Original Article

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Recovery of xylan from *Acacia mearnsii* using ultrasound-assisted alkaline extraction

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Abstract: Novel and effective biorefinery methods are necessary to fractionate lignocellulosic biomass into separate components for the valorization of these components into value-added products. For example, the xylan fraction can be used as an adhesive, a thickener, an additive to plastics, an emulsifier, a drug carrier, and as a precursor of prebiotic xylooligosaccharides. Unfortunately, conventional xylan extraction procedures require lengthy extraction periods and are characterized by poor product purity and yields. In this study, a non-conventional ultrasound-assisted alkaline extraction method was developed for the effective extraction of xylan from *Acacia mearnsii*, an industrially relevant hardwood in South Africa. The effect of the additional ultrasonication step on the conventional alkaline extraction of xylan was evaluated. After this, basic structural analysis was conducted on both acacia xylan extracts and commercially available beech-derived xylan. Composition analysis data showed the acacia-derived glucuronoxylan to have an Ara:GluA:Xyl ratio of 0.5:1:5, while that from beechwood had a 0:1:4 ratio. Ultrasound-assisted alkaline extraction also gave a better yield (24%) than conventional alkaline extraction (21%) and had a quicker extraction period of 1 h versus 4 h. Finally, the acacia-derived xylan exhibited antioxidant potential, with an EC50 value of ≈1.5 mg/mL against 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH). In conclusion, ultrasound-assisted alkaline extraction was successfully employed to extract high-purity, bioactive xylan from acacia. The purified acacia xylan is a suitable replacement for birchwood glucuronoxylan, which is no longer commercially available, or for expensive beechwood substitute for numerous applications. © 2023 The Authors. *Biofuels, Bioproducts and Biorefining* published by Society of Industrial Chemistry and John Wiley & Sons Ltd.

Key words: acacia wood chips; glucuronoxylan; hemicellulose fractionation; ultrasonication

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Introduction

Wood biomass has long been utilized as a source of energy for heating and cooking, and for industrial uses such as paper and pulp making, the formulation of extracts for dust control, traditional medicine, and making furniture.[1] *Acacia mearnsii* trees occur naturally in Australia and are the most widespread invasive alien trees in South Africa.[2,3] Acacia species are well known for their invasiveness, and studies have reported their negative effects on native tree species, causing a serious threat to biodiversity.[3] In South Africa, *A. mearnsii* is used for various industrial applications including the use of its bark in the tanning process.[1]

Wood shavings accumulate during wood processing, leading to environmental concerns. However, because of their high cellulose and hemicellulose content, these shavings have the potential to be used in biorefineries, where polymers like xylan, important in the production of value-added products such as prebiotic xylooligosaccharides (XOS), with promising health benefits, can be produced.[4,5] Interestingly, XOS derived from hardwoods have been shown to exhibit antioxidant activity, particularly those that are aldouronic acids or substituted with 4-*O*-methyl-D-glucuronic acid (MeGlc*p*A) side chains.[6] Due to xylan's nondigestibility in the stomach, it can be used as a colon-specific drug-delivery system. It has also been shown that xylan can be used as an additive in papermaking and textile printing.[7]

Hardwoods such as acacia, aspen, beech, and birch are mostly composed of xylans in the form of glucuronoxylans as the primary hemicellulose.[8–10] The xylans represent 10–35% of the total dry mass in these hardwoods.[11,12] The hardwood xylans consist of the main chain of β-1,4-linked D-xylopyranosyl (Xyl*p*) residues, which are substituted by residues of MeGlc*p*A in a molar ratio of 5–20:1 (Xyl*p*: MeGlc*p*A).[13,14] In some cases, the Xyl*p* residues may also be esterified by acetyl groups at a degree of 0.4–0.6 in positions *O*-2 or *O*-3 and/or *O*-2,3.[10,14]

The complex nature of wood biomass, with extensive interactions such as covalent and hydrogen bonding between the constituents, makes the xylan extraction a tedious and economically unfavourable process.[15] Xylans are conventionally fractionated from lignocellulosic biomass by alkaline (KOH or NaOH) or by dimethyl sulfoxide extraction with or without the combination of chlorite or hydrogen peroxide.[15] Methods such as the use of alkali achieve hemicellulose fractionation by breaking the ether and ester bonds between lignin and hemicellulose, disrupting the plant cell-wall structure, and leading to the release of the hemicellulose into the solution.[16]

In addition to finding alternative xylan sources to the exuberant beechwood xylan13, novel extraction procedures that are economically viable are therefore required, as conventional xylan extraction procedures lead to environmental pollution.[17] Alkali extraction conditions can also degrade the fractionated hemicellulose through β-elimination reactions that split glycosidic bonds.[17] Ultrasound technology has gained increasing attention in phytochemical extraction from biomass over the past few decades, as it offers high yields and reduced processing time.[18] Ultrasonication facilitates polysaccharide extraction through the generation of cavitation bubbles that collapse in the liquid medium, enhancing mass and heat transfer, improving the activity of reactant molecules, and increasing the probability of their collision with each other.[16]

In this study, *A. mearnsii* was evaluated as a potential alternative source to beechwood for the extraction of 4-*O*-methyl-D-glucuronoxylan. We also attempted to improve the time and yield of the conventional alkaline xylan extraction method through the addition of an ultrasonication step. Finally, a comparison of extraction yields of the two procedures and structure elucidation of the extracted xylan was conducted.

Materials and methods

Materials

Beechwood-derived xylan, monosaccharide estimation kits (K-ARGA, K-GLUC, K-URONIC, and K-XYLOSE), a recombinant GH11 xylanase (Xyn11A) from *Neocallimastix patriciarum* were purchased from Megazyme (Bray, Ireland). All other reagents used were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany). Wood chips (length × width × thickness of 4 cm × 3 cm × 0.5 cm) of *Acacia mearnsii* were kindly provided by Mondi Limited (Durban, South Africa).

Preparation of acacia wood chips for xylan extraction

Before the extraction procedure, the wood chips were passed three times through a Trimtech garden vacuum blower with the vacuum or mulch speed set at level 6. The mulched wood chips were then crushed into fine residue by crushing the chips twice for up to 2 min each run using a Waring commercial blender. The fine residues were then used as the biomass for the extraction procedure.

Composition analysis of acacia wood

A composition analysis of the acacia wood sample was conducted to evaluate if it is a viable source for xylan extraction.

The powder derived from the pulverization of acacia wood chips was acid hydrolyzed using a modified sulfuric acid method by the National Renewable Energy Laboratory (NREL) at the Department of Wood Science, Stellenbosch University, South Africa.[19] After the hydrolysis the fractions were filtered to remove the insoluble lignin from the sugar solutions. The insoluble fraction was kept in an oven at 50°C until no further change in the mass of the fraction was apparent. The Klason lignin (KL) content was determined by measuring the mass of the solid residue after overnight drying at 105°C. The acid-soluble lignin (ASL) and monosaccharide content were analyzed using the filtrate, and ASL was determined by measuring the absorbance at 205 nm by ultraviolet-visible (UV-visible) spectroscopy, and the monosaccharides (glucose, xylose, arabinose, galactose, and mannose) were analyzed using high-performance anion exchange chromatography (HPAEC) with an ICS-5000 ion-chromatography system (Dionex) equipped with an electrochemical detector following the procedure described previously.[20]

Alkaline and ultrasound-assisted extraction of xylan

The extraction of xylans from acacia was performed using the alkaline extraction method with minor modifications.[21] Briefly, 20 g of acacia powder was added to 200 mL of an alkaline solution of 1% (w/v) borate containing 10% (w/v) sodium hydroxide, followed by sonication (40 kHz, 300 W) for 10 min at 60°C using a 9 L digital ultrasonic cleaner (Eins Sci, Johannesburg, South Africa). After sonication, the solution was incubated in a shaking incubator (Labcon, Petaluma, CA, USA) at 60°C for 3 h, with agitation at 200 rpm. The residual biomass was removed by filtering the suspension through a muslin cloth. The filtrate was then neutralized to pH 5.0 using glacial acetic acid. The xylan in the filtrate was precipitated through the addition of three volumes (600 mL) of ice-cold ethanol (95% v/v), followed by overnight incubation at 4°C. The conventional alkaline extraction method was also carried out as described above with the exception of the 10 min ultrasonication step. Xylan extraction using the ultrasound-assisted alkaline procedure was evaluated over a range of incubation times, 0, 0.5, 1, 2, and 3 h, post-sonication for 10 min as described previously. The xylan extraction yield obtained at these various extraction durations was determined.

Extraction yield calculation

The xylan extraction yield was calculated by dividing the dry weight (g) of the xylan extract by the dry weight of the acacia wood powder used in the extraction procedure and multiplying the result by 100%.

Carbohydrate composition of xylan

Xylan's total carbohydrate content was estimated using the phenol-sulfuric acid method described previously.[22] Xylose was used as a suitable standard.

Monosaccharide composition of the carbohydrate fraction of xylan

The monosaccharide composition of the xylan extracted from acacia was determined by 2 mol L^{-1} trifluoroacetic acid (TFA) hydrolysis in a dry digital bath (Eins Sci) for 2 h at 120°C. The residual TFA was evaporated from the sample by keeping it in a hot-air oven at 80°C for 1 h. The dried sample was then resuspended in deionized water for sugar analysis. Quantitative analysis of arabinose, glucose, glucuronic acid, and xylose content was performed enzymatically using sugar determination kits according to the manufacturer's instructions.

Phenolic composition of xylan

The total amount of contaminant phenolics in the xylan extract was determined using a modified Folin–Ciocâlteu method described previously, using a 0.1% (w/v) polysaccharide solution dissolved in dH_2O.[23] Gallic acid was used as a phenolic standard.

Protein composition of xylan

Bradford's method was used to determine the amount of protein contaminant in the xylan extract, using a 0.1% (w/v) polysaccharide solution dissolved in dH_2O.[24] Bovine serum albumin was used as a protein standard.

Ferulic acid composition of xylan

Some of the arabinofuranosyl (Araf) substituents in xylans are known to be linked to ferulic acid by ester bondings, tethering this hemicellulose type to the lignin in lignocellulosic biomass. In this study, the following method was used to determine the extent of Araf esterification by ferulic acid. The ferulic acid content of the xylan samples was determined by measuring the absorbance of a 0.1% (w/v) polysaccharide solution dissolved in dH_2O at 325 nm (λ_{max} of ferulic acid). Ferulic acid was used as a suitable standard.

Xylan dispersibility

The dispersibility of the xylan was tested by mixing 1% (w/v) of the solid fraction with 1 mL of deionized water in an Eppendorf tube under 25 rpm agitation using a Benchmark H2024 rotary mixer (Sayreville, NJ, USA) at 25°C for 5 h,

followed by 20 min at 99°C. Prepared suspensions were then centrifuged for 5 min at 12 000×*g*. The supernatant was removed, while the non-dispersible pellet of xylan was dried at 40°C overnight. The tubes were weighed, and the mass of the dispersible fraction was determined.

Fourier transform infrared spectroscopic analysis of xylan

A Spectrum 100 Fourier transform infrared (FTIR) spectrometer system (Perkin Elmer, Waltham, MA, USA) was used to analyze the xylan samples for present functional groups. Each sample was pressed uniformly and tightly against the sample spotting surface using a spring-loaded anvil. Fourier transform infrared spectra were obtained by averaging four scans from 4000 to 650 cm^{-1}. Baseline and attenuated total reflectance (ATR) corrections for penetration depth and frequency variations were carried out using the Spectrum One software supplied with the equipment.

Nuclear magnetic resonance spectroscopic analysis of xylan

The extracted xylan samples were analyzed by H^1 nuclear magnetic resonance (NMR) spectroscopy using a Bruker Avance III 400 MHz spectrometer with a double resonance broadband (BBI) probe (Bruker, Karlsruhe, Germany). For NMR analysis, 15 mg of xylan was suspended in 1 mL of D_2O (99.96%) (Merck), and the spectra were recorded at ambient temperature. The spectra were processed and analyzed using TopSpin NMR software, version 3.6.5 (Bruker).

X-ray powder diffraction analysis of xylan

The crystallinity of the xylan samples was determined by X-ray diffraction (XRD) using Cu K radiation (1.5405 Å, nickel filter) on a Bruker D8 Discover equipped with a proportional counter. The samples were scanned from 2θ of 5 to 60° with a step size of 0.02 per second°. The relative crystallinity of the xylans was calculated by dividing the area of the diffraction peak at 2θ = 17–21° by the total area under this peak.[25]

Thermogravimetric analysis of xylan

The xylan samples were subjected to thermogravimetric analysis (TGA) to evaluate the thermal degradation properties (thermogravimetric and differential thermal properties). The changes in enthalpy and weight loss were monitored using a PerkinElmer, Pyris Diamond thermal analyzer. Approximately 6 mg of xylan was added into an alumina crucible and heated in the range of 30–700°C with a heating rate of 10°C min^{-1}, while continuously flushing the apparatus with argon at a flow rate of 60 mL/min at the atmospheric pressure.

Intrinsic viscosity determination of xylan

The intrinsic viscosity [η] of each xylan in dL g^{-1} was measured at 25°C by using a Cannon-Manning semi-micro capillary viscometer (Size 50, 9721-Y53) (State College, PA, USA). Briefly, a 2 mg mL^{-1} stock solution for the xylan samples was prepared in 50 mmol L^{-1} NaCl. The viscosity measurements were performed on the diluted samples with different concentrations (0.25, 0.5, 0.75, and 1.0 mg/mL). First, the relative viscosity (η_r) of the xylan was calculated using the following equation:

$$\eta_r = \frac{\eta}{\eta_s} \quad (1)$$

where η_r is the relative viscosity, η is the xylan solution viscosity (mPa s), and η_s is the solvent viscosity. Finally, inherent viscosity (η_{inh}) was calculated using the equation:

$$\eta_{inh} = \frac{ln\eta_r}{C} \quad (2)$$

where η_r is the relative viscosity and C is the xylan concentration (g dL^{-1}). Inherent viscosity values were plotted against polymer solution concentrations and intrinsic viscosity (the limit of inherent viscosity as the concentration approaches zero dilution) was determined from extrapolation of the curve according to the Kraemer method.[26]

Bioactivity of xylan – antioxidant activity of xylan

Freshly prepared 0.1 mmol L^{-1} 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical reagent (methanol used as a solvent) was used to evaluate the radical scavenging antioxidant potential of the xylan samples. The xylan samples (0.15 mL of 0.1–2 mg mL^{-1} solutions) were mixed with 0.15 mL of the stable DPPH radical in an ethanol solution. Ascorbic acid (0.025–0.1 mg/mL) was used as an antioxidant control in the assay. The mixture of ethanol (0.15 mL) and sample (0.15 mL) served as blank, and the control solution was prepared by mixing deionized water (0.15 mL) and DPPH radical solution (0.15 mL). The changes in colour (from deep violet to light yellow) were read at 517 nm after 30 min of reaction using a UV-visible spectrophotometer. The scavenging activity percentage (AA%) of the xylan samples was calculated using the equation below, and ascorbic acid was determined using the attained absorbances and used to obtain their EC50 values:

$$AA\% = \frac{(control - sample)}{control} \times 100 \quad (3)$$

Evaluation of xylanase activity on xylan

The potential suitability of extracted acacia xylan as a viable substrate for xylanase activity assays was evaluated by estimating the activity of Xyn11A. The enzyme assay was set up in a microtiter plate with 1% (w/v) xylan substrate dissolved in 50 mmol L^{-1} sodium citrate buffer (pH 5.0) and allowed to incubate for 30 min at 50°C. Reducing sugar release by Xyn11A from the xylan was then estimated using the dinitrosalicylic assay,[27] with xylose used as a suitable standard.

Data analysis

A one-way ANOVA was used to determine significant differences between samples using GraphPad Prism 8.

Results and discussion

Xylan extraction from acacia

The chemical composition of the acacia wood revealed that the biomass contained a high fraction of cellulose, followed by xylan and lignin, on a dry mass basis (Table 1). In comparison with other hardwoods grown in plantations across South Africa, such as poplar (14.84% xylan)[28] and eucalyptus (9.20% xylan),[20] and the current source of commercial glucuronoxylan, beech (21.35%),[29] acacia has a significantly higher xylan content (23.88%). This finding makes acacia a suitable source for the production of xylan for use in numerous sectors such as papermaking, pharmaceutical, and food industries.

Alkaline and ultrasound-assisted alkaline extraction processes were carried out to obtain high xylan yields from acacia. The yields were 21 ± 0.25% and 24 ± 0.25%, respectively. Based on an ANOVA, the addition of the ultrasonication step significantly improved xylan extraction ($P < 0.05$) from acacia in comparison with the use of alkali alone. These yields corresponded to xylan recoveries from acacia (amount of extracted xylan from theoretically available xylan in the wood, as determined from its composition analysis) of 87% and 79% in the case of the alkaline- and ultrasound-assisted alkaline extraction, respectively. The extraction time optimization study for the ultrasound-assisted alkaline method showed that 60 min post-sonication was the time required for optimal extraction of xylan from acacia, as there was no difference in the extraction yield at this time point in comparison with 180 min (3 h) (Fig. 1). The incubation periods of the alkaline- and incubation time-optimized ultrasound-assisted alkaline extraction were significantly different (P-value <0.05); 4 and 1 h, respectively. A similar study showed that arabinoxylan could be extracted from wheat bran in a 60% shorter time (from 60 to 10 min) when ultrasonication was applied.[30] In summary, the ultrasound-assisted alkaline extraction procedure was significantly more effective ($P < 0.05$) at xylan extraction than the conventional alkaline conditions alone.

Table 1. Chemical composition (%) of acacia wood (dry mass basis).

Glucan	Xylan	Arabinan	Total lignin
48.75	23.88	Nd	22.38

Abbreviation: Nd, not detected.

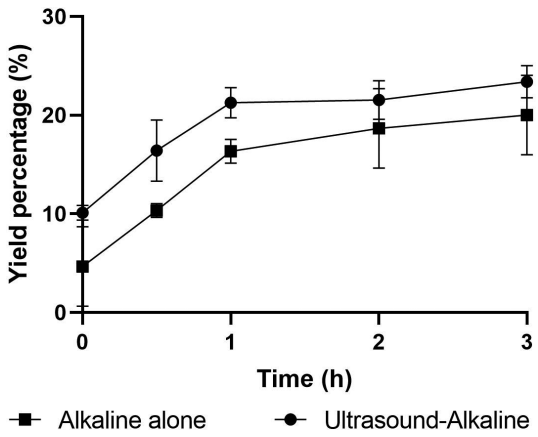

Figure 1. Effect of incubation time during alkaline and ultrasound-assisted alkaline extraction on the xylan yield. Values are represented as means ± standard deviations, $n = 3$.

The most probable mechanism for ultrasonic treatment enhancement of the extraction procedure is the intensification of mass transfer and easier access of the solvent to the vegetal cells of the biomass.[31] Ultrasonication generates cavitation bubbles that collapse near cell walls, leading to cell disruption coupled with good solvent penetration into the cells.[32,33] Overall, these effects of ultrasonication on the biomass and solvent lead to easier extractability of the xylan by alkali from the matrix of the acacia wood. Similar to our findings, a study on the extraction of xylan from a hardwood, *Ailanthus altissima*, showed that ultrasound-alkali treatment was more effective than alkali treatment alone at hemicellulose solubilization, although it also lowered lignin content from the biomass.[16]

Composition of acacia xylan

Composition analysis of the carbohydrate content of the xylan extracted from acacia by both alkaline and

Table 2. Composition (%) of xylan extracted by different methods.

Xylan source	Total sugar	Xyl*p*	MeGlc*p*A	Ara*f*	Ferulic acid	Phenolics	Protein
Alkaline xylan	87 ± 1.50	74.0 ± 0.57	17.5 ± 0.35	8.0 ± 0.98	1.4 ± 0.19	6.1 ± 0.03	Nd
Ultrasound-alkaline xylan	79 ± 3.25	75.0 ± 0.54	19.7 ± 0.95	6.2 ± 0.12	0.6 ± 0.08	6.1 ± 0.21	Nd
beech xylan	91 ± 4.00	92.9 ± 0.67	6.2 ± 0.50	0.8 ± 0	Nd	6.6 ± 0.11	Nd

Note: Monosaccharide content percentages are reported based on total sugar values. Values are represented as means ± standard deviations, $n = 3$.
Abbreviations: Ara*f*, arabinofuranoside; MeGlc*p*A, 4-*O*-methyl-glucuronic acid; Xyl*p*, xylopyranoside. Nd, not detected.

ultrasound-assisted alkaline methods exhibited an Ara*f* to MeGlc*p*A to Xyl*p* percentage composition of 6.2–8%, 17.5–19.7% and 74–75% (a ratio of 0.5:1:4). This was higher than the percentage for the commercial beechwood-derived xylan, which was 0.8%, 6.2% and 92.9% (a ratio of 0:1:15). Interestingly, beech xylan extracted by dimethyl sulfoxide (DMSO) was reported to have a MeGlc*p*A to Xyl*p* ratio of 1:14–18,[34] while *Paulownia elongate* has a MeGlc*p*A to Xyl*p* ratio of 1:20.[35] On the other hand, KOH extracted *A. mangium* xylan reportedly has a ratio of 1:10, with traces of Ara*f* and Gal*p* residues.[10] It is unclear whether the differences are a result of the preferential dissolution of more substituted fractions of the original xylan by the methods employed in this study compared to that used commercially for beechwood xylan extraction or whether acacia xylan inherently has a higher proportion of methyl-glucuronic acid substituents than beechwood xylan.

Total sugar composition analysis of the alkaline and ultrasound-assisted alkaline extracted acacia xylan showed that 80% or more of the weight of the extracts was carbohydrate (Table 2). Finally, none of the xylan samples exhibited protein content, and they all had minor phenolic (≈6%) and ferulic acid content (>2%), except for beech which did not have ferulic acid content (Table 2). Overall, these composition analysis data confirmed that the two procedures that were compared both succeeded in extracting relatively pure xylan from acacia.

Xylan dispersibility

The extent and pattern of acetylation and sugar residue substitution of hemicelluloses are known to affect their solubility and/or dispersibility. As much as the Xyl*p*: MeGlc*p*A ratios of the acacia and beech xylan differed markedly, the extent to which they were non-dispersible was comparable, 11–13% (P-value >0.05). Aspen xylan was also shown to be sparingly soluble in cold water but highly soluble in hot water, owing to the extent of its acetylation, which prevented the hyperentanglement of xylan polymer chains.[25] Similarly, another study reported that native glucuronoxylan from aspen and that which was partly acetylated were partially soluble in hot water.[36] Another study showed that locust bean gum, with a mannopyranosyl (Man*p*):galalactopyranosyl (Gal*p*) ratio of 4:1, and konjac glucomannan, linear polymer with Man*p* interspersed by glucopyranosyl (Glc*p*) at a ratio of 2–3:1, exhibited non-dispersibility of about 30% and 10%, respectively. Acetylation of these mannans at degrees of about 0.3 (locust bean gum) and 0.15 (konjac glucomannan) led to increased non-dispersibility of about 35%.[37,38] Overall, the non-dispersibility of the xylan samples was similar to that of other amorphous hemicellulosic polysaccharides and hardwood xylans reported in the literature and, therefore, the xylan samples can be considered water soluble.

Fourier transfer infrared analysis of xylan

The functional groups contained in the structure of the extracted xylan were determined by recording the FTIR spectra (Fig. 2). The broad peak between 3600 and 3000 cm^{-1} in all xylan samples corresponds to the presence of –OH groups that are linked through intra- and inter-molecular hydrogen bonding.[7] The peak at 2900 cm^{-1} is assigned to the C–H stretching vibration of alkanes in xylan.[7] The peak at 1700 cm^{-1} is characteristic of the C=O stretching of the ester bonds or carboxylic acid groups.[39] The prominent peak at 1100 cm^{-1} is assigned to C–O and O–C stretching of the ester bonds with some contribution of the OH bending mode, which is characteristic of the glycosidic groups. The peak at 1000 cm^{-1} belongs to the C–O–C stretching of the pyranoside rings in xylan.[39–41] Finally, the small, sharp peak at 900 cm^{-1} is typical of β-glycosidic linkages between sugar residues in polysaccharides.[39,40] Overall, FTIR successfully identified the key organic functional groups and some chemical bonds present in the main and side chains of acacia xylan (Fig. 2).

Nuclear magnetic resonance analysis of xylan

The structure of the extracted xylan was determined by recording the ^{1}H NMR spectra (Fig. 3). The strong signal at δ 4.70 ppm originated from the residual solvent. Chemical

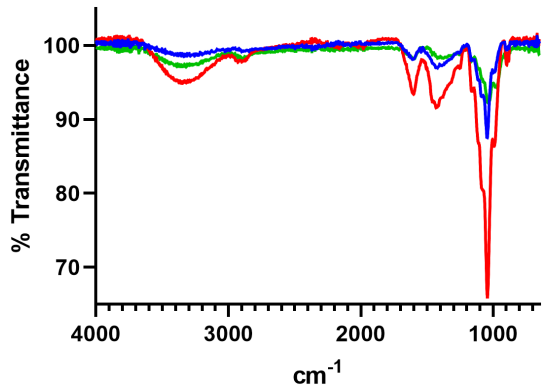

Figure 2. Fourier transform infrared spectra of xylan samples. Red is beechwood xylan, and green and blue are alkaline and ultrasound-assisted alkaline extracted xylan from acacia, respectively.

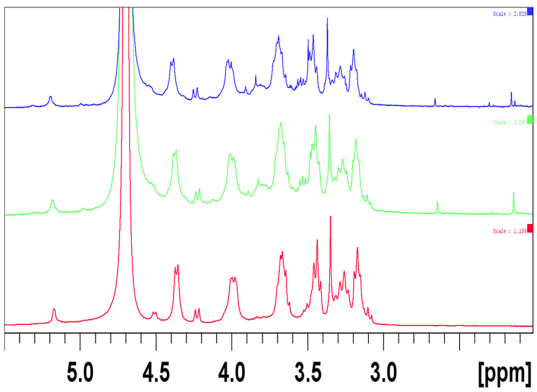

Figure 3. Nuclear magnetic resonance spectra of xylan samples. Red is beechwood xylan, and green and blue are alkaline and ultrasound-assisted alkaline extracted xylan from acacia, respectively.

shifts corresponding to non-substituted xylose residues δ 3.15 (H2), 3.22 ($H5_{axial}$), 3.42 (H3), 3.64 (H4), and 3.95 ppm ($H5_{eqitorial}$), were observed for all the xylan samples.[39,42] Proton shifts related to a 4-*O*-methyl glucuronic acid residue linked to a xylose residue (XG) were identified at δ 3.69 (H4), 3.98 ($H5_{equitorial}$) and 4.47 ppm (H1).[43] Finally, proton shifts corresponding to a 4-*O*-methyl-glucuronic acid residue were also noted at δ 3.33 ($-OCH_3$), 3.34 (H4), 3.43 (H2), 3.48/3.53 ($H6_{equitorial/axial}$), 3.64 (H3), 4.43 ($H5_{equitorial}$) and 5.13 ppm (H1). These ^{1}H NMR results from the extracted xylan are characteristic of β-1,4-linked xylose residues with 4-*O*-methyl-glucuronic acid residue substituents, such as *Acacia nilotica*[42] and beech.[44]

Interestingly, the *Acacia mearnsii* xylan extracted in this study also showed shifts at δ 3.85 (H3), 4.15 (H4), and 5.31 ppm (H1), indicating the presence of minor linkages between the arabinofuranosyl and xylose residues (AG), which were absent in beech xylan. The presence of these peaks corroborated the composition analysis data, which showed the presence of arabinose content in the acacia xylan and not in the beech xylan (Table 2). These signals are characteristic of arabinoxylan originating from grasses, such as wheat, barley, rice, and oat spelt.[40] Finally, the acacia xylan extracts also showed shifts at δ 2.15 ppm, characteristic of 2-*O*-acetyl-Xylp or 3-*O*-acetyl-Xylp,[34,45] which were absent in beech xylan. Overall, NMR spectroscopy corroborated composition analysis data regarding the issue of whether acacia wood xylan is *O*-acetyl-4-*O*-methylglucuronoxylan with minor Ara*f* substituents (Fig. 3).

X-ray diffraction analysis of xylan

A broad major peak around 20° was noted for all xylan samples assessed, indicating some short-range order in

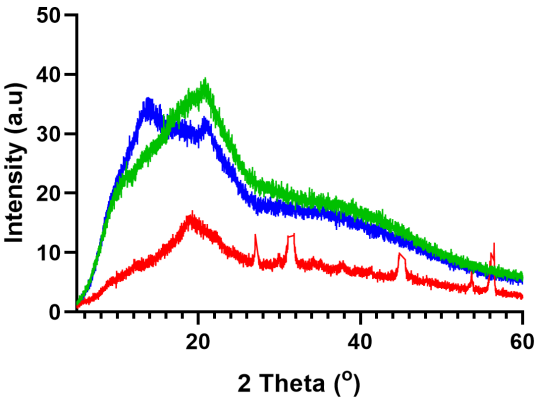

Figure 4. X-ray diffraction analysis of xylan samples. Red is beechwood xylan, and green and blue are alkaline and ultrasound-assisted alkaline extracted xylan from acacia, respectively.

the amorphous structure of glucuronoxylan (Fig. 4). This is characteristic of hardwood xylan and has been reported on beech[46] and birch xylans.[41] The relative crystallinities of the xylans were 0.29, 0.29, and 0.32 for beech, alkaline, and ultrasound-assisted alkaline-extracted acacia xylan, respectively. The relative crystallinities of the samples analyzed in the present study were similar to that of an aspen-derived xylan, with a value of 0.37.[25]

Thermogravimetric analysis of xylan

Thermogravimetric analysis and derivative thermogravimetry (DTG) analysis showed that the xylan samples lost weight slightly at approximately 50–180°C (Fig. 5). This weight loss could be attributed to the loss of moisture and volatile compounds from the samples.[47] The samples further

underwent a second but massive loss in weight between 200 and 400°C (Fig. 5(a)). The DTG curves were similar, with an exothermal peak at 240–280°C for the xylan samples (Fig. 5(b)). This stage of TGA is associated with the decomposition and depolymerization of biomass such as hemicellulose.[47] The xylan samples essentially showed a pyrolysis kinetic that follows the three-Gaussian distributed activation energy model, which is characteristic of hemicellulosic polysaccharides.[48]

The beech xylan exhibited a somewhat higher onset decomposition temperature than the acacia xylan. We suppose that the lower substituents on the beech xylan, as shown by composition analysis data, may cause this xylan to undergo a higher degree of hyperentanglement, forming more short-range order in its amorphous structure, leading to the marginally observed decomposition stability compared to the highly substituted acacia xylan. There was about 20–30% solid residue remaining from the pyrolysis of the xylan samples at 600°C. We suppose this to derive from inorganics introduced during the extraction procedure, such as salts, and decomposition remains from the biomass.[39,43]

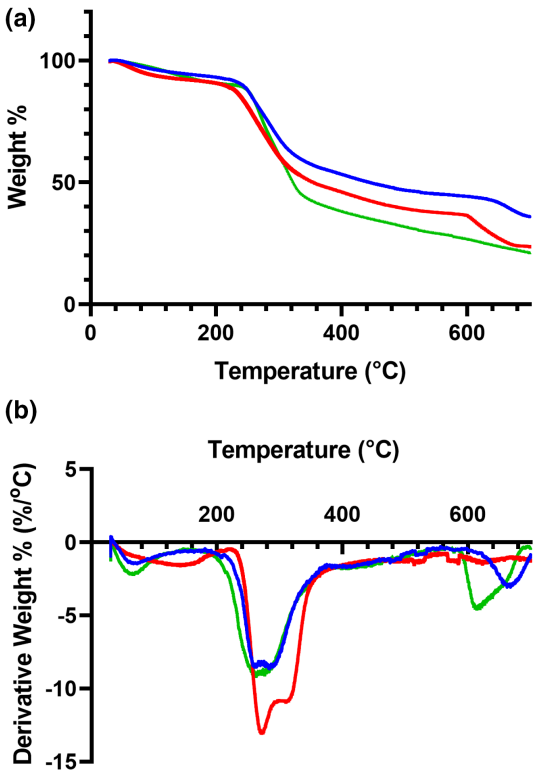

Figure 5. (a) Thermogravimetric analysis and (b) derivative thermogravimetry analysis of the xylan samples. Red is beechwood xylan, and green and blue are alkaline and ultrasound-assisted alkaline extracted xylan from acacia, respectively.

Intrinsic viscosity and viscosity average molecular weight of xylan

The relationship of $[\eta]$ and viscosity average molecular weight (M_v) is given by the Mark–Houwink–Sakurada equation ($[\eta] = K_\eta M_v^{\alpha}$), where K_η and α are both constants for a particular polymer-solvent system.[49] The $[\eta]$s of the xylans were 1.137, 0.998, and 0.989 for beech, alkaline, and ultrasound-assisted alkaline-extracted acacia xylan, respectively. The values of the Mark–Houwink exponent α are 0–0.5, 0.5–0.8, and 0.8–1.8 for spherical, random coil, and rod conformations, respectively.[49] The constant for alkaline extracted glucuronoxylan (with M_v of 21.5 kDa and MeGlc*p*A to Xyl*p* ratio of 1:6.8) from *Dolichos lablab* L. hull is 0.70.[50] Using the information mentioned above, the M_v were calculated and found to be 25.7 kDa, 21.4 kDa and 21 kDa for beech, alkaline, and ultrasound-assisted alkaline extracted acacia xylan, respectively. Several studies have reported that beech xylan has a molecular weight of around 20 kDa; 21.5 kDa,[51] 20.84 kDa,[52] and 11.1 kDa.[34] On the other hand, *Acacia mangium*-derived xylan was reported to be 28 kDa,[10] which is similar to the weight reported in the current study.

Bioactivity of xylan – antioxidant activity

Ascorbic acid, a model antioxidant compound, exhibited an EC50 of 0.06 mg/mL (344 µmol L^{-1}) against DPPH (Fig. 6). The acacia xylan exhibited an EC50 value (the concentration of a compound that gives half-maximal response) of ≈1.5 mg/mL, whereas the beech xylan did not display any antioxidant capacity. Interestingly, a recent study showed that acidic XOS derived from beech xylan exhibited higher antioxidant activity (measured by the ABTS·+ discolouration assay) than the unmodified xylan, whereas neutral XOS displayed no antioxidant activity.[51] We suppose the antioxidant activity exhibited by acacia xylan may be due to the enrichment in acidic groups (MeGlc*p*A and 2-*O*-acetyl-Xylp or 3-*O*-acetyl-Xylp) compared to beech xylan with lower content of these acidic groups. Previous studies have confirmed uronic acid[6,51,53] and ferulic acid[53] substituents to be contributors to the antioxidant profile of xylan and its oligosaccharides. Considering that no further purification or purity evaluation was conducted on the xylan extracts, we cannot completely rule out the possibility that an impurity, besides protein and phenolics, which were not detected in the xylan, may have contributed to the antioxidant activity of the xylan.

Evaluation of xylanase activity on xylan

The applicability of the acacia-derived xylan as a xylanase assay substrate substitute for beech xylan was evaluated using

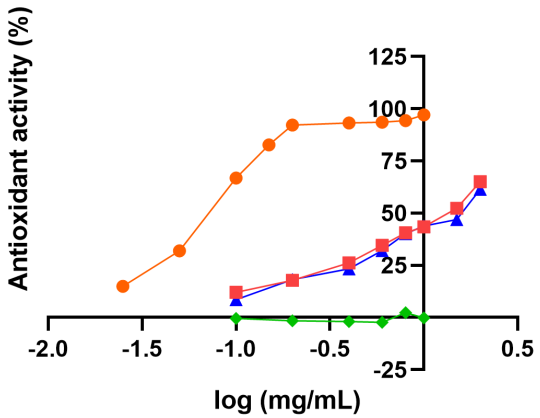

Figure 6. A sigmoid antioxidant dose-responses curve of xylan samples; alkaline extracted acacia (red squares), ultrasound-assisted alkaline extracted acacia (blue triangles), and beech (green diamonds), against 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) to determine the EC50:50% maximal effective concentration. Ascorbic acid (orange circles) was used as a positive control. Values are represented as means ± standard deviations, n = 3.

a GH11 xylanase, Xyn11A. Xylanases are used in numerous industrial applications, including fruit-juice processing, paper production, and biofuels. Interestingly, Xyn11A displayed over 2.5-fold more activity on the commercially available beech-derived xylan (27.91 U/mg) than the acacia-extracted xylan (≈10.8 U/mg) (Table 3). This was not surprising because the acacia xylan contains more Araf and MeGlc*p*A substituents than beech, which could have been reported to sterically hinder the activity of glycoside hydrolase (GH) family 11 derived xylanases.[13,54] Similarly, another study showed that the *N. patriciarum* Xyn11A has a lower catalytic rate for wheat flour arabinoxylan (39% α-linked L-*Araf*) than acid-debranched wheat flour arabinoxylan (26% α-L-*Araf*), whereas other xylanases, such as those from *Aspergillus niger* and *Cellulomonas mixtus*, exhibited the highest activity on beech glucuronoxylan (10% 4-O- MeGlc*p*A) among the three substrates.[14] This further proves that a high level of xylan substitution tends to hinder xylanase activity, particularly from GH11. Overall, these results confirmed that the extracted xylan could be used as a substrate for the screening and biochemical characterization of xylanases.

Comparison of the xylan extracted by the two methods

Based on the chemical profile, supported by carbohydrate and ferulic acid content (Table 2), and physical properties, denoted by XRD (Fig. 4) and TGA (Fig. 5), it could be concluded that the addition of the ultrasound cavitation step during alkaline extraction of xylan from acacia led to no structural changes in the fractionated hemicellulose. Biologically, the two extracted xylans behaved similarly when used as a xylanase substrate (Table 3) and employed as an antioxidant against DPPH (Fig. 6), further supporting the view that the two products exhibit similar chemical and structural profiles. This finding was not surprising, as previous studies have shown that polysaccharides such as corn xylan could be extracted with ultrasound without observation of substantial changes in its structure, molecular properties, or biological activity.[55]

Table 3. Enzyme specific activity of Xyn11A on acacia-derived xylan and commercial beech xylan.

Xyn11A specific activity (U/mg)		
Alkaline xylan	Alkaline-ultrasound xylan	Beech xylan
10.07 ± 0.5	11.56 ± 0.4	27.91 ± 0.9

Note: Values are represented as means ± standard deviations, n = 3.

Conclusions

Ultrasound-assisted alkaline extraction of xylan from acacia was demonstrated to be more effective than the conventional alkaline extraction method: 24% versus 21% extraction yield and extraction periods of 4 versus 1 h for alkaline and ultrasound-assisted alkaline extraction, respectively. Carbohydrate composition and structural analysis showed that the extracted xylan is composed of a D-xylose backbone substituted by 4-*O*-methyl-D-glucuronic acid residues and minor arabinofuranosyl residues, which is unusual in hardwood xylans. Overall, the structural and chemical properties of the extracted xylan were similar to those of beech xylan. Interestingly, acacia xylan exhibited antioxidant activity, which may be useful for good health status when used as an additive in the food and feed sector.

Author contributions

Claudious Gufe: Methodology, visualization, formal analysis, investigation, writing – original draft. Mapitsi S. Thantsha: Conceptualization, supervision, formal analysis, validation, writing – review and editing. Samkelo Malgas: Conceptualization, formal analysis, methodology, funding acquisition, supervision, project administration, visualization, writing – original draft, writing – review and editing.

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Conflicts of interest

There are no conflicts to declare.

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