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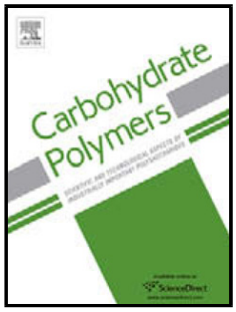

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PII: S0144-8617(19)30980-4

DOI: https://doi.org/10.1016/j.carbpol.2019.115313

Reference: CARP 115313

To appear in:

Received Date: 6 May 2019

Revised Date: 6 September 2019

Accepted Date: 9 September 2019

Please cite this article as: Fernández PV, Zelaya VM, Cobello L, Vega AS, Ciancia M, Glucuronoarabinoxylans and other cell wall polysaccharides from shoots of *Guadua chacoensis* obtained by extraction in different conditions, *Carbohydrate Polymers* (2019), doi: https://doi.org/10.1016/j.carbpol.2019.115313

Glucuronoarabinoxylans and other cell wall polysaccharides from shoots of *Guadua chacoensis* obtained by extraction in different conditions

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Highlights

* Major amounts of glucuronoarabinoxylans (GAX) were obtained from bamboo shoots.
* Exhaustive alkaline extraction and alternative extraction with DMSO were performed.
* GAX showed acetylation mainly on C3, but also on C2 of some xylose units.
* Arabinogalactans and pectins, mixed linkage glucans and xyloglucans were obtained.
* The whole cell wall polysaccharide system from this raw material was characterized.

Abstract

Guadua is a genus of woody bamboos, native to the American continent, which comprises economically important species. Cell wall polysaccharides from young shoots of *Guadua chacoensis* showed glucuronoarabinoxylans (GAX) as the major hemicellulosic components (65%, of the recovered carbohydrates by extraction with aqueous solutions), which were obtained in major quantities with 1M KOH (molar ratio Xyl:Ara:GlcA, 100:28:8). AGP and pectin polymers, as well as mixed linkage glucans and xyloglucans were present in smaller amounts (16% and 15%, respectively). Alternative extraction of GAX with solutions of DMSO allowed the characterization of alkali labile substituents, in particular, acetyl groups, which were linked mainly to C3 or, to a lesser extent, to C2 of some of the xylose residues. Besides, small amounts of phenolic compounds, released by saponification, were detected, linked to C5 of some α-L-arabinofuranose units. These results could be relevant for their use as a new resource for the food industry.

Keywords: bamboo shoot; glucuronoarabinoxylan; *Guadua chacoensis*, acetylation pattern, cell wall, grasses

1. Introduction

Grasses represent the major source of energy ingested directly or indirectly by humans (Vogel, 2008). They also have other important uses, as forages, fibers, and biofuels. Their production leaves a large amount of biomass available, comprising different plant organs and tissues, very rich in cell

wall components. Besides a certain degree of variability (e.g. due to taxonomic, phenological, environmental factors), grasses share a common model for their primary cell walls, known as Type II, as opposed to Type I cell walls present in dicot and noncommelinoid monocots. Type II primary cell walls are rich in cellulose and hemicellulosic polysaccharides, mainly glucuronoarabinoxylans (GAX), but also mixed linkage β-glucans (MLG) and small amounts of xyloglucans (XG), while pectins and structural proteins are relatively scarce (Vogel 2008). GAX have a (1→4)-β-D-xylopyranose backbone, with α-L-arabinofuranose and α-D-glucuronic acid side chains; they can also bear acetyl groups on C2 and/or C3 of some of the xylose units, and ferulic acid attached to some of the arabinose units. MLG are linear polymers of glucose that contain both β-(1→3)- and β-(1→4)-linkages, present in the cell walls of grasses. XG also have a (1→4)-β-D-glucopyranose backbone, substituted with α-D-xylose on C6 of some glucose units (Vogel, 2008). XG from grasses usually lack galactose and fucose, which appear on the side chains of XG from dicots (Carpita, 1996). GAX comprise 17-35 % of the grass biomass, varying depending on their source (Tye, Lee, Abdullah, & Leh, 2016).

Nowadays, this is an active field of research, which comprises the development of new uses for compounds derived from the most common crops, as well as the exploration of alternative grass resources, as some woody bamboo species. For example, great efforts are being undertaken to optimize bioethanol production including, not only cellulose, but also xylans. Mixing different fermenting microorganisms is an alternative method in ethanol production, known as simultaneous saccharification and cofermentation (SSCF). This approach allows the mixed-culture microbes to commence the continuous process without sugars separation (Aditiya, Mahlia, Chong, Nur, & Sebayang, 2016). Besides, xylans have been investigated as raw material for preparation of biofilms and biocomposites, and for production of xylooligosaccharides with prebiotic, antioxidant, and other biological activities (Deutschmann & Dekker, 2012, Singh, Banerjee, & Arora 2015; Naidu, Hlangothi, & John 2018).

As one of the fastest growing plants, woody bamboos represent an interesting source of biomass for

traditional and new uses. Bamboo shoots are the new culms that arise from the rhizomes containing nodes and internodes in a miniaturized form (Choudhury, Sahu, & Sharma, 2012; Nirmala, Bisht, & Haorongbam, 2011). Although a total amount of 1670 bamboo species has been described, only around 200 species are utilized for their edible shoots (Nirmala, Bisht, & Haorongbam, 2011; Soreng et al., 2017), especially in Asia. These structures bring together desirable nutritional properties (high dietary fiber, low fat, and rich mineral content), and they are also a natural source for the extraction of antioxidant compounds (Chung, Cheng, Lin, & Chang, 2012). The cell walls of bamboo shoots from some species were studied. Chang, Chang, Chang, & Yeh (2013) analyzed some aspects of cell wall variations along different growth stages of the shoots from *Dendrocalamus latiflorus* Munro, using both chemical and immunohistological methods, but *Phyllostachys* Siebold & Zucc. is possibly the most explored genus, regards the cell wall components. Xylans, arabinogalactans, and α-glucans were reported as major water soluble polysaccharides (Maekawa & Kitao, 1973; Maekawa 1975a, b). In addition, different hemicellulosic components from *P. edulis* (Carrière) J. Houz. were characterized: xyloglucans, arabinoxylans, and mixed-linkage glucans (Ishii & Hiroi, 1990a, b; Edashige & Ishii, 1998), and a boron-rhamnogalacturonan-II complex was isolated (Kaneko, Ishii, & Matsunaga, 1997). More recently, water soluble polysaccharides, possibly a mixture of pectic and hemicellulosic polymers, from the shoots of *P. praecox* C. D. Chu & C. S. Chao were obtained, and their prebiotic activity was evaluated (He et al., 2016). Also, the structure of different GAX, showing a broad structural variability, and other cell wall polysaccharides from *P. aurea* Carrière ex Rivière & C. Rivière were described (Zelaya et al., 2017).

Guadua Kunth is a genus native to the American continent (Judziewicz, Clark, Londoño, & Stern, 1999). *Guadua angustifolia* Kunth has economic importance in tropical regions, and *G. chacoensis* (Rojas) Londoño & P. M. Peterson seems to have similar economic potential (Londoño & Peterson, 1992; Lizarazu, Rúgolo de Agrasar, & Vega, 2013); however, there are no studies available about the cell wall of its shoots and culms. Recently, perennial leaves of *G. chacoensis* were evaluated as

a promising food source for ruminants (Panizzo, Fernández, Colombatto, Ciancia, & Vega, 2017), and important amounts of cellulose and GAX, as well as small quantities of mixed linkage glucans and pectins were found.

In this work, characterization of the major carbohydrate components of cell walls from shoots of *G. chacoensis* obtained in different extraction conditions was carried out. First, the water soluble cell wall components were studied, and the residue obtained was treated in two different ways: A classical extraction sequence comprising aqueous solutions allowed exhaustive solubilization of different cell wall components, leaving a final residue composed by cellulose as the only major carbohydrate. Extraction was also carried out with DMSO solutions, which allowed separation and characterization of GAX, including their acetylation pattern, taking into account that degree of acetylation and the position of these substituents can modify the physicochemical properties of polysaccharides, and it can vary between plants, cell types and stages (Pauly & Ramirez 2018). Our results show that shoots of *G. chacoensis* constitute an interesting resource, particularly for the major quantities GAX, which have many possible industrial applications.

2. Experimental

2.1 Plant Material

2.1.1. Plant Species

Young shoots of *Guadua chacoensis* (Rojas) Londoño & P. M. Peterson (Poaceae, Bambosoideae, Bambuseae), cultivated at the Botanical Garden of the School of Agriculture (University of Buenos Aires), were used in this study. A voucher specimen was deposited at Gaspar Xuarez Herbarium (BAA; Thiers, 2017), according to the following specification: ARGENTINA. Buenos Aires: Cult. en el Jardín Botánico de la Facultad de Agronomía, 12 Jun 2008, *A. S. Vega & T. San Martín 17* (BAA).

2.1.2. Sampling.

A pool of young shoots of similar size (30-35 cm long), was collected during spring of 2014 and 2015. Emerging shoots were surrounded by a shoot shell, which was removed before selection of shoot material used in this study **(Figure 1A and B).**

2.2 Study of cell wall polysaccharides

Fresh shoots were cut in small pieces, dried in an oven at 50º C, and then milled in a high-speed disintegrator (Arcano FW 100, 60-200 mesh). The milled material was extracted with 70% EtOH (80g/ L) for 4 h at room temperature, giving a residue (AIR: alcohol insoluble residue). The AIR was treated with α-amylase (type VI-B from bovine pancreas; Sigma); a suspension of the sample (40 mg/mL) in phosphate buffer 0.1M at pH 6.9, containing 5-6 mg of the enzyme, was kept 24 h at room temperature with constant agitation. The suspension was centrifuged (3,000 rpm, at 20ºC, for 25 min) giving a product that was recovered from the supernatant after freeze-drying, and a residue, which was treated in a similar form once more. In this way, extracts W-a and W-b were obtained. The residue obtained from the enzymatic treatment (AIR/α-amylase) was treated in two different ways **(Figure 1C)**.

2.2.1. Extraction with aqueous solutions

The AIR/α-amylase was sequentially extracted at room temperature with 0.05 M cyclohexane diamine tetraacetic acid (CDTA) at pH=6, 0.05 M Na_2CO_3, and 1M and 4M KOH solutions (40g /L) in a same way (twice with each solvent), giving 8 extracts (CDTA-a and CDTA-b, Na_2CO_3-a and Na_2CO_3-b, 1M KOH-a and 1M KOH-b, 4M KOH-a and 4M KOH-b) and a final residue (FR) (Fry, 1988). All the extracts and residues were dialyzed (MWCO 6-8,000) against tap water for 48 h, then against distilled water for further 24 h, and finally freeze dried. Extracts obtained with CDTA solutions were also dialyzed against 0.5 M CH_3CO_2Na to achieve complete removal of the chelator.

2.2.2. Extraction with DMSO solutions

The AIR/α-amylase was extracted with DMSO (0.1g/mL) at 80ºC for 3 h. After cooling to room temperature, the supernatant was separated by centrifugation and dialyzed (MWCO 6-8000) against tap water for 5 days, then against distilled water for further 24 h, and finally freeze dried. This procedure was repeated once more. Both extracts were analyzed together as extract D1. The residue was further extracted with an 8.4% LiCl solution in DMSO (Petruš, Gray, & BeMiller, 1995), at 80ºC for 24 h (0.1g/mL). Finally, extraction was repeated in the same way, but the temperature was raised to 100ºC. In this case, a precipitate appeared in the dialysis bag, which was separated by centrifugation. This product was obtained in a 1.0% yield regards the cell wall material, and had low content of carbohydrates (13.8%), being glucose the major monosaccharide component (69%). The supernatant was freeze dried and analyzed as extract D3.

2.2.3. Purification of W-a

Extract W-a was treated with protease. A suspension of the sample (2 mg/mL) in Tris buffer 0.15M at pH 7.8, containing the enzyme (Type XIV Protease form *Streptomyces griseus*), was kept 24 h at room temperature with constant agitation, and then dialyzed (MWCO 6-8,000), giving a product containing 29.6 % of carbohydrates and 48% recovery. A second treatment was done in the same way, giving W-aP, which was constituted by 32.1 % of carbohydrates, and represented only 13.8 % of W-a.

2.2.4. General methods of analysis

Total carbohydrates content was analyzed by the PhOH–H_2SO_4 method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956); for these determinations, samples were prepared considering them as insoluble material (Ahmed & Labavitch, 1977). Lignin content was estimated as the sulfuric acid insoluble residue: the sample (5-10 mg) was treated 10 min with concentrated sulfuric acid (1 mL); then water was added (1 mL) and the sample was stirred overnight at room temperature. After centrifugation, the residue was washed with water and then with ethanol, and finally dried at and weighted until constant weight. Total nitrogen was estimated, after total degradation of the sample,

by conversion to N_2. The gas mixtures were separated by gas chromatography (GC) with a Porapak column in Carbo Erba EA 1108 chromatograph (Milan, Italy), using a thermic detector, and the total protein content was expressed using a factor of 6.25. Hydroxyproline content was determined according to Leach (1960), after hydrolysis of the samples with HCl 6M (24 h, 105ºC). Dot-blot assay was performed with β-Glc Yariv phenylglycoside reagent to localize arabinogalactan proteins (AGPs) due to its specificity, and α-Man Yariv phenylglycoside reagent was used as a negative control, while gum arabic was used as standard (Yariv, Rapport, & Graf, 1962). Total phenolics (cell wall phenolic esters, conjugated phenolic acids and free phenolics) were determined following the method of Bunzel et al. (2000). After saponification, the phenolic content was evaluated in the supernatant using the Folin–Ciocalteu technique (Shui & Leong, 2006). Free phenolics were determined in the same way, dissolving the samples in methanol (1mg/ml), without previous saponification. Gallic acid (Merck, Germany) was used as standard, and results were expressed as µg of gallic acid equivalent (GAE) per mg of sample. Bound phenolics were calculated as the difference between total and free phenolics.

The monosaccharide composition was determined by hydrolysis and conversion of the monosaccharides to the corresponding alditol acetates, by dissolving the samples in TFA 13M (37°C, 1 h), followed by dilution of the acid to 11.5 M heating at 100°C for 1 h, and further dilution to 2 M to achieve the regular hydrolysis conditions for insoluble polysaccharides (Morrison, 1988); the hydrolysate was derivatized to the corresponding alditol acetates. In some cases, the method comprising reductive hydrolysis and acetylation was carried out (Stevenson & Furneaux, 1991) (see Table 1). Galacturonic, glucuronic, and 4-*O*-methylglucuronic acids were quantified by derivatization to the corresponding N-propylaldonamide acetates, which were separated by GC, by a modification of the methods of Lehrfeld (1987) and Walters & Hedges (1988), as described before (Zelaya et al., 2017). *Myo*-inositol was used as internal standard and a factor of recovery was applied for each sugar (1.57 for GlcA and 4-*O*-Me-GlcA, and 2.29 for GalA). For extracts from the extraction procedure carried out with solutions of DMSO, uronic acids content was estimated by the

method of Filisetti-Cozzi & Carpita (1991); in these cases, samples were prepared considering them as insoluble material (Ahmed & Labavitch, 1977). The acetyl content was determined according to Hestrin (1949).

2.2.5. Methylation analysis

For soluble polysaccharides (10 mg), methylation was carried out in DMSO (1 mL) using finely powdered NaOH as base (Ciucanu & Kerek, 1984), the mixture was agitated vigorously for 1h, then cooled in an ice bath, and CH_3I (0.5 mL) was added drop by drop. The mixture was kept at room temperature for 1 h, and then it was poured into water (10 mL), and finally, dialyzed (MWCO 3,500) and freeze dried. Prior to methylation, extracts obtained with 1M and 4M KOH solutions and residue FR, which were insoluble in DMSO, were dissolved in an 8.4% LiCl solution in DMSO (Petruš et al., 1995). The methylated samples were derivatized to the alditol acetates as described for the native polysaccharides. Two or three methylation sequences were carried out to achieve permethylation of the samples.

2.2.6. Analysis of the monosaccharide composition by GC and GC-MS

GC of the derivatized samples, as well as those of the partially methylated alditol acetates were carried out on an Agilent 7890A gas-liquid chromatograph (Avondale PA, USA) equipped with a flame ionization detector and fitted with a fused silica column (0.25 mm id x 30 m) WCOT-coated with a 0.20 μm film of SP-2330 (Supelco, Bellefonte PA, USA). Chromatography was performed as follows: (a) from 200°C to 230°C at 1°C min^{-1}, followed by a 30 min hold for alditol acetates; (b) from 235°C to 248°C at 2°C min^{-1} followed by a 30 min hold for uronic acids quantitation; (c) from 160°C to 210°C at 1°C min^{-1}, from 210°C to 230°C at 2°C min^{-1}, followed by a 30 min hold for partially methylated alditol acetates arising from methylation analyses. N_2 was used as the carrier gas at a flow rate of 1 mL min^{-1} and the split ratio was 80:1. The injector and detector temperature was 300ºC.

GC–MS of the alditol acetates, N-propylaldonamide acetates, and partially methylated alditol

acetates was performed on an Agilent 7890A gas-liquid chromatograph interfaced to a GCMSQP 5977A mass spectrometer. Chromatography was performed on the SP-2330 capillary column as described above, using He as carrier gas. Mass spectra were recorded over a mass range of 30–500 amu. N-Propylaldonamide acetates were identified as explained by Zelaya et al. 2017. Partially methylated alditol acetates were identified based on previously published mass spectra (Carpita & Shea, 1989), and to differentiate between the partially methylated monosaccharides derived from different hexoses and pentoses, the published retention times were considered (Shea & Carpita, 1988). GC-MS analyses of samples are given as supplementary material (Supplementary Figure S1).

2.2.7. Nuclear Magnetic Resonance spectroscopy

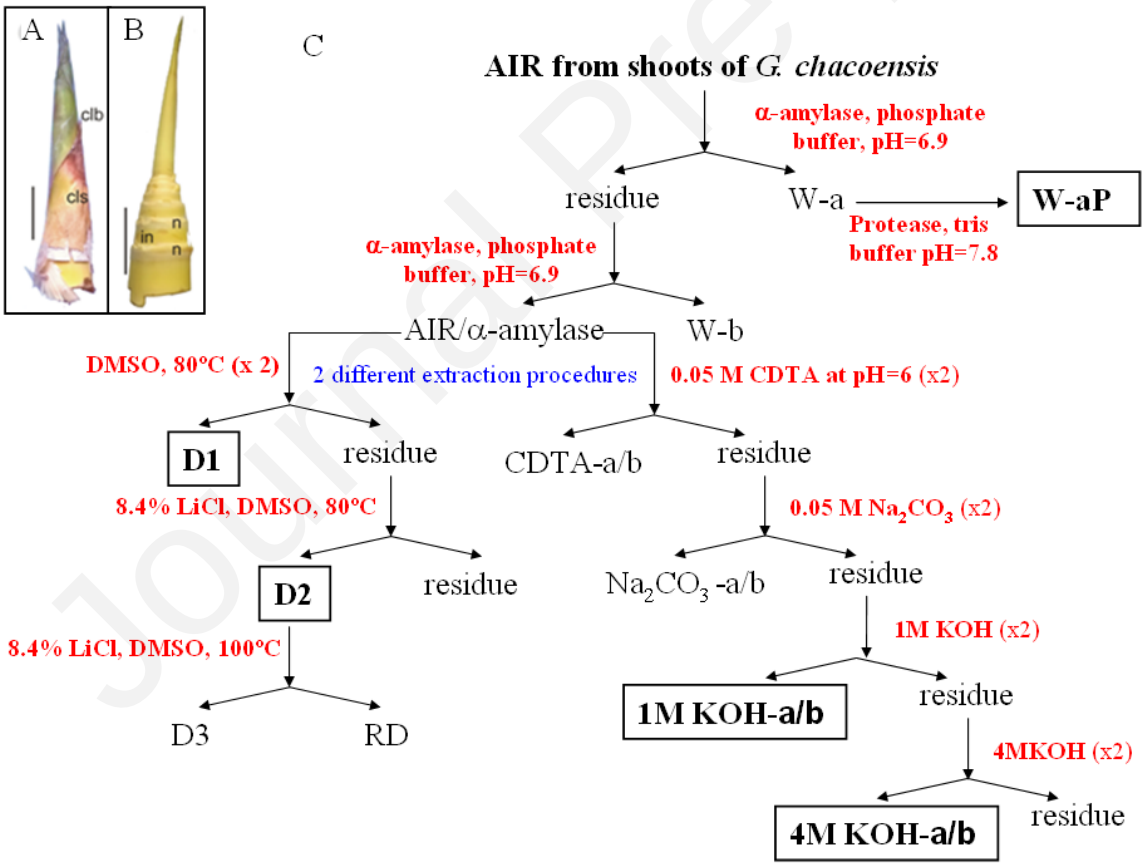
The sample (10 mg), previously exchanged with deuterium by three repeated evaporations in D_2O, was dissolved in D_2O (1 mL) and placed in 5-mm tubes. Spectra were recorded at room temperature on a Bruker Avance II 500 spectrometer (Karlsruhe, Germany). For ^{1}H NMR experiments the parameters were: a spectral width of 6.25 kHz, a 76º pulse angle, an acquisition time of 3 s, a relaxation delay of 3 s, for 32 scans. For 125 MHz proton decoupled ^{13}C NMR experiments the parameters were: a spectral width of 29.4 kHz, a 51.4º pulse angle, an acquisition time of 0.56 s, a relaxation delay of 0.6 s, for 25000 scans. Signals were referenced to internal acetone at 2.21 ppm for ^{1}H NMR and 31.1 ppm for ^{13}C NMR experiments, respectively. Pulse sequences for ^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC and HMBC techniques were supplied by the spectrometer manufacturer; spectra were recorded at room temperature and they were obtained at a base frequency of 500 MHz for ^{1}H and 125 MHz for ^{13}C.

3. Results and discussion

3.1. Extraction of cell wall polysaccharides of shoots from *G. chacoensis* and characterization of the extracts

The general extraction sequence from the alcohol insoluble residue (AIR) is shown in **Figure 1C**. AIR was treated with α-amylase in a phosphate buffer at pH 6.9 sequentially twice, obtaining extracts W-a and W-b **(Table 1)**, and a residue AIR/α-amylase. Both extracts plus residue AIR/α-amylase together were considered as the total cell wall polymers material.

Figure 1. A. Young shoot from *Guadua chacoensis*, external view. B. Young shoot devoid of culm leaves. References: clb, culm leaf blade; cls, culm leaf sheath; n, node. Scale bar 5 cm. C. Extraction sequences of cell wall polysaccharides from shoots of *Guadua chacoensis*. Extractions were carried out at room temperature, unless otherwise indicated. Extraction solvents are shown in red. Extracts studied in detail are highlighted in boxes. AIR=alcohol insoluble residue; CDTA= cyclohexane diamine tetraacetic acid.



To obtain and characterize the different polysaccharide components, AIR/α-amylase was treated in

two different ways:

1) An extraction comprising, in the first place, CDTA solutions, and then, alkaline solutions of increasing strength, was carried out, obtaining 8 extracts and a final residue (FR). FR showed glucose (>97%) as the only monosaccharide present in significant quantities (**Table 1**), being cellulose the major carbohydrate component (see later, **Table 2**), showing that the extraction procedure was exhaustive. Extracts obtained by this procedure represented 22.3% w/w of the cell wall material. The highest yields were obtained for 1M KOH-a, which gave xylose and arabinose as major monosaccharide components. On the other hand, low yields of the extracts obtained with CDTA and Na_2CO_3 aqueous solutions agree with the typical Type II cell wall found in grasses (Gibeaut & Carpita, 1993; Carpita, Ralph, & McCann, 2015). These extracts also showed very low content of total carbohydrates, so they were not studied further.

2) An extraction sequence comprising DMSO solutions, in order to determine the structure of cell wall polysaccharides, specially, GAX, including their alkali-labile substituents. This extraction sequence was not exhaustive, as low yields were obtained, and, besides cellulose, additional cell wall material remained insoluble, being detected in the final residue, RD (see later, **Table 4**).

3.2. Structural characterization of water soluble polysaccharides from *G. chacoensis*

The first extracts obtained from the shoots of *G. chacoensis*, W-a and W-b, had similar composition, with very low total carbohydrate content, and together, they represented about 12% of the cell wall (**Table 1**).

Table 1 - Yields and analyses of the extracts and residues obtained from the shoots of *Guadua chacoensis* by extraction with aqueous solutions.

Extract/Residue	Yield	TC	Lignin	Monosaccharide composition (mol %)[g]

	(%)	(%)[c]	(%)[d]	Total protein (%)	Rha	Ara	Xyl	Man	Gal	Glc	GalA	GlcA	4-*O*-Me GlcA
AIR	84.4[a]	46.4	13.7	nd[e]	tr[f]	13.0	21.0	2.1	6.0	53.7	2.9	1.3	-
W-a	8.1[b]	12.7	6.5	33.8	10.0	24.5	9.3	1.1	37.7	9.9	4.4	3.1	-
W-b	3.8	13.0	24.6	nd	7.8	26.8	13.8	tr	31.9	13.4	3.5	2.6	0.3
CDTA-a	0.8	27.8	1.8	30.5	9.6	14.4	17.3	4.3	20.7	28.5	4.2	0.9	0.2
CDTA-b	0.2	23.3	19.6	nd	8.5	22.5	18.9	2.9	15.3	25.8	5.4	0.6	0.2
Na_2CO_3-a	2.1	9.3	21.7	43.4	3.2	19.4	35.4	2.0	13.1	20.3	3.7	1.3	1.6
Na_2CO_3-b	1.1	9.9	15.4	nd	2.7	19.4	30.2	2.0	12.9	24.4	5.1	1.8	1.6
1M KOH-a	10.3	31.7	5.0	19.4	tr	16.7	60.4	-	6.2	9.2	2.8	3.8	0.8
1M KOH-b	5.3	38.2	6.9	nd	tr	13.4	62.3	tr	3.2	15.7	2.6	2.8	0.6
4M KOH-a	1.2	47.9	-	8.1	-	11.9	49.9	tr	tr	34.8	0.9	1.9	0.6
4M KOH-b	1.3	38.0	-	nd	tr	3.8	21.1	tr	2.9	67.3	2.6	2.3	-
FR	26.8	60.0	-	< 2.5	-	tr	tr	tr	tr	97.2	2.3	0.5	-

[a] Expressed as percentage of dry matter. [b] For this, and the following fractions, values express yield as percentage of cell wall (AIR treated with α-amylase + W-a + W-b). [c] TC: total carbohydrate content. [d] Lignin was estimated as the sulfuric acid insoluble residue. [e]Not determined. [f] Percentages lower than 1% are given as traces (tr), except for uronic acids. [g]For extracts obtained with water, CDTA and Na_2CO_3 solutions, derivatization was carried out as described by Stevenson & Furneaux (1991).

Purification of W-a by treatment with protease gave W-aP, with higher carbohydrate content (32.1%) (**Figure 1C**). Methylation analysis of W-aP (**Table 2**) showed mostly 6-linked and 3,6-linked galactose residues, together with 5-linked and terminal arabinofuranose units, typical of Type II arabinogalactans. In view of these linkages, the presence of hydroxyproline was investigated, and it was found that it represented 1.9% w/w of total protein content of W-a. In addition, a positive reaction with Yariv β-glucosyl reagent was observed in a dot blot assay (Yariv, Rapport, Graf, 1962), confirming the presence of arabinogalactan-proteins (AGPs) in this sample. Besides, 4-linked galactose units, which are typical of arabinogalactans from pectins (Carpita, 1996) and terminal non reducing galactose units were also present. Arabinose terminal units and 5-linked furanosic residues could also be part of the pectin structures.

Small amounts of terminal, 4-, 3,4- and 2,3,4-linked xylopyranose residues, characteristic of arabinoxylans, were detected.

Table 2 - Methylation analysis of the major extracts and the final residue obtained from shoots of *G. chacoensis* with aqueous solutions.

Monosaccharide[a,b]	Structural Unit	W-aP	1M KOH		4M KOH		FR[d]
			a	b	a	b	
2,3,5 Ara	Ara*f*(1→	15.2[c]	14.5	12.4	7.0	3.2	-
2,5 Ara	→3)Ara*f*(1→	1.0	-	-	-	-	-
2,3 Ara	→5)Ara*f*(1→	8.9	3.3	1.7	1.4	0.8	-
3,5 Ara	→2)Ara*f*(1→	0.4	-	-	-	-	-
Ara	→2,3,5)Ara*f*(1→	0.8	-	-	3.9	-	-
2,3,4 Xyl	Xyl*p*(1→	0.2	1.3	2.1	4.6	1.1	-
2,3 Xyl	→4)Xyl*p*(1→	5.1	57.6	59.6	40.6	14.3	-
2/3 Xyl	→3/2,4)Xyl*p*(1→	3.0	4.8	3.4	2.7	4.2	-
Xyl	→2,3,4)Xyl*p*(1→	1.5	0.8	0.5	3.6	2.5	-
2,3,4,6 Gal	Gal*p*(1→	10.4	6.6	3.4	tr	1.0	tr
2,4,6 Gal	→3)Gal*p*(1→	0.9	-	-	-	2.0	-
2,3,4 Gal	→6)Gal*p*(1→	13.1	-	-	-	-	-
2,3,6 Gal	→4)Gal*p*(1→	7.9	-	-	-	-	-
2,4 Gal	→3,6)Gal*p*(1→	5.4	-	-	-	-	-
Gal	→2,3,4,6)Gal*p*(1→	2.1	-	-	-	-	
2,3,4,6 Glc	Glc*p*(1→	6.9	1.2	-	5.2	-	1.3
2,4,6 Glc	→3)Glc*p*(1→	0.7	-	-	2.6	2.6	-
2,3,6 Glc	→4)Glc*p*(1→	-	5.5	10.4	15.5	59.1	75.8
2,3 Glc	→4,6)Glc*p*(1→	1.2	-	3.6	5.4	2.4	4.8
Glc	→2,3,4,6)Glc*p*(1→	1.3	3.2	1.9	7.2	5.9	7.2

[a]Methylated at the positions indicated. [b] Only traces of 2,3-linked Rha*p* were detected in the analysis. [c] Mol % of the individual extracts or residues, considering only the neutral carbohydrates. [d] FR gave small amounts of 3,4- (4.5%) and 3,4,6-linked Glc*p* (1.6%).

NMR spectra of W-aP (**Figure 2, Table 3)** showed signals of 6- and 3,6-linked β-D-galactopyranose units, in agreement with methylation analysis. Terminal and 5-linked α-L-arabinofuranose units were found, with anomeric signals at δ 110.3/5.17 and 108.4/5.00, respectively, corresponding to arabinogalactan structures (Hromádková, Smestad Paulsen, Polovka, Kostálová, & Ebringerová, 2013). Signals of the other atoms of these structural units were also assigned (Shakhmatov, Atukmaev, & Makarova, 2016). The small signals at 104.7/4.62 and 104.9/4.59 could correspond to

6-(3,6)-linked β-D-galactopyranose units or to 4-linked β-D-galactopyranose units. Both structural units were detected by methylation analysis, but, due to their low content it was not possible to assign them univocally. For 3,6-linked galactans, the difference in δ of the anomeric signal of β-D-galactopyranose units, depending the glycosidic linkage in which it is involved, was clearly determined before (Bilan, Vinogradova, Shashkov, & Usov, 2007; Shakhmatov et al. 2016). In addition, signals of α-D-galacturonic acid residues, arising from a homopolymeric backbone (homogalacturonan, HG), as well as from chains comprising alternating 4-linked α- D-galacturonic acid and 2-linked α- L-rhamnopyranose residues (rhamnogalacturonan I, RGI), were detected (anomeric signals at δ 99.5/5.11 and 98.0/5.01, respectively) (Mikshina et al., 2012; Shakhmatov, Udoratina, Atukmaev, & Makarova, 2015). A small signal at δ 99.5/5.27 was assigned to the rhamnose units (Figure 2), the presence of these units was confirmed by the characteristic signals at δ 17.7/1.27 and 17.7/1.24, due to C6/H6 of these units, with or without side chains linked to C4, respectively (Mikshina et al., 2012).

Although the presence of small amounts of GAX in W-aP was well established from methylation analysis (**Table 2**), and by traces of glucuronic acids found in the monosaccharide composition (**Table 1**), they were not detected in the NMR spectra, possibly due to differences in their solubility.

Figure 2. HSQC NMR spectrum of W-aP: (A) anomeric region in detail; (B) region comprising the remaining carbohydrate signals. References: Ara: α-L-arabinofuranose, Gal: β-D-galactopyronose, α-L-rhamnopyranose, and GalA: α-D-galacturonic acid. Cn/Hn refers to the position of the corresponding pair C/H in the sugar ring. T: terminal unit. Numbers before sugar indicate linkages of the structural unit (*i.e.*, C1/H1 6Gal (1→3) corresponds to the anomeric pair C/H of 6-linked Gal*p* units linked to C3 of another Gal*p* unit).

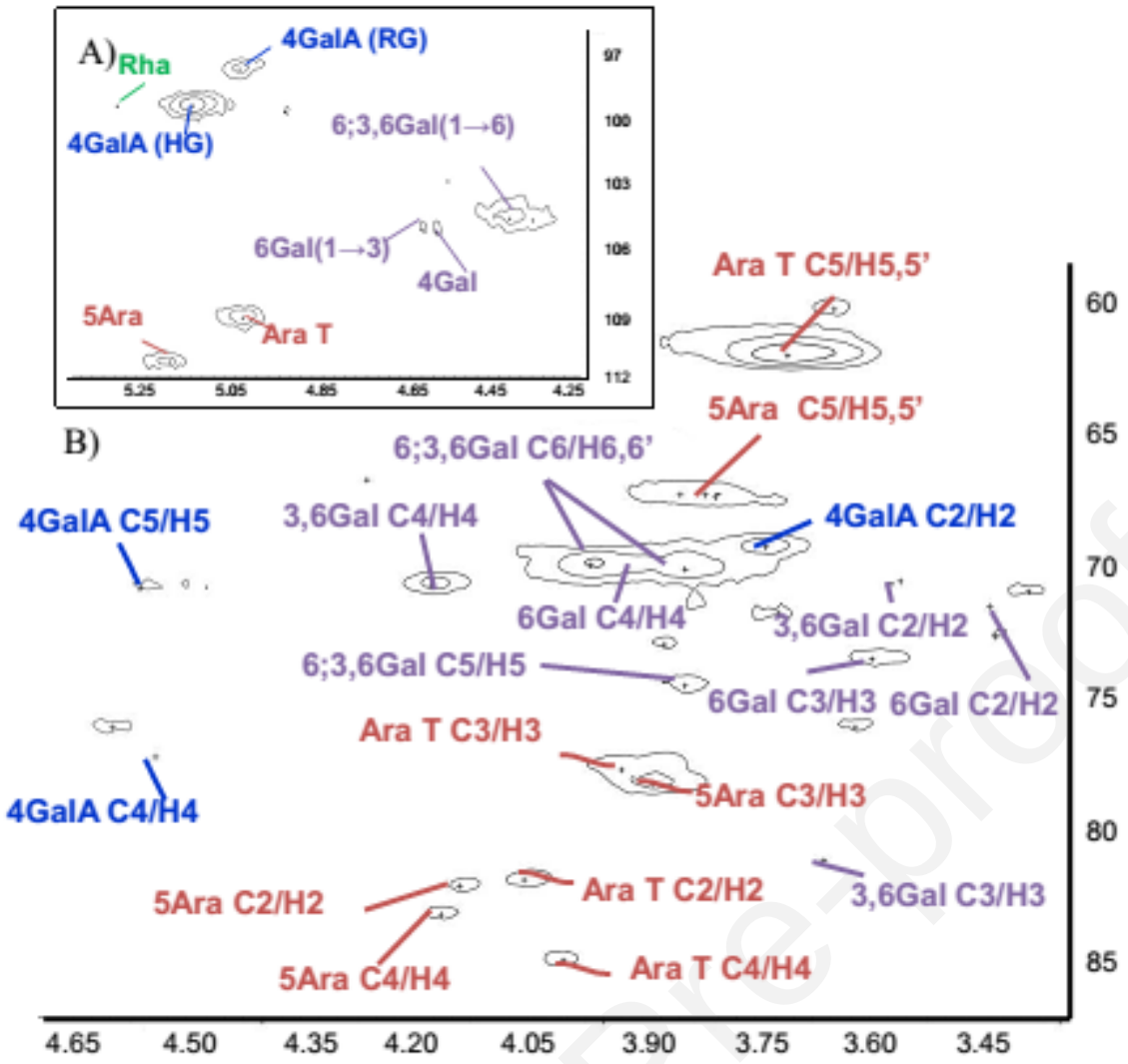


Table 3 - Signal assignments of the major structures detected in the NMR spectra of W-aP, the first water extract from shoots of *G. chacoensis*, after treatment with protease.

Structural unit	**Chemical shifts, ppm[b]**					
	C1/H1	**C2/H2**	**C3/H3**	**C4/H4**	**C5/H5,5´**	**C6/H6,6´**
Pectin backbone						
4-linked α-D-GalA (linked to Rha)	97.9/5.02					174.6
4-linked α-D-GalA	99.5/5.11	69.4/3.74	68.8/4.07	77.1/4.54	71.0/4.56	174.6
2(2,4)-Rha	99.5/5.27					17.7/ 1.24
Arabinogalactans						
T α-L -Ara*f*	110.3/5.17	81.9/4.05	77.8/3.93	85.0/4.05	62.1/3.75,3.69	
5-linked α-L -Ara*f*	108.4/5.00	82.2/4.14	77.5/3.87	83.3/4.16	67.2/3.85,3.82	

6-linked β-D-Gal*p*-(1→6)[c]	104.4/4.43	71.8/3.45	73.6/3.59	70.1/3.91	74.5/3.87	70.2/3.96
3,6-linked β-D-Gal*p*-(1→6)[c]	104.1/4.43	70.9/3.56	81.2/3.60	70.8/4.18	74.5/3.87	70.2/3.96
6-linked β-D-Gal*p*-(1→3)[c]	104.7/4.62[c]					
4-linked β-D-Gal*p*	104.9/4.59[c]					

[a] Assignment was confirmed based on previous work (Hromádková et. al., 2012, Mikshina et al., 2012; Shakhmatov et al. 2015, 2016). [b] For 3,6-linked β-D-galactans, δ of the anomeric signal varies if it is linked to C3 or C6 of the other unit (Bilan et al. 2007; Shakhmatov et al. 2016). [c]Assignment could be interchanged.

As expected for Type II walls, the cell wall material studied here was low in pectins, which were obtained in small quantities, mainly in the first extracts. Pectins from grasses were reported to comprise HG and RG I, and it was previously suggested that highly substituted GAX could be closely associated with them. Interactions between these polymers were supposed to control wall loosening activities (Carpita et al. 2015). In W-a, small amounts of GAX were extracted with pectins, suggesting a possible interaction between these polymers in the cell wall.

3.3. Hemicelluloses extracted with alkaline aqueous solutions from *G. chacoensis*

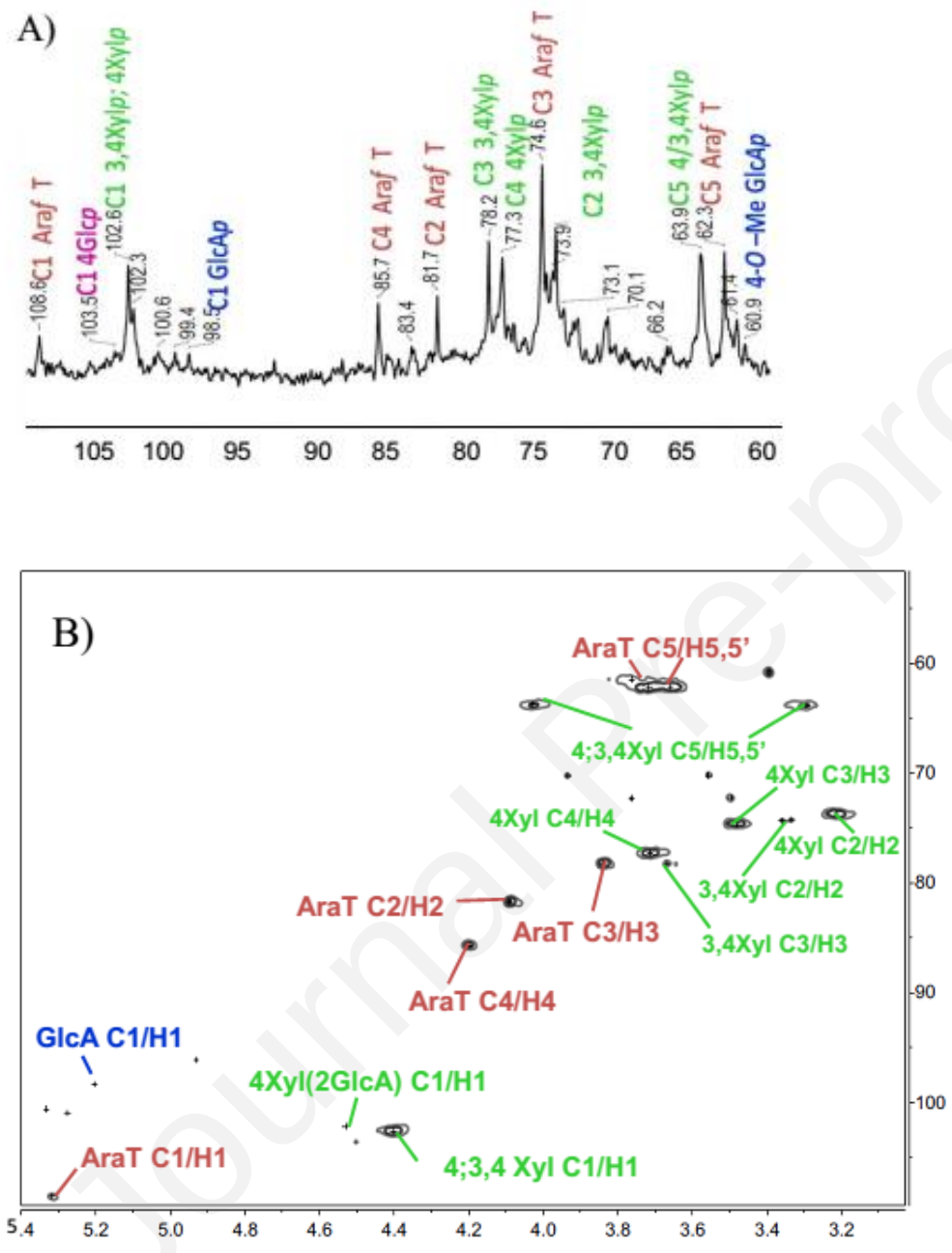
Methylation analysis of extracts obtained with alkaline solutions (**Table 2**) showed that all of them were composed by major amounts of hemicelluloses. The first one, 1M KOHa was constituted by glucuronoarabinoxylan as the major carbohydrate component, while the amount of glucans increased in the following extracts of the sequence. A degree of branching of 9.9 and 6.7% for the first and second extracts, respectively (calculated from the ratio between molar percentages of mono- plus 2x di-substituted units and total xylose units) was found for 1M KOH-a and 1M KOH-b. The side chains correspond to terminal α-L-arabinofuranose and α-D-glucuronic acid (and its 4-*O*-methylated derivative), as major structures (**Tables 1 and 2**). The small amount of disubstituted

4-linked xylose units possibly corresponds to residues bearing two arabinose side chains, as there have been no reports of both arabinose and glucuronic acid being substituted onto the same xylose unit (Kozlova, Mikshina, & Gorshkova, 2012).

In addition, terminal galactose (**Table 2**) was detected in small amounts, which was attributed to part of the xylan structure (see later, NMR analysis of this extract). The fact that, not only terminal, but also a small amount of 5-linked arabinofuranose units were detected suggests that some of these units could be substituted by the terminal galactose residues. Small amounts of terminal galactose were frequently detected by methylation of GAX from different sources, however, they were only assigned in a few cases to a defined structure. These units were found to be linked to C5 of α-L-arabinofuranose or to C4 of β-D-xylopyranose units, in both cases as part of GAX side chains (Wilkie & Woo, 1977; Saulnier, Marot, Chanliaud, & Thibault, 1995). Our results are in agreement with the first option, but with our data, the second substitution pattern could not be discarded. Analysis of the NMR spectra of 1M KOH-a (**Figure 3A** and **B**) showed major signals corresponding to 4-linked β- D-xylopyranose units, partially substituted on C3 with terminal α-L-arabinofuranose units (Zelaya et al. 2017). Minor signals in the anomeric region at δ 98.5/5.20 and 102.3/4.53, corresponding to α-D-glucuronic acid residues and to 4-linked β-D-xylopyranose units with substitution on C2, respectively, were also detected (Evtuguin, Tomás, Silva, & Neto, 2003; Guo et al., 2011). In addition a signal at δ 99.4 was clear in the ^{13}C NMR spectrum and it could be tentatively attributed to the anomeric carbon of terminal α-D-galactopyranose units of the GAX. Finally, a small signal at δ 103.4/4.50 was tentatively assigned to 4-linked β-D-glucopyranose units that could correspond to small amounts of xyloglucans (Ding et al. 2016) or mixed linkage glucans (Cui, Wood, Blackwell, & Nikiforuk, 2000).

Figure 3. (A) ^{13}C NMR spectrum of 1MKOH-A. (B) HSQC NMR spectrum of 1MKOH-a. References: Ara: arabinofuranose, Xyl: xylopyranose, GlcA: glucuronic acid, and 4-*O*MeGlcA:4-*O*-

methyl-D-glucuronic acid. Cn or Cn/Hn refers to the position of the corresponding C or pair C/H in the sugar ring. T: terminal unit. Numbers before sugar indicate linkages of the structural unit (*i.e.*, C1/H1 4;3,4 Xyl corresponds to the anomeric pair C/H of 4-linked and 3,4-linked xylopyranose units).



Comparing the major GAX from bamboo shoots of *G. chacoensis* (1M KOH-a) with that from *Phyllostachys aurea*, previously studied by our group (Zelaya et al. 2017), the former presented a significantly lower degree of ramification. This conclusion is based on the ratio Ara/Xyl (0.28 for 1M KOH-a and 0.67 for the fraction obtained in similar conditions from *P. aurea*), and on the fact

that similar amounts of uronic acids were obtained in both cases. This structural characteristic determines their solubility behavior, susceptibility to enzymes, and other functional properties, and, consequently, their possible industrial applications. Moreover, spacing of single stubs of arabinose and glucuronic acid units of GAXs could determine porosity and surface charge, functionally replacing the predominant pectic substances in Type I cell walls (Carpita et al. 2015).

Finally, extracts 4M KOH-a and 4M KOH-b presented less amounts of GAX, and a predominance of glucans, obtained in major quantities in 4M KOH-b (with a content of ~67 mol% of glucose). Small amounts of 3-linked glucose units, detected by methylation analysis, indicated the existence of mixed linkage glucans, although a small quantity of xyloglucan could also be present (Carpita, 1996). The latter polysaccharide, which is the major hemicellulose of dicotyledonous plants, is present in grasses as a minor cell wall component, including bamboo shoots (Edashige & Ishii, 1998). The significant amounts of 4,6-linked glucose units, as well as terminal xylose units detected by methylation analysis, mainly in 4M KOH-a confirmed the presence of these polymers (**Table 2**).

To have a general view of the whole material extracted, approximate calculations of the amounts of each polysaccharide type obtained were carried out, and these results are shown in **Supplementary Table S1**. These values also permitted estimation of the approximate amounts of the different types of polysaccharides obtained by this extraction procedure: GAX, 65%; AGP+Pectin, 16%; and XG+MLG, 15%. In addition they showed the distribution of the major quantities of the polysaccharide types in the different extracts, namely, GAX in 1MKOH-a and b, and 4MKOH-a (88, 82, and 64%, respectively); AGP+Pectins, in W-a and W-b (74 and 67%, respectively); and XG+MLG in 4MKOH-b (70%).

3.4. Extraction of *G. chacoensis* with DMSO solutions. Characterization of alkali-labile substituents of the GAX backbone.

With the aim of further characterizing the GAX from *G. chacoensis*, AIR/α-amylase was extracted twice with DMSO at 80ºC for 3 h. Preliminary analyses (not shown) showed very similar composition for both extracts, which were obtained in small yields, so they were worked up together as extract D1. The residue from this extraction procedure was further extracted with an 8.4% LiCl solution in DMSO at 80ºC for 24 h (Petruš et al. 1995) to give extract D2 and a residue **(Figure 1C, Table 4**), which still comprised 14% of xylose and small amounts of sugars other than glucose. Another extraction in similar conditions, but at 100ºC, gave extract D3 and the residue RD, which did not change significantly in monosaccharide composition regards the previous residue (12.2% of xylose). Hence, it was assumed that the extraction with this solvent was exhaustive, although small amounts of polysaccharides other than cellulose were present in RD. Only D1 and D2 were chosen for further studies.

Table 4 - Yields and analyses of the extracts and residue obtained from the shoots of *Guadua chacoensis* by extraction with DMSO solutions.

Extract/Residue	Yield (%)[b]	TC (%)[c]	Uronic Acids[d] (%)	Acetylation (%)	Phenolics (μg/mg)[f]		Neutral Monosaccharides composition (mol %)					
					free	linked	Rha	Ara	Xyl	Man	Gal	Glc
D1	2.9	54.2	5.9	14.3	8.6	0.5	1.9	30.0	22.1	2.9	15.7	27.4
D2	3.3	41.0	7.7	14.8	5.1	3.8	tr[g]	22.8	28.0	3.8	5.9	39.5
D3[a]	1.0	47.5	8.3	8.5	20.1	0.0	4.4	12.0	23.7	7.4	13.1	36.2
RD	41.9	60.1	6.6	nd[e]	8.8		tr	4.9	12.2	3.2	2.0	76.9

[a]3.2% of Fuc was detected in this extract. [b] Values express yield as percentage of cell wall (AIR treated with α-amylase plus the soluble material recovered from the aqueous phase after this treatment). [c] TC: total carbohydrate content. [d] D1 comprised 2.7 % of galacturonic acid, while in D2 and RD only traces of this uronic acid were detected. [e] nd=not determined. [f]Expressed as μg of gallic acid per mg of the sample. The amount of linked phenolics was determined as difference between the total amount obtained after saponification of the sample, and the free phenolics. [g]Percentages lower than 1% are given as traces (tr).

Monosaccharide composition of these extracts showed major amounts of GAX, but also important quantities of other polysaccharides, like pectins, confirmed by the small percentages of galacturonic acid and rhamnose detected, glucans (MLG and XG), and mannans.

Only low quantities of phenolics were attached by ester linkages to the cell walls (**Table 4**). Although in small amounts, similar to those reported for other Poaceae, these substituents have been previously studied in detail in some grasses, and they are believed to be an important structural characteristic of GAX. They comprise ferulic acid, and, to a lesser extent, other phenolic acids, and they enable crosslinking between GAX molecules, as well as linkages between GAX and other cell wall polymers, providing structural support to the plant, enhancing insect and disease resistance, and they were also reported to regulate cell elongation (Schendel, Becker, Tyl, & Bunzel, 2015).

Methylation analysis of D1 and D2 showed the partially methylated monosaccharides expected, taking into account data described above (section 3.3.), and considering that the methylation procedure involved alkaline medium, and the consequent ester hydrolysis (**Table 5**). Some differences between these extracts are worth to note: 1) The degree of ramification of the xylan backbone was lower in D2 (molar ratio monosubstituted+2xdisubstituted/total Xyl of 0.48 and 0.26 for D1 and D2, respectively), 2) A high degree of undermethylation was observed, but only for glucose partially methylated derivatives in D2; this result suggests different structures for glucans of both samples (see later), 3) The high amounts of galactose derivatives in D1 comprised terminal, and 6-, 3,6-, and 4-linked galactose units, while in D2, only a small amount of galactose derivatives was found, only as 4-linked units.

Table 5- Methylation analysis of the extracts obtained from shoots of *G. chacoensis* with DMSO solutions.

Monosaccharide[a]	**Structural Unit**	**D1**[b]	**D2**[b]
2,3,5 Ara[c]	Ara*f*(1→	12.5	13.4
2,3 Ara	→5)Ara*f*(1→	9.9	5.3
3,5 Ara	→2)Ara*f*(1→	-	1.6

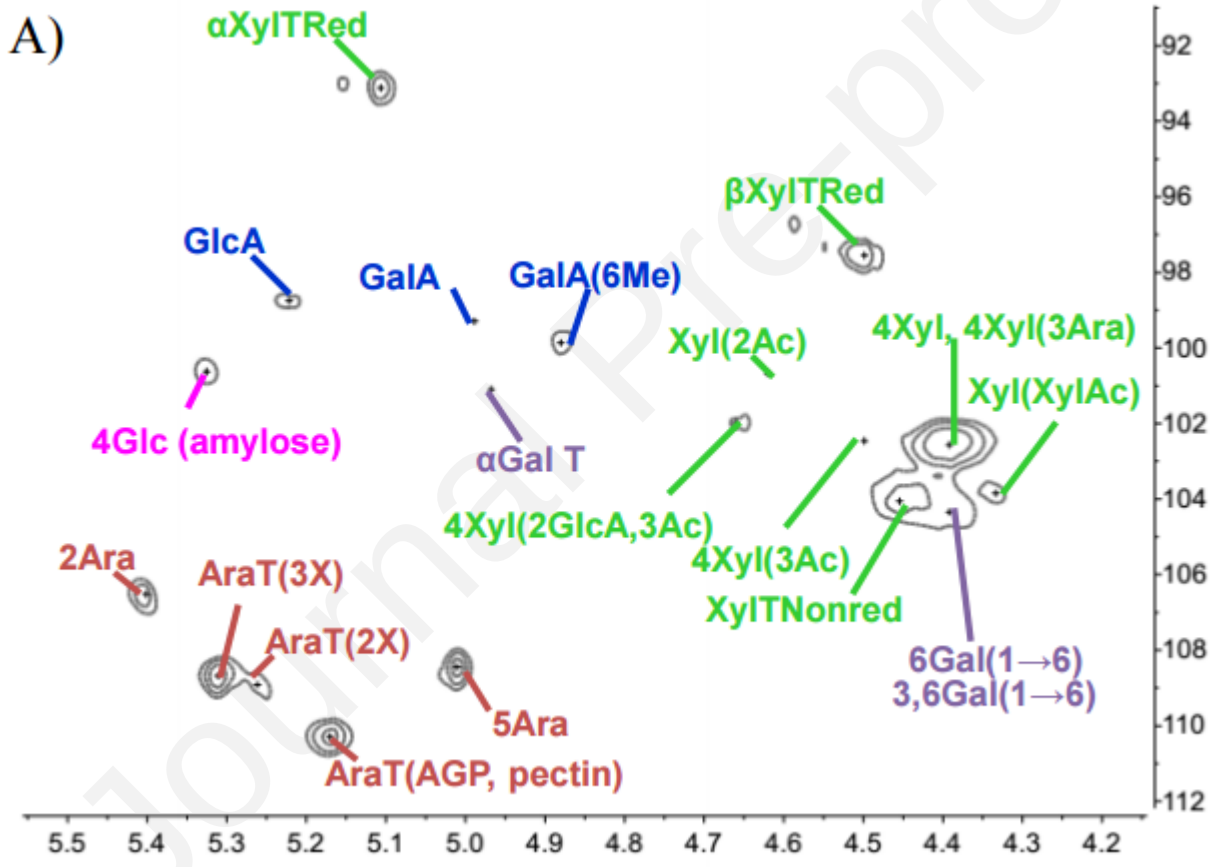
Ara	→2,3,5)Ara*f*(1→	7.2	1.6
2,3,4 Xyl	Xyl*p*(1→	-	2.1
2,3 Xyl	→4)Xyl*p*(1→	14.1	19.6
2/3 Xyl	→3/2,4)Xyl*p*(1→	5.6	5.1
Xyl	→2,3,4)Xyl*p*(1→	2.5	1.1
2,3,4,6 Gal	Gal*p*(1→	4.4	-
2,3,4 Gal	→6)Gal*p*(1→	3.2	-
2,3,6 Gal	→4)Gal*p*(1→	3.3	4.9
2,4 Gal	→3,6)Gal*p*(1→	2.3	-
Gal	→2,3,4,6)Gal*p*(1→	1.7	-
2,3,4,6 Glc	Glc*p*(1→	14.7	4.6
2,4,6 Glc	→3)Glc*p*(1→	1.2	1.5
2,3,6 Glc	→4)Glc*p*(1→	5.4	-
3,4,6 Glc	→2)Glc*p*(1→	1.5	1.2
2,3 Glc	→4,6)Glc*p*(1→	1.1	13.8
Glc	→2,3,4,6)Glc*p*(1→	3.5	18.4
2,3,6-Man	→4)Manp(1→	1.6	-
Man	→2,3,4,6)Man*p*(1→	1.4	-

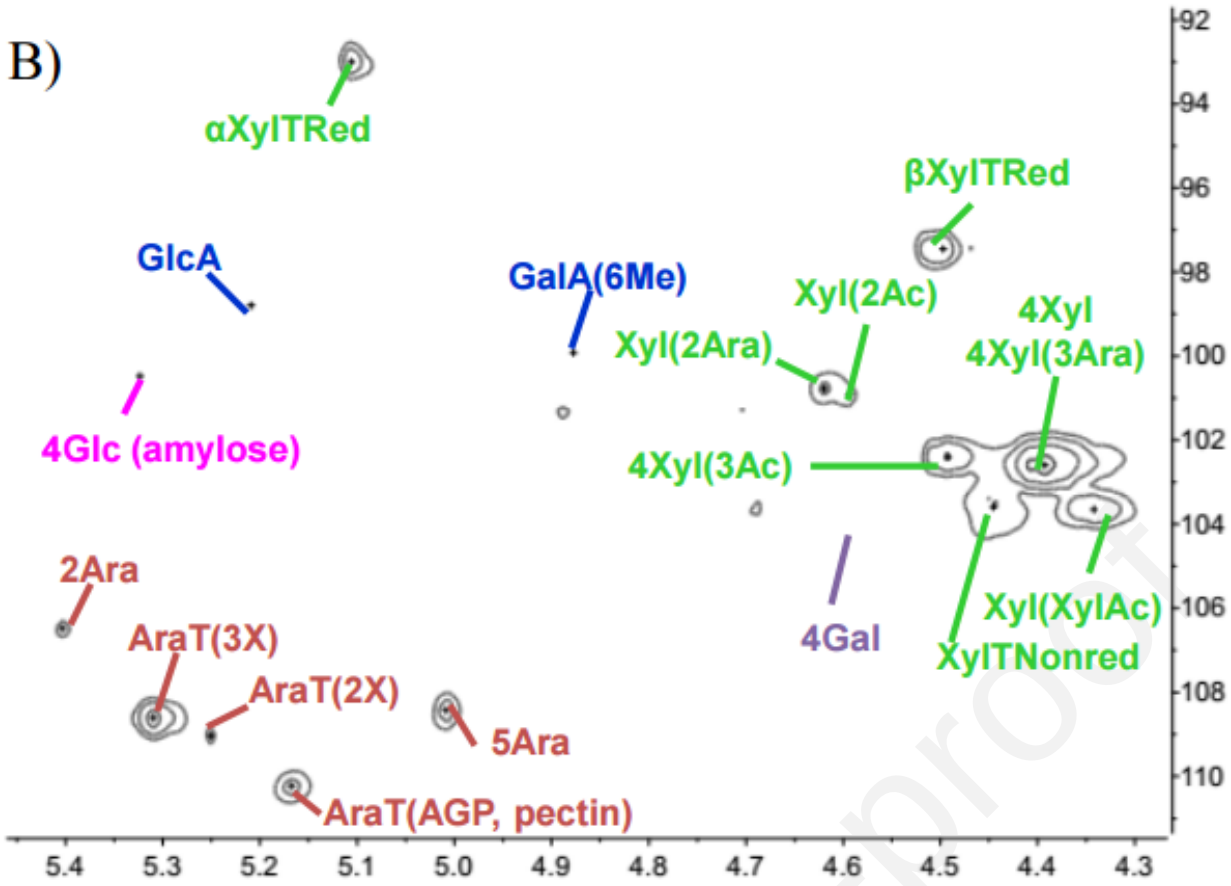
[a]Methylated at the positions indicated. [b]Mol % of the individual extracts or residues, considering only the neutral carbohydrates. [c]The amount of terminal α-L-arabinofuranose residues from GAX and from pectins was estimated from the ^{1}H NMR signals at δ 5.31 and 5.17 (6.8 and 5.7 %, respectively for D1, and 10.0 and 3.4 %, respectively, for D2).

When both samples were prepared for NMR (10 mg/mL D_2O solutions), most of the material in extract D1 was dissolved (73% of the recovered material), however, only a small amount of D2 was soluble (21% of the recovered material) in these conditions. The monosaccharide composition of the soluble and insoluble materials was analyzed for each extract. For D1, the amount of glucose was 23 and 57 %, while for D2, it was 4 and 71 %, for the soluble and insoluble fractions, respectively (**Supplementary Table S2**). These results suggested that in the soluble fractions, glucose derived principally from partially hydrolyzed α-glucans, which were not completely degraded during the treatment of AIR with α-amylase (this was confirmed by analysis of the NMR spectra, **Figure 4**), while in the insoluble moieties, glucose derived from β-glucans, possibly, mixed linkage glucans and small amounts of xyloglucans, scarcely soluble in water. Hence, in spite of the yields of the soluble fractions, they were considered representative of the GAX present in the corresponding extract.

HSQC NMR spectra of these soluble fractions from extracts D1 and D2 were very complex, as shown by the presence of many signals in the anomeric region (**Figure 4**, the complete HSQC NMR spectrum of D2, as well as the ^{13}C NMR spectrum of D2 are shown in **Supplementary Figure S1)**.

Figure 4. Anomeric region of the HSQC NMR spectra of (A) D1 and (B) D2. References: Ara: arabinofuranose, Xyl: xylopyranose, Glc: glucopyranose, GlcA: glucuronic acid, and GalA: galacturonic acid. T: terminal unit. Red: reducing end. Nonred: non-reducing end. Ac: acetyl group. Me: methyl ester. In parenthesis, position and/or type of substituent are indicated.





Some of the signals were previously found in the spectra analyzed before in this work. The signal at δ 102.6/4.40 was the most important, and it was previously assigned to 4-linked xylopyranose units, partially substituted by α-L-arabinofuranose side chains on C3; the anomeric signal of the latter residue is also clear at δ 108.6/5.31, and the rest of the signals of these units were assigned in spectra of both samples (**Table 6**). Close to the latter signal, there was another one at δ 108.9/5.26, which was assigned to terminal α-L-arabinofuranose linked to C2 of some xylopyranose units (Hromádková et al. 2013). Besides, only one clear signal was observed in both spectra at δ 98.8/5.21 assigned to the anomeric carbon of α-D-glucuronic acid, linked to C2 of some xylose units of the xylan backbone. The fact that the signal of the 4-*O*-methyl substituent at δ 60.8/3.38 was important, suggests predominance of the 4-*O*-methylated derivative of glucuronic acid. Some small signals of other carbons of this unit, as well as those of the corresponding xylose residues (only clear in D1), were detected in the spectra. These spectra also showed small signals assigned to terminal reducing and non reducing xylose units; their anomeric signals were present at δ 97.6/4.50

and 93.1/5.11, and at 104.1/4.45, respectively, some signals of the other carbon atoms of these units were identified, like those corresponding to C5/H5,5`, which appeared in clean regions of the spectra (**Table 6**). In the spectrum of D1, the latter signal is partially overlapped with a signal at δ 104.4/4.39, which corresponds to (1→3)(1→6)-β-D-galactan structures, as well as that at δ 104.9/4.62, similar to those found in W-aP (**Table 3**), this assignment was confirmed by results from methylation analysis of D1 (**Table 5**). In addition, in the spectrum of D1, the anomeric signals corresponding to α-D-galacturonic acid and its methyl esterified derivative were clear at δ 99.3/4.99 and 99.9/4.88, respectively. The characteristic signal at δ 53.9/3.73, which derived from the methyl ester, was detected in the spectra of both extracts (Shakhmatov et al., 2016). It is important to note that there were some differences between signals due to galacturonic acid residues in these spectra and those detected in W-aP (**Figure 3, Table** 3). These differences were attributed the absence of methyl esters, at least in significant quantities in the homogalacturonan chains of the water extract.

The signals of C1/H1 of terminal, 5-linked, and 2-linked α-L-arabinofuranose units from arabinogalactan structures were clear in both spectra.

Table 6 - Signal assignments of the major structures detected in the NMR spectra obtained by dissolving extracts D1 and D2 from shoots of *G. chacoensis* in water (D_2O, 10 mg/mL solutions).

Structural units[a]	**Chemical shifts, δ**[c]					
	C1/H1	**C2/H2**	**C3/H3**	**C4/H4**	**C5/H5,5´**	**C6/H6,6´**
Glucuronoarabinoxylan						
4-linked β-D-Xyl*p*	102.5/4.40a	73.8/3.20	74.6/3.47	77.3/3.70	63.8/3.28, 4.00	
4-linked β-D-Xyl*p* with tAra*f* on C3	102.5/4.40a	74.5/3.35b	78.2/3.66	74.6/3.74	63.7/3.28, 4.00	
T α-L-Ara*f* linked to C3 of β-D-Xyl*p*	108.7/5.31	81.7/4.08c	78.2/3.83	85.7/4.19	62.2/3.73,3.80	
4-linked β-D-Xyl*p* substituted on C2 with (4Me)GlcA and Ac on	102.0/4.66	75.0/3.58	74.9/5.00			

C3						
4-linked β-D-Xyl*p* substituted on C3 with Ac	102.4/4.49	71.9/3.39	76.1/4.91	76.5/3.85	63.9/3.38,4.04	
4-linked β-D-Xyl*p* (linked to Xyl-Ac)	103.9/4.33	73.7/3.11				
4-linked β-D-Xyl*p* substituted on C2 with Ac	100.9/4.59d	74.4/4.62				
4-linked β-D-Xyl*p* with tAra*f* on C2 and C3	100.8/4.62d	81.7/4.08c	78.2/3.83	85.7/4.19	62.2/3.73,3.80	
T α-L-Ara*f* linked to C2 of β-D-Xyl*p*	109.0/5.26					
T α-D-4MeGlcA*p*	98.7/5.21		73.6/3.74	83.2/3.11		174.7e
4-linked D-Xyl Red α	93.1/5.10				59.8/3.84	
4-linked D-Xyl Red β	97.5/4.50				63.4/3.95, 3.30	
Tβ-D-Xyl*p*	104.0/4.45	74.2/3.30b	76.6/3.36	70.2/3.57	66.0/3.88, 3,24	
2-linked α-L-Ara*f*[b]	106.5/5.40	85.6/4.27	76.2/4.02	85.0/4.02f	61.8/3.85	
T α-D-Gal*p*	101.1/4.97					
AGPs/pectins						
5-linked α-L-Ara*f*[b]	108.4/5.01	82.4/4.14	77.5/3.87	83.3/4.17	67.7/3.81,3.72	
t α-L-Ara*f* arabinogalactan	110.2/5.17	81.9/4.05	77.8/3.93	84.8/4.05f	62.1/3.75,3.63	
6-linked β-D-Gal*p*(1→6)[b]	104.5/4.39	71.8/3.45	73.6/3.59	69.4/4.07	74.5/3.87	70.3/3.97,3.85
3,6-linked β-D-Gal*p*(1→6)[c]	104.4/4.39	70.9/3.58	81.2/3.67	69.4/4.07	74.5/3.87	70.3/3.97,3.85
6-linked or 3,6-linked β-D-Gal*p* (1→3)[c]	104.9/4.62					
4-linked β-D-Gal*p*	104.6/4.57					
4-linked α-D-GalA*p*	99.3/4.99					174.0e
4-linked α-D-GalA*p* 6Me	99.9/4.88					

[a]Signal of 4-*O*-Me-GlcA at δ 60.8/3.38. Acetyl signal at δ 21.3/2.05-2.10 (CH_3). C6/H6 of 2-linked Rha and 2,4-linked Rha at δ 17.7/1.24 and 17.5/1.31. Methyl ester of GalA at δ 53.9/3.73. Signals of phenolics at δ 57.0/3.85 (due to methoxyl groups) and, only in D2, in the range δ 110-150/6.5-8.0. Signal at δ 100.6/5.33 from contaminant 4-linked α-glucans. [b] With the evidence obtained here it was not possible to determine whether these units belong to the GAX or to the AGP/pectin. [c]For 3,6-linked β-D-galactans, δ of the anomeric signal varies if it is linked to C3 or C6 of the other unit (Bilan et al. 2007; Shakhmatov et al. 2016). [c]For a,b,c,d,e,f, assignments could be interchanged.

Although signals corresponding to anomeric carbon of rhamnose were not detected, the presence of the characteristic signals at δ 17.9/1.24 and 17.5/1.31, due to C6/H6 indicated small amounts of 2-linked and 2,4-linked rhamnose units, respectively, in these extracts (Mikshina et al., 2012).

Regards the alkali labile substituents on the GAX, the signals expected for ferulic acids were not clear in the spectrum of D1, with the exception of those of methoxyl groups, which were found at δ 57.0/3.85. Also, a small signal at δ 65.8/4.33 was attributed to C5 of the α-L-arabinofuranose units linked to ferulic acid (Schendel et al., 2015). On the other hand, in the spectrum of D2, small signals of ferulic acids were found. These results were in agreement with the small quantity of these compounds detected in these extracts (**Table 4**).

The most important differences found in these spectra compared to those obtained from the alkaline extracts were detection of the positions of acetylation. The assignment of the corresponding signals was carried out taking into account structural studies on glucuronoxylans from wood species (Evtuguin et al., 2003; Gonçalves, Evtuguin, & Domingues, 2008; Marques, Gutiérrez, del Río, & Evtuguin, 2010). Those xylans are less complex than xylans from grasses, because they do not have arabinose side chains, so in those cases, it was possible to quantify the acetyl groups linked to different positions from the ^{1}H NMR spectra. In extracts from shoots of *G. chacoensis*, the degree of acetylation was determined colorimetrically (Hestrin 1949) (**Table 4**). On the other hand, an approximate value of the total degree of acetylation was also estimated from the ^{1}H NMR spectra only for D2 (molar ratio Xyl:acetyl groups, 1.00:0.35). For D1, the presence of important amounts of β-D-galactans, having anomeric ^{1}H NMR signals in the same range as those from β-D-xylans, made these calculations impossible (**Table 6, Figure 4**).

For both extracts, it was found that the most important acetylation position was on C3 of the xylopyranose residues. All the signals of this structure were clear in the spectra of both extracts, mainly in that of D2. In addition, it was reported that this acetylation pattern produced a displacement of the anomeric ^{13}C NMR signal of the neighboring unit to lower fields, while the ^{1}H

NMR signal was oppositely displaced. An important peak in the spectrum of D2 at δ103.8/4.33 was assigned to this unit, only minor changes were reported for the other signals of this unit.

Acetylation on C2 was also observed (**Table 6**), but units acetylated on both positions were not detected. A signal in the region corresponding to the secondary carbons substituted by acetyl groups (around δ 72-77 for ^{13}C and δ 4.40-5.10, for ^{1}H) was found at δ 74.9/5.00, which was more important in the spectrum of D1. It was attributed to acetylation on C3/H3 of β-D-xylose units having (4-*O*-methyl)-α-D-glucuronic acid side chains linked to C2 of the same unit. This speculation is reinforced by the fact that there was a small displacement of the anomeric signal of the xylose units substituted with glucuronic acid side chains regards the value found in the spectra of 1M KOH-A (from δ 102.3/4.53 in1M KOH-a to δ 102.0/4.66 in D1, **Figures 3** and **4**).

It should be noted that *O*-acetyl-groups can migrate in aqueous solutions to non-substituted hydroxyl groups within the same glycosyl residue by a chemical, non-protein mediated mechanism, forming an equilibrium of acetylation on the various positions available (Gille & Pauly 2012). In xylo-oligosaccharides obtained from hydrothermally treated *Eucalyptus* wood, it was proved that part of the 2-*O*-acetyl substituents were migrated to the 3-*O*-position of the same xylosyl residue (Garrote, Domínguez, & Parajó 1999). However, it is important to bear in mind that *O*-acetyl migration was only observed within the same xylosyl residue (Kabel et al. 2003). In this paper, the first extracts (W-a and W-b) were obtained with aqueous solutions at room temperature, and the residue of this extraction procedure was extracted with DMSO at higher temperatures. Hence, although the possibility of a certain degree of acetyl migration should be considered, extraction conditions used in this work were very different to those reported for acetyl migration (Garrote et al. 1999).

It has been reported that secondary cell walls of vascular plants seem to generate a specific xylan decoration pattern as acetate and glucuronic acid are found spaced on even-numbered residues in the xylan backbone. It was suggested that a correct *O*-acetylation pattern is required for addition of

glucuronic acid residues (Pauly & Ramirez, 2018). However, the substitution pattern of GAX from grasses is more complex due to the presence of arabinose side chains, and it is not so deeply understood. In our study, predominance of C3 *O*-acetylation and lower degree of C2 *O*-acetylation were detected, but no evidence of diacetylation was found. Besides, the possibility of acetylation of xylose units bearing glucuronic acid side chains on C2 should be considered.

In summary, extraction of cell wall polysaccharides using DMSO solutions allowed the characterization of alkali labile substituents, showing a small amount of phenolics linked to the polysaccharides, in particular, to C5 of some of the α-L-arabinofuranose units. In addition, it was possible to characterize the acetylation pattern of the GAX backbone, with predominance of acetyl groups linked to C3 of some β-D-xylopyranose units, but also to C2. These results revealed details about the complex structure of GAX from shoots of *G. chacoensis*, which are key aspects determining their solubility, enzymatic degradability, solution viscosity, and gel formation, and, in consequence, their potential applications.

4. Conclusions

The major hemicellulosic components of young shoots from the native woody bamboo *Guadua chacoensis* were glucuronoarabinoxylans, extracted with the highest yields with 1 M KOH solutions, as previously found for other grasses. These GAX, which were obtained in different quantities in all the extracts analyzed, comprised polysaccharides with different structural parameters, such as composition and patterns of ramification, and these differences were determinant of the variations observed in their extraction behaviour. Only a moderate degree of substitution by terminal α-L-arabinofuranose units was found in the alkaline extracts, comparing with GAX obtained in alkaline conditions from other sources. This structural characteristic could be an advantage to use of this material for production of xylo-oligosaccharides by xylanases. In addition, other polysaccharides were detected and characterized, namely, arabinogalactan proteins,

pectins, mixed linkage glucans, and xyloglucans, which were present in minor but significant quantities, contributing with cellulose, to the whole cell wall carbohydrates system. An alternative extraction with solutions of DMSO solutions gave a different view of GAX structures from this resource, by the characterization of substituents lost during the conventional extraction methodology. Taking into account the scarce information available about this species, every contribution to its exhaustive knowledge could be of benefit to both basic and applied fields.

Acknowledgements

P.V.F., A.S.V., and M.C. are Research Members of the National Research Council of Argentina (CONICET). The authors are indebted to J.M. Estevez for the dot-blot assay with the Yariv reagent.

Funding: This work was supported by the National Agency for Promotion of Science and Technology (ANPCYT) [PICT 2013-2497]; the National Research Council of Argentina (CONICET) [PIP 11220130100762CO 2014-2016, PUE 22920160100068CO]; and the University of Buenos Aires [UBACyT 20020120100069BA and 20020120300004BA].

References

Aditiya, H.B., Mahlia, T.M.I., Chong, W.T., Nur, H., & Sebayang, A.H. (2016). Second generation bioethanol production: A critical review. *Renewable and Sustainable Energy Reviews, 66*, 631-653.

Ahmed, A.E.R., & Labavitch, J.M. (1977). A simplified method for accurate determination of cell wall uronide content. *Journal of Food Biochemistry, 1*, 361-365.

Bilan, M.I., Vinogradova, E.V., Shashkov, A.S., & Usov, A.I. (2007) Structure of a highly pyruvylated galactan sulfate from the Pacific green alga *Codium yezoense* (Bryopsidales, Chlorophyta). *Carbohydrate Research, 342*, 586–596.

Bunzel, M., Ralph, J., Marita, J., Stainhart, H. (2000). Identification of 4-O-5-coupled diferulic acid from insoluble cereal fiber. *Journal of Agricultural and Food Chemistry 48,* 3166–3169.

Carpita, N.C. (1996). Structure and biogenesis of the cell wall of grasses. *Annual Review of Plant Physioogy and Plant Molecular Bioogy, 47,* 445–76.

Carpita, N.C., Ralph, J. & McCann, M.C. (2015). The cell wall. In B.B. Buchanan, W. Gruissem, & R.L. Jones (Eds.). *Biochemistry & Molecular Biology of Plants* ($_2$nd ed.) (pp 45-110). Oxford: John Wiley & Sons, Ltd.

Carpita N.C. & Shea, E.M. (1989). Linkage structure of carbohydrates by gas chromatography-mass spectrometry of partially methylated alditol acetates. In C.J. Biermann & G.D. McGinnis (Eds.). *Analysis of carbohydrates by GLC and MS* (pp.157-216). Boca Raton: CRC Press, Inc.

Chang, W.J., Chang, M.J., Chang, Z.T., Yeh, T.F. (2013). Chemical composition and immunohistological variations of a growing bamboo shoot. *Journal of Wood Chemistry and Technology 33,* 44-155.

Choudhury D., Sahu, J.K., & Sharma, G.D. (2012). Value addition to bamboo shoots: a review. *Journal of Food Science and Technology,49(4),* 407–414.

Chung, M.J., Cheng, S.S., Lin, C.Y., & Chang, S.T. (2012). Profiling of volatile compounds of

Phyllostachys pubescens shoots in Taiwan. *Food Chemistry, 134,* 1732–1737.

Ciucanu I., & K. Kerek (1984). A simple and rapid method for the permethylation of carbohydrates. *Carbohydrate Research, 134,* 209-217.

Coelho, E., Rocha, M.A.M., Moreira, A.S.P., Domingues, M.R.M., &.Coimbra M.A (2016). Revisiting the structural features of arabinoxylans from brewers' spent grain. *Carbohydrate Polymers, 139,* 167-176.

Cui, W., Wood, P.J., Blackwell, B., & Nikiforuk, J. (2000). Physicochemical properties and structural characterization by two-dimensional NMR spectroscopy of wheat b-D-glucan—comparison with other cereal β-D-glucans. *Carbohydrate Polymers, 41,* 249–258.

D´Ambrogio de Argüeso, A. 1986. *Manual de técnicas en histología vegetal*. Hemisferio Sur, Buenos Aires.

Deutschmann,R. & Dekker, R.F.H. (2012). From plant biomass to bio-based chemicals: Latest developments in xylan research. *Biotechnology Advances 30(6),* 1627-1640.

Ding, H.H., Cui, S.W., Goff, H.D., Chen, J., Guo, Q., & Wang, Q. (2016). Xyloglucans from flaxseed kernel cell wall: Structural and conformational characterization. *Carbohydrate Polymers 151,* 538–545.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., & Smith, F. (1956), Colorimetric method of determination of sugars and related substances. *Analytical Chemistry, 28,* 350-356.

Ebringerová, A., Hromádková, Z., Alfodia, J., & Hrıbalova, V. (1998). The immunologically active xylan fromultrasound-treated corn cobs: extractability, structure and properties. *Carbohydrate Polymers, 37,* 231–239.

Evtuguin, D.V., Tomás, J.L., Silva, A.M.S., & Neto, C.P. (2003). Characterization of an acetylated heteroxylan from *Eucalyptus globulus* Labill. *Carbohydrate Research 338,* 597–604.

Edashige, Y. & Ishii, T. (1998). Hemicellulosic polysaccharides from bamboo shoot cell-walls. *Phytochemistry, 49,* 1675-1682.

Filisetti-Cozzi, T.M.C.C. & Carpita, N.C. (1991). Measurement of uronic acids without interference from neutral sugars. *Analytical Biochemistry 197,* 157–162.

Fry, S.C. (1988). *The growing plant cell wall: chemical and metabolic analysis.* Longman Scientific & Technical: Harlow, Essex.

Garrote, G., Domínguez, H., & Parajó, J.C. (1999). Mild autohydrolysis: an environmentally friendly technology for xylooligosaccharide production from wood. *Journal of Chemical Technology and Biotechnology 74,* 1101-1109.

Gibeaut, D.M. & Carpita, N.C. (1993). Structural models of primary cell wall in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant Journal, 3,* 1-30.

Gonçalves, V.M.F., Evtuguin, D.V., & Domingues, M.R. (2008). Structural characterization of the acetylated heteroxylan from the natural hybrid *Paulownia elongata/Paulownia fortune*, *Carbohydrate Research 343,* 256–266.

Guo, Q., Cui, S.W., Wang, Q., Hu, X., Wu, Y., Kang, J., & Yada, R. (2011). Structure characterization of high molecular weight heteropolysaccharide isolated from *Artemisia sphaerocephala* Krasch seed. *Carbohydrate Polymers 86*, 742– 746.

Hromádková, Z., Smestad Paulsen, B., Polovka, M., Kostálová, Z., & Ebringerová, A. (2013). Structural features of two heteroxylan polysaccharide fractions from wheat bran with anti-complementary and antioxidant activities. *Carbohydrate Polymers 93,* 22–30.

He, S., Wang, X., Zhang, Y., Wang, J., Sun, H., Wang, J., Cao, X., & Ye, Y. (2016). Isolation and prebiotic activity of water-soluble polysaccharides fractions from the bamboo shoots (*Phyllostachys praecox*). *Carbohydrate Polymers, 151,* 295-304.

Hestrin, S. (1949). The reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine, and its analytical applications. *Journal of Biological Chemistry 180,* 249-261.

Ishii, T. & Hiroi, T. (1990a). Isolation and characterization of feruloylated arabinoxylan oligosaccharides from bamboo shoot cell-walls. *Carbohydrate Research, 196,* 175-183.

Ishii, T. & Hiroi, T. (1990b). Linkage of phenolic acids to cell-wall polysaccharides of bamboo shoot. *Carbohydrate Research, 206,* 297-310.

Judziewicz, E.J., Clark , L.G., Londoño, X. & Stern, M.J. (1999). *American bamboos*. Smithsonian Institution Press, Washington.

Kabel, M.A., de Waard, P., Schols, H.A., Voragen, A.G.J. (2003). Location of *O*-acetyl substituents in xylo-oligosaccharides obtained from hydrothermally treated *Eucalyptus* wood. *Carbohydrate Research 338,* 69–77**.**

Kaneko, S., Ishii, T., & Matsunaga, T. (1997). A Boron-Rhamnogalacturonan-II complex from bamboo shoot cell walls. *Phytochemistry, 44,* 243-248.

Kozlova, L., Mikshina, P., & Gorshkova, T. (2012). Glucuronoarabinoxylan extracted by treatment with endoxylanase from different zones of growing maize root. *Biochemistry* (Moscow) *77*, 395–403.

Leach, A. A. (1960). Notes on a modification of the Neuman & Logan method for the determination of hydroxyproline. *Biochemistry Journal*, *74*, 70–71.

Lehrfeld, J. (1987). Simultaneous gas-liquid chromatographic determination of aldoses and alduronic acids. *Journal of Chromatography, 408*, 245-253.

Lizarazu, M.A., Rúgolo de Agrasar , Z.E., & Vega, A.S. (2013) A new species of *Guadua* (Poaceae, Bambusoideae, Bambuseae) and synopsis of the genus in Argentina and neighboring regions. *Systematic Botany, 38*, 1062-1075.

Londoño, X., & Peterson, P.M. (1992). *Guadua chacoensis* (Poaceae: Bambuseae), its taxonomic identity, morphology, and affinities. *Novon, 2,* 41-47.

Maekawa, E., & Kitao, K. (1973). Water soluble polysaccharides in bamboo shoot. *Agricultural and Biological Chemistry, 37*, 2445-2447.

Maekawa, E. (1975a). Isolation and fractionation of water soluble polysaccharides from bamboo shoot. *Agricultural and Biological Chemistry, 39,* 2281-2289.

Maekawa, E. (1975b). The structure of water soluble polysaccharides isolated from bamboo shoot.

Agricultural and Biological Chemistry, 39, 2291-2296.

Marques, G., Gutiérrez, A., del Río, J.C., & Evtuguin, D.V. Acetylated heteroxylan from *Agave sisalana* and its behavior in alkaline pulping and TCF/ECF bleaching. *Carbohydrate Polymers 81,* 517–523.

Mikshina, P.V., Gurjanov, O.P., Mukhitova, F:K., Petrova, A.A., Shashkov, A.S., Gorshkova, T.A. (2012). Structural details of pectic galactan from the secondary cell walls of flax (*Linum usitatissimum* L.) phloem fibres. *Carbohydrate Polymers 87,* 853–861.

Morrison, I.M. (1988). Hydrolysis of plant cell walls with trifluoroacetic acid. *Phytochemistry, 27*, 1097-1100.

Naidu, D.S., Hlangothib, S.P., & John, M.J. (2018). Bio-based products from xylan: A review. *Carbohydrate Polymers, 179*, 28–41.

Nirmala, C., Bisht, M.S., & Haorongbam, S. (2011). Nutritional properties of bamboo shoots: potential and prospects for utilization as a health food. *Comprehensive Reviews in Food Science and Food Safety, 10,* 153-169.

Panizzo, C., Fernández, P.V., Colombatto, D., Ciancia, M., & Vega, A.S. (2017). Anatomy, nutritional value, and cell wall chemical analysis of foliage leaves in *Guadua chacoensis* (Poaceae, Bambusoideae, Bambuseae), a promising source of forage. *Journal of the Science of Food and Agriculture, 97,* 1349–1358.

Petruš, L., Gray, D. G., & BeMiller, J. N. (1995). Homogeneous alkylation of cellulose in lithium chloride/dimethyl sulfoxide solvent with dimsyl sodium activation. A proposal for the mechanism of cellulose dissolution in LiCl/Me2SO. *Carbohydrate Research,268,* 319–323.

Saulniet, L., Marot, C., Chanliaud, E., & Thibault, J.-F. (1995). Cell wall polysaccharide interactions in maize bran. *Carbohydrate Polymers 26,* 279-287.

Schendel, R.R., Becker, A., Tyl, C.E., & Bunzel, M. (2015). Isolation and characterization of feruloylated arabinoxylan oligosaccharides from the perennial cereal grain intermediate wheat grass (*Thinopyrum intermedium*). *Carbohydrate Research 407,* 16-25.

Shakhmatov, E.G., Udoratina, E.V., Atukmaev, K.V., & Makarova, E.N. (2015). Extraction and structural characteristics of pectic polysaccharides from *Abies sibirica*. *Carbohydrate Polymers, 123,* 228–236.

Shakhmatov, E.G., Atukmaev, K.V. & Makarova, E.N. (2016). Structural characteristics of pectic polysaccharides and arabinogalactan proteins from *Heracleum sosnowskyi* Manden. *Carbohydrate Polymers 136,* 1358–1369.

Shea, E.M. & Carpita N.C. (1988). Separation of partially methylated alditol acetates on SP-2330 and HP-1 vitreous silica capillary columns. *Journal of Chromatography*. 445, 426-428.

Shui, G. & Leong, L. P. (2006). Residue from star fruit as valuable source for functional food ingredients and antioxidant nutraceuticals. *Food Chemistry 97,* 277–284.

Singh, R.D., Banerjee, J., & Arora, A. (2015). Prebiotic potential of oligosaccharides: A focus on xylan derived oligosaccharides. *Bioactive Carbohydrates and Dietary Fibre, 5,* 19-30.

Soreng, R. J., Peterson, P. M., Romaschenko, K., Davidse, G., Teisher, J. K., Clark, L. G., Barberá, P., Gillespie, L. J., & Zuloaga, F. O. (2017). A worldwide phylogenetic classification of the Poaceae (Gramineae) II: An update and a comparison of two 2015 classifications. *Journal of Systematics and Evolution, 55,* 259-290.

Stevenson, T.T. & Furneaux, R.H. (1991). Chemical methods for the analysis of sulphated galactans from red algae. *Carbohydrate Research, 210,* 277-298.

Thiers, B. (2017). *Index Herbariorum: A global directory of public herbaria and associated staff.* New York Botanical Garden's Virtual Herbarium. Available from: http://sweetgum.nybg.org/ih/ (accessed: 25 January 2017).

Tye, Y.Y.; Lee, K.T., Abdullah, W.N.W., Leh, C.P. (2016). The world availability of non-wood lignocellulosic biomass for the production of cellulosic ethanol and potential pretreatments for the enhancement of enzymatic saccharification. *Renewable Sustainable Energy Reviews 60,* 155–172.

Vogel, J. (2008) Unique aspects of grass cell wall. *Current Opinion in Plant Biology 11,* 301-307.

Walters, J.S., & Hedges, J.I. (1988). Simultaneous determination of uronic acids and aldoses in plankton, plant tissues, and sediment by capillary gas chromatography of N-hexylaldonamide and alditol acetates. *Analytical Chemistry, 60,* 988-994.

Wilkie, K.C.B. & Woo, S.-L. (1977). A heteroxylan and hemicellulosic materials from bamboo leaves and a reconsideration of the general nature of commonly occurring xylans and other hemicelluloses. *Carbohydrate Research 57,* 145-162.

Yariv, J., Rapport, M.M., & Graf, L. (1962). The interaction of glycosides and saccharides with antibody to the corresponding phenylazoglycoside. *Biochemical Journal, 85,* 383-388.

Zelaya, V.M., Fernández, P.V., Vega, A.S., Mantese, A.I., Federico, A.A., & Ciancia, M. (2017). Glucuronoarabinoxylans as major cell walls polymers from young shoots of the woody bamboo *Phyllostachys aurea*. *Carbohydrate Polymers, 167,* 240-249.