Indian Journal of Chemistry
Vol. 46B, March 2007, pp. 471-477

HPAEC and MALDI-TOF-MS analysis of oligosaccharides generated from sesame meal by enzymatic hydrolysis

Kaushik Chattopadhyay, Partha Ghosh, Pradyot Ghosal & Bimalendu Ray*

Natural Products Laboratory, Department of Chemistry, The University of Burdwan, Barddhaman 713 104, India

E-mail: bimalendu_ray@yahoo.co.uk

Received 2 November 2005; accepted (revised) 5 September 2006

Polysaccharides obtained from depectinated sesame meal by extraction with 1*M* KOH (1OH) have been fractionated by anion exchange chromatography yielding a neutral xyloglucan rich population (1OHaq). Size exclusion chromatography of 1OHaq has resulted in one peak between K_{av} 0.3 to 0.6, corresponding to an apparent molecular weight of 85 ± 25 kDa. The structure of xyloglucan has been investigated by enzyme degradation with endo-(1→4)-β-D-cellulase followed by analysis of the resulting fragments by chemical methods as well as high performance anion exchange chromatography (HPAEC) and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS). The result shows that the xyloglucan present in 1OHaq fraction contains hexa(**1**)-, hepta(**2**)-, octa(**3**)-, nona(**4,5**)- and deca(**6**)-saccharides as the building sub-units. Hydrolysis of 4*M* KOH extracted material (4OH) with endo-β-(1→4)-D-xylanase and analysis of the derived oligosaccharides shows that sesame meal xylan is composed of a β-(1→4)-linked-D-xylopyranose backbone substituted with 4-*O*-methyl-D-glucuronic acid residue.

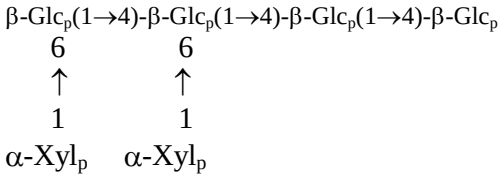
Keywords: Sesame meal, enzyme hydrolysis, oligosaccharides, GC, GC-MS, MALDI-TOF-MS, HPAEC

IPC: Int.Cl.[8] C07C

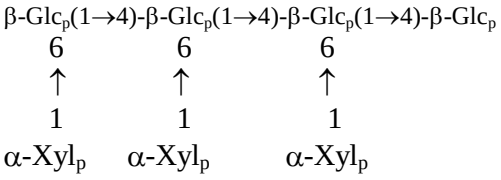
In a preceding paper[1] it was shown that 43% of the defatted sesame meal (DM) could be solubilised during sequential extraction with dil HCl (A), sodium chlorite (Sc), dil KOH (OH), 1*M* KOH (1OH) and 4*M* KOH (4OH). Partial chemical characterization of the polysaccharides present in "A", "Sc", "OH" and "4OH" fractions revealed the presence of arabinan, arabinogalactan protein, rhamnogalacturonan I, xyloglucan and xylan. The present study reports on the structural features of polysaccharides present in 1*M* KOH extracted fraction (1OH). In particular, isolation of a xyloglucan rich pool (1OHaq) by anion exchange chromatography of 1OH fraction and structural elucidation of oligosaccharides generated by endo-glucanase digestion of "1OHaq" fraction is described (**Scheme I**). Structural analysis of the resulting oligosaccharides was carried out by combination of chemical, chromatographic and matrix-assisted laser desorption ionisation-time of light-mass spectrometric techniques. In addition, structural features of xylans present in 4OH fraction will also be reported.

Results and Discussion

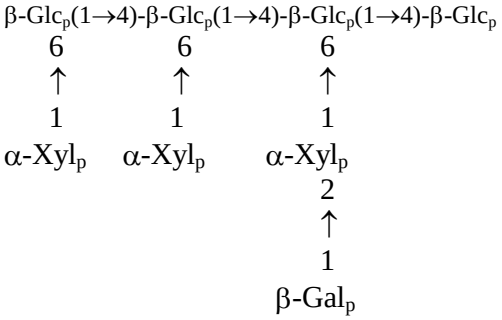
Isolation and size exclusion chromatography of a xyloglucan rich fraction. Hemicellulosic polysaccharides were obtained from *Sesamum indicum* meal by extraction with 1- and 4*M* KOH (named "1OH" and"4OH", respecively) as described[1]. FT-IR spectrum (**Figure 1**) of these fractions showed absorption bands at 1641, 1556, 1418 and 1051 cm^{-1}, which are indicative of hemicelluloses[2]. The broad band between 3600 and 3000 cm^{-1}, corresponding to vibrations of the hydroxylic band as well as the methyl and methylene group vibrations around 2927 cm^{-1} were present in the spectrum of both polymers. The small band at 891cm^{-1} characteristics of β-glycosidic linkages between the sugar units[3] was also observed. Sugar composition of 1OH fraction indicates the probable presence of xyloglucan and xylan type polysaccharides[1]. But composition analysis by simple acid hydrolysis may yield ambiguous information[4]. So, attempts have been made to purify the polysaccharides present in 1*M* KOH extracted fraction (1OH), by passing it through DEAE-Sepharose FF column. On total sugar basis the



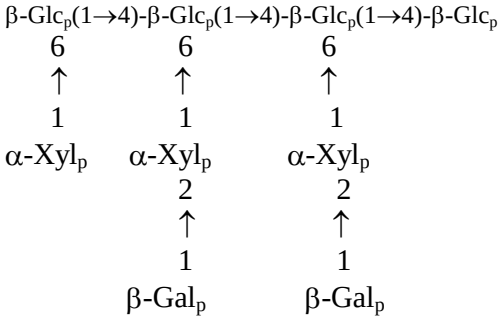
XXGG (1)



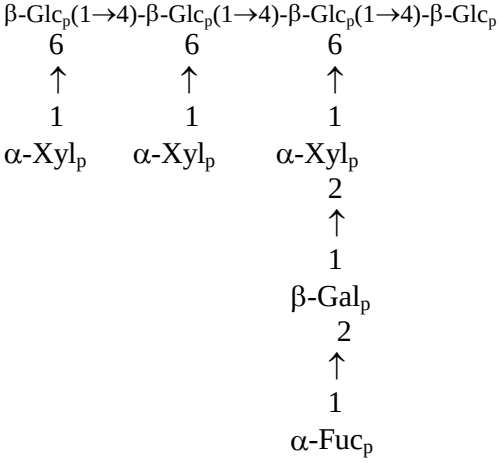
XXXG (2)



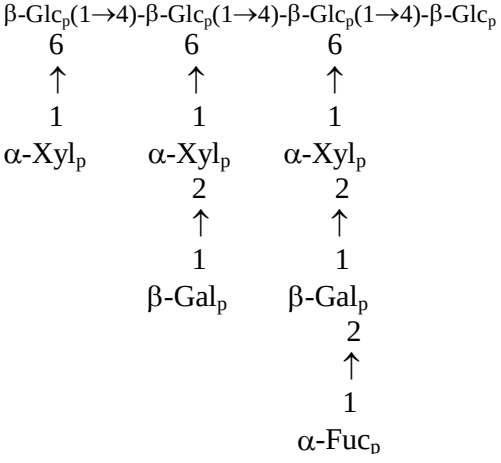
XXLG (3)



XLLG (5)



XXFG (4)



XLFG (6)

Scheme I

recovery yield from the anion exchanger was 89%. Moreover, 41% of the recovered sugar material was eluted with water (designated as 1OHaq) and the bound material (59%) was eluted from the column by using salt gradient. Sugar compositional analysis of the non-retained fraction (1OHaq) shows the presence of glucose and xylose as the major constituent sugars together with smaller amount of other sugars (**Table I**). Therefore, 1OHaq fraction contains xyloglucan type polysaccharide. The polysaccharide present in this fraction was further characterized with respect to their molecular weight as determined by gel permeation chromatography on Sephacryl S-1000. The elution pattern shows a broad peak between

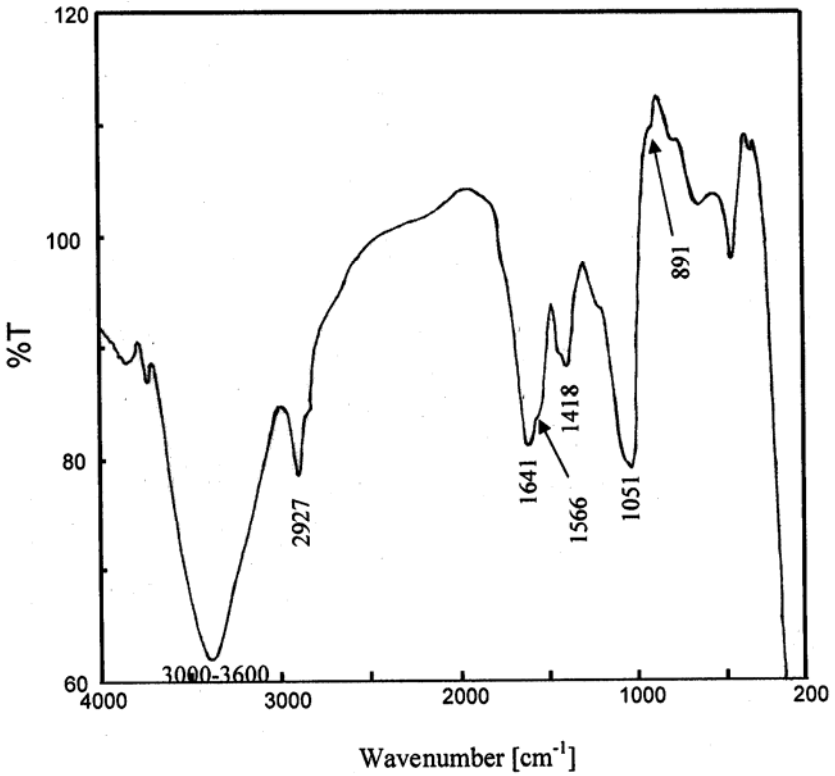


Figure 1 — FT-IR spectra of polysaccharide fraction (1OH) isolated from *Sesamum indicum* meal by 1M KOH

Table I — Sugar composition (mol %) of fractions generated from hemicellulosic polysaccharides of sesame meal by digestion with endo-glucanase and endo-xylanase, and of 1OHaq fraction (see text for identification of fractions

	Ara	Rha	Fuc	Xyl	GalA	Man	Gal	Glc
1OHaq	5	tr	4	32	4	3	12	40
1XGose	2	nd	3	38	tr	1	12	44
1GRM	6	tr	tr	33	5	4	14	38
4Xose	7	tr	tr	79	tr	tr	2	12

tr: trace
nd: not detected

K_{av} 0.3 to 0.6 (**Figure 2**), corresponding to an apparent molecular weight of 85 ± 25 kDa.

The structure of xyloglucan and xylan rich fractions (1Ohaq and 4OH, respectively) were further investigated by hydrolyzing them with specific endoglycosidase and analyzing the resulting fragments by GC, GC-MS, HPAE-PAD chromatography and MALDI-TOF-mass spectrometry.

Structure analysis of the xyloglucan: **Degradation by endo-(1→4)-β-D-glucanase**. It is well known that endo-(1→4)-β-D-glucanase cleaves (1→4)-β-D-glucosidic linkages of xyloglucan next to an unbranched glucose residue[4], without damaging side chains. The xyloglucan rich fraction 1OHaq was incubated with endo-(1→4)-β-D-glucanase and the glucanase resistant material (1GRM) was separated from the digest by precipitation with aqueous 80% ethanol. Endo-glucanase generated xyloglucan oligomers (1XGose) were recovered from the supernatant.

Sugar composition of endo-(1→4)-β-D-glucanase generated oligosaccharides (1XGose). As shown in **Table I**, glucose and xylose residues are the major monosaccharides of the xyloglucan-derived oligomeric fraction (1XGose). Galactose and fucose are the other neutral sugars found in this fraction. The sugar composition is thus consistent with the presence of galactoxyloglucan. Sugar composition of the

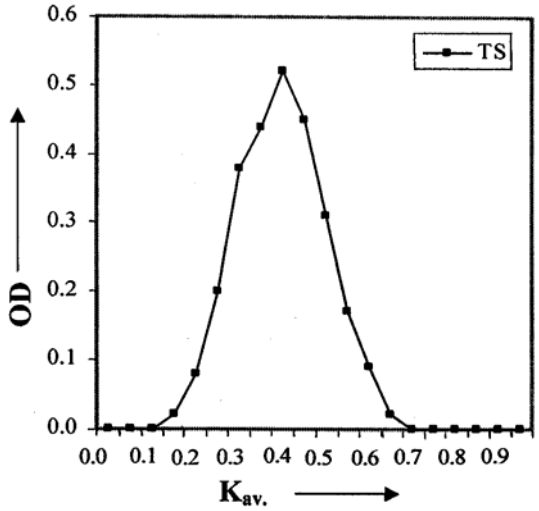


Figure 2 — SEC elution profile of a xyloglucan rich pool (1OHaq) isolated from *Sesamum indicum* meal. TS = Total sugar

glucanase-resistant material (1GRM), is similar to that of 1OHaq fraction. It should be noted that the ratio of xylose to glucose for 1XGose fraction is 0.9. It is therefore likely that the xylan present in 1OHaq was partially hydrolysed by endoxylanase present as contaminant in the commercial endoglucanase preparation used in this study.

Glycosyl linkage composition of xyloglucan-derived oligosaccharides (1XGose). The methylation analysis data of xyloglucan-derived oligosaccharides are shown in **Table II**. The presence of (1→4,6)- and (1→4)-linked glucopyranosyl residues, typical for the cellulosic backbone of xyloglucan is revealed. Terminal fucose, galactose and xylose (all) and (1→2)-linked xylose and (1→2)-linked galactose are also present. Together, these results demonstrate the presence of xyloglucan thus confirming sugar compositional data. In addition, (1→4)-linked xylose is present. These data confirm the results of sugar compositional analysis where degradation of xylan by commercial endoglucanase preparation has been inferred.

Matrix-Assisted Laser Desorption Ionization-Time of Flight- mass spectrometry (MALDI-TOF MS). MALDI-TOF-mass spectrometry, because of its sensitivity and applicability to the analysis of mixtures, is a convenient tool for the structural analysis of highly branched xyloglucan oligosaccharides. This technique was applied to the analysis of the endo-glucanase generated oligosaccharides. The mass spectrum of 1XGose fraction indicates the presence of two xyloglucan 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 (**Figure 3**). Taking into consideration the specificity and mode of action of the endo-(1→4)-β-D-glucanase, sugar composition and linkage analysis data of fragments present in 1XGose fraction and molecular masses of the known xyloglucan oligosaccharides[6-8], tentative structures for the xyloglucan-derived oligomers are proposed (**Figure 3**). For example, *m/z* value of 953 corresponds to Hex_4Pent_2 and it is, therefore, assigned as XXGG **1** named according to Fry *et al*., 1993 (ref. 5). Similarly, 1085 assigned as XXXG **2**, 1247 as XXLG **3**, 1393 as XXFG **4**, 1440 as XLLG **5** and 1555 as XLFG **6** (**Scheme I**). Although mass spectroscopy cannot distinguish stereoisomers,

Table II — Methylation analysis of oligosaccharides generated from *Sesamum indicum* meal (see text for the identification of fraction) by endo-glucanase treatment

Methylation products	*m/z* values	Peak area[a]
2,3,5-Ara[b]	43, 45, 102, 118, 129, 161 and 205	2
2,3,4-Xyl	43, 101, 102, 117, 118, 161 and 162	27
2,3-Xyl	43, 87, 102, 118, 129, 189 and 233	5
3,4-Xyl	43, 88, 101, 117, 130 and 190	8
2,3,4,6-Gal	43, 45, 87, 102, 118, 129, 145, 161, 162 and 205	2
3,4,6-Gal	43, 45, 71, 88,101, 129, 130, 145, 161, 190 and 205	5
2,3,4-Fuc	43, 72, 88,102, 1115, 118, 131, 162 and 175	4
2,3,4-Glc	43, 87, 102, 118, 129, 162, 189 and 233	6
2,3,6-Glc	43, 45, 87, 102, 113, 118, 129, 162, 173 and 233	3
2,3-Glc	43, 102, 118, 127, 162, 201, 261 and 305	38

[a]Percentage of total area of the identified peaks.
[b]2,3,5-Ara denotes 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylarabinitol, *etc*.

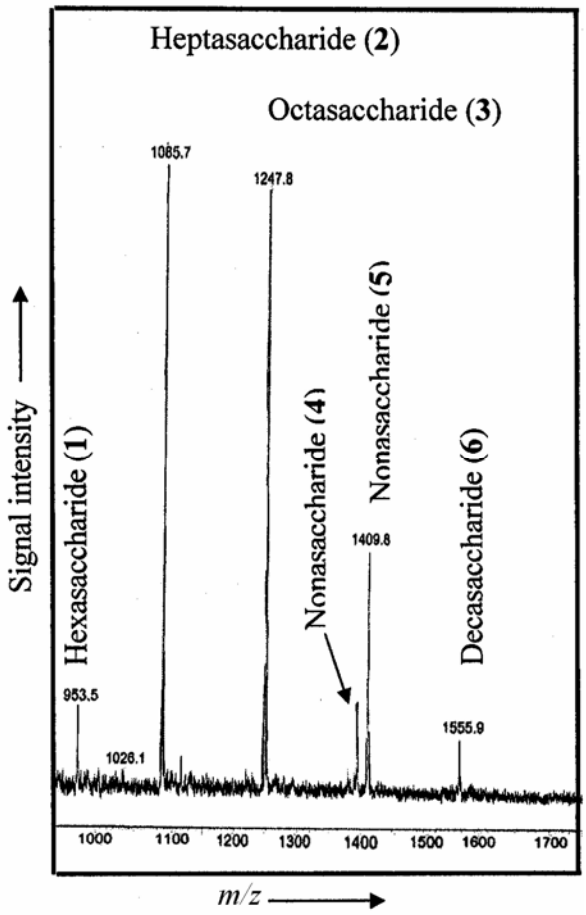


Figure 3 — MALDI-TOF mass spectrum of oligosaccharides generated from a xyloglucan rich pool (1OHaq) of *Sesamum indicum* meal by endo-β-(1→4)-D-glucanase digestion

but sugar compositional and glycosidic compositional analysis indicates the presence of xyloglucan derived oligosaccharides.

High Performance Anion Exchange-Pulse Amperometric Detection (HPAE- PAD)-chromatography. HPAE-PAD chromatographic analysis of the 1XGose fraction corroborates the results obtained from MALDI-TOF-Mass spectrometry. Indeed, the HPAEC-PAD chromatography elution profile (**Figure 4**) of 1XGose fraction shows the presence of five peaks having different intensity. Retention times of four of these peaks are similar to xyloglucan oligosaccharides XXXG, XXFG, XXLG + XLFG and XLLG, respectively generated from *Arabidopsis thaliana*[9], *Argania spinosa*[10], *Benincasa hispida*[11] and *Brassica compestris*[12] xyloglucan by endo-glucanase digestion.

Structure analysis of xylan

Information on the structure of xylan present in 4OH fraction was obtained by treating this fraction

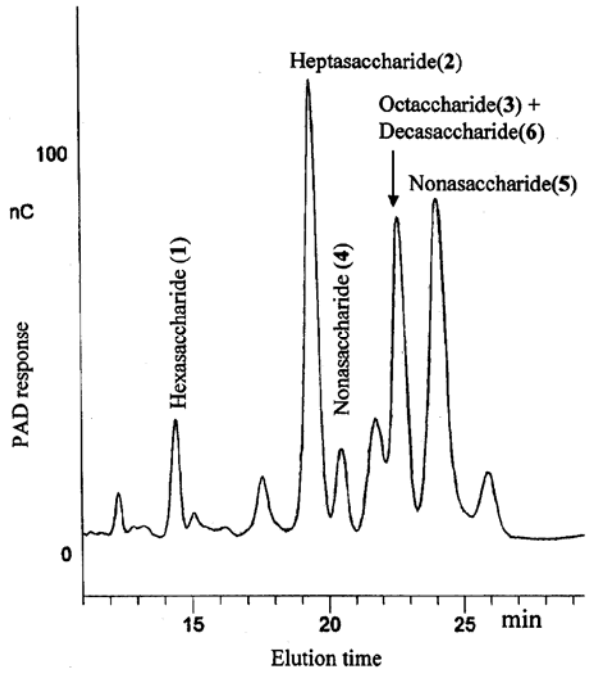


Figure 4 — HPAE-PAD chromatographic elution profile of heptasaccharide **2**, nonasaccharide **4**, mixture of octasaccharide **3** and decasaccharide **6,** and nonasaccharide **5** generated from *Sesamum indicum* meal using endo-β-(1→4)-D-glucanase degradation

with endo-(1→4)-β-D-xylanase, an enzyme specific for β-D-xylan. Sugar compositional analysis of the xylan-derived oligomers (4Xose) showed the presence of xylose residues (79 mol%) together with smaller amount of arabinose, glucose and galactose residues (**Table I**).

MALDI-TOF-mass spectrum of 4Xose fraction showed one major peak at *m/z* 760, which corresponds to one 4-O-MeGlcA linked to five xylose residues (**Figure 5**). The peak at 782 and 798 corresponding to the potasiated and di-sodiated pseudomolecular ions of the said oligosaccharide was also present. It also gives 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 have also been detected (data not shown). Therefore, the acidic xylan present in 4OH fraction has a classical structure similar to those present in other sources [10-12].

Experimental Section

Isolation of polysaccharides. The 1- and 4*M*-KOH extracted materials (designated as "1OH" and "4OH", respectively) were obtained from *Sesamum indicum* meal as described previously[1].

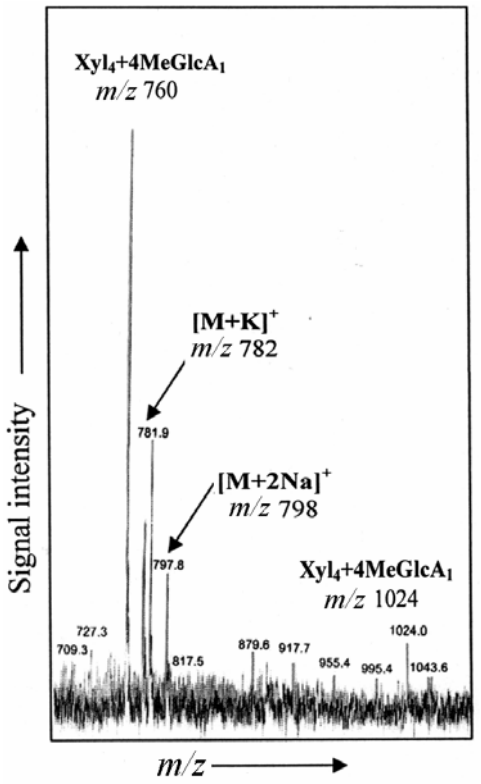


Figure 5 — MALDI-TOF mass spectrum of oligosaccharides generated from 4M KOH soluble fraction (4OH) of *Sesamum indicum* meal after degradation by endo-β-(1→4)- D –xylanase

Anion-exchange chromatography. Fraction (1OH) was submitted to anion-exchange chromatography on DEAE-Sepharose FF column (7 mL, Bio-RAD, AcO^-) equilibrated previously with water. After loading with sample, the column was eluted with the same solvent (30 mL) at a flow rate of 30 mL/hr to obtain the non-retained fraction (named 1OHaq). Bound materials were eluted from the column by using salt gradient.

Size Exclusion Chromatography (SEC). SEC of the 1OHaq fraction was done on a Sephacryl S-1000 column (90 × 2.6cm, BioRad) calibrated with standard dextrans (molecular-weight range of 10 000 to 1000 000 kDa) using 500 mmole sodium acetate (*p*H 5.0) at a flow rate of 20 mL/hr as described[13].

Preparation of xyloglucan oligosaccharides. Fraction 1OHaq (1 mg) was dissolved in 1 mL of 50 mmole NaOAc (*p*H 5.0) and the mixture incubated with 5 units of endo-glucanase (Megazyme International, Ireland) for 24 hr at 37°C with constant shaking. The glucanase resistant material was then precipitated in 80% ethanol (v/v), removed by centrifugation and lyophilised (1GRM). The soluble fraction containing the xyloglucan oligosaccharides was concentrated under a stream of nitrogen at 40°C and finally lyophilised (1XGose).

Preparation of xylan oligosaccharides. Hydrolysis of the 10 mg of xylan rich fraction (4OH) was performed in 5 mL of 10 mmole NaOAc (*p*H 5.0) using 40 units of endo-xylanase (Megazyme International, Ireland) at 37°C for 24 hr 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 (4Xose) were recovered by concentrating the supernatant under a stream of nitrogen at 40°C and lyophilising the concentrated solution.

Sugar analysis. Total sugars were determined by the phenol-sulfuric acid assay using glucose as standard[14]. The neutral sugar compositions of fractions were determined after hydrolysis with sulfuric acid (2*M*, 100°C, 2hr), reduction and acetylation[15]. Alternatively, the polysaccharides in the samples were hydrolyzed using trifluoro acetic acid (2*M*, 2 hr at 110°C), followed by an 18 hr methanolysis at 80°C with dry 2*M* methanolic-HCl. The generated methyl glycosides were converted into their TMS-derivatives[7] and separated by gas chromatograph (GC) with H_2 as carrier gas as described[10].

Methylation analysis. The pool of oligosaccharides (1XGose) generated from *Sesamum indicum* meal xyloglucan was permethylated according to Ciucanu and Kerek[16]. Permethylated material was extracted, dried, hydrolysed, converted into its partially methylated alditol acetates (PMAA) and was separated by GC and analysed by MS using a GC-MS as described previously[10-12].

HPAE- PAD chromatography. Fragments present in 1XGose fraction was 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^{-1}) with the following NaOAc gradient in 100 mmole NaOH as described[11].

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

MALDI-TOF mass spectrometry. MALDI TOF mass spectrometry in reflectron mode was performed using a Micromass (Manchester, UK) Tof spec E MALDI-TOF mass spectrometer. 2,5-dihydroxybenzoic acid (10 mg/mL) was used as matrix.

Conclusion

In conclusion, the study shows the presence of xyloglucan in 1*M* KOH extracted fraction (1OH) of *Sesamum indicum* meal. The molecular weight of this polymer is higher than the xyloglucan of mustard meal[12]. Sesame xyloglucan contains a hepta(**2**)-, an octa(**3**)- and a nona(**5**)-saccharides as major building sub-units. The enzyme-derived oligosaccharides are available for biological activity tests.

It is also shown that the acidic xylan present in 4*M* KOH extracted fraction (4OH) has a backbone of β-(1→ 4)-linked xylosyl residues substituted with 4-*O*-MeGlcA residues, as observed for many other higher plants[6,7,24].

Hemicellulosic polysaccharides have considerable potential for application in foods[17-19], pharmaceuticals[20-22], and in paper and cotton[6,23] industries. Since sesame is an important commercial crop, further studies on purified polymers will be of interest from scientific as well as industrial purposes.

Acknowledgement

This work was supported by CSIR (Grant no. 01(1962)/05/EMR-11) to B. R. The authors thank Prof P Lerouge University of Rouen, France for extending MALDI-MS and HPAEC analysis facility.

References

1 Ghosh P, Ghosal P, Thakur S, Lerouge P, Loutelier-Bourhis C, Driouich A & Ray B, *Food Chem*, 90, **2005**, 719.
2 Kacurakova M, Capek P, Sasinkova V, Wellner N & Ebringerova A, *Carbohydr Polym*, 43, **2000**,195.
3 Gupta S, Madan R S & Bansal M C, *Tappi J*, 70, **1987**, 113.
4 Fry S C, *J Exp Bot*, 40, **1989**, 1.
5 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, *Physiol Plant*, 89, **1993**, 1.
6 Sims I M, Munro S L A, Currie G, Craik D & Bacic A, *Carbohydr Res*, 293, **1996**, 147.
7 York W S, Kolli V S K, Orlando R, Albersheim P & Darvill A G, *Carbohydr Res*, 285, **1996,** 99.
8 Hisamatsu M, York W S, Darvill A G & Albersheim P, *Carbohydr Res*, 227, **1992,** 45.
9 Lerouxel O, Choo T S, Seveno M, Usadel B, Faye L, Lerouge P & Pauly M, *Plant Physiol*, 130, **2002,** 1754.
10 Ray B, Loutelier-Bourhis C, Lange C, Condamine E, Driouich A & Lerouge P, *Carbohydr Res*, 339, **2004**, 201.
11 Mazumder S, Lerouge P, Loutelier-Bourhis C, Driouich A & Ray B, *Carbohydr Polym*, 59, **2005**, 231.
12 Ghosh P, Ghosal P, Thakur S, Lerouge P, Loutelier-Bourhis C, Driouich A & Ray B, *Carbohydr Polym*, 57, **2004**, 7.
13 Mazumder S, Morvan C, Thakur S & Ray B, *J Agric Food Chem*, 52, **2004**, 2356.
14 Dubois M, Gilles K A, Hamilton J K, Rebers P A & Smith F, *Anal Chem*, *28*, **1956,** 350.
15 Blakeney A B, Harris P, Henry R J & Bruce A B, *Carbohydr Res*, 113**, 1983,** 291.
16 Ciucanu I & Kerek F, *Carbohydr Res*, 131, **1984**, 209.
17 Fooks L J & Gibson G R, *FEMS Microbiol Ecol*, 39, **2002,** 67.
18 Garrote G, Dominguez H & Pajaro J C, *Carbohydr Polym*, 52, **2002,** 211.
19 Kabel M A, Carvalheiro F, Garrote G, Avgerinos E, Koukios E, Pajaro J C, Girio F M, Schols H A & Voragen A G J, *Carbohydr Polym*, 50, **2002,** 47.
20 Burgalassi S, Chetoni P & Saettone M F, *E J Pharm Biopharm*, 42, **1996,** 385.
21 Kato Y, Uchida J, Ito S & Mitsuishi Y, *Int Cong Ser*, 1223, **2001**,161.
22 Miyazaki S, Kawasaki N, Endo K & Attwood D, *J Pharm Pharmacol*, 53, **2001**,1185.
23 Glicksman M, in *Food Hydrocolloids*, Vol III, (CRC Press, Boca Raton, Florida, USA), **1986**, p.191.
24 Izydorezyk M S & Biliaderis C G, *Carbohydr Polym*, 28, **1995,** 33.