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A Structural Characterization of the Lignins from Sugarcane (Saccharum spp. L.) Bagasse and Straw

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Abstract

The structure of the lignins of sugarcane bagasse and straw was investigated. The lignins were characterized both *in situ* and in isolated preparations (Milled-Wood Lignin, MWL, and Cellulolytic Lignin, CEL) by Py-GC/MS and 2D-NMR. It was concluded that they are *p*-hydroxyphenyl-guaiacyl-syringyl lignins with associated *p*coumarates and ferulates. 2D-NMR indicated that the main substructures present are β–*O*–4´-ethers, followed by β–5´ phenylcoumarans and with lower amounts of β–β´ resinols and β–1´ spirodienones.

Keywords: Sugarcane; *Saccharum sp.*; Bagasse; Straw; Lignin.

Introduction

Sugarcane (*Saccharum spp.* L.) bagasse and straw are wastes from the process of sugar extraction, and are abundant and low-cost materials of the alcohol and sugar industries. Sugarcane bagasse and straw are composed of cellulose (ca. 41%), hemicelluloses (ca. 39%) and lignin (19-21%), with high amounts of extractives (2.4-4.8%) and ash (ca. 5%), and are attractive feedstocks to produce second–generation bioethanol and other value-added products in the context of the lignocellulosic biorefinery. The conversion of lignocellulosic biomass to bioethanol involves saccharification of carbohydrates to fermentable reducing sugars and then fermentation of these free sugars to ethanol. However, the presence of lignin limits the accessibility of enzymes to cellulose, thus reducing the efficiency of the hydrolysis [1]. Pretreatment of lignocellulosic materials to remove or modify the lignin is therefore needed to enhance the hydrolysis of carbohydrates. The efficiency of pretreatment methods is highly dependent on the lignin structure, and hence the knowledge of the structure of the lignin polymer in sugarcane bagasse and straw is important to develop appropriate pretreatment methods for lignin modification and/or removal. In this paper, we report the structural characteristics of the lignins in sugarcane bagasse and straw by analytical pyrolysis (Py-GC/MS) and 2D-NMR. The knowledge of the composition and structure of the lignin of sugarcane will help to maximize the exploitation of these important agroindustrial as a feedstocks for biofuels and other biorefinery products.

Experimental

Samples

Sugarcane *(Saccharum spp.* L.*)* bagasse and straw were supplied by the University of Viçosa (MG, Brazil). Klason lignin content was estimated as the residue after sulphuric acid hydrolysis according to the TAPPI method T222 om-8. The acidsoluble lignin was determined, after the insoluble lignin was filtered off, by UV-spectroscopic determination at 205 nm wavelength using 110 L cm^{-1} g^{-1} as the extinction coefficient (TAPPI method UM 250).

Isolation of 'Milled-Wood' Lignin (MWL) and Cellulolytic lignin (CEL)

'Milled-wood´ lignin (MWL) was extracted from finely ball-milled (50 h) plant material, free of extractives and
hot water soluble material, using dioxane-water (9:1, v/v), followed by evaporation of the solvent, and purified as described [2]. The final yields ranged from 5-15%. Cellulolytic lignin (CEL) preparations were isolated by enzymatically saccharifying polysaccharides as described [3]. Cellulysin cellulase (Calbiochem), from *Trichoderma viride* was used. The final yields were higher than 90%.

Analytical pyrolysis

Pyrolysis (ca. 100 μg) was performed with a 3030 micro-furnace pyrolyzer (Frontier Labs) connected

to an Agilent 7820A GC using a DB-1701 capillary column (60 m x 0.25 mm i.d., 0.25 μm film thickness) and an Agilent 5975 mass selective detector. The pyrolysis was performed at 500 ºC. The oven was programmed from 45 ºC (4 min) to 280 ºC (10 min) at 4 ºC min^{-1}. Helium was the carrier gas (1 mL min^{-1}).

Results and Discussion

The abundance of the main constituents (water solubles, acetone extractives, Klason lignin, acid-soluble lignin, holocellulose, α-cellulose, and ash) of sugarcane bagasse and straw are shown in **Table 1**.

Table 1. Abundance of the main constituents (% dry-weight) of sugarcane bagasse and straw.

	Sugarcane bagasse	Sugarcane straw
Water soluble extractives	1.4 ± 0.2	2.2 ± 0.2
Acetone extractives lipids	0.8 ± 0.1	1.3 ± 0.1
Klason lignin	17.8 ± 0.6	17.0 ± 0.2
Acid-soluble lignin	2.2 ± 0.2	1.9 ± 0.2
Holocellulose (α-cellulose)	75.8 ± 0.5 (40.1 ± 0.2)	72.9 ± 0.7 (37.9 ± 0.3)
Ash	2.0 ± 0.1	4.7 ± 0.5

MWL and CEL preparations were isolated from sugarcane bagasse and straw according to traditional lignin isolation procedures [2,3], and were subsequently analyzed by Py-GC/MS and 2D-NMR. The pyrograms of sugarcane basse and straw, and of their corresponding MWL and CEL preparations are shown in **Figure 1**. The identities and relative molar abundances of the released compounds are listed in **Table 2**.

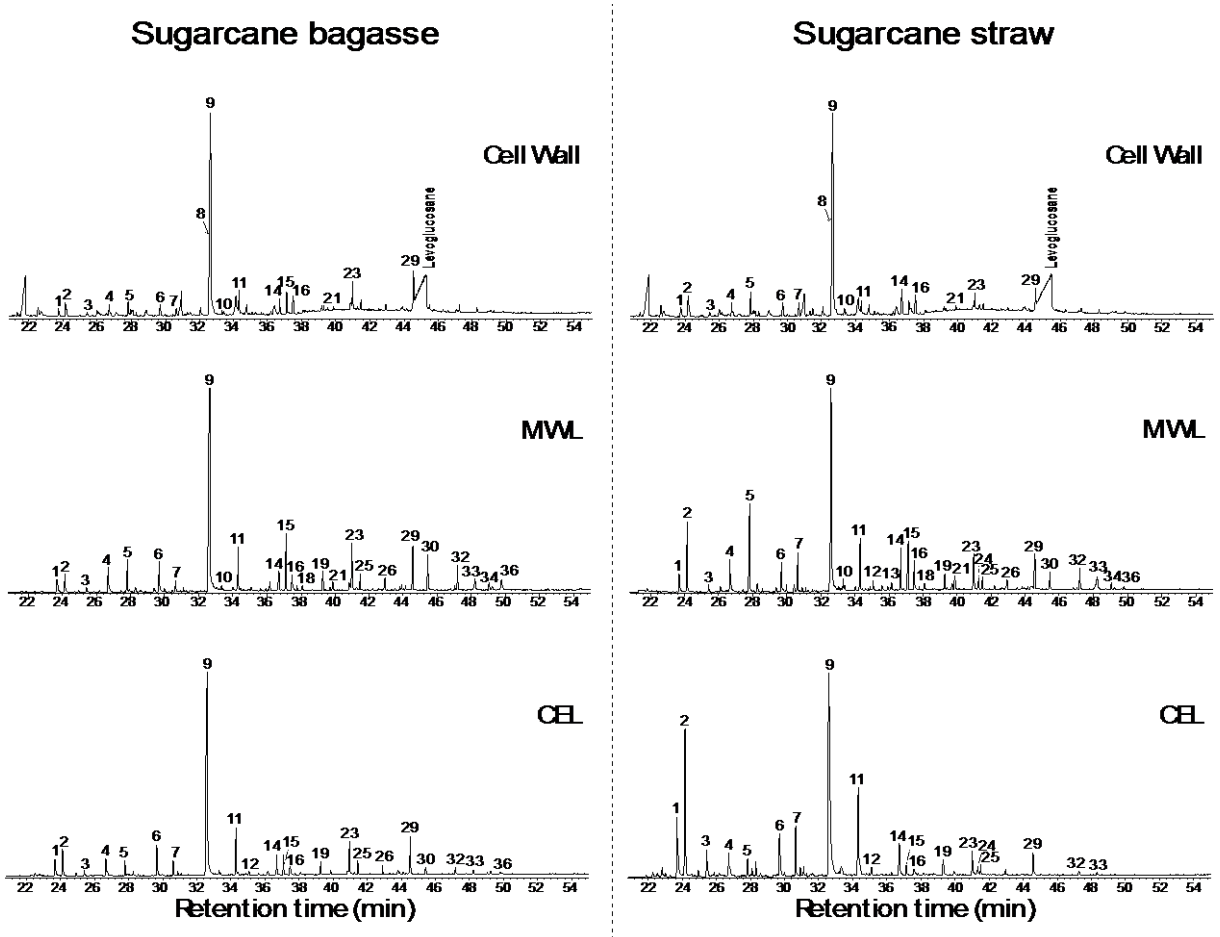

Figure 1. Py-GC/MS of sugarcane bagasse and straw (whole cell-walls), and their isolated MWL and CEL lignin preparations. The identities and relative abundances of the released compounds are listed in **Table 2**.

Table 2. Identities and relative molar abundances of the compounds released after Py-GC/MS of sugarcane bagasse and straw (whole cell walls) and their isolated MWL and CEL preparations.

		Sugarcane bagasse			Sugarcane straw		
	Compounds	CW	MWL	CEL	CW	MWL	CEL
1	phenol	2.46	2.67	4.12	3.06	3.05	10.17
2	guaiacol	2.54	2.18	3.55	3.87	8.03	13.59
3	3-methylphenol	1.05	0.66	0.97	1.06	0.83	3.32
4	4-methylphenol	2.49	4.21	2.84	3.10	4.38	2.91
5	4-methylguaiacol	2.33	2.98	1.70	4.00	8.87	1.36
6	4-ethylphenol	1.48	3.67	3.97	2.14	2.87	4.38
7	4-ethylguaiacol	0.83	0.99	1.44	1.75	2.80	3.44
8	4-vinylguaiacol	10.99	3.30	7.09	16.97	8.72	9.09
9	4-vinylphenol	53.27	41.72	47.40	43.85	29.98	31.30
10	eugenol	0.42	0.35	0.49	0.78	0.77	0.59
11	syringol	3.13	4.17	5.47	2.08	4.26	7.74
12	*cis*-isoeugenol	0.27	0.35	0.37	0.46	0.73	0.55
13	*trans* 4-propenylphenol	0.46	1.02	0.65	0.48	0.81	0.29
14	*trans*-isoeugenol	1.75	1.55	2.12	3.13	3.03	2.61
15	4-methylsyringol	2.39	5.10	1.93	1.70	3.49	0.72
16	vanillin	2.36	1.88	1.34	2.58	2.52	0.91
17	propynylguaiacol	0.17	0.36	0.13	0.21	0.26	0.09
18	propynylguaiacol	0.27	0.51	0.15	0.29	0.55	0.08
19	4-ethylsyringol	0.48	1.24	1.08	0.31	0.91	1.05
20	vanillic acid methyl ester	0.00	0.25	0.09	0.00	0.44	0.00
21	acetovanillone	0.48	0.89	0.54	0.76	1.28	0.42
22	4-hydroxybenzaldehyde	0.00	1.35	0.67	0.00	0.30	0.00
23	4-vinylsyringol	2.97	3.97	3.08	1.92	2.52	1.65
24	guaiacylacetone	0.24	0.21	0.22	0.55	0.83	0.49
25	4-allylsyringol	0.87	1.06	1.06	0.57	0.67	0.48
26	*cis*-4-propenylsyringol	0.51	0.81	0.66	0.30	0.58	0.27
27	propinylsyringol	0.00	0.29	0.44	0.45	0.05	0.07
28	propinylsyringol	0.00	0.30	0.18	0.15	0.03	0.06
29	*trans*-4-propenylsyringol	3.19	3.22	3.28	2.00	2.08	1.56
30	syringaldehyde	0.62	3.20	0.78	0.00	1.26	0.14
31	syringic acid methyl ester	0.12	0.27	0.11	0.08	0.11	0.00
32	acetosyringone	0.65	1.82	0.72	0.36	1.37	0.30
33	syringylacetone	0.62	1.21	0.39	0.40	0.93	0.22

34	propiosyringone		0.19	0.45	0.17	0.10	0.34	0.06
35	syringyl vinyl ketone		0.19	0.28	0.35	0.25	0.15	0.09
36	*trans*-coniferaldehyde		0.21	1.26	0.43	0.28	0.22	0.00
37	*trans*-sinapaldehyde		0.00	0.27	0.00	0.00	0.00	0.00
		S/G[a]	1.1	1.7	1.3	0.5	0.5	0.5

[a]All G- and S-derived peaks were used for the estimation of the S/G ratio, except 4-vinylguaiacol (which also arises from ferulates), and the analogous 4-vinylsyringol.

Pyrolysis of the whole cell-walls of sugarcane bagasse and straw released compounds from carbohydrate and lignin moieties, as well as from *p*-hydroxycinnamates. Among the lignin derived phenols, the pyrograms showed compounds derived from *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) lignin units as well as from the cinnamic acid esters in the wall. The most prominent cinnamate or lignin-derived compounds released were 4-vinylguaiacol (8) and 4-vinylphenol (9) with important amounts of other lignin-derived compounds such as phenol (1), guaiacol (2), 4-methylguaiacol (5), syringol (11), 4-methylsyringol (15) and 4-vinylsyringol (23). However, the high amounts of 4-vinylphenol released upon pyrolysis is mostly due to the presence of *p*-coumarates which decarboxylates efficiently under pyrolytic conditions [4–7]. Similarly, 4-vinylguaiacol, which is present in high abundance among the pyrolysis products of the whole cell-walls, also arises from ferulates after decarboxylation upon pyrolysis.

Pyrolysis of the MWL and CEL preparations released a similar distribution of cinnamate- and lignin-derived compounds as from their respective whole cell-walls, except for the much lower relative abundance of 4-vinylguaiacol (8). The most prominent compound in the pyrograms of the MWL and CEL preparations was still 4-vinylphenol (9), derived largely from the *p*-coumarate esters acylating lignin sidechains, and as also occurred in the pyrolysis of their whole cell-walls. The presence of *p*-hydroxycinnamates in the whole cell walls, as well as in the isolated lignins, however, could be addressed by pyrolysis in the presence of a methylating agent, tetramethylammonium hydroxide (data not shown). The relative abundances of *p*-hydroxycinnamates (*p*-coumarate/ferulate ratio) present in the whole cell-walls and in their isolated lignins revealed that ferulate is mostly attached to the carbohydrates while *p*-coumarate is primarily attached to the lignin polymer, as occur in other grasses [6,7].

It is obvious then that these vinyl compounds cannot be used for the estimation of the lignin H:G:S composition upon Py-GC/MS, as the major part of them do not arise from the core lignin structural units but from *p*-hydroxycinnamates. A rough estimation of the S/G ratio of the lignins in sugarcane bagasse and straw and their isolated MWL and CEL lignins was, however, performed by ignoring 4-vinylguaiacol (and the analogous 4-vinylsyringol), and revealed that sugarcane bagasse lignin is enriched in S-lignin units (S/G of 1.1–1.7) whereas the lignin from sugarcane straw is enriched in G-lignin units (S/G of 0.5) (**Table 2**).

Additonal analysis by 2D-NMR revealed that the main lignin substructures present were β–*O*–4′ aryl ethers followed by smaller amounts of phenylcoumarans, resinols and spirodienones. The S/G ratios obtained by 2D-NMR closely matched those determined by Py-GC/MS, indicating a predominance of S-lignin units in sugarcane bagasse lignin and a predominance of G-lignin units in sugarcane straw lignin. The spectra indicated that *p*-coumarates are acylating the γ-position of the lignin side-chains, as also occurs in other grasses [6–8]. The spectra also revealed that the flavone tricin was incorporated into the structure of these lignins, as also occurred in other grasses [6,7].

Conclusions

Analysis of sugarcane bagasse and straw and their isolated MWL and CEL lignin preparations indicated that they are H-G-S lignins with some amounts of associated *p*-coumarates (acylating the γ-position of the lignin moiety) and ferulates (associated to the carbohydrates). Whereas the lignin in sugarcane bagasse is more enriched in S-lignin units, the lignin from sugarcane straw is enriched in G-lignin units. The main lignin inter-unit linkages present were the β–*O*–4′ aryl ethers, followed by phenylcoumarans, resinols and spirodienones. The flavone tricin was also found to be incorporated into these lignins, as also occurs in other grasses.

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