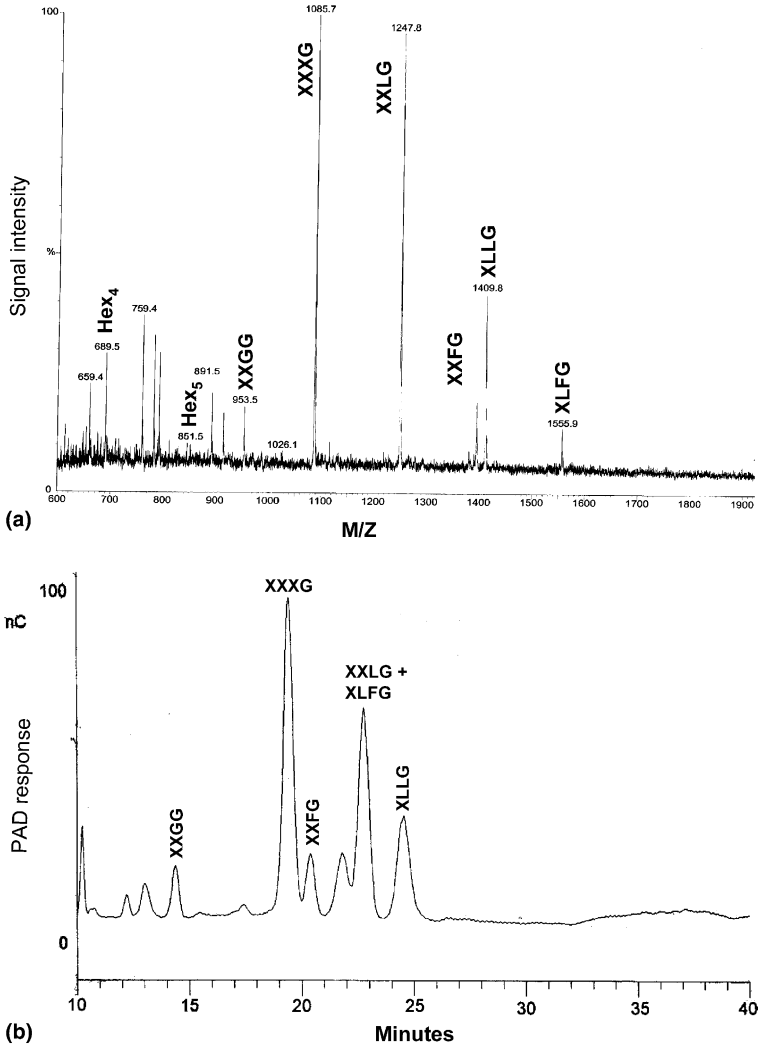

Fig. 3. (a) MALDI-TOF mass spectrum and (b) HPAEC elution pattern of the 4 M KOH soluble fraction (4OH) of *S. indicum* seed cake after degradation by endo-glucanase.

composition and molecular masses of the known xyloglucan oligosaccharides (XGose), tentative structures for the XG-derived oligomers are proposed (Fig. 3(a)). For example, the m/z value of 1087 corresponds to Hex_4Pent_3 and it is, therefore, assigned as XXXG. Similarly, 1247 as XXLG and/or XLXG, 1393 as XXFG or XFXG and 1555 as XLFG or XFLG. The mass spectrum for the 1XGose was similar to that of the 4XGose fraction and therefore, not shown. Besides, a series of ions having a mass difference of 162 Da was also observed in the mass spectrum of the 4XGose with a DP ranging from four to as much as six. The sugar analysis of 4XGose fraction shows the presence of very high amount of mannose residues. Therefore, these pseudomolecular ions, which lack pentosyl units, probably originated from mannan-derived oligomers.

The HPAEC–PAD elution profile (Fig. 3(b)) of the 4XGose fraction contains three major peaks and their retention times are similar to those of XXXG, XXLG + XLFG and XLLG, respectively, generated from *Arabidopsis thaliana* (Lerouxel et al., 2002) and *Argania spinosa* (Ray et al., 2004). Based on the HPAE–PAD chromatographic data, and assuming that PAD response of the XGose are identical, we conclude that XXXG, XXLG + XLFG and XLLG represent the major building units of *S. indicum xyloglucan* in a ratio of 1:0.9:0.3.

3.8. Structure of xylan

Information was sought on the structure of xylan present in the 1OH fraction obtained by treating this fraction with endo-(1 → 4)-β-D-xylanase, an enzyme specific for β-D-xylan. Sugar compositional analysis of the xylan-derived oligomers (1Xose) showed the presence of xylose residues (91 mol%), together with smaller amount of arabinose, glucose and galactose residues and trace amounts of glucuronic acid (GlcA) and 4-*O*-methyl glucuronic acid (4-*O*-MeGlcA) residues.

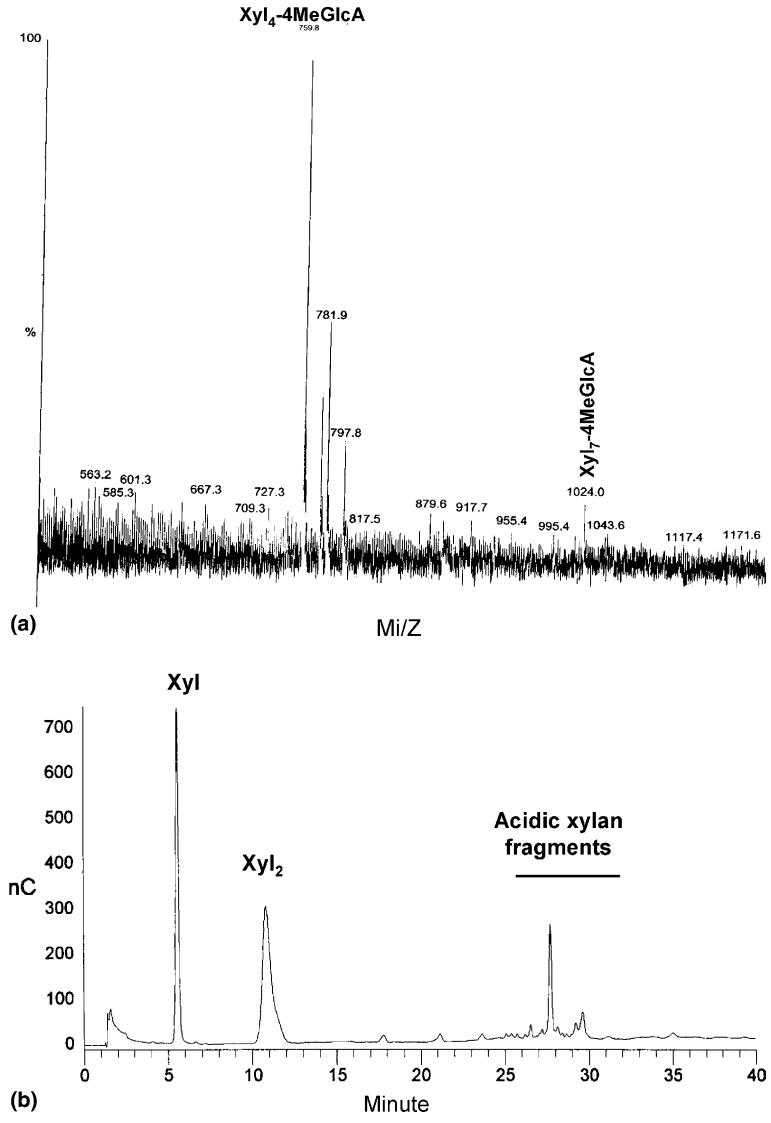

Fig. 4. (a) MALDI-TOF mass spectrum and (b) HPAE-PAD chromatographic elution profile of the 1Xose fraction obtained from 1OH fraction of *S. indicum* seed meal after endoxylanase degradation (see text for the identification of fractions).

MALDI-TOF-mass spectrum of the 1Xose fraction showed one major peak at m/z 760 which corresponds to a 4-*O*-MeGlcA linked to four xylose residues (Fig. 4(a)). Peaks at m/z 775, 782 and 798 correspond to potassiated, disodiated and dipotassiated ions, respectively, of the same oligomer. It also showed another small peak at $[M + Na]^+$ 1024, corresponding to oligomers containing one 4-*O*-MeGlcA and six pentose residues. Peaks at $[M + Na]^+$ 173 and 305, corresponding to xylose and xylobiose, were also detected (data not shown). Although mass spectroscopy can not distinguish stereoisomers, xylose accounted for 91 mol% of the sugar detected in the xylanase generated oligomeric fraction.

As shown in Fig. 4(b), the HPAE–PAD chromatography elution profile of 1Xose indicates the presence of monomeric xylose (40%), xylobiose (46%) and acidic oligosaccharides (14%) mainly composed of pentose and uronic acid.

4. Conclusions

To summarise, the study showed the presence of arabinan, RGI, xylan, XG and cellulose in *S. indicum* meal. We have shown, for the first time, that DM contains AGPs, a class of plant proteoglycans with many pharmacological and food-industry applications.

We also show (1) that dilute-acid (HCl) and alkali (KOH) are efficient as well as a low cost reagents for the isolation of polymers from DM, (2) that XG is of XXXG type, and contains XXXG, XXFG, XXLG and XLLG as major oligomeric building sub-units and (3)

xylan has a classical structure with a backbone of β-(1 → 4)-linked xylosyl residues substituted with 4-*O*-methyl glucuronic acid.

Moreover, the enzyme-derived oligosaccharides are available for biological activity tests. Both the further structural characterisation of the enzyme-generated oligosaccharides and their biological activity testing will be of interest.

Hot water-extracted material from defatted sesame seed has a hypoglycemic effect (Takeuchi et al., 2001). These studies showed that hot water-extracted material contained, inter alia, an acidic arabinogalactan, a class of pectic substance with pharmacological activities (Paulsen, 2001; Yamada, 1994). Since sesame is an important commercial crop, biological activity studies on purified polymers will be of interest from scientific point of view as well as for industrial purposes.

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