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ISOLATION AND STRUCTURAL CHARACTERIZATION OF LIGNIN-CARBOHYDRATE COMPLEXES FROM SISAL AND ABACA FIBERS

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ABSTRACT

Lignin carbohydrate complexes (LCC) were isolated from sisal and abaca. Two LCC fractions, namely GL and XL, were isolated in a quantitative manner. GL fractions were enriched in glucans and depleted in lignin whereas XL fractions were depleted in glucans but were enriched in xylans and lignin. The lignin moieties in the different LCC fractions of sisal and abaca are structurally different from each other, with a lignin that is enriched in S-units, less condensed, that is preferentially associated to xylans, and a lignin with more G-units, more condensed, that is preferentially associated to glucans. In addition, the analyses indicated that the acetate groups attached to the γ-carbon of the lignin side-chains in abaca and sisal were completely hydrolyzed and removed during the isolation procedure.

I. INTRODUCTION

Lignin, cellulose and hemicelluloses are the three major structural components of plant cell-walls. However, selective separation or fractionation of these components is not an easy task because they are physically entangled with one another in the cell-wall [1]. In addition, chemical linkages can also occur between these components, forming the so-called lignin–carbohydrate complexes (LCCs) [1,2]. The linkage types and numbers are still not well understood, although it is generally accepted that there are three types of lignin-carbohydrate linkages present in wood, namely phenyl glycosides, esters, and benzyl ethers [1].

However, and in order to investigate LCCs structure, a clear and complete fractionation with proper preservation of the bonds between the lignin and carbohydrate must be obtained. Recently, a simple fractionation method was established to isolate LCCs from lignocellulosic biomass based on mild milling and dissolution using a mixture of dimethyl sulfoxide (DMSO) and 50% aqueous tetrabutylammonium hydroxide (TBAH) [3,4]. Two LCC fractions, a glucan-lignin (GL) and a xylan-lignin (XL) fractions can be quantitatively obtained, while a glucomannan-lignin (GML) fraction was additionally obtained from softwoods. It was claimed that this method can isolate all cell-wall components, including lignin, in a chemically unaltered form. In this work, we have applied the LCC isolation method recently developed [3,4] to two plants that are known to have lignins with high extent of acetylation, as sisal (*Agave sisalana*) and abaca (*Musa textilis*). The structure of the lignin as well as the preservation of the acetylated lignin units was monitored by using a modification of the so-called Derivatization Followed by Reductive Cleavage (DFRC) degradation method [5].

II. EXPERIMENTAL

Plant samples

The plant samples selected for this study consist of leaf fibers of sisal (*Agave sisalana*) and abaca (*Musa textilis*). The fibers were finely ground to sawdust using a knife mill before analysis.

Lignin-carbohydrate complex (LCC) fractionation

The LCC fractionation was performed according to the method previously described [3,4]. A ball-milled sample (ca. 3 g) was completely dissolved in a mixture of 30 ml DMSO and 30 ml TBAH (40% w/w in water). Then, the clear solution was dispersed into 500 ml deionized water until a precipitate was formed. The precipitate was washed with deionized water until neutral pH, and was then freeze-dried to obtain the glucan-lignin fraction (GL). The xylan-lignin fraction (XL) was obtained, after separation of the precipitate, by neutralizing the solution with HCl, followed by dialysis and freeze-drying. The barium hydroxide step detailed in [4] was skipped since glucomannan is not a major hemicellulose in these plants.

DFRC (derivatization followed by reductive cleavage) degradation

A modification of the standard DFRC method by using propionyl instead of acetyl reagents (DFRC´) was used [5], and the detailed protocol has been published elsewhere [6].

III. RESULTS AND DISCUSSION

Sisal and abaca were fractionated according to the LCC fractionation approach previously developed [3,4]. Two different LCC fractions, namely a glucan-lignin (GL) and a xylan-lignin (XL) fraction, were efficiently and quantitatively isolated from sisal and abaca. The yields of the GL and XL fractions isolated from sisal and abaca, as well as the Klason lignin content and the composition of the sugars in these fractions, are detailed in **Table 1**. In sisal, GL and XL fractions accounted for 68.5% and 31.5%, respectively, whereas in abaca GL and XL fractions accounted for 78.8% and 21.2%, respectively. Interestingly, the total of both fractions amounted to nearly 100% of the initial material, indicating that the fractionation was quantitative. In both plants, the yields of GL were higher than XL, as also occurred in hardwoods and softwoods [3,4]. In addition, in both plants, the GL fractions were enriched in glucan and depleted in lignin, while the XL fractions were depleted in glucan and enriched in xylan and lignin. Hence, in sisal, GL was enriched in glucan (88.4% of the sugars were glucose) with low Klason lignin content (7.8%), whereas XL was enriched in xylose (89.4% of the sugars) with a high Klason lignin content (24.1%). In abaca, GL was also enriched in glucose (95% of all the sugars) and depleted in lignin (4.4% Klason lignin), whereas XL was enriched in xylose (72.6% of all sugars) and presented a high Klason lignin content (29.4%).

Table 1. Yield of GL and XL fractions isolated from sisal and abaca, and content of klason lignin and carbohydrates.

	Sisal		Abaca	
	GL	XL	GL	XL
Yield (%)	68.5	31.5	78.8	21.2
Klason lignin (%)	7.8	24.1	4.4	29.4
Carbohydrates (relative %)				
Arabinose	1.5	0.6	0.3	3.9
Xylose	9.0	89.4	4.1	72.6
Mannose	0.9	2.6	0.5	15.0
Galactose	0.2	0.3	0.1	0.3
Glucose	88.4	7.1	95.0	8.2

The lignins from sisal and abaca are known to be highly acetylated at the γ-carbon of the lignin side-chain [6-8]. Since it might exist the possibility of some structural modifications of the lignin due to the use of TBAH, a strong basic salt that may alter the structure of the lignin during the fractionation, the occurrence of naturally acetylated lignin in the GL and XL fractions isolated from sisal and abaca was analyzed in detail. For this, we used a modification of the so-called Derivatization Followed by Reductive Cleavage (DFRC) method, that cleaves α-ether and β-ether linkages but leaves γ-esters intact allowing the analysis of native γ-acylated lignin, by using propionyl instead of acetyl reagents (DFRC′) [5]. The chromatograms of the DFRC′ degradation products of the XL and GL fractions isolated from sisal and abaca are shown in **Figure 1**. The chromatograms of the DFRC′ degradation products of the MWL isolated from the same plants are also shown for comparison.

The MWL from sisal and abaca released the *cis* and *trans* isomers of guaiacyl (*c*-G and *t*-G) and syringyl (*c*-S and *t*-S) lignin monomers in different proportions, arising from normal (γ-OH) units in lignin. In addition, the presence of originally γ-acetylated guaiacyl (*c*-Gac and *t*-Gac) and syringyl (*c*-Sac and *t*-Sac) lignin units could be clearly observed in the chromatograms of the DFRC′ of the MWL isolated from sisal and abaca (**Figure 1**). The relative abundances of γ-OH and γ-acetylated lignin units (G, Gac, S and Sac), as well as the percentage of acetylated Gac- and Sac- units and the S/G ratios, in the GL and XL fractions isolated from sisal and abaca, and in their respective MWLs, are shown in **Table 2**. In both MWLs, the acetylation occurred predominantly on syringyl units, as has been observed in most lignins [6-9]. The high extent of γ-acetylation of sisal MWL included both S units (80%) and G units (48%), whereas in the case of abaca MWL γ-acetylation occurred predominantly on S units (84%) and only a minor degree of acetylation was observed on G units (4%), in agreement with previous studies [6-8]. The S/G ratios of the MWL from sisal and abaca, as determined by DFRC′, were 1.4 and 1.3, respectively.

The analyses indicated that the composition of the lignin moiety in the XL and GL fractions isolated from sisal and abaca were completely different from each other and from the respective MWL. Sisal XL fraction presented a lignin enriched in S-units (S/G ratio 2.8), whereas sisal GL fraction presented a lignin with more G-units (S/G ratio 1.0). Likewise, abaca XL fraction presented a lignin enriched in S-units (S/G ratio of 3.3), whereas abaca GL fraction presented a lignin with more G-units (S/G ratio of 1.1). Similar results were also found in the LCC fractions of eucalyptus [3] and spruce [4]. This fact suggests that the lignin moiety in the different LCC fractions

of sisal and abaca are structurally different from each other, and indicates the presence of two types of lignins in each plant, a lignin that is enriched in S-units, less condensed, that is preferentially associated to xylans, and a lignin with more G-units, more condensed, that is preferentially associated to glucans.

Interestingly, XL and GL fractions only released lignin monomers arising from normal (γ-OH) units in lignin. The γ-acetylated guaiacyl (*c*-Gac and *t*-Gac) and syringyl (*c*-Sac and *t*-Sac) lignin units were completely absent in the chromatograms. This indicates that the acetate groups acylating the γ-carbon of the lignin side-chain have been extensively hydrolyzed during LCC fractionation, most probably due to the use of TBAH, a strong basic salt. Therefore, this LCC fractionation seems to affect the structure of lignins, particularly those having a large extent of γ-acylation. Acetylated lignin units are known to occur in all angiosperms, including mono- and eudicotyledons [6-12]. Likewise, it can be envisaged that other groups (*p*-coumarates and *p*-hydroxybenzoates) that are also acylating the γ-carbon of the lignin in other plants [10-12] may behave similarly and suffer from hydrolysis during the LCC fractionation. Moreover, we also observed that acetates attached to carbohydrates (as in xylans) were also be hydrolyzed during LCC fractionation.

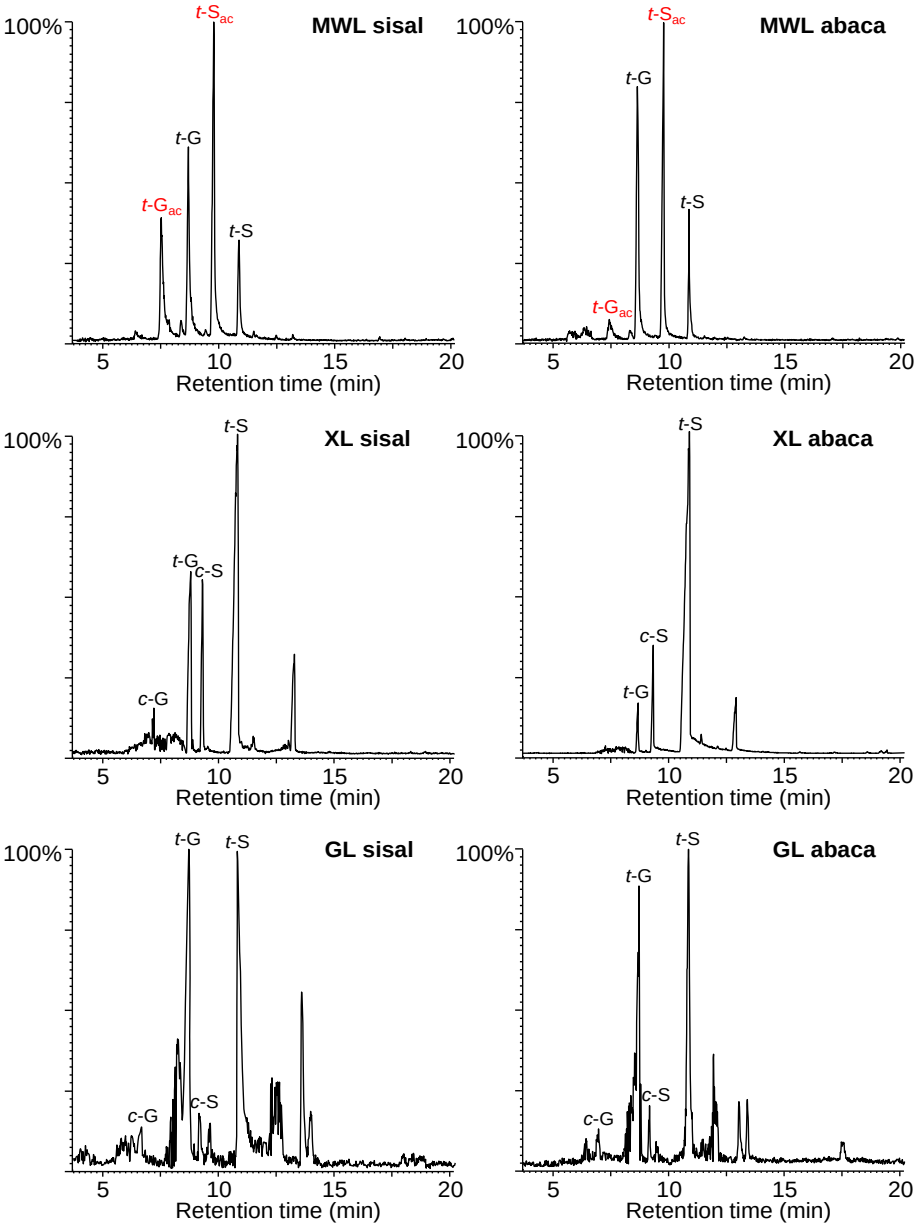

Figure 1. Reconstructed Ion Chromatograms (*m/z* 222+236+252+266) of the DFRC′ degradation products of XL fractions isolated from sisal and abaca. The DFRC′ degradation products of the respective MWL are shown for comparison. *c*-G, *t*-G, *c*-S and *t*-S are the *cis*- and *trans*-guaiacyl and syringyl monomers, respectively. *c*-Gac, *t*-Gac, *c*-Sac and *t*-Sac are the originally γ-acetylated *cis*- and *trans*-guaiacyl and syringyl monomers, respectively.

Table 2. Relative abundance (%) of γ-OH and γ-acetylated lignin units (G, Gac, S and Sac), percentage of acetylated G and S units and S/G ratios, in the GL and XL fractions isolated from sisal and abaca, and in their respective MWLs, as determined upon DFRC′

	Sisal			Abaca		
	MWL	GL	XL	MWL	GL	XL
Gac	20	0	0	2	0	0
G	22	50	26	43	47	23
Sac	46	0	0	46	0	0
S	12	50	74	9	53	77
% acetylated G units	48	0	0	4	0	0
% acetylated S units	80	0	0	84	0	0
S/G ratio	1.4	1.0	2.8	1.3	1.1	3.3

IV. CONCLUSIONS

Sisal and abaca were fractionated into different LCC fractions. Two different LCC fractions, namely a glucan-lignin (GL) and a xylan-lignin (XL) fraction, were quantitatively isolated. The GL fractions were enriched in glucan and depleted in lignin, while the XL fractions were depleted in glucan and enriched in xylan and lignin. The structural characteristics of the lignins in the XL fractions, in particular the extent of acetylation of the γ-carbon of the side-chain, were studied by DFRC that indicated that the acetate groups that are natively present in these lignins were completely hydrolyzed and removed during the LCC fractionation.

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