ELSEVIER

Carbohydrate Polymers 47 (2002) 253–257

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Characterization of free sugars and xyloglucan-type polysaccharides of two tropical legumes

J.C. Onweluzo[a], H.P. Ramesh[b], R.N. Tharanathan[b,*]

[a]*Department of Food Science and Technology, University of Nigeria, Nsukka, Nigeria*
[b]*Department of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore 570013, Karnataka, India*

Received 14 September 2000; revised 24 October 2000; accepted 11 January 2001

Abstract

The major alcohol-soluble sugars in the dehulled flours of *Detanium microcarpum* and *Mucuna flagellipes* were glucose, fructose sucrose, raffinose and stachyose. Sucrose was the predominant sugar. Extraction with 10% trichloroacetic acid of the alcohol-insoluble residue gave a highly viscogenic mucilaginous polysaccharide complex, which upon anion exchange and gel permeation chromatographies gave a xyloglucan-type polysaccharide, having a 1,4-linked D-glucan backbone with branch-off residues composed of 1,6-linked D-xylose and some non-reducing terminal glucose moieties. The degree of branching was relatively high in *D. microcarpum* fraction.

Keywords: Free sugars; Legume seeds; Mucilage; Polysaccharides; Xyloglucan

1. Introduction

Endogenous polysaccharides of plant origin have long been recognized as functional food ingredients (Tharanathan, 1995; Tharanathan, Muralikrishana, Salimath & Raghavendra Rao, 1987). Such a functional role is evident from the fact that sources of gums have expanded beyond the natural plant exudates to include chemically modified and synthetic products. All known natural gums have been extensively studied and structurally characterized. Being an energy reserve polysaccharide, like starch and galactomannans, xyloglucans are of frequent occurrence in many plant species. As a component of dietary fiber, xyloglucans may possess nutritional attributes of physiological significance and value. In the native state, they are highly associated with the cellulose microfibrils forming a rigid network structure (Hayashi, 1989). In the plant, their presence helps in wall loosening in response to auxin action (Hayashi, Wong & Maclachlan, 1984). O-Acetyl substitution in the back bone is shown to increase the elasticity of primary cell walls, which helps in elongation growth in plants (O'Neill, Morris, Selevendran, Sutherland & Taylor, 1986). Because of remarkably regular substitution pattern on the glucan backbone, a repeating unit of Glc_4-Xyl_3 has been postulated in the xyloglucan of *Hymenaea courbaril* cotyledons (Buckeridge, Crombie, Mendes, Reid & Gidley, 1997). Tamarind seed xyloglucan has been studied extensively (Kooiman, 1967).

Some of the tropical legume seeds such as *Detanium microcarpum* (DM) and *Mucuna flagellipes* (MF) have found considerable usage in Nigeria and a few other West African countries (Onweluzo, Obanu & Onnoha, 1994). Their seed flour is used for thickening soups and stews. Except for some preliminary work (Onweluzo et al., 1994), no systematic study has been done on the chemical nature and structural feature of the highly viscogenic mucilaginous polysaccharides of these two legume seeds.

2. Materials and methods

2.1. Materials

Seeds of DM and MF were purchased from a local market in Nsukka, Nigeria. Dehulling was done by cracking and water soaking (1 h at room temperature) and manually peeling off the husk. The wet cotyledons were dried and ground to 52 μm size.

2.2. Ethanol-soluble sugars

The defatted (by treatment with petroleum ether-hexane, 1:1 at 40°C for 3 h) flour was boiled with aqueous ethanol (80%, 1:20 w/v, 1 h, thrice), centrifuged and the pooled

* Corresponding author. Tel.: +91-821-514876; fax: +91-821-517233.
E-mail address: tharanathan@yahoo.co.uk (R.N. Tharanathan).

0144-8617/02/$ - see front matter
PII: S0144-8617(01)00188-6

Table 1
Composition (%) of soluble sugars in alcoholic extracts of the dehulled flours

Sample	Sugars[a]					
	Glc	Fru	Suc	Raf	Sta	Unidentified
DM	0.08	1.61	9.35	0.48	0.35	–
MF	0.88	0.50	8.75	2.48	3.08	0.10

[a] Identified by HPLC.

extracts were deionized by passage through Dowex-50 (H^+) and Dowex-1 (OH^-) resins, and concentrated by flash evaporation. Sugars in the deionized extracts were analyzed by HPLC.

2.3. Extraction of polysaccharides

The alcohol-insoluble residues from the above were repeatedly extracted ($\times 4$) in the cold (4°C) with 10% trichloroacetic acid (1:50, w/v) for 4 h with good stirring. The extracts were separated by centrifugation and to the combined extracts was added acetone (3 vol) and the precipitated material was recovered after a preliminary dialysis and lyophilization.

2.4. Isolation of starch

From the trichloroacetic acid-insoluble residue, starch was recovered by the aqueous steeping and sieving method (Madhusudhan & Tharanathan, 1995). The crude starch was purified by repeated treatments with diluted NaOH to pH 9.0 for 1 h followed by 0.1 M NaCl–toluene (10:1, v/v) for 30 min. The purified starch was water washed and dried by solvent exchange method.

2.5. Fractionation of the mucilaginous polysaccharide

To an aqueous solution (0.3%) of the polysaccharide was added ethanol with stirring to incipient turbidity (Whistler & Sannella, 1965). The precipitate thus formed was centrifuged out. Addition of ethanol to the supernatant was continued and the precipitates recovered at each step were solvent washed and dried.

The major fractions, DM-2 (37.9% EtOH concentration) and MF-2 (56.2% EtOH concentration) were subjected to column chromatography on DEAE cellulose (CO_3^{2-}) ($44 \times 3\ cm^2$) and elution with water, 0.2–0.4 M $(NH_4)_2CO_3$ and 0.4 M NaOH and the individual fractions were assayed for total sugar by the phenol–H_2SO_4 method (Rao & Pattabiraman, 1989), pooled, dialyzed and recovered by freeze drying.

The water eluted neutral polysaccharides were subjected to GPC on a precalibrated (with T-series dextrans of known MW) column of Sepharose CL-2B ($100 \times 1.5\ cm^2$) and eluted with water ($18\ ml\ h^{-1}$). Fractions (3 ml) were analyzed for total sugars and their approximate MW computed.

Size exclusion chromatography on E-linear and E-1000 (Waters Associates, Milford, USA, ss, 3.9 mm i.d. × 30 cm) μ-Bondagel columns connected in series with a guard column was performed on a Shimadzu HIC-6A chromatograph (Madhusudhan & Tharanathan, 1996).

2.6. Analysis

Polysaccharides were acid hydrolyzed with either 2M TFA (120°C for 4 hr) or 72% H_2SO_4 method and derivatized into alditol acetates before GC analysis. For precise quantitation, the molar response factor for each of the sugar was determined using myo-inositol as an internal standard. The various analytical determinations were performed as described before (Managala & Tharanathan, 1999; Ramesh & Tharanathan, 1998). GalA was determined by the method of Blumenkrantz and Asboe-Hansen (1973). The specific and intrinsic viscosities of aqueous solutions (0.1–0.5%) of polysaccharides were determined by extrapolation to zero concentration, respectively, from the plots of relative viscosity (η_r) and reduced viscosity (η_0) against concentration. Permethylation of the polysaccharide fractions was carried out by the Hakomori procedure using CH_3I and dymsil anion (Hakomori, 1964). After purification by filtration through SEP PAK C_{18} cartridges, the *O*-methylpolysaccharides were depolymerized and converted to permethylated alditol acetates and analyzed by GC-MS in a high performance quadrupole Shimadzu mass spectrometer QP-5000 fitted with a SP-2380 fused silica capillary column (30 m × 0.32 mm i.d., 0.02 μ) and operating at 150–200°C at $2°\ min^{-1}$, 70 eV, mass range 40–400 amu and 4 ms $scan^{-1}$.

2.7. Liquid chromatography

Quantitative sugar analysis was done on a μ-Bondapack NH_2 column (25 cm × 1.5 mm i.d.) in a Shimadzu HPLC system consisting of a LC-6A pump, RI detector and a Shimadzu CR-4A Chromatopac integrator. The mobile phase consisted of acetonitrile–water (75:25, v/v). An isocratic elution at a flow rate of $1\ ml\ min^{-1}$ was used at 40°C.

3. Results and discussion

Major sugars in the alcoholic extracts of the dehulled flours of DM and MF were glucose, fructose, sucrose, raffinose and stachyose with traces of maltose (Table 1). Sucrose was the predominant sugar in both. This sugar profile is reminiscent of those present in several of the commonly consumed legume flours (El Faki, Bhavanishankar, Tharanathan & Desikachar, 1983; Jaya, Naik & Venkatraman, 1979). The content of raffinose (2.5%) in MF was comparable to that reported in chickpea alcohol solubles (2.2%),

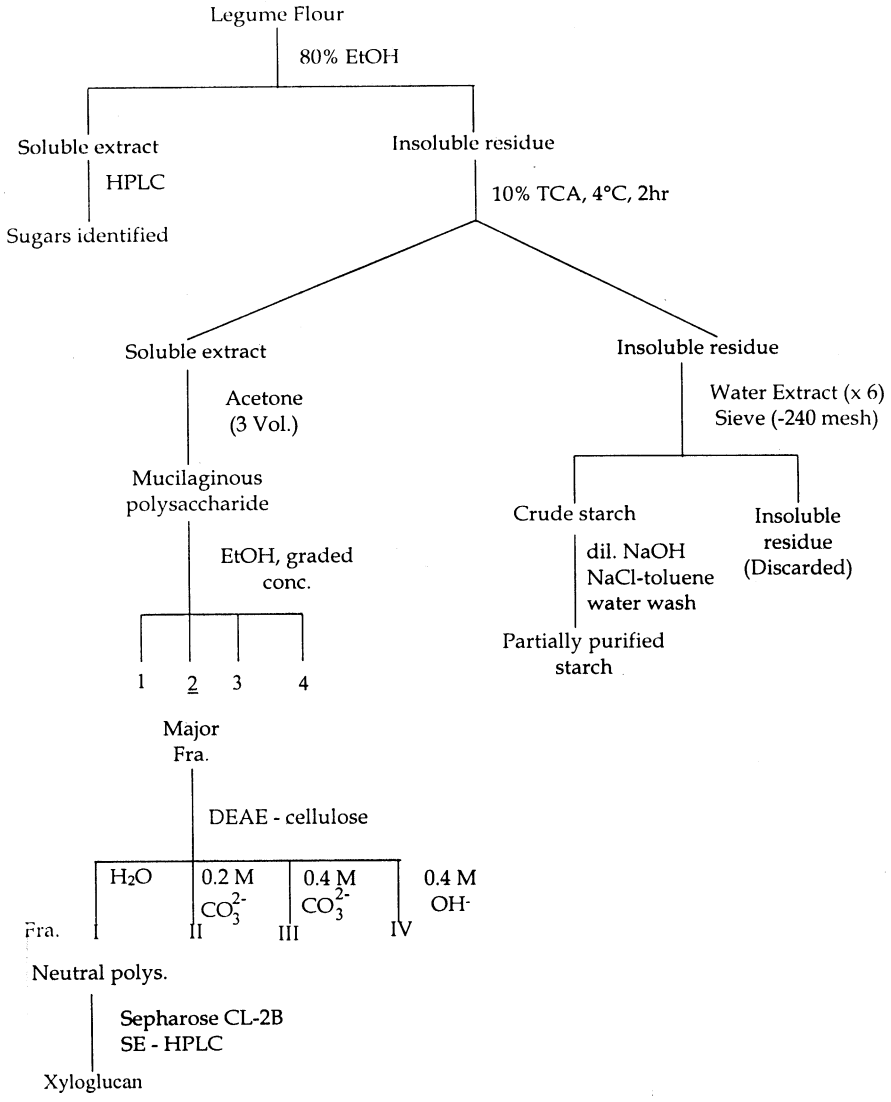

Fig. 1. Scheme of extraction of xyloglucan from legume flour.

whereas the stachyose content was much higher in MF than in other legume flours. DM had a higher (free) sugar content than MF. Raffinose and stachyose were several folds higher in MF, which partially accounts for its relatively higher flatulence property. Nevertheless, as these legume flours are used in small quantities mainly as a functional food ingredient to build up uniform consistency, their usage may not pose any serious nutritional problem.

The various polysaccharide fractions belonging to starch and non-starch type were sequentially extracted from the alcohol-insoluble residues of DM and MF by a scheme depicted in Fig. 1. Extraction with 10% trichloroacetic acid gave a higher yield of soluble polysaccharides from DM (65%) than from MF (36.7%). They were fibrous and dissolved in water to give thixotropic solutions. At concentrations of 0.5% and above the MF polysaccharide showed

Table 2
Proximate composition (%) of polysaccharides of DM and MF

Polysaccharide	Yield	Protein	Total sugars	Sugars[a]				
				GalA	Ara	Xyl	Gal	Glc
DM	65.1	3.1	76.8	1.2	1.8	25.6	16.0	53.6
MF	36.7	4.6	86.2	1.6	1.1	27.4	18.2	50.7

[a] Quantitated by GC.

Table 3
Sugar composition (%) by GLC of ethanol precipitated fractions

Sample	EtOH concentration (%)	Total sugars	Yield	Sugars[a]					
				Ara	Xyl	Man	Gal	Glc	Un-identified
DM-1	23	98	25.4	1.3	31.9	3.4	13.9	43.9	–
DM-2	37	100	44.4	0.9	33.5	–	13.3	51.9	–
DM-3	58	95	11.4	0.5	26.2	–	18.3	50.5	
DM-4	67	100	1.6	3.4	29.7	6.0	17.4	42.4	
MF-1	43	65	10.9	2.3	15.7	–	11.2	29.7	3.1
MF-2	56	82	75.9	0.5	23.2	1.1	11.7	40.9	1.9
MF-3	66	85	4.2	0.9	26.0	–	15.1	39.6	2.2

[a] Quantitated by GC.

non-Newtonian characteristics. A higher degree of water of hydration and as a consequence, the build-up of high viscosity was observed more in MF than in DM. It was reported that the associated proteins present in them may also contribute to their hydration power and viscosity gain (Onweluzo et al., 1994). The polysaccharides were composed of xylose, galactose and glucose in more or less comparable concentrations (Table 2). Chromatographic evidence showed the presence of galacturonic acid in these polysaccharides.

The content of starch in both the legume flours was very little, ~2–4% and upon microscopic examination, tiny spherical starch granules appeared, which were birefringent. Majority of the granules showed a close association with cell wall debris. The latter was difficult to remove as repeated water washings of the starchy mass gave opalescent viscous extracts. The partially purified starch had a blue value of 0.09 and 0.02, respectively for DM and MF, and amylose and amylopectin contents of ~25 and 74%.

Upon sequential precipitation from aqueous solution (~0.3%) with graded concentrations of ethanol (Table 3) four fractions in DM and three in MF were obtained. The major fraction (No. 2) in DM came out at ~38% EtOH, whereas it was at 56% EtOH concentration in MF, which might probably indicate subtle variations in the hydrophilic–lipophilic balance of these mucilages. Their sugar composition showed xylose, galactose and glucose in a relative proportion of 2.5:1:3.9 and 2.0:1:3.5 in DM-2 and MF-2, respectively. The content of arabinose in MF-2 was less than that of DM-2, and in addition, small amounts of mannose and an unidentified component were also seen in the former.

Anion exchange chromatography of DM- 2 gave ~37% of water eluted neutral polysaccharide, contrary to only ~7% from MF-2. The former was composed of xylose, galactose and glucose in the ratio 1:1.4:4.8, whereas in the latter, these sugars were present in 1:1.3:15.2 ratio, respectively (Table 4). The sugar profile of the fraction from DM-2 was similar to xyloglucan-type polysaccharides found in tamarind (Kooiman, 1967), field bean (Salimath & Tharanathan, 1982) and several other sources. GPC and size exclusion chromatography of these two neutral poysaccharides gave a major peak (~95%) indicating their homogeneity. These fractions of DM-2 and MF-2 had MW values of 100 and 398 kDa, respectively and contained xylose and glucose in the ratio of 1:4 with only traces of galactose.

Permethylation followed by depolymerization, alditol (^{1}H) acetate derivatization and GC-MS analysis (Table 5) revealed the pattern of the glycosidic linkages. However, their relative proportion in DM-2 and MF-2 fractions varied considerably. The low comparison in the proportion of terminal to branching sugar residues indicates substantial loss of the former during sample preparation steps.

Table 4
Sugar composition (%) of DEAE cellulose fractions derived from DM-2 and MF-2

Fractions eluted with	Total sugars	Yield	Sugars[a]				
			Ara	Xyl	Man	Gal	Glc
DM-2							
Water	96	37.0	0.9	12.9	–	18.4	61.8
0.2 M CO_3^{2-}	95	6.7	–	10.9	14.1	8.5	56.5
0.4 M CO_3^{2-}	100	6.5	1.2	9.9	2.0	20.3	65.1
0.4 M OH^-	100	37.3	–	19.9	–	16.2	63.9
MF-2							
Water	80	6.8	–	4.3	–	5.8	65.5
0.2 M CO_3^{2-}	100	36.6	1.4	13.3	–	29.2	56.0
0.4 M OH^-	100	54.2	0.8	18.4	10.4	13.7	56.2

[a] Quantitated by GC.

Table 5
Permethylation analysis of polysaccharide fractions derived from DM-2 and MF-2

O-methyl ether	Relative proportion[a]		Diagnostic fragments (*m/z*)	Mode of linkage
	DM-2 fractions	MF-2 fractions		
2,3,4 – Me_3-Xyl	4.80	1.55	205, 162, 161, 145, 129,. 118, 101	D-Xyl-(1 →
2,3,4,6-Me_4-Glc	1.00	1.00	205, 145, 162, 161, 129, 118, 102, 101	D-Glc-(1 →
2,3,4,6-Me_4-Gal	0.50	T	205, 145, 162, 161, 129, 118, 102, 101	D-Gal-(1 →
2,3,6-Me_3-Glc	5.41	1.58	233, 173, 162, 118, 101, 87	-4)-D-Glc-(1 →
2,3-Me_2-Glc	14.64	3.68	261, 201, 162, 127, 118, 101	-4,6)-D-Glc-(1 →

[a] Relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. T: Traces.

Identification of 2,3,6-tri-*O*-methyl-D-glucose suggests a (1,4)-linked glucan backbone having side chain branching with non-reducing terminal residues mostly of (1,6)-linked xylopyranosyl, and some galactosyl and glucosyl moieties. The degree of branching is relatively of much lower magnitude in MF. Similar structural features have been reported for xyloglucans from several other plant sources (Kooiman, 1967; Salimath & Tharanathan, 1982). Assuming a glucose backbone, the degree of polymerization (DP) was 21 for DM-2 and 6 for MF-2, which is not consistent with the reported DP of xyloglucans. (1-2)-Xylp was not found by methylation analysis, probably indicating the absence of glycosidically linked galactose to the C2 of xylose.

Acknowledgements

JCO acknowledges receiving a post-doctoral fellowship under the CSIR-TWAS exchange programme.

References

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry*, *54*, 484–489.

Buckeridge, M. S., Crombie, H. J., Mendes, C. J. M., Reid, J. S. G., Gidley, M. J., & Vieira, C. C. J. (1997). A new family of oligosaccharides from the xyloglucan of *Hymenaea courbaril* L. (Leguminosae) cotyledons. *Carbohydrate Research*, *303*, 233–237.

El Faki, H. A., Bhavanishankar, T. N., Tharanathan, R. N., & Desikachar, H. S. R. (1983). Flatus effect of chick pea (*Cicer arietinum*), cow pea (*Vigna sinensis*) and horse gram (*Dolichos biflorus*) and their isolated carbohydrate fractions. *Nutrition Reports International*, *27* (921), 929.

Hakomori, S. (1964). A rapid permethylation of glycolipid and polysaccharide catalyzed by methylsulfinyl carbanion in dimethyl sulfoxide. *The Journal of Biochemistry*, *55*, 205 –208.

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

Hayashi, T., Wong, Y., & Maclachlan, G. (1984). Pea xyloglucan and cellulose, II. Hydrolysis by pea *endo*-1,4-β-glucanases. *Plant Physiology*, *75*, 605–610.

Jaya, T. V., Naik, H. S., & Venkatraman, L. V. (1979). Effect of germinated legumes on the rate of in vitro gas production by *Clostridium perfringens*. *Nutrition Reports International*, *20*, 393–401.

Kooiman, P. (1967). The constitution of the amyloid from seeds of *Annona muricata* L. *Phytochemistry*, *6*, 1665–1673.

Madhusudhan, B., & Tharanathan, R. N. (1995). Legume and cereal starches — why differences in digestibility? — Part II. Isolation and chracterization of starches from rice (*O. sativa*) and ragi, (finger millet, *E. coracana*). *Carbohydrate Polymers*, *28*, 153–158.

Madhusudhan, B., & Tharanathan, R. N. (1996). Structural studies of linear and branched fractions of chickpea and finger millet starches. *Carbohydrate Research*, *284*, 101–109.

Managala, S. L., & Tharanathan, R. N. (1999). Structural studies of resistant starch derived from processed (autoclaved) rice. *European Food Research and Technology*, *209*, 38–42.

O'Neill, M. A., Morris, V. J., Selvendran, R. R., Sutherland, I. W., & Taylor, I. T. (1986). Structure of the extracellular gelling polysaccharide produced by Enterobacter (NCIB 11870) species. *Carbohydrate Research*, *148*, 63–69.

Onweluzo, J. C., Obanu, Z. A., & Onuoha, K. C. (1994). Functional properties of some lesser known tropical legumes. *Journal of Food Science and Technology*, *31*, 302–306.

Ramesh, H. P., & Tharanathan, R. N. (1998). Structural characteristics of a mixed linkage β-D-glucan from sorghum (*Sorghum bicolor*). *Carbohydrate Research*, *308*, 239–243.

Rao, P., & Pattabiraman, T. N. (1989). Reevaluation of the phenol–sulfuric acid reaction for the estimation of hexoses and pentoses. *Analytical Biochemistry*, *181*, 18–22.

Salimath, P. V., & Tharanathan, R. N. (1982). Structural features of two amyloids from the hemicellulosic fraction of field bean (*Dolichos lablab*) hulls. *Carbohydrate Research*, *107*, 103–109.

Tharanathan, R. N. (1995). Polysaccharide gums of industrial importance: a review. *Journal of Scientific and Industrial Research*, *54*, 512–523.

Tharanathan, R. N., Muralikrishana, G., Salimath, P. V., & Raghavendra Rao, M. R. (1987). Plant carbohydrates — an overview. *Proceedings of Indian Academy of Sciences (Plant Science)*, *97*, 81–155.

Whistler, R. L., & Sannella, J. L. (1965). In R. L. Whistler, *Fractional precipitation with ethanolMethods in Carbohydrate Chemistry*, V. New York: Academic.