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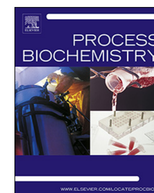

Structure of peanut shell xylan and its conversion to oligosaccharides

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ABSTRACT

We present here that peanut shell, an underutilized agro-residue, is suitable for the isolation of xylan and the production of prebiotic xylooligosaccharides (XOS). Two different alkaline extraction procedures coupled to delignification were applied for the extraction of xylan. A one-step extraction in the presence of sodium hypochlorite produced xylan I (14.8% yield) contaminated with other hemicelluloses, mainly xyloglucan. A two-step extraction of sodium chlorite-delignified material yielded much purer polysaccharide, assigned as xylan II (15.5% yield). This polysaccharide was characterized by NMR, and MALDI ToF MS analysis was performed on xylooligosaccharides generated by various xylanases. The polysaccharide appears to be a glucuronoxylan similar to a hardwood glucuronoxylan, however, with a relatively higher degree of substitution of the main chain with 4-*O*-methyl-D-glucuronic acid (Xyl:MeGlcA ratio ∼6–7). Consequently, the peanut shell glucuronoxylan hydrolysates produced by GH10, GH11 and GH30 xylanases contained considerable portion of acidic xylooligosaccharides. A process for the production of peanut shell xylooligosaccharides from alkali extracted peanut shell xylan using a thermophilic *Thermomyces lanuginosus* GH11 xylanase has been proposed.

1. Introduction

Peanut shell represents an abundant agro-residue which has not been sufficiently explored. The Food and Agriculture Organizationof the United Nations reports that peanut production worldwide corresponds to about 46 million tons, of which shells account for 20% of the entire weight [1]. In South Africa alone, approximately 22,000 tons of peanut shells are produced annually [2] and exploitation of this underutilized biomass offers a great potential for added value, besides waste management. Peanut shells contain ∼25% of their total dry weight as hemicellulose [3], which is predominantly xylan. Literature presents several examples of the use of peanut shells, either intact or after mechanical pretreatment. They are primarily used as a carbon source for the production of plant biomass-degrading enzymes [4,5], and in powder form, as an adsorbent for sequestering heavy metals from aqueous solutions [6], and in combination with corn stover as a material for the production of briquettes [3]. Peanut shells pretreated by wet oxidation, dilute-acid or ionic liquids can serve as a potential source of sugars fermentable to ethanol, following enzymatic hydrolysis [1,7–10]. However, majority of the peanut shells are still either dumped or burnt as waste.

The present work focuses on utilizing the unexplored peanut shell biomass, isolation of its major hemicellulose i.e., xylan, its structural characterization and possibility of its enzymatic conversion to prebiotic xylooligosaccharides (XOS). XOS not only serve as a selective carbon source for health-promoting bacteria in the human digestive tract [11] but may also have many other positive effects, such as improvement of mineral absorption and prevention of gut infections [12,13]. XOS have also been used as functional and water binding food additives [14,15].

Thus far, there are no detailed studies dedicated to xylan present in peanut shells. In this work, alkaline extraction of deacetylated peanut shell xylan and its first structural characterization by NMR spectroscopy and enzymatic hydrolysis is reported. This, together with the analysis of XOS generated by three types of endoxylanases suggests that we have isolated a glucuronoxylan with unusually high content of 4-*O*-methyl-D-glucuronic acid side residues. In the final phase of completion of this manuscript, a new report emerges which suggest peanut shells as a source of bioactive oligosaccharides [16], however, the nature of xylan has not been studied. To the best of our knowledge, this is the first report on the characterization of xylan from peanut shells and its enzymatic conversion to XOS.

Abbreviations: Xyl, *p*β-D-xylopyranosyl residue; MeGlcA, 4-*O*-methyl-D-glucuronic acid; GH, glycoside hydrolase; Xyl_n, β-1,4-xylooligosaccharide of 'n' Xyl residues; $MeGlcA^{i}Xyl_n$, aldouronic acid containing one residue of MeGlcA linked to Xyl*p* residue marked in Xyl_n with 'i' which is the number of Xyl*p* residue counted from the reducing end 'i' would be equal to 1 if MeGlcA would be linked to the reducing end Xyl 2 if MeGlcA would be linked to the second Xyl*p* residue from the reducing end etc.); Glc, glucose; Gal, galactose; Man, mannose; Ara, arabinose; Fuc, fucose; Rha, rhamnose

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2. Materials and methods

2.1. Biomass collection

Peanut shells were obtained from a peanut processing plant in Durban, KwaZulu Natal, South Africa during the autumn season (April). The shells were washed, dried to constant weight at a temperature of 60 °C, ground in a ball mill grinder and sieved. The fraction consisting of particles smaller than 0.35 mm was collected and used for xylan isolation.

2.2. Extraction of xylan from peanut shells

Two different alkali extractions were employed for xylan isolation. For the first procedure, i.e., one-step NaClO/NaOH extraction, 50 g of powdered shells were stirred with 125 ml of 1% NaClO solution for 1 h at room temperature. The wet material was washed several times with distilled water and soaked in 15% NaOH for 24 h at room temperature, as described by Chapla et al. [17]. The mixture was filtered through a muslin cloth followed by neutralizing with glacial acetic acid, and the filtrate was precipitated by 3 volumes of ice-cold 95% ethanol. The neutralized precipitate was centrifuged at a speed of $8800 \times g$ for 10 min at 4 °C, washed with 75% ethanol and dried at 60 °C till constant weight was reached.

Xylan II was extracted according to the procedure of Ebringerová et al. [18]. Briefly, powdered peanut shells (50 g) were soaked in 500 ml of distilled water and heated to a temperature of 50 °C. Acidification with glacial acetic acid (7 ml) was followed by the gradual addition of sodium chlorite (35 g). The mixture was heated at 70 °C for 1 h in a protected fume hood to liberate lignin and coloured materials. This was followed by extensive washing with water. The neutral wet cake was suspended in 350 ml of 1% NH_4OH at room temperature for 2 h. The extract was then filtered off and discarded. The remaining solid material was extracted with 5% NaOH at room temperature for 2 h under occasional stirring. The mixture was then filtered through a muslin cloth; the solid material extraction was repeated, and the two filtrates were combined and mixed with 3 volumes of 95% ethanol to precipitate the polysaccharide. The precipitate thus formed was collected by filtration, followed by washing with 75–80% ethanol containing 2% acetic acid to neutralize the residual alkali and subsequently washed several times with 80% ethanol. Finally, the precipitated polysaccharide, assigned as xylan II, was suspended in water and freeze-dried.

2.3. Monosaccharide composition of isolated polysaccharides

Neutral sugars were quantified by GLC in the form of alditol acetates after hydrolysis of the samples with 2 M trifluoroacetic acid (TFA) for 1 h at 120 °C [19]. The uronic acid content of the extracted xylan was determined using H_2SO_4/3-hydroxybiphenyl reagent [20].

2.4. Enzymes used in the study

GH11 family xylanase from the thermophilic fungus *T. lanuginosus* SSBP was purified as already described [21]. *Cellvibrio mixtus* GH10 xylanase, *Clostridium thermocellum* GH16 endo-1,3(4)-β-glucanase and *Paenibacillus* sp. GH5 endo-xyloglucanase were obtained from Megazyme (Bray, Ireland). GH30 xylanase from *Erwinia chrysanthemi* was supplied by Prof. James F. Preston (University of Florida, Gainesville, FL, USA). *Bacillus subtilis* α-amylase was procured from Sigma-Aldrich (St. Luis, MO, USA). Xylanase activity was determined on beechwood glucuronoxylan (Sigma-Aldrich, USA) following the formation of reducing groups quantified by Somogyi-Nelson procedure [22]. One unit of xylanase activity is defined as the amount of μmol equivalents of xylose generated in 1 min. Other enzymes were applied in units reported by the supplier.

Table 1
Molar ratio of monosaccharides in peanut shell xylan.

Xylan preparation	Xyl	MeGlcA	Glc	Gal	Man	Ara	Fuc	Rha
Xylan I (NaClO/NaOH one-step extraction)	1.00	0.13	0.14	0.09	0.02	0.12	0.02	0.03
Xylan II (NaOH extraction after $NaClO_2$ delignification and NH_4OH treatment)	1.00	0.14	0.009	0.02	0.003	0.04	0.001	0.01

2.5. Enzymatic degradation of peanut shell xylan and beechwood glucuronoxylan

Isolated peanut shell xylan II and commercial beechwood glucuronoxylan were dissolved (1%, w/v) in 50 mM sodium citrate buffer (pH 6.5) and incubated in a water bath at 50 °C for 24 h with *T. lanuginosus* GH11 xylanase (10 U/g). The citrate buffer was replaced with sodium acetate buffer (pH 5.5) for reaction mixtures of GH10 and GH30 xylanases (10 U/g) and was incubated at 35 °C for 24 h. In the search for co-extracted polysaccharides, both xylan I and xylan II were treated with other pure glycoside hydrolases under similar conditions. The hydrolysis products of xylan II were analysed by thin layer chromatography (TLC) and MALDI-TOF MS as described below. The GH11 hydrolysate of xylan II was also subjected to ^{1}H NMR spectroscopy.

2.6. TLC analysis of enzymatically released carbohydrates

TLC analysis was carried out on silica gel-coated aluminium sheets (Merck, Darmstadt, Germany) and developed in a solvent system of n-butanol/ethanol/water (10:8:5, v/v). Appropriately diluted samples and standards were spotted (3 μl) and detected using orcinol reagent (1% in 10% H_2SO_4 in ethanol). Standards of linear XOS were procured from Megazyme Int. (Bray, Ireland). Shorter aldouronic acids in the peanut shell xylan hydrolysate were identified using hydrolysates of beechwood glucuronoxylan by GH10 and GH11 xylanases containing $MeGlcA^3Xyl_3$ and $MeGlcA^3Xyl_4$ as the predominant acidic products, respectively [23]. Supporting information on the nature of acidic oligosaccharides was obtained by NMR spectroscopy and MALDI-TOF mass spectrometry.

2.7. NMR spectroscopy and MALDI-TOF mass spectrometry

For NMR analysis, all samples were freeze-dried twice from D_2O. ^{1}H NMR spectra were measured at 25 °C using automatic chemical shift calibration in D_2O on either AVANCE III HD X 600 MHz equipped with a Triple inverse TCI H-C/N-D-05-Z cryo probe, or AVANCE III HD 400 MHz with a broad band BB-(H-F)-D-05-Z liquid N_2 Prodigy probe (all from Bruker BioSpin, Rheinstetten, Germany), using a pre-saturation *zgpr* sequence, with a pre-saturation delay of 2 s, a r.f. 90° pulse and an acquisition time of 2.5 s.

Analysis of oligosaccharides was done by MALDI-ToF MS as previously described [24]. An Ultraflex MALDI-TOF/TOF instrument (Bruker Daltonics GmbH, Bremen, Germany) equipped with a nitrogen 337 nm laser beam was operated in reflectron positive acquisition mode and controlled using the Flex Control 3.3 software package. Ions of sodium adducts of xylan fragments were marked using monosaccharide codes while ions of fragments of xyloglucan were marked using the one letter nomenclature for xyloglucan-derived oligosaccharides [25].

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