ALESSANDRO GUARINO LINO

COMPOSIÇÃO QUÍMICA E ESTRUTURAL DA LIGNINA E LIPÍDIOS DO BAGAÇO E PALHA DA CANA-DE-AÇÚCAR

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Agroquímica, para obtenção do título de *Doctor Scientiae*.

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BIOGRAFIA

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RESUMO

LINO, Alessandro Guarino, D.Sc., Universidade Federal de Viçosa, setembro de 2015. **Composição química e estrutural da lignina e lipídios do bagaço e palha da cana-de-açúcar.** Orientador: Claudio Ferreira Lima. Coorientador: Jorge Luiz Colodette.

Nesse trabalho, nos reportamos uma extensiva e detalhada caracterização das ligninas no bagaço e palha de cana-de-açúcar. Com esse propósito, nos usamos poderosas metodologias analíticas, incluindo pirólise analítica acoplada a cromatografia gasosa e espectrometria de massa (Pi-CG/EM), métodos espectroscópicos como ressonância magnética nuclear (2D-NMR) e derivatização seguida por clivagem redutiva (DFRC), para delinear as diferenças de composição e estrutura entre estas duas ligninas. Esses dados vão ajudar a maximizar o aproveitamento desses importantes resíduos agroindustriais. O bagaço em estudo é composto por holocelulose (75,8%), lignina total (20%), cinzas (2%) e extrativos (2,2%). A palha teve composição química semelhante comparada a do bagaço, tendo holocelulose (72,9%), lignina total (18,9%), cinzas (4,7%) e extrativos (3,5%). Enquanto que a lignina do bagaço é rica em siringil (H:G:S de 2:38:60), a lignina da palha é rica em guaiacil (H:G:S de 4:68:28). As diferenças de composição foram também refletidas nas relativas abundancias das diferentes ligações. As ligninas do bagaço prevaleceram principalmente β–*O*–4´subestruturas aril-alquil-éter (representando 83% das ligações medidas no RMN) seguido de quantidades menores de β-5´ (fenilcumarânico, 6%) e outras subestruturas condensadas. A lignina a partir da palha tem menor quantidade de ligações β–*O*–4′ aril-alquil-éter (75%), mas os níveis relativamente mais altos de fenilcumarânico (β-5´, 15%) e dibenzodioxina (5-5/4-O β, 3%), correspondendo com uma lignina enriquecida de unidades G. A composição de fitoquímicos lipofílicos no bagaço e palha foi investigada em detalhe por cromatografia gasosa e espectrometria de massa. A composição dos lipídios a partir do bagaço e palha de cana-de-açúcar foram completamente diferentes umas das outras. Os extrativos de bagaço de cana foram dominados por *n*-aldeídos (cerca de 48% de todos os lipídios identificados) e *n*-álcoois graxos (ca. 23%) com pequenas quantidades de *n*-ácidos graxos (10%) e cetonas esteroides (14 %), os extratos de palha de cana de açúcar foram fortemente dominados por *n*-ácidos graxos (representando cerca de 60% de todos os compostos identificados) com quantidades significativas de compostos esteroides, particularmente esteróis (10%) e esteroides cetonas (14%). Tocoferóis e triterpenos também foram encontrados, sendo particularmente abundante entre os extrativos da palha da cana. Bagaço e palha de cana-de-açúcar podem ser

considerados como matérias-primas promissoras para obtenção de combustíveis e produtos químicos de interesse industrial.

ABSTRACT

LINO, Alessandro Guarino, D.Sc., Universidade Federal de Viçosa, September, 2015. **Chemical composition and structural of lignin and lipids from bagasse and straw sugarcane.** Adviser: Claudio Ferreira Lima. Co-Adviser: Jorge Luiz Colodette.

In this paper, we report a more extensive and detailed structural characterization of the lignins in sugarcane bagasse and straw. For this purpose, we used powerful analytical methodologies, including analytical pyrolysis coupled to gas chromatography and mass spectrometry (Py-GC/MS), spectroscopic methods such as nuclear magnetic resonance (2D-NMR) spectroscopy, and derivatization followed by reductive cleavage (DFRC), to delineate the compositional and structural differences between these two lignins. Such data will help to maximize the exploitation of these important agro-industrial wastes as feedstocks for the production of second-generation bioethanol and other biobased products. The bagasse studied is composed of holocellulose (75.8%) Total lignin (20%), ash (2%) and extractives (2.2%). The straw had similar composition compared to the bagasse, having holocellulose (72.9%) Total lignin (18.9%), ash (4.7%) and extractives (3.5%). Whereas the lignin from bagasse is rich in syringyl (H: G: S 2:38:60), the lignin from straw is rich in guaiacyl (H: G: S 4:68:28). The compositional differences were also reflected in the relative abundances of the different linkages. Bagasse lignin was primarily β–*O*–4´ alkyl-aryl ether substructures (representing 83% of NMR-measurable linkages), followed by minor amounts of β-5´ (phenylcoumarans, 6%) and other condensed substructures. The lignin from straw has lower levels of β–*O*–4′ aril-alquil-éter (75%) but higher relative levels of phenylcoumarans (β-5´, 15%) and dibenzodioxocins (5-5/4-O-β, 3%) consistent with a lignin enriched in G-units. The composition of lipophilic phytochemicals in sugarcane bagasse and straw was investigated in detail by gas chromatography and mass spectrometry. The composition of the lipids from sugarcane bagasse and straw were completely different from each other. While the extracts of sugarcane bagasse were dominated by n-aldehydes (ca. 48% of all identified lipids) and n-fatty alcohols (ca. 23%) with lower amounts of n-fatty acids (10%) and steroid ketones (14%), the extracts from sugarcane straw were strongly dominated by n-fatty acids (accounting for ca. 60% of all identified compounds) with significant amounts of steroid compounds, particularly sterols (10%) and steroid ketones (14%). Tocopherols and triterpenols were also found, being particularly abundant among the extractives of sugarcane

straw. Sugarcane bagasse and straw can be considered as promising feedstocks to obtain fuels and chemicals of industrial interest.

1. INTRODUÇÃO

Na última década há uma crescente busca da utilização dos resíduos agroindustriais, devido a incessante demanda das atividades agrícolas. O acúmulo destes resíduos gera a deterioração do meio ambiente e perda de recursos, com contribuição significante para o problema da reciclagem e conservação da biomassa. Diversos processos são desenvolvidos para utilização desses materiais, transformando-os em compostos químicos e produtos com alto valor agregado como álcool, enzimas, papel, energia, ácidos orgânicos, aminoácidos, etc. A maior parte da energia produzida no Brasil vem das hidrelétricas, mas com as secas que o país vem enfrentando as atenções se voltam para fontes alternativas como a biomassa da cana-de-açúcar. Segundo a Aneel (2014), somente 7% da energia produzida no Brasil vem da biomassa (bagaço de cana-de-açúcar, lenha, carvão vegetal entre outros). A cana-de-açúcar é cultivada em todas as regiões geográficas do Brasil. O seu cultivo continua crescendo em áreas próximas às usinas e em dezenas de novos empreendimentos que estão sendo instalado nas últimas safras, em áreas do oeste de São Paulo, Minas Gerais, Goiás, Mato Grosso do Sul, Mato Grosso, norte e nordeste brasileiro.

O Brasil é o maior produtor de cana-de-açúcar do mundo, seguido por Índia e Austrália. Planta-se cana no Centro-Sul e no Norte-Nordeste do país, o que permite dois períodos de safra no ano. A produção de cana-de-açúcar no Brasil tem crescido nos últimos anos, podendo atingir na safra 2015/2016 um total de aproximadamente 600 milhões de toneladas (UNICA - União da Indústria de Cana-de-Açúcar, 2015). No Brasil, o bagaço e a palha da cana são subprodutos disponíveis em maior quantidade. O bagaço e a palha da cana são uma abundante fonte de material lignocelulósico, sendo uma alternativa significante para o aumento da produção de etanol (SANTOS et al., 2012). Apresenta também as seguintes características: facilidade de obtenção e transporte, alta concentração de carboidratos e baixo custo de colheita e armazenagem (PANDEY et al., 2000). A produtividade média de cana-de-

açúcar no Brasil é de 85 toneladas por hectare, sendo que para cada tonelada de cana processada são gerados cerca de 140 kg de palha e 140 kg de bagaço em base seca, ou seja, 12 toneladas de palha e 12 toneladas de bagaço (CTC – Centro de Tecnologia Canavieira, 2014). Considerando que toda glicose vai ser convertida em etanol, o aproveitamento integral da cana-de-açúcar (colmo, palha e bagaço) poderá aumentar significativamente a produção de etanol por hectare, passando dos atuais 7.000 L para aproximadamente 14.000 L, sem necessidade de expansão da área cultivada (Ripoli et al., 2010; Felipe, 2010). Consequentemente, a não utilização dessa biomassa é desperdício energético. Grande parte do bagaço e da palha produzido é utilizada pelas próprias usinas no aquecimento de caldeiras e geração de energia elétrica. Hoje, essa biomassa tem sido alvo de vários estudos visando seu potencial energético no que diz respeito à produção de biocombustível, porém, seu uso não está restrito a esse fim. Devido à grande quantidade produzida e as suas características físicas e químicas, esses materiais encontram um vasto campo de utilização, dentre eles, na produção de ração animal, na indústria química, na fabricação de papel, papelão e aglomerados, como material alternativo na construção civil, na produção de biomassa microbiana e mais recentemente, na produção de álcool via bagaço e palha da cana.

A tecnologia de conversão de biomassa lignocelulósica em açúcares fermentáveis para a produção de etanol vem sendo considerada como uma alternativa promissora para atender à demanda mundial por combustíveis liquidos. Apesar de já existirem tecnologias disponíveis para o processamento da celulose, a maioria esbarra em dificuldades técnicas ou econômicas.

A composição química do bagaço apresenta pequenas variações em função da variedade da cana empregada e da localização geográfica dos locais de cultivo. O bagaço é composto por celulose, polioses (hemiceluloses) e lignina, como principais componentes macromoleculares. A celulose é o principal componente da parede celular e representa 41 a

44% do bagaço, a lignina representa de 20 a 22%, as hemiceluloses representam 25 a 27% e o restante, cerca de 10%, são componentes minoritários de baixo peso molecular, extrativos e substâncias minerais (ICIDCA, 1990). A palha da cana-de-açúcar, sendo toda a parte aérea da planta menos os colmos industrializáveis, é constituída basicamente de celulose, hemicelulose e lignina, na proporção aproximada de 40, 30 e 25%, respectivamente. Trabalhos realizados por Silva (2009), com a palha da cana *in natura* mostraram que o material apresenta 38% de celulose, 29% de hemicelulose e 24% de lignina. Aguilar *et al.* (1989), verificaram que a palha da cana apresenta um teor de cinzas entre duas e quatro vezes maior do que o bagaço da cana, variando em função do local de coleta do material, condições climáticas, estágio de desenvolvimento vegetativo e cultivar (Gomez, et al. 2010; Paes, 2005). A principal dificuldade encontrada na análise de composição química de materiais lignocelulósicos é o fato de que as macromoléculas presentes na parede celular (celulose, hemicelulose e lignina), se encontram intimamente associadas. Devido à íntima associação recalcitrante existente entre os três componentes poliméricos da biomassa, a liberação dos polissacarídeos como fonte de açúcares fermentáveis para produção de etanol está entre as mais importantes e urgentes prioridades nas áreas de pesquisa e desenvolvimento do etanol celulósico (Zhang, 2008).

Apesar das enormes quantidades de trabalho dedicadas ao uso do bagaço e palha de cana de açúcar como matérias-primas para a produção de bioetanol de segunda geração e outros bioprodutos, as estruturas de suas ligninas não foram estudadas em detalhe. Portanto, neste trabalho, apresentamos uma caracterização estrutural mais ampla e detalhada das ligninas no bagaço e palha de cana-de-açúcar. Para este fim, utilizou-se metodologias analíticas importantes, incluindo o método de degradação térmica, pirólise analítica acoplada a cromatografia gasosa e espectrometria de massa (Pi-CG / EM), técnicas espectroscópicas tais como heteronucleares de ressonância magnética bidimensional nuclear (2D-RMN), e o

método de degradação química de diagnóstico, derivatização seguida por clivagem redutiva (DFRC), para delinear as diferenças de composição e estruturais entre estas duas ligninas. Esses dados vão ajudar a maximizar o aproveitamento desses resíduos agro-industriais importantes como matérias-primas para a produção de bioetanol de segunda geração e outros produtos de base biológica.

Neste trabalho estudou-se a composição e a estrutura do bagaço e palha da cana-de-açúcar utilizando-se a pirólise acoplada à cromatografia gasosa e espectrometria de massas (Pi-CG/EM), derivatização seguida por clivagem redutiva e ressonância magnética nuclear bidimensional (2D - NMR), com o objetivo de elucidar a composição e estrutura da lignina e dos compostos lipofílicos no bagaço e palha da cana-de-açúcar. Esta informação irá ajudar a maximizar a exploração desta importante cultura agrícola como matéria-prima para os biocombustíveis e outros produtos de biorrefinaria.

2. REFERENCIAL TEÓRICO

2.1. Materiais lignocelulósicos de interesse industrial

A biomassa vegetal é a principal fonte de materiais renováveis da Terra e, portanto, tem um grande potencial como matéria-prima para a produção de celulose, bioenergia e outros produtos de interesse industrial. A biomassa vegetal e em particular materiais lignocelulósicos estão disponíveis em grandes quantidades e a baixo custo. São gerados através de práticas agrícolas e florestais, indústrias de celulose, indústrias de madeira e muitas agroindústrias contribuindo com o problema da poluição ambiental.

A composição química apresentada pelos materiais fibrosos é o que possibilita as indústrias converterem essa biomassa em diferentes produtos de valor agregado, como energia, biocombustíveis e bioprodutos (Ragauskas et al., 2006; Sarkar et al, 2012;. Somerville et al., 2010). Materiais fibrosos como palha (composta pela parte foliar da cana e porção apical dos colmos), bagaço da cana-de-açúcar, bambu ou capim-elefante apresentam bons valores de celulose em sua constituição (del Río et al., 2012; Zanine et al., 2007;

Tamolang et al., 1980). A cana-de-açúcar é largamente utilizada no Brasil pela indústria sucroalcooleira. Entretanto, a geração de resíduos proveniente dessas indústrias é inevitável, sendo que os mais representativos resíduos gerados são o bagaço e a palha. Além disso, essas matérias-primas são abundantes, ricas em compostos lignocelulósicos e para sua aquisição demandam pouco investimento financeiro, o que é altamente vantajoso em processos de biorrefinaria visando à produção de etanol (Wolf, 2011; Canilha et al., 2010).

2.2. Cana-de-açúcar

A cana-de-açúcar é uma planta que pertence ao gênero Saccharum L.. Há pelo menos seis espécies do gênero, sendo a cana-de-açúcar cultivada um híbrido multiespecífico, recebendo a designação *Saccharum spp*. As espécies de cana-de-açúcar são provenientes do Sudeste Asiático. A cana-de-açúcar é conhecida por suas características peculiares: uma planta fina de formato cilíndrico, folhas grandes e pode alcançar até seis metros de altura.

Introduzida no período colonial, a cana-de-açúcar se transformou em uma das principais culturas da economia brasileira. O Brasil não é apenas o maior produtor de cana. É também o primeiro do mundo na produção de açúcar e etanol e conquista, cada vez mais, o mercado externo com o uso do biocombustível como alternativa energética.

Responsável por mais da metade do açúcar comercializado no mundo, o país deve alcançar taxa média de aumento da produção de 3,25%, até 2018/19, e colher 47,34 milhões de toneladas do produto, o que corresponde a um acréscimo de 14,6 milhões de toneladas em relação ao período 2007/2008. Para as exportações, o volume previsto para 2019 é de 32,6 milhões de toneladas (MAPA, 2015).

O etanol, produzido no Brasil, a partir da cana-de-açúcar, também conta com projeções positivas para os próximos anos, devidas principalmente, ao crescimento do consumo interno. A produção projetada para 2019 é de 58,8 bilhões de litros, mais que o dobro da registrada em 2008. O consumo interno está projetado em 50 bilhões de litros e as exportações em 8,8 bilhões (MAPA, 2015).

A cana-de-açúcar é reconhecida como uma das melhores alternativas para a produção da bioenergia. As tecnologias convencionais já permitem a produção do etanol e da energia elétrica em padrões ambientais altamente sustentáveis. Esse balanço pode ser ainda mais satisfatório com o domínio tecnológico de novos processos, como a hidrolise lignocelulosica e gasificação dos resíduos energéticos.

A alta produtividade agrícola e a diversificação industrial permitem maior solidez aos empreendimentos, quando comparados a outras cadeias de bioenergia. Essa solidez econômica pode se reproduzir sob a forma de oferta de melhor remuneração e maior proteção aos trabalhadores envolvidos nas diferentes etapas da cadeia produtiva.

Além disso, a possibilidade de geração descentralizada de energia permite a extensão dos benefícios sociais para as comunidades locais no entorno dos projetos agroindustriais. Ao se consolidar como fonte de suprimento energético, a cana também pode se tranformar em vetor de promoção do desenvolvimento local, especialmente nos países tropicais.

A exitosa experiência brasileira deve ser aprimorada, de forma a melhor se adequar as necessidades dos países menos desenvolvidos. Para tanto, é preciso avançar no domínio das tecnologias de produção e uso do etanol em menor escala, onde já existem no mercado microdestilarias capazes de operar com relativa eficiência, chegando ao etanol dentro das especificações técnicas requeridas para o mercado de combustíveis.

Desta mesma forma, a indústria começa a desenvolver equipamentos adequados ao uso, como fogões, e os geradores a etanol, em substituições aos tradicionais geradores a diesel. Quanto a isso, o etanol apresenta algumas vantagens, especialmente, as facilidades de produção em grande escala e o fato d não ser perecível, o que facilita o armazenamento.

Esse conjunto de possibilidades deve permitir a construção de uma nova imagem para a cana-de-açúcar. De uma cultura historicamente associada á exclusão social, ela pode se consolidar em alternativa sólida para a promoção do desenvolvimento local em diversas regiões carentes, onde a insegurança alimentar está diretamente associada à insegurança energética.

2.3. Bagaço da cana-de-açúcar

É o mais significativo resíduo da indústria sucroalcooleira em volume (97 milhões de toneladas) e a cada ano estima-se que gere um excesso de 12 a 15 milhões de toneladas (Rezende et al., 2002 citado por Maciel, 2006).

É constituído fisicamente, assim que extraído o caldo da cana-de-açúcar, de quatro frações: material fibroso (45%), água (51% a 49%), sólidos não solúveis (2 a 3%), sólidos solúveis e extrativos (2 a 3%) (Triana et al., 1990).

A composição química do bagaço apresenta pequenas variações em função da variedade da cana empregada e da localização geográfica dos locais de cultivo. O bagaço é composto por celulose, polioses (hemiceluloses) e lignina, como principais componentes

macromoleculares. A celulose é o principal componente da parede celular e representa 41 a 44% do bagaço, a lignina representa de 20 a 22%, as hemiceluloses representam 25 a 27% e o restante, cerca de 10%, são componentes minoritários de baixo peso molecular, extrativos e substâncias minerais (ICIDCA, 1990). O bagaço da cana sem extrativos apresenta em torno de 42,4% de celulose, 25,2% de hemiceluloses, sendo as xilanas a mais expressiva hemicelulose do bagaço, 19,6% de ligninas e 1,6% de cinzas, base bagaço seco (Brienzo et al., 2009). Valores reportados por Maciel (2006) apontam teores de 45, 35 e 15%, respectivamente, para a celulose, hemiceluloses e lignina. Teores de cinzas tradicionalmente encontrados para madeiras (Mori et al., 2003; Brito e Barrichelo, 1978) são mais baixos que os encontrados em bagaço da cana, o que pode ser justificado pela natureza botânica do vegetal; que por apresentar rápido crescimento e imobiliza nutrientes na biomassa. Grande parte das cinzas do bagaço é representada pela sílica. Em estudo realizado por Boechat (2010), este reporta que aproximadamente 1,8% do bagaço são constituídos de sílica.

Sobre os usos futuros do bagaço diversos estudos apontam a viabilidade de produção de energia elétrica excedente e sua posterior comercialização à rede – mediante a modernização dos sistemas atuais de cogeração e utilização do excedente de bagaço -, além do uso das fibras como matéria-prima para o incremento de produção de etanol, por processo de hidrólise ácido-enzimático diluído, ou para a produção de outros biocombustíveis ou químicos, por processos de gaseificação, pirólise rápida ou BTL - Biomass to Liquid.

2.4. Palha da cana-de-açúcar

A palha corresponde às porções foliares e às ponteiras da cana, que, na maior parte das vezes, é colhida juntamente com os colmos, mas deixados no próprio lugar de plantio para devolver ao solo parte dos nutrientes minerais imobilizados durante o crescimento (Almeida, 2008). Os processos convencionais de colheita manual, com queima prévia ou mecânica, visam exclusivamente ao aproveitamento do colmo da cana. Em ambos os casos, o aproveitamento da palha não faz parte do processo de colheita (Ripoli et al., 2010). No entanto, ciente dos danos ao meio ambiente causados pelas queimadas dos canaviais, essa realidade vem se modificando, seja com a intervenção de órgãos públicos ou por meio de representantes do setor sucroenergético (Magalhães, et al., 2010). Com o Decreto Federal n. 2.661/98 que estabelece o fim gradativo da queima da cana-de-açúcar até 2017, haverá aumento na disponibilidade de palha para ser recuperada e posteriormente utilizada como

nova fonte de biomassa para produção de etanol celulósico (Gomez, et al., 2010; Ripoli, et al., 2010).

Tendo em vista a área cultivada de cana-de-açúcar no país, a geração de palha chega a alcançar 97 milhões de toneladas. A palha da cana-de-açúcar representa 15% do peso dos colmos da cana madura, ou 12% quando seca (Ripoli, et al., 2000; Abramo, et al., 1993). Em termos energéticos a palha representa 1/3 da energia potencial da cana-de-açúcar que, atualmente, é subaproveitada (CTC, 2014; Rabelo, et al., 2010; Abramo, et al., 1993). O tecido vegetal da palha de cana apresenta os mesmos componentes químicos que o bagaço ou a madeira. A palha da cana-de-açúcar, sendo toda a parte aérea da planta menos os colmos industrializáveis, é constituída basicamente de celulose, hemicelulose e lignina, na proporção aproximada de 40, 30 e 25%, respectivamente. Trabalhos realizados por Silva (2009) com a palha da cana in natura mostraram que o material apresenta 38% de celulose, 29% de hemicelulose e 24% de lignina. Aguilar et al. (1989), verificaram que a palha da cana apresenta um teor de cinzas entre duas e quatro vezes maior do que o bagaço da cana, variando em função do local de coleta do material, condições climáticas, estágio de desenvolvimento vegetativo e cultivar (Gomez, et al., 2010; Paes, et al., 2005). A compreensão da complexidade estrutural desses materiais lignocelulósicos requer o conhecimento das propriedades físico-químicas de cada um dos seus componentes para se ter a noção exata do seu potencial energético.

Apesar do alto potencial energético da palha, o seu uso não é considerado viável nos ciclos de cogeração, sendo os altos custoa de operação e transportes apontados como um dos principais entraves. Ademais, o fato de as usinas terem alcançado a autossuficiência energética cm o uso do bagaço, não necessitando, assim, incrementar as produções de vapor e energia elétrica para o uso interno, não incentiva o aproveitamento da palha para fins energéticos.’

Nesse contexto, a palha (quando não se tem a queima do canavial previamente à colheita) tem como única finalidade a proteção do solo. Segundo Rossetto et al. (2010), diversos estudos mostram os benefícios da manutenção da palhada nos canaviais, de forma a minimizar a degradação do sol. Os autores informam que a degradacao pode ser física, química e biológica e que, neste cenário, a manutenção do colchão de palha pode contribuir com cada um desses diferentes processos. Os autores apontam a redução da erosão (remoção das partículas mais finas do solo pela ação da chuva e do vento), manutenção da umidade do solo (redução da evaporação d água) e reposição de nutrientes como os principais benefícios

para a manutenção da palhada no campo. Entretanto, ainda é um tema controverso e sem resposta a determinação da quantidade mínima e ideal de palhada a ser deixada no campo.

Apesar ainda de não ser considerada um resíduo energético, essa visão deverá sofrer alterações em futuro próximo, haja vista os esforcoes, bem como os resultados obtidos, nos estudos da rota enzimática (processos de hidrolise), por meio da qual se pretende empregar o bagaço para aumentar a produtividade de etanaol nas usinas. Nesse cenário, a palha é apontada como uma possível fonte energética substituta do bagaço nos sistemas de cogeração.

2.5. Estrutura e composição química dos materiais lignocelulósicos

Os materiais lignocelulósicos estão constituídos majoritariamente por três polímeros estruturais, celulose e hemiceluloses e polímero aromático lignina, presentes na parede celular vegetal.

As propriedades extraordinárias das fibras lignocelulósicas e a morfologia e funcionalidade da parede celular são o resultado do alto grau de organização, estruturação e coordenação destes polímeros. Uma parte minoritária dos materiais lignocelulósicos consiste em compostos de baixo peso molecular solúveis em água ou em solventes orgânicos, assim como pequenos conteúdos de proteínas e sais minerais (Sjöström, 1993; Fengel e Wegener, 1984).

2.5.1. Celulose

A celulose, representada na Figura 1, é o polissacarídeo mais abundante na natureza, sendo base estrutural das células das plantas, é a mais importante substância natural produzida pelos organismos vivos. A porcentagem de celulose nas plantas varia dependendo da origem. O isolamento da celulose é fortemente influenciado pelas substâncias que acompanham a celulose na parece celular. Compostos como gorduras, ceras, proteínas e pectina podem ser facilmente removidos por extração com solventes orgânicos e álcalis diluídos. Sua cadeia se constitui de unidades de anidroglicose, unidas através de ligações β-1,4-glicosídicas, resultando num polímero de alto peso molecular e com grau de polimerização variando de 1.000 a 15.000. As cadeias de celulose nas paredes celulares das plantas são arranjadas compactamente, de modo que suas fibras apresentam regiões nitidamente cristalinas, uma consequência do número grande de ligações de hidrogênio que

resulta numa forte interação entre suas moléculas. Os grupos funcionais da cadeia de celulose responsáveis por essas interações são grupos hidroxilas.

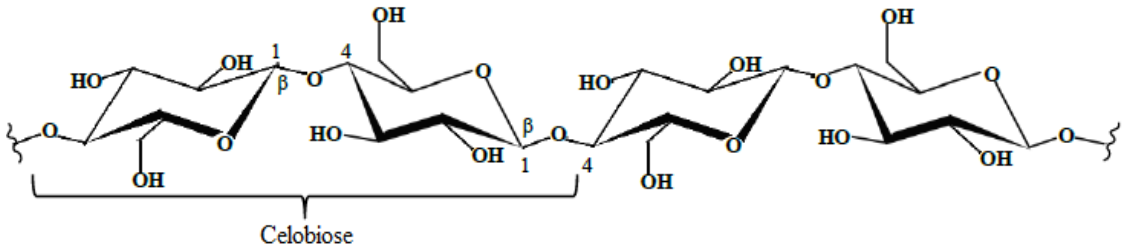

Figura 1. Estrutura de uma cadeia de celulose.

Moléculas de celulose são completamente lineares e tem forte tendência para formar ligações de hidrogênio inter e intramoleculares (Figura 2). Feixes de moléculas de celulose se agregam na forma de microfibrilas na qual regiões altamente ordenadas (cristalinas) se alternam com regiões menos ordenadas (amorfas). As microfibrilas se unem formando fibrilas e estas constroem as fibras celulósicas. Como consequência dessa estrutura fibrosa a celulose possui alta resistência à tração e é insolúvel na maioria dos solventes.

Os grupos hidroxilas (OH) são responsáveis pelo comportamento físico e químico da celulose, sendo capazes de formar dois tipos de ligações de hidrogênio, em função do seu posicionamento na unidade glicosídica. Existem ligações de hidrogênio entre grupos OH de unidades glicosídicas adjacentes da mesma molécula de celulose, que são ligações intramoleculares, responsáveis por certa rigidez das cadeias unitárias. Também ocorrem ligações entre grupos OH de moléculas adjacentes de celulose, constituindo as chamadas ligações intermoleculares, estas ligações são responsáveis pela formação das estruturas supramoleculares e são apresentadas. A Figura 2 ilustra a interação entre as moléculas de celulose formando microfibrilas.

A dissolução da celulose é possível por meio de conversões heterogêneas em ésteres (celulose nitrato e celulose acetato) ou éteres (metilceluloses, caboximetilceluloses) solúveis. Pode também ser diretamente dissolvida em ácidos concentrados. Contudo, o tratamento com ácidos pode conduzir a uma clivagem hidrolítica das cadeias de celulose, gerando produtos de degradação como ésteres ou adição de compostos. (Fengel e Wegener, 1984).

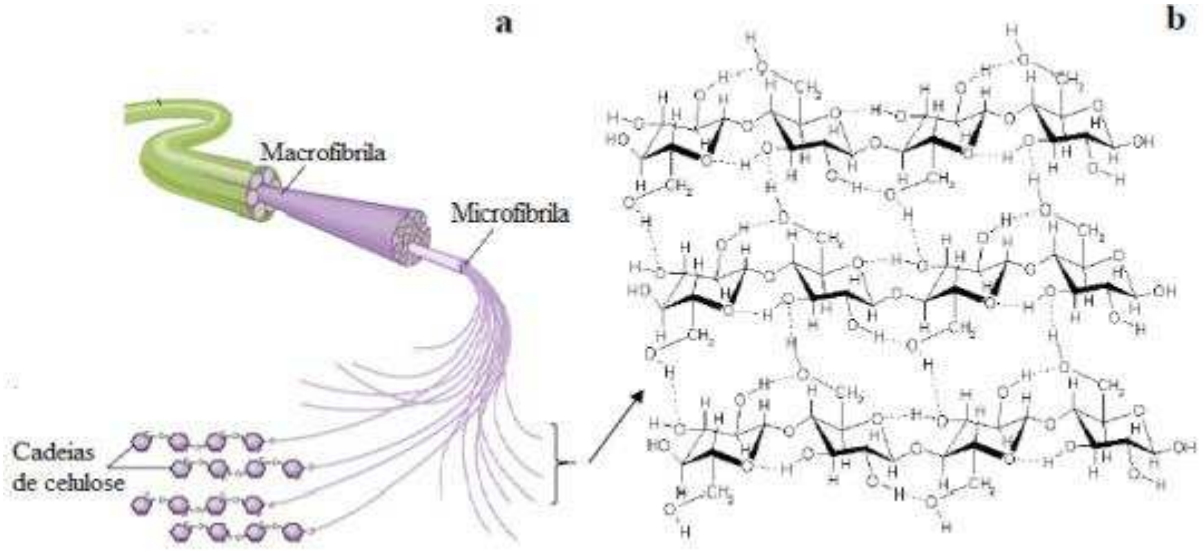

Figura 2. (a) Estrutura de cadeias de celulose em micro e macrofibrilas. (b) interações mediante ligações de hidrogênio e entre as cadeias (Prinsen, 2013).

2.5.2. Hemicelulose

Hemiceluloses são polissacarídeos não celulósicos também denominados polioses. Estão associadas à celulose e à lignina nos tecidos vegetais. As hemiceluloses diferem da celulose pela composição de várias unidades de açúcar, com cadeias moleculares menores e ramificadas. Enquanto a celulose, como substância química, contém exclusivamente a D-glicose como unidade fundamental, as polioses são polímeros, em cuja composição podem aparecer condensadas em proporções variadas, as seguintes unidades de açúcar: β-Dxilose, β-D-manose, β-D-glicose, α-L-arabinose, α-D-galactose, ácido β-Dglicourônico, ácido β-D-galactourônico e ácido α-D-4-metilglicourônico. As estruturas dos principais açúcares estão mostradas na Figura 3. Deve-se sempre lembrar que o termo hemicelulose não designa um composto químico definido, mas sim uma classe de componentes poliméricos presentes em vegetais fibrosos, possuindo cada componente propriedades peculiares. Como no caso da celulose e da lignina, o teor e a proporção dos diferentes componentes encontrados nas polioses (hemiceluloses) de madeira variam grandemente com a espécie e, provavelmente, também de árvore para árvore (Teleman, 2009). Como observado na Figura 3, algumas das unidades de açúcares que compõem as polioses possuem cinco átomos de carbono, sendo denominadas pentoses, outras, porém, possuem seis átomos de carbono, sendo denominadas hexoses. Os polímeros formados pela condensação de pentoses são chamados pentosanas, e

os formados por hexoses, hexosanas. As polioses não são, portanto um composto químico definido, mas sim uma classe de compostos poliméricos presentes em vegetais fibrosos, possuindo cada um destes componentes propriedades características. Madeiras de folhosas e coníferas diferem não só na porcentagem do total de polioses, mas também na porcentagem individual de cada açúcar que compõem as polioses. Coníferas possuem uma maior proporção de unidades de manose e galactose quando comparado às folhosas. Estas, por sua vez, apresentam uma maior proporção de unidades de xilose e grupos acetila que o encontrado em coníferas. No caso específico do bagaço da cana, o principal açúcar constituinte das polioses é a xilose (Fengel e Wegener, 1984).

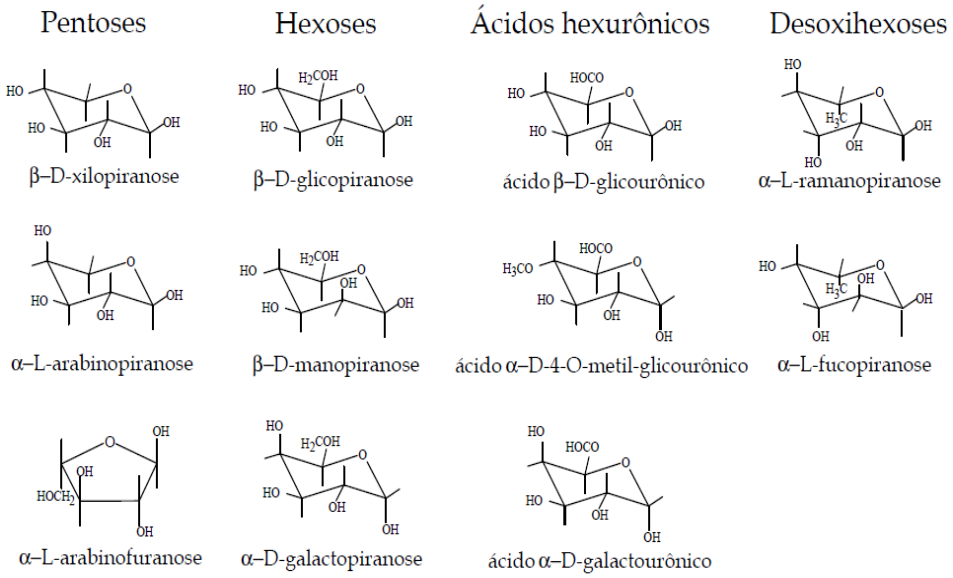

Figura 3. Fórmulas dos açucares componentes das polioses.

2.5.3. Lignina

A lignina é um polímero aromático composto por unidades de 4-fenilpropano. Tem uma estrutura macromolecular heterogênea e pode conter três tipos de unidades aromáticas: unidades p-hidroxifenil (H), guaiacil (G) e siringil (S), que não tem nenhum grupo metoxil (H) ou tem um (G) ou dois (S) grupos metoxil nas posições C3 e C5 da unidade aromática (Figura 4).

A composição da lignina depende de sua origem vegetal. De fato, as ligninas de madeiras de fibras longas (coníferas), madeiras de fibras curtas (folhosas) e gramíneas possuem estruturas básicas muito diferentes entre elas. Assim, a lignina de coníferas apresenta principalmente de unidades G, as folhosas unidades G e S em diversas proporções,

e a lignina em gramíneas, como a cana-de-açucar, a formação da lignina envolve a polimerização dos três tipos de unidades (lignina H-G-S) (del Río et al., 2005; Barbosa, 2008). A lignina representa entre 25 e 33% da biomassa seca em madeiras de coníferas, e entre 18 e 34% em madeiras folhosas (Aitken et al.,1988). É o segundo polímero mais abundante na Terra, atrás da celulose. Lignina é uma substância amorfa, de natureza aromática e muito complexa, presente na parede celular e na lamela média dos vegetais (Rowell, 2005). Atua como componente essencial da madeira, promovendo o fortalecimento das paredes celulares, facilitando o transporte de água e impedindo a degradação da parede polissacarídea (Hatfield, R. e Vermerris, 2001). A concentração da lignina é alta na lamela média e baixa na parede secundária. Por causa da sua espessura, pelo menos 70% da lignina das coníferas é, entretanto, localizada na parede secundária. A parede secundária das madeiras de compressão das coníferas pode apresentar concentração de lignina entre 55 e 88%. Os valores são bastante similares para madeiras de folhosas. Quando o processo de lignificação é completado, geralmente coincide com a morte da célula formando o que se denomina tecido de resistência. Daí concluir-se que a lignina é um produto final do metabolismo da planta (Terashima et al., 1993; Saka e Goring, 1985).

2.5.3.1. Biossíntese da lignina

A biossíntese da lignina nas plantas é realizada pela rota do ácido chiquímico, sendo este convertido em ácido prefênico e, posteriormente, transformado nos aminoácidos tirosina e fenilalanina. Esses dois aminoácidos são os pontos de partida para a produção dos álcoois p-cumarílico, coniferílico e sinapílico (Figura 4). Esses três precursores são a base do polímero da lignina, lignina p-hidroxifenila (H) derivada do álcool p-cumarílico, Guaiacila (G) derivada do álcool coniferílico e a Siringila (S) do álcool sinapílico (Boerjan et al., 2003; Fengel e Wegner, 1984; Sarkanen, 1971).

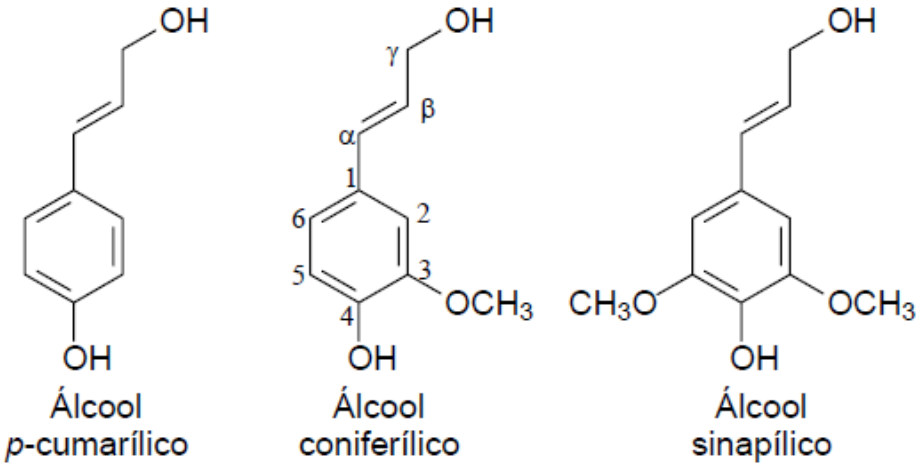

Figura 4. Precursores primários da lignina.

Esses precursores entram em contato com enzimas desidrogenases (peroxidases e lacases), conduzindo a abstração inicial de um hidrogênio radicalar no fenol, o que dá início a todo o processo de polimerização nos sítios de lignificação (Freudenberg e Neish, 1968). As ligações para a formação da lignina podem ocorrer nos átomos de carbono da cadeia lateral do propano, no núcleo aromático e na hidroxila fenólica. A ligação de principal ocorrência entre as unidades de fenilpropano é do tipo β-O-4 (éter-arila). Além dessa ligação, outros tipos podem ocorrer como as ligações 4-O-5, 1-O-4, α-O-4, 5-5, 1-5, β -5, β-1, β-β e as ligações condensadas nos carbonos 2 e 6 (Figura 4).

A presença de diferentes unidades precursoras e o elevado número de combinações possíveis entre as unidades precursoras faz com que a estrutura da macromolécula de lignina seja bem mais complexa que a apresentada pela celulose e pelas polioses. Os estudos visando esclarecer a estrutura da lignina empregam métodos de isolamento suaves procurando preservar ao máximo as ligações presentes (Lin e Dence, 1992).

Uma das classificações possíveis para a lignina é estabelecida em função das espécies vegetais e dos padrões aromáticos de substituição, como segue:

- Lignina de Coníferas: São mais homogêneas, contendo quase que exclusivamente unidades guaiacila (Ligninas-G);

- Ligninas de Folhosas: Apresentam quantidades equivalentes de grupos guaiacila e siringila, e pequenas unidades p-hidroxifenila (Ligninas-GS);

- Ligninas de Gramíneas: Apresentam maior quantidade de unidades p-hidroxifenila que o encontrado em madeiras (coníferas ou folhosas), mas sempre em proporção menor que as outras unidades (Ligninas-GSH).

Na literatura há uma série de modelos de ligninas, todos construídos a partir de análises de grupos funcionais e espectroscópicas. Na Figura 5 é apresentado modelo estrutural da lignina proposto por Nimz (1981).

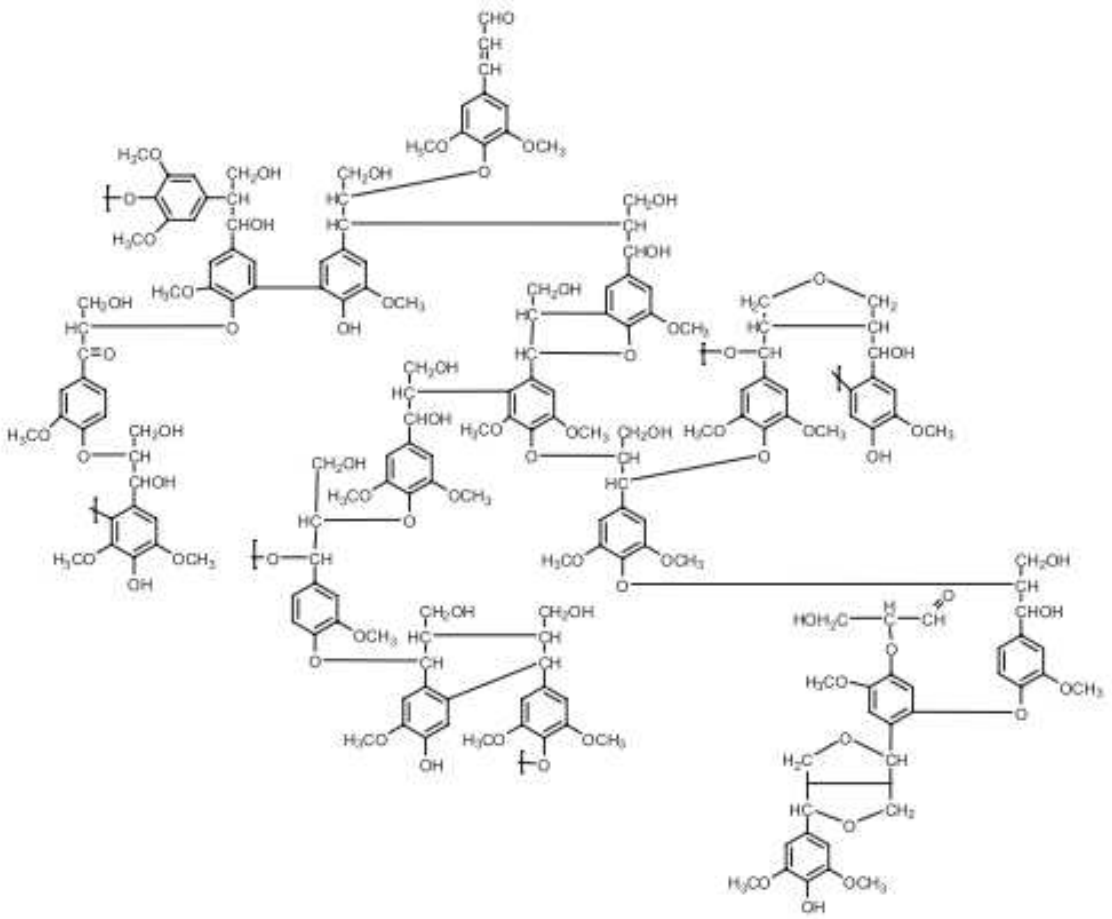

Figura 5. Esquema de uma estrutura química da lignina de folhosas (Nimz, 1981).

O comportamento químico da lignina é governado, principalmente, pela presença de diferentes grupos funcionais encontrados na macromolécula. A Figura 5 revela a presença de éteres (alifáticos e aromáticos), álcoois alifáticos e benzílicos, fenóis e em menor proporção grupos carbonila (aldeídos, cetonas e ésteres).

Ainda, as diferentes ligações como ligações do tipo éter ou tipo carbono carbono determinam a escolha das condições experimentais a serem utilizadas quando se deseja o isolamento, a caracterização e o estudo das propriedades químicas das ligninas. (Curvelo, 1992; Fengel e Wegener, 1984).

A determinação de grupos funcionais, como hidroxilas alifáticas e aromáticas, grupos éter, grupos carbonila e metoxila, para a elucidação da estrutura da lignina, pode ser feita por

vários métodos químicos e físicos, como espectroscopia na região do ultravioleta visível, espectroscopia na região do infravermelho, ressonância magnética nuclear (RMN de 1H e RMN de 13C), espectrometria de massas, em combinação com cromatografia gasosa, pirólise acoplada à espectrometria de massa, e também os métodos químicos como acetilação para determinação de hidroxilas alifáticas e totais e determinação de grupos carbonila pela reação com cloridrato de hidroxilamina (del Río et al., 2012; Rencoret et al., 2011; Lin e Dence, 1992; Fengel e Weneger, 1984).

2.5.3.2. Constituintes minoritários

Os componentes minoritários incluem uma variedade de compostos orgânicos, sendo que nenhuma espécie vegetal os contém em sua totalidade. A presença relativa destes é governada por uma série de fatores, entre os quais merece maior destaque os de natureza genética e climática (D'Almeida, 1988).

Os constituintes menores não residem na parede celular da planta e dividem-se, basicamente, em duas classes. A primeira classe engloba materiais conhecidos como extrativos por serem extraíveis em água (hidrossolúveis extraíveis), solventes orgânicos neutros (extraíveis lipofílicos), ou volatilizados por arraste de vapor. A segunda classe engloba materiais que não são comumente extraíveis com os agentes mencionados, como, por exemplo, compostos inorgânicos, proteínas e substâncias pécticas. Esses constituintes minoritários são frequentemente responsáveis por determinadas características da planta, como cor, cheiro, resistência natural ao apodrecimento, sabor e propriedades abrasivas (D'Almeida, 1988).

Os principais grupos químicos que compreendem as substâncias de baixo peso molecular são: os compostos aromáticos (fenólicos), as substâncias mais importantes deste grupo são os compostos tanínicos que podem ser divididos em: taninos hidrolisáveis e flobafenos condensados, além de outras substâncias como estilbenos, lignanas e flavonóides e seus derivados; Terpenos, englobam um grande grupo de substâncias naturais, quimicamente podem ser derivados do isopreno. Duas ou mais unidades de isopreno constituem os mono - sesqui - di - tri - tetra e politerpenos; Ácidos alifáticos, ácidos graxos saturados e insaturados são encontrados principalmente na forma dos seus ésteres com glicerol (gordura e óleo) ou com álcoois (ceras). O ácido acético é ligado às polioses como um grupo éster. Ácido di e hidroxi-carboxílico ocorrem principalmente como sais de cálcio; Álcoois, as maiorias dos

álcoois alifáticos na madeira ocorrem com componentes ésteres, enquanto que os esteróis aromáticos, pertencentes aos esteróides, são principalmente encontrados como glicosídeos.

3. OBJETIVOS

3.1 Objetivo geral

A presente tese aborda o estudo da composição química e estrutural dos principais constituintes do bagaço e da palha da cana-de-açúcar. Teve-se um interesse especial nos extraíveis lipofílicos e no polímero lignina, já que ambos constituintes apresentam importância quando o material lignocelulósico é utilizado para a produção de celulose e papel, biocombustíveis e outros produtos de valor agregado. Este estudo permitirá avaliar o potencial destes materiais lignocelulósicos (bagaço e palha) e servem como ponto de partida para obter um melhor aproveitamento industrial mediante processos de biorrefinaria.

3.1 Objetivo específico

- ✓ Identificar as principais técnicas analíticas de identificação da lignina do bagaço e da palha da cana-de-açúcar;
- ✓ Estudar detalhadamente a composição química da lignina do bagaço e palha da cana;
- ✓ Identificar as principais ligações presentes na lignina da palha e do bagaço da cana;
- ✓ Estudar detalhadamente a composição dos lipídios presentes no bagaço e palha da cana;
- ✓ Comparar as diferenças entre a composição química da lignina presentes na palha e no bagaço da cana-de-açúcar.

4. PROCEDIMENTOS EXPERIMENTAIS

4.1. Matéria-prima

A matéria-prima utilizada nos experimentos foi à palha e o bagaço da cana-de-açúcar cultivar RB867515 fornecidas por uma usina de etanol de médio porte localizados na região da cidade de São Pedro dos Ferros, Minas Gerais, proveniente de uma mesma safra (2013/2014).

Figura 6. Ilustração (A) Palha cana-de-açúcar e (B) Bagaço cana-de-açúcar.

4.2. Procedimentos

4.2.1. Preparação das amostras

As amostras foram fornecidas por uma usina de etanol de médio porte localizados na região da cidade de São Pedro dos Ferros, em Minas Gerais, Brasil. As plantas de cana-de-açúcar foram recolhidas e limpas no local. A cana recolhida foi processada por uma moenda para a retirada do caldo. O bagaço resultante foi transportado para o laboratório onde foi seco ao ar e moído utilizando um moinho de facas IKA (Janke & Kunkel, Staufen, Alemanha) para ter uma boa homogeneização do material. A palha, material heterogêneo composto por folhas e ponteiras da cana-de-açúcar, foi triturada no mesmo equipamento utilizado para triturar o bagaço a fim de promover uma melhor homogeneização do material. Após a moagem, as serragens resultantes (de palha e bagaço) foram secas ao ar na sala de temperatura controlada durante pelo menos 24 horas, e, em seguida, armazenadas em recipientes hermeticamente fechados.

4.2.2. Composição Química

Os diferentes métodos analíticos utilizados para determinar a composição química dos materiais lignocelulósicos são descritos nos itens seguintes deste trabalho. As amostras moídas (serragem) foram secas ao ar na sala de temperatura controlada (23 ± 1ºC e UR% de

50 ± 2%) durante pelo menos 24 horas. O teor de cada constituinte químico foi expresso com base em matéria seca. Determinou-se a umidade em cada teste realizado. Todas as análises foram realizadas em triplicatas para cada tipo amostra (palha e bagaço).

$$Umidade\ (U, \%) = (m - m') / m \times 100$$

Onde:

m = massa úmida

m' = massa seca

4.2.3 Determinação do conteúdo de cinzas

O teor de cinzas foi determinado de acordo com a Norma Tappi 211 om-93. Pesaram cadinhos de porcelana previamente limpos com HCl concentrado e aquecidos durante seis horas a 575 ºC em um forno de mufla (Heraeus, modelo M110). Pesou-se cerca de 200 mg de matéria seca (m) nos cadinhos de massa conhecida. Os cadinhos com as amostras foram aquecidos durante seis horas a 575 ºC em um forno de mufla. O resíduo (c) foi determinado por gravimetria e se calculou o teor de cinzas (C) como % de matéria seca. Todas as análises foram realizadas em triplicatas para cada tipo amostra (palha e bagaço).

$$Cinzas\ (C, \%) = c / m \times 100$$

4.2.4 Determinação do conteúdo dos lipofílicos extraíveis

Os compostos extraíveis lipofílicos se extraem com acetona em um extrator do tipo Soxhlet durante oito horas. Considera-se o peso da amostra seca (sem umidade). A solução com os compostos lipofílicos extraídos é seca em um rotoevaporador até a secagem completa. A quantidade de matéria seca foi determinada por gravimetria.

Os extratos lipofílicos obtidos são dissolvidos em $CHCl_3$ para posterior analises por cromatografia gasosa (CG) e cromatografia gasosa/espectrometria de massas (CG/EM).

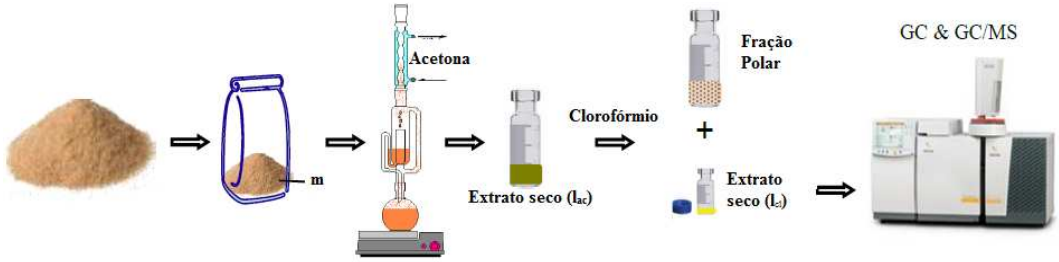

Figura 7. Determinação do conteúdo e da composição química dos extraíveis lipofílicos.

4.2.4.1 Analises dos extratos lipofílicos mediante CG e CG/EM

Os compostos extraíveis lipofílicos foram analisados por CG e CG/EM. As colunas capilares selecionadas para as analises de lipídios por CG foram de menor comprimento (5m), já que proporcionam uma eluição e uma separação adequada dos lipídios de alto peso molecular (Prinse, et al., 2012; Gutiérrez et al., 1998). Para as análises por CG e CG/EM é essencial que os compostos existentes na amostra sejam suficientemente voláteis, por isso é necessário recorrer a métodos de derivatização, tais como a sinalização, quando os componentes a analisar não são voláteis. A sinalização dos grupos hidróxidos de alcoóis, esteróis entre outros, se realizaram com BSTFA. Para este fim, após a secagem da amostra foi adicionado 0,2 mL de BSTFA e 0,1 mL de piridina. A mistura foi então aquecida a 70º C durante duas horas e seca com nitrogênio. Posteriormente dissolveu-se em clorofórmio para ser analisada por CG e CG/EM.

As análises cromatográficas dos extratos lipofílicos foram feitas em um cromatógrafo de gases HP 5890 (Hewlett Packard, Hoofddorp, Netherlands) equipado com injetor split/spliless e um detector de ionização de chamas FID e uma coluna capilar de alta temperatura, com tubo de sílica fundida DB5-HT (J&W, comprimento 5 m, diâmetro interno 0,25 mm e espessura de filme 0,1μm). O programa de aquecimento do forno começou a 100 ºC (1min), seguindo de um aumento de temperatura ate 350 ºC (3min) a 15º C/min. As temperaturas do injetor e do detector se mantiveram a 300 ºC e 350 ºC, respectivamente. O gás de arraste que se utilizou foi hélio e a injeção se realizou em modo splitless.

As análises mediante CG/EM foram feitas em um cromatógrafo de gases Varian Star 3400 acoplado a um detector íon trap (Varian Saturn 400), usando uma coluna capilar de sílica fundida DB-5HT (J&W, comprimento 15m, diâmetro interno 0,25mm e espessura de filme 0.1 μm). As amostras, com uma concentração de 2,5 mg / ml, foram injetadas com um injetor programável (Varian 8200). A temperatura do injetor durante a injeção da amostra foi

60 ºC, e 0,1 min após a injeção foi programada para 380 ºC com uma razão de 200 ºC/min e mantendo-se até ao final da analise (10 min). O forno aqueceu de 120 ºC (1 min) a 380 ºC (5 min) a 10 ºC/min. A linha de transferência se manteve a 300 ºC. O gás de arraste utilizado foi o gás hélio a uma taxa de 2 mL/min. A identificação de cada componente se fez por comparação de seus espectros de massa com os espectros de massa existentes nas bibliotecas (Wiley e Nist). Os picos cromatográficos foram quantificados a partir de suas áreas nos cromatogramas e o fator de diluição utilizado para preparar a dissolução nos frascos de análises.

4.2.4.2. Determinação do conteúdo de hidrossolúveis extraíveis

Para determinar o teor de compostos hidrossolúveis pesaram-se aproximadamente dois gramas de material lignocelulósico previamente extraído com acetona para eliminar os compostos lipofílicos extraíveis a uma extração com água destilada a 100º C durante três horas de acordo com a norma Tappi T207 om-88 (Figura 8).

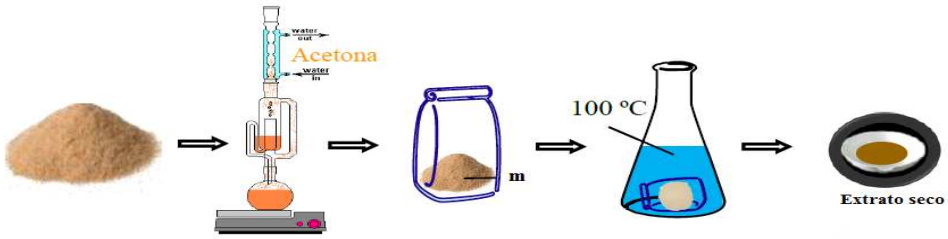

Figura 8. Determinação do conteúdo de hidrossolúveis extraíveis.

Hidrossolúveis extraíveis (HE %) = he / [m × (100-U)/100 × (100 + L_{ac})/100] × 100

O extrato foi concentrado através de rotoevaporação a 95º C sob pressão reduzida. O concentrado foi transferido a cadinhos de porcelana que foram secos a 100º C até peso constante (he). Calculou-se o conteúdo por rendimento gravimétrico como % de matéria seca inicial (HE). A umidade (U) foi levada em consideração no momento de pesar a amostra (extraída com acetona) e se recalculou o peso da amostra com conteúdo de lipofílicos extraíveis previamente determinados (L_{ac}).

4.2.5. Analise elementar (C, N, H, S)

Essa técnica está baseada na combustão de uma amostra do material a ser analisado para a determinação das quantidades de carbono, enxofre, hidrogênio e nitrogênio; a quantidade de oxigênio (O) é obtida por diferença, conforme a equação 1. As análises elementares foram realizadas pela Universidade de Sevilha, utilizando um analisador elementar modelo EA LECO CHNS-932.

$$\%O = 100 - \%C - \%H - \%N - \%S - Cinzas \quad \text{(Equação 1)}$$

4.2.6. Determinação do conteúdo de carboidratos

4.2.6.1. Determinação do conteúdo de holocelulose

A holocelulose representa a fração total de polissacarídeos, celulose mais hemicelulose. O conteúdo de holocelulose foi determinado segundo o método de WISE (1946). A 5 g do material a ser analisado sem extraíveis se adiciona 160 mL de água e aquece em banho Maria a aproximadamente 80 ºC. Uma vez aquecidos, adiciona-se 1,5 g de clorito de sódio e 10 gotas de ácido acético glacial. Agita-se periodicamente e a cada hora se adiciona outras doses de clorito sódico e ácido acético (normalmente, quatro vezes). A solução foi filtrada e a holocelulose foi lavada com acetona, água quente e novamente com acetona. Determinou-se o resíduo seco (hc) e determinou o conteúdo como % da matéria seca inicial (HC), através da equação 2.

$$Holocelulose\ (HC, \%) = hc / [m \times (100-U)/100 \times (100+L_ac+HE)/100] \times 100 \quad \text{(Equação 2)}$$

4.2.6.2. Determinação do conteúdo de α-celulose

A α-celulose em geral indica a celulose de alto peso molecular não degradada. A α-celulose se dissocia como um resíduo insolúvel a partir da holocelulose por extração das hemiceluloses com dissolução alcalina.

Determinou-se o conteúdo de α-celulose conforme a norma Tappi T 203 OS-61. A 3 g de holocelulose adiciona-se 75 mL de NaOH 17,5% em algumas etapas. Primeiro adiciona-se 15 mL (1 min), depois 10 mL (0,75 min) e finalmente 10 mL (0,25 min) agitando sempre.

Deixa-se repousar por um minuto e em seguida adiciona-se 10 mL (2,5 min) e repita mais três vezes. Depois se deixa repousar por 30 min, adiciona-se 100 mL de água destilada e agita vigorosamente. Deixa-se repousar por 30 min, e filtrou-se a solução com um filtro de poro nº 2. Lavou-se com 25 mL de NaOH 8,3% e lavou-se 5 vezes com 50 mL de água destilada, e finalmente com 400 mL de água. Depois se lavou o resíduo no filtro com acido acético (2mol/L). Finalmente lavou-se com água destilada até se obter um filtrado neutralizado. Secou-se o filtro e determinou-se a quantidade de α-celulose por gravimetria (ac) e determinou o conteúdo como % da matéria seca inicial (AC, %) de acordo com a equação 3.

$$\alpha\text{-celulosa (AC, \%)} = ac / [\, m \times (100-U)/100 \times (100+L_{ac}+HE)/100] \times 100 \quad \text{(Equação 3)}$$

4.2.7. Determinação do conteúdo de lignina total

O conteúdo de lignina é a soma do conteúdo de lignina solúvel e lignina insolúvel em ácido (lignina Klason).

4.2.7.1. Determinação do conteúdo de lignina Klason

Determinou-se a lignina Klason de acordo com a norma Tappi T222 om-98 com algumas modificações. Primeiro eliminou os extraíveis lipofílicos e hidrossolúveis do material lignocelulosico. Pesou-se 300 mg da amostra seca e colocou em frascos de vidro pirex junto com 3 mL de H_2SO_4 72% e se manteve em banho Maria a 30 ºC durante 1 hora. Posteriormente diluiu-se com 84 mL de água para obter a concentração de ácido de 4% e colocou-se em uma autoclave durante uma hora a 120 ºC. Depois de esfriar e repousar, a solução foi filtrada através de um filtro de kitasato de poro nº 3 previamente pesado. O composto hidrolisado foi guardado para as análises de lignina ácido solúvel. O filtro que contém a lignina ácido insolúvel foi lavado com água destilada até obter um filtrado de pH neutro. Secou-se o filtro durante 4 horas a 100 ºC para sua determinação gravimétrica (lik). O conteúdo final (LIK) de lignina Klason determinou-se subtraindo o conteúdo de cinzas (C_{lik}) e proteínas ($N_{lik} \times 6.25$) no resíduo insolúvel (Equação 4).

$$LIK(\%) = [lik \times (100 - C_{lik} - (N_{lik} \times 6.25))/100]/[m \times (100-U)/100 \times (100+L_{ac}+HE)/100] \times 100 \quad \text{(Equação 4)}$$

4.2.7.2. Determinação do conteúdo em lignina solúvel em ácido

Para a determinação do conteúdo de lignina ácido solúvel (LAS) mediu-se a absorbância do hidrolisado obtido da hidrólise ácida nas análises da lignina Klason em cubetas de quartzo a 205 nm. Para o branco preparou uma solução de 4% de H_2SO_4. Determinou-se a média de três medições pontuais de absorbância e calculou-se o conteúdo de acordo com a equação 5.

LAS (%) = (A × V × f) / [ξ × (m × (100-H)/100 × (100 + L_{ac} + HS)/100)] × 100 (Equação 5)

A (AU/cm)	Absorbância a 205 nm	m (g)	Peso da amostra
V (L)	Volume final do hidrolisado	U (%)	Umidade da amostra
F	Fator de diluição	Lac (%)	Conteúdo de extraíveis lipofílicos
ξ (AU/cm g)	Coeficiente de extinção (110)	HS (%)	Conteúdo de extraíveis hidrossolúveis

4.3. Moinho de bolas e isolamento da lignina "de madeira moída" (MWL)

A MWL foi obtida de acordo com o procedimento de Björkman (1956). Cerca de 40 g de material livre de extrativo foi finamente moído em um moinho de bolas Retsch PM100 (Restch, Haan, Alemanha), utilizando um frasco de ágata e 20 bolas ágata esféricas de dois centímetros de diâmetro. A lignina do material moído foi então extraída com dioxano-água, 96: 4 (v/v) (20 mL de solvente/g de fibra moída), e a MWL isolada posteriormente purificada como descrito por del Río et al. (2012). A amostra obtida foi à lignina Björkman, que conservou a 4 ºC sem contato com a luz e o ar. As amostras de MWL foram analisadas por Py-GC/MS e 2D-NMR.

4.4. Técnicas analíticas para a análise de lignina

4.4.1. Pi-CG/EM

A Pi-CG/EM (Pirólise acoplada a cromatografia gasosa e espectrômetro de massa) é um método que transforma compostos complexos não voláteis em uma mistura de fragmentos voláteis pela decomposição térmica na ausência de oxigênio (Fullerton e Franich, 1983; Meier e Faix, 1992). A pirólise normalmente é realizada a temperaturas entre 400 e 800 ºC. Analisam-se os produtos resultantes da pirólise da lignina em um cromatógrafo de gases acoplado a um espectrômetro de massa. A energia térmica presente faz ocorrer à dissociação de forma controlada das ligações éter entre as unidades de lignina, e em certa proporção

também das ligações carbono-carbono, obtendo uma mistura de compostos fenólicos voláteis, nos quais os grupos metoxilas se mantêm unidos. Isto permite deduzir de que tipo de unidade de lignina se produz determinado composto da pirólise e determinar a proporção das unidades H, G e S. A Pi-CG/EM apresenta diversas vantagens frente a outros métodos. É uma técnica analítica rápida, que necessita pouca quantidade de amostra e uma simples preparação da mesma. Também apresenta vantagem frente aos métodos clássicos de análise de lignina, pois não é necessário isolar a lignina da amostra, permitindo sua análise *in situ*.

Para as análises de lignina por Pi-CG/EM utilizou aproximadamente 0,1mg da amostra de lignina WCW (whole-Cell-wall) e lignina isolada MWL. A pirólise foi feita em um pirolisador EGA/PY-3030D microfurnace pyrolyzer (Frontier Laboratories Ltd.), que operou a 500 ºC durante 10 s, conectado a um equipamento de CG/EM (Agilent 7820) com uma coluna capilar DB-1701 (comprimento 60m, diâmetro 0,25 mm e espessura do filme 0,25µm). A temperatura do forno de CG inicial foi de 45 ºC (4 min), aumentando de 45 a 280 ºC na taxa de 4 ºC/min, permanecendo nessa temperatura por 10 min. O detector de massas operou com ionização por impacto de elétrons (70 eV). O fluxo do gás de arraste (He) foi de 2mL/min. Para a pirólise na presença de hidróxido de tetrametilamónio (Pi/TMAH), cerca de 0,1 mg de amostra foi misturada com 0,5 mm^3 de TMAH (25%, m/m, em metanol), a pirólise foi efetuada a 500 °C, e os produtos introduzidos analisados como descrito acima. Identificação dos compostos liberados foi feita por comparação do seu espectro de massa com os das bibliotecas Wiley e NIST e com os relatados na literatura (Faix et al. 1990; Ralph e Hatfield, 1991). Calcularam-se as áreas molares, se normalizou a 100%, e fez uma média entre as repetições das pirólises. O desvio padrão foi inferior a 5%.

4.4.2. 2D – NMR

Através dos espectros 2D é possível obter a simplificação de espectros complexos que consistem de muitas bandas ou picos sobrepostos; uma melhor resolução espectral através da expansão dos picos sobre a segunda dimensão e, a identificação de várias interações inter e intramoleculares através da correlação seletiva dos picos . O experimento com análises de 2D-NMR foi realizado em um instrumento AVANCE III 500 MHz (Bruker, Karlsruhe, Alemanha), nas instalações de RMN do centro de pesquisa da Universidade de Sevilha.

Para as análises da lignina isolada (MWL), se dissolveram 40 mg das amostras de bagaço e palha de cana-de-açúcar em 0,75 mL de dimetil sulfóxido deuterado (DMSO-*d*6) em tubos de NMR. Para as análises da lignina da amostra WCW dissolveram 50 mg material

lignocelulósico (moída e livre de extrativo) em 0,75 mL de DMSO-*d6*.(Kim et al., 2008; Rencoret et al., 2009).

Os sinais de correlação foram calibrados em relação ao sinal do solvente (δC 39.5; δH 2.49 para DMSO). Identificação dos sinais liberados por HSQC e foram atribuídos por comparação com a literatura (Ralph e Landucci, 2009, 2010; Kim et al., 2008; Rencoret et al., 2012; del Río et al. 2012;).

4.4.3. DFRC

Um novo método denominado de derivatização seguida por clivagem redutiva ou clivagem seletiva (originalmente *derivatization followed by reductive clivage* – DFRC) promove a derivatização da parede celular com brometo de acetila em ácido acético, solubilizando a lignina. Na sequência, a lignina derivatizada é submetida a uma clivagem redutiva utilizando-se o zinco permitindo a análise de sua composição monomérica. Esta técnica tem sido utilizada para se estudar a composição unitária da lignina devido à excelente recuperação dos monolignóis (S, G e H) comparados com outros métodos de extração. O método DFRC da informação quantitativa da composição das unidades H, G e S, presentes na subestrutura eterificadas (Lu e Ralph, 1997a, 1997b). A inclusão de um padrão interno nas análises de CG/EM, permite quantificar a proporção de unidades eterificadas.

A degradação DFRC foi realizada de acordo com o método desenvolvido por Lu e Ralph (1997, 1999). Cerca de 5 mg de amostras de lignina foram agitadas durante duas horas a 50 ºC com brometo de acetila em ácido acético 8:92 (v/v) e tratado com zinco em pó (50 mg) por 40 minutos em temperatura ambiente. Passo seguinte foi a adição de 10 mL de diclorometano, 10 mL de solução saturada de cloreto de amônio. O pH da fase aquosa foi ajustado para menos que 3 com HCl diluído (3%). A fase aquosa foi extraída três vezes com diclorometano. Adicionou-se sulfato de sódio anidro a fase orgânica para assegurar a ausência de umidade e em seguida filtrou-se. A solução foi decantada para um balão para posterior evaporação rotativa para obter-se o produto seco.

O resíduo foi acetilado por 1 hora em 1,1 mL de diclorometano contendo 0,2 mL de ácido acético anidro e 0,2 mL de piridina. Todos componentes voláteis foram removidos completamente com coevaporação com etanol P.A. a vácuo (4 vezes consecutivas). O produto final foi mantido sem água, longe da luz e em baixa temperatura. Os produtos de degradação da lignina acetilada foram analisados por CG/EM utilizando espectros de massa e os tempos de retenção relativos para autenticar os monômeros DFRC e seus conjugados de p-

coumarato como descrito por Lu e Ralph (1997). A fim de avaliar a presença grupos de ligninas acetiladas naturalmente, o método DFRC modificado (chamado DFRC') foi utilizado como descrito por del Río et al.(2007).

Os produtos obtidos foram analisados utilizando um cromatografo CGEM-QP2010plus (Shimadzu Co.,Japan), com uma coluna capilar DB-5MS de 30 m × 0.25 mm diâmetro. Se utilizou tetracosano como padrão interno. O programa de temperatura iniciou a 140 °C (1 min) aumentando 3°C/min até 250 °C, depois se elevo até 280 °C (10 °C/min) e finalmente a 300 ºC (20 ºC/min) que se manteve durante 18 min. As temperaturas do injetor e da linha de transferência se estabeleceram a 250 ºC e 310 ºC, respectivamente. O gás de arraste foi o He (1 mL/min.). A quantificação dos monômeros liberados (acetilada e propilados) foi realizada usando o tetracosano como padrão interno e assumindo semelhanças dos monômeros acetilados relatados anteriormente por Lu e Ralph (1997). Os rendimentos molares dos produtos da lignina degradada foram determinados com base no peso molecular dos respectivos compostos acetilados ou propilados.

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6. RESULTADOS E DISCUSSÃO

Os resultados obtidos durante a Tese e a discussão dos mesmos são apresentados em forma de publicações.

Publicação I. Jose C. del Río, Alessandro G. Lino, Jorge L. Colodette, Claudio F. Lima, Ana Gutierrez, Angel T. Martínez, Fachuang Lu, John Ralph, Jorge Rencoret. Differences in the chemical structure of the lignins from sugarcane bagasse and straw. Biomass and Bioenergy 81 (2015) 322 e 338.

Publicação II. Jose C. del Río, Alessandro G. Lino, Jorge L. Colodette, Claudio F. Lima, Ana Gutierrez, Gisela Marques. Lipophilic phytochemicals from sugarcane bagasse and straw. Industrial Crops and Products 77 (2015) 992–1000.

Publicação III. Alessandro G. Lino, Jorge Rencoret, Ana Gutiérrez, Jorge L. Colodette, Claudio F. Lima, Ángel T. Martínez, José C. del Río (2014). Structural characterization of the lignins from sugarcane bagasse and straw. Proceedings of the 13th European Workshop on Lignocellulosics and Pulp, Seville. pp. 519–522.

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Research paper

Differences in the chemical structure of the lignins from sugarcane bagasse and straw

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ABSTRACT

Two major residues are produced by the sugarcane industry, the fibrous residue following juice extraction (bagasse), and the leftover harvest residue (straw). The structure of the lignins from these residues were studied by pyrolysis coupled to gas chromatography-mass spectrometry (Py-GC/MS), nuclear magnetic resonance (NMR), and derivatization followed by reductive cleavage (DFRC). Whereas the lignin from bagasse has a syringyl- (S)-rich *p*-hydroxyphenyl (H):guaiacyl (G):syringyl (S) composition of 2:38:60, the lignin from straw is guaiacyl- (G)-rich (H:G:S of 4:68:28). The relative abundances of the different interunit linkages also reflected these compositional differences. The lignin from bagasse was mostly made up by β–*O*–4′ alkyl-aryl ether units (representing 83% of all NMR-measurable linkages), followed by minor amounts of phenylcoumarans (6%) and other condensed units. The lignin from straw has lower levels of β-ether (β–*O*–4′ alkyl-aryl ether linkages, 75%) but higher relative levels of phenylcoumarans (β–5′, 15%) and dibenzodioxocins (5–5/4–*O*–β, 3%), consistent with a lignin enriched in G-units. Both lignins are extensively acylated at the γ-carbon of the lignin side-chain (42% and 36% acylation in bagasse and straw), predominantly with *p*-coumarates but to a minor extent with acetates. Although *p*-coumarates are preferentially attached to S-units in both lignins, acetates are attached preferentially to G-units. Tetrahydrofuran structures diagnostically arising from β–β coupling and cross-coupling of sinapyl alcohol and sinapyl *p*-coumarate were found in both lignins, indicating that sinapyl *p*-coumarate acts as a monomer participating in lignification. The flavone tricin was also found in the lignins from sugarcane bagasse and straw, as also occurs in other grasses.

Keywords: NMR; DFRC; lignification; tricin; lignin acylation; *p*-coumarates

1. Introduction

Concerns about declining fossil fuel reserves and global climate changes caused by increasing greenhouse gas emissions from burning fossil fuels, together with securing energy supply, have focused attention on the use of lignocellulosic biomass as a source for the production of biofuels, biochemicals, and bio-based products in the context of the so-called Lignocellulosic biorefinery [1]. Lignocellulosic biomass is the major source of renewable materials on Earth, is widely available at low cost and represents a potential source of renewable energy and bio-based materials alternative to fossil fuel resources [2].

Sugarcane (*Saccharum spp.*) is a perennial monocotyledonous plant belonging to the Gramineae (Poaceae) family that has gained considerable attention in the last years due to its great potential for biofuel production. The plant originates from Asia but it is well adapted to most tropical and subtropical climates where it has become one of the most important bioenergy crops. Its C4 photosynthesis makes it one of the most efficient species in carbon conversion and one of the most productive amongst all cultivated crops. Sugarcane is the main feedstock for sugar production, accounting for nearly two thirds of the world's production and is also the main raw material for the production of ethanol, with Brazil leading the world's production [3-5]. World sugarcane production was estimated to be around 1.6 billion tons in 2012, about 570 million tons produced in Brazil alone, with an average productivity of 68 tons/ha [6, 7]. During the harvest of sugarcane, the leaves and tops are left in field, whereas the stalks are transported to the mill where they are crushed to extract the sugar juice for the production of sugar and ethanol. Two major residues are produced by the sugarcane industry, the fibrous residue following juice extraction (named bagasse), and the left over harvest residue (straw). The wastes from the sugarcane agro-industry are produced in large quantities, about 280 million tons of bagasse and straw per year [7], and they are expected to increase in the near future as this crop expands and new industrial plants are implemented.

There is an increasing trend nowadays towards the more efficient use of agro-industrial wastes, including sugarcane bagasse and straw, as a means to avoid wasting profitable material and to maximize the use of agricultural resources. Sugarcane bagasse and straw are lignocellulosic materials basically composed of cellulose, hemicelluloses and lignin, with lower amounts of extractives and ash, and are therefore attractive feedstocks for the production of new products, including the production of second-generation bioethanol due to their high carbohydrates content and their essential independence from competition with food/feed demands [5-10]. Currently, in most of the sugar industries, only the sugar juice is fermented to ethanol, whereas the bagasse and straw are mostly used for the generation of heat and also to produce electricity. The production of second-generation bioethanol from these 'waste' materials could be advantageous for the sugarcane industries as they can use the same infrastructures used for the production of first-generation bioethanol that are already in place. Converting bagasse and straw to ethanol requires an enzymatic saccharification of the carbohydrates to fermentable reducing sugars and their subsequent

fermentation to ethanol or other liquid fuels. However, the cell-wall architecture, and especially the presence of lignin, reduces the accessibility of enzymes to cellulose, thus decreasing the efficiency of the hydrolysis. Therefore, costly and harsh pretreatment processes are needed in order to circumvent cell-wall recalcitrance by removing or cleaving and redistributing the lignin in order to render the polysaccharides more accessible to the enzymatic hydrolysis [7-14]. Pretreatment is the most important limiting step in the production of ethanol from lignocellulosic materials. The efficiency of the pretreatment process is highly dependent on both the lignin content and structure, and therefore knowledge of the structure of the lignin polymers in sugarcane bagasse and straw is important to develop appropriate pretreatment methods for delignification. However, bagasse and straw residues differ in their physical nature and chemical composition, and therefore the required delignification pretreatments will be different for each residue. As the lignin is the main factor conferring recalcitrance of the cell-walls to saccharification, understanding the composition and structure of the lignins from sugarcane bagasse and straw is paramount.

Lignin is a complex aromatic heteropolymer produced by the oxidative combinatorial coupling of three main monolignols, *p*-coumaryl, coniferyl and sinapyl alcohols, differing in their degree of methoxylation [15, 16]. When incorporated into the lignin polymer, these monolignols produce *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) lignin units, respectively, generating a variety of structures and linkages within the polymer, including β–*O*–4′ alkyl-aryl ethers, phenylcoumarans, resinols, spirodienones and dibenzodioxocins, among others. The lignin composition varies between plants from different taxa and even between different tissues from the same plant. Generally, hardwood lignins are composed of S and G units in different ratios, softwood lignins are composed essentially of G units with minor amounts of H units, and grass lignins include the three units, with H-units still comparatively minor. In addition, the *p*-hydroxycinnamates (*p*-coumarates and ferulates) widely occur in the grass polymers (hemicellulosic polysaccharides and lignin), with *p*-coumarates mostly acylating the γ-OH of the lignin side-chain, and predominantly on S-units, whereas ferulates and diferulates acylate arabinosyl residues of arabinoxylan chains and participate in both polysaccharide-polysaccharide and lignin-polysaccharide cross-coupling reactions [17].

Despite the huge amounts of work devoted to the use of sugarcane bagasse and straw as feedstocks for the production of second-generation bioethanol and other bioproducts [5-14], the structures of their lignins have not been studied in detail to date, although some studies regarding the structure of bagasse lignin have been published in recent years [18-20], and a systematic study of lignin deposition during sugarcane stem development has also been recently performed [21]. Therefore, in this paper, we report a more extensive and detailed structural characterization of the lignins in sugarcane bagasse and straw. For this purpose, we used powerful analytical methodologies, including the thermal degradative method, analytical pyrolysis coupled to gas chromatography and mass spectrometry (Py-GC/MS), spectroscopic methods such as heteronuclear bidimensional nuclear magnetic resonance (2D-NMR) spectroscopy, and the diagnostic chemical degradative method, derivatization followed by reductive cleavage (DFRC), to delineate the compositional and structural differences between

these two lignins. Such data will help to maximize the exploitation of these important agro-industrial wastes as feedstocks for the production of second-generation bioethanol and other biobased products.

2. Materials and methods

2.1. Samples

Sugarcane bagasse and straw were collected at harvesting age from high performance sugarcane plantations and were supplied by a mid-size ethanol mill located in the São Pedro dos Ferros county, MG, Brazil. The processing of sugarcane involved two steps. The first was the crushing of sugarcane stalks in a semi-industrial chipper; the fragments were then processed by milling to extract the sugarcane juice. The resulting residue (bagasse) was used for the subsequent characterization studies. Straw, heterogeneous material composed of leaves and tops of sugarcane, was crushed in the same equipment used to grind the stalks in order to have a better homogenization of the material. The samples were air-dried and milled using an IKA knife mill (Janke & Kunkel, Staufen, Germany). Samples of around 50 g of sugarcane bagasse and straw were extracted with acetone in a Soxhlet for 16 h and then with hot water at 100 ºC for 3 h. The Klason lignin content was measured as the residue after sulphuric acid hydrolysis of the pre-extracted material according to the TAPPI method T222 om-8 [22]. The Klason lignin content was then corrected for ash, and for proteins, determined from the N content obtained by CHN elemental analysis in a LECO CHNS-932 Elemental Analyzer (LECO Corp., St. Joseph, Mich.) and using a 6.25 factor [23] . The acid-soluble lignin content was measured, after the insoluble lignin was filtered off, by UV-spectroscopic determination at 205 nm using 110 L/cm/g as the extinction coefficient, according to Tappi method UM 250 [22]. The holocellulose was isolated from pre-extracted samples by delignification for 4 h using the acid chlorite method [24]. The content on α-cellulose was determined by removing the hemicelluloses from the holocellulose by alkaline extraction [24]. Finally, the ash content was estimated as the residue after 6 h of heating at 575 ºC. Three replicates were used for each sample.

2.2. Ball milling and Isolation of ‘milled-wood’ lignins (MWLs)

The MWLs were obtained according to the classical procedure [25]. Around 40 g of extractives-free material were finely ball-milled in a Retsch PM100 planetary ball mill (Restch, Haan, Germany) at 400 rpm (36 h) using an agate jar and 2 cm x 20 balls. This ball-milled material was used directly for whole-cell-wall-NMR to determine compositional and structural features of the entire lignin fraction. The ball-milled material was then extracted with dioxane-water, 96:4 (v/v) (20 mL of solvent/g of milled fiber). The solution was centrifuged and the supernatant was then evaporated to dryness at 40 °C at reduced pressure. The obtained residue (raw MWL) was then redissolved into a solution of acetic acid/water, 9:1 (v/v) (25 mL of solvent/g of raw MWL). The lignin in the solution was precipitated into

water and the precipitate was separated by centrifugation, milled in an agate mortar and then dissolved in a solution of 1,2-dichloroethane:ethanol, 2:1, (v/v). The mixture was then centrifuged to remove any insoluble material. The lignin in the supernatant was precipitated in diethyl ether, and the precipitate was separated by centrifugation. This residue was then suspended in diethyl ether, centrifuged and finally suspended in petroleum ether. The final purified MWL sample was washed with *n*-hexane and recovered by centrifugation and dried under a N_2 current. The final yields ranged from 10-15% of the original Klason lignin content.

2.3. Pyrolysis coupled to gas chromatography and mass spectrometry (Py-GC/MS)

Pyrolysis of the whole cell-walls and of their isolated MWLs (ca. 0.1 mg) were performed in an EGA/PY-3030D micro-furnace pyrolyzer (Frontier Laboratories Ltd., Fukushima, Japan) connected to a GC 7820A (Agilent Technologies, Inc., Santa Clara, CA) and an Agilent 5975 mass-selective detector (EI at 70 eV). The column used was a 60 m x 0.25 mm i.d., 0.25 μm film thickness, DB-1701 (J&W Scientific, Folsom, CA). The pyrolysis was performed at 500 ºC. The oven temperature was programmed from 45 ºC (4 min) to 280 ºC (10 min) at 4 ºC/min. Helium was the carrier gas (2 mL/min). For the pyrolysis in the presence of tetramethylammonium hydroxide (Py/TMAH), 0.1 mg of sample was mixed with 0.5 μL of TMAH (25%, w/w, in methanol), and the pyrolysis was carried out as described above. The compounds released were identified by comparison of their mass spectra with those of the Wiley and NIST libraries and with those reported in the literature [26,27], and when possible, by comparison with the retention times and mass spectra of authentic standards. Peak molar areas were calculated for the lignin degradation products, the summed areas were normalized, and the data for two repetitive analyses were averaged and expressed as percentages.

2.4. Nuclear magnetic resonance (NMR) spectroscopy

For the NMR of the whole cell-walls of sugarcane bagasse and straw, around 50 mg of finely divided (ball-milled) extractives-free samples were swollen in 0.75 mL DMSO-d_6 according to the method previously described [28,29]. In the case of the isolated MWLs, around 40 mg of underivatized or acetylated sample were dissolved in 0.75 mL of DMSO-d_6 or 0.75 mL of $CDCl_3$, respectively. NMR spectra were recorded at 25 ºC on a AVANCE III 500 MHz instrument (Bruker, Karlsruhe, Germany) equipped with a cryogenically-cooled z-gradient triple-resonance probe. HSQC (heteronuclear single-quantum coherence) experiments used Bruker's 'hsqcetgpsisp2.2' adiabatic pulse program with spectral widths of 5000 Hz (from 10 to 0 ppm) and 20625 Hz (from 165 to 0 ppm) for the ^{1}H- and ^{13}C-dimensions. The number of collected complex points was 2048 for the ^{1}H-dimension with a recycle delay of 1 s. The number of transients was 64, and 256 time increments were recorded in ^{13}C-dimension. The $^{1}J_{CH}$ used was 140 Hz. Processing used typical matched Gaussian apodization in ^{1}H and a squared cosine-bell in ^{13}C. Prior to Fourier transformation,

the data matrices were zero-filled up to 1024 points in the ^{13}C-dimension. The central solvent peaks were used as internal references (δ_C/δ_H 39.5/2.49 for DMSO and δ_C/δ_H 70.0/7.26 for $CHCl_3$). For heteronuclear multiple bond correlation (HMBC) acquisition experiments, the long range *J*-coupling evolution time used was 80 ms.

HSQC cross-signals were assigned by comparison with the literature [28-36]. A semi-quantitative analysis of the volume integrals of the HSQC cross-correlation signals was performed. As the volume integral depends on the particular $^1J_{CH}$ value, as well on the T_2 relaxation time, absolute quantitation is impossible but relative integrals (between spectra) allow valid comparisons. Thus, the integration of the cross-signals that correspond to chemically analogous carbon-proton pairs was performed separately for the different regions of the HSQC spectra. For these signals, the $^1J_{CH}$ coupling value is similar and integrals can be used semi-quantitatively to estimate the relative abundance of the different species; the use of the adiabatic HSQC variant of the experiment additionally minimizes *J*-coupling differences. In the aliphatic oxygenated region, the relative abundances of side-chains involved in inter-unit linkages or present in terminal units were estimated from the C_α/H_α correlations to avoid possible interference from homonuclear ^1H–^1H couplings, except for substructures **A_{ox}** and **I/I'**, for which C_β/H_β and C_γ/H_γ correlations had to be used. In the aromatic/unsaturated region of the spectra, C_2–H_2 correlations from H, G and S lignin units and from *p*-coumarates and ferulates and C_6/H_6 correlations for tricin were used to estimate their relative abundances.

2.5. Derivatization followed by reductive cleavage (DFRC)

The DFRC degradation was performed according to the protocol previously developed [37-40]. Around 5 mg of lignin samples were stirred for two hours at 50 ºC with acetyl bromide in acetic acid, 8:92 (v/v). The solvents and the excess of acetyl bromide were removed in a rotary evaporator. The products were then dissolved in dioxane/acetic acid/water (5:4:1, v/v/v), and powdered Zn (50 mg) was added. After 40 min stirring at room temperature, the mixture was then transferred into a separatory funnel with dichloromethane and saturated ammonium chloride. The pH of the aqueous phase was adjusted to less than 3 by adding 3% HCl, the mixture was vigorously mixed and the organic layer was then separated. The water phase was extracted twice more with dichloromethane. The joined dichloromethane fractions were dried over anhydrous Na_2SO_4 and the filtrate was evaporated in a rotary evaporator. The residue was acetylated for 1 h in 1.1 mL of dichloromethane containing 0.2 mL of acetic anhydride and 0.2 mL pyridine. The acetylated lignin degradation products were collected after rotary evaporation of the solvents, and subsequently analyzed by GC/MS using mass spectra and relative retention times to authenticate the DFRC monomers and their *p*-coumarate conjugates as described [37-40]. To assess the presence of naturally acetylated lignin units, the described modification of the standard DFRC method by using propionylating instead of acetylating reagents (DFRC´) was used in the present study [41,42].

The GC/MS analyses were performed with a GCMS-QP2010plus instrument (Shimadzu Co., Kyoto, Japan) using a capillary column (DB-5MS 30 m × 0.25 mm I.D., 0.25 μm film thickness). The oven was heated from 140 ºC (1 min) to 250 ºC at 3 ºC/min, then ramped at 10 ºC/min to 280 ºC (1 min) and finally ramped at 20 ºC/min to 300 ºC, and held for 18 min at the final temperature. The injector was set at 250 ºC and the transfer line was kept at 310 ºC. Helium was used as the carrier gas at a rate of 1 mL/min. Quantitation of the released individual (acetylated and propionylated) monomers was performed using tetracosane as internal standard and by assuming response factors similar to those of the acetylated monomers reported previously [Lu and Ralph, 1997a]. The molar yields of the released compounds were determined on the basis of molecular weights of the respective acetylated (or propionylated) compounds.

3. Results and discussion

3.1. Composition of the main constituents of sugarcane bagasse and straw

The abundances of the main constituents (water soluble material, acetone extractives, Klason lignin, acid-soluble lignin, holocellulose, α-cellulose, and ash) of sugarcane bagasse and straw are shown in **Table 1**. The composition is in the range of values previously published for similar samples, except for the much higher content of extractives reported in previous papers [6,43]. Bagasse and straw have a high content of carbohydrates (75.8% and 72.9%) and low lignin contents (20% and 18.9%). The composition of the lignins in both sugarcane residues were analyzed *in situ* by Py-GC/MS and 2D-NMR. In addition, and for a thorough structural characterization, the MWLs were isolated from the sugarcane residues by aqueous dioxane extraction from finely ball-milled samples according to the classical lignin isolation protocol [25] and subsequently analyzed in detail by Py-GC/MS, 2D-NMR and DFRC.

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

	Sugarcane bagasse[a]	Sugarcane straw[a]
Water-soluble material	1.3 ± 0.2	2.1 ± 0.2
Acetone extractives	0.9 ± 0.1	1.4 ± 0.1
Klason lignin[b]	17.8 ± 0.6	17.0 ± 0.2
Acid-soluble lignin	2.2 ± 0.2	1.9 ± 0.2
Holocellulose (α-cellulose)[c]	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

[a] Average of three replicates

[b] Corrected for proteins and ash

[c] The α-cellulose contents are shown in parentheses

3.2. Lignin composition of sugarcane bagasse and straw as observed by Py-GC/MS

The pyrograms of the whole cell-walls of sugarcane bagasse and straw and of their corresponding MWLs are shown in **Figure 1**. The identities and relative molar abundances of the released phenolic compounds are listed in **Table 2**. Pyrolysis of the whole cell-walls of sugarcane bagasse and straw released compounds derived from carbohydrate and lignin moieties, as well as from *p*-hydroxycinnamates. Among the lignin-derived phenols, the pyrograms showed compounds derived from the H, G and S lignin units as well as from the *p*-hydroxycinnamate esters present in the cell-wall. The most prominent *p*-hydroxycinnamate and/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-methylphenol (**4**), 4-methylguaiacol (**5**), syringol (**11**), 4-methylsyringol (**15**), 4-vinylsyringol (**23**) and *trans*-4-propenylsyringol (**29**). As usually occurs in the pyrolysis of grasses, the high amounts of 4-vinylphenol and 4-vinylguaiacol released upon pyrolysis are mostly due to *p*-coumarates and ferulates after decarboxylation [35,36,44,45]. Pyrolysis of the isolated MWLs released a similar distribution of phenolic compounds as from their respective whole cell-walls, except for the much lower relative abundances of 4-vinylguaiacol (**8**), largely derived from ferulates attached to carbohydrates that are essentially absent from the MWLs. The most prominent compound in the pyrograms of the MWLs was still 4-vinylphenol (**9**), derived essentially from *p*-coumarate esters on lignin.

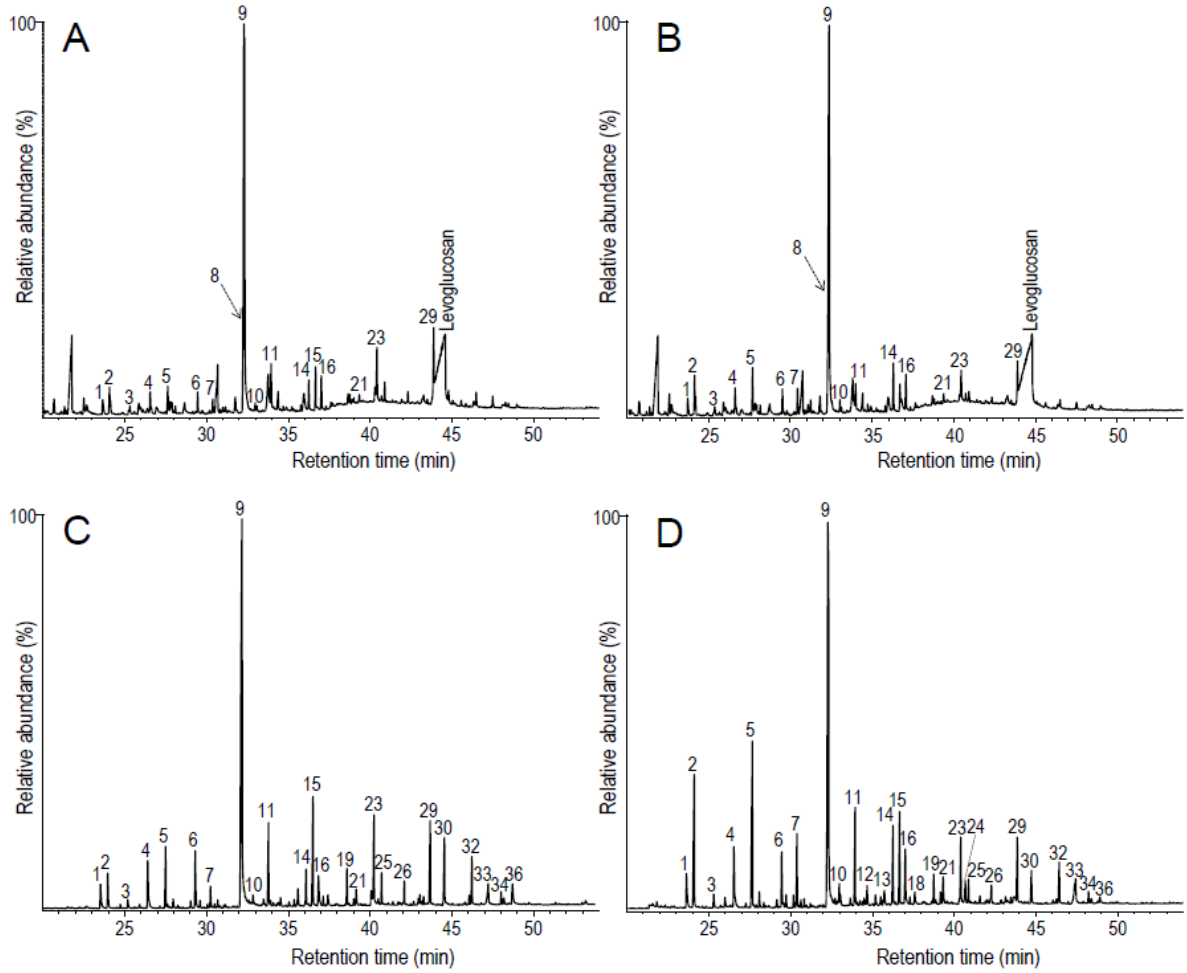

Figure 1. Py-GC/MS chromatograms of the whole cell-walls of sugarcane bagasse (A) and straw (B), and of the MWLs from sugarcane bagasse (C) and straw (D). The identities and relative abundances of the released phenolic compounds are listed in Table 2. Levoglucosan arises from carbohydrates.

Table 2 – Identities and relative molar abundances of the compounds released after Py-GC/MS of whole cell-walls (CW) of sugarcane (*Saccharum spp.*) bagasse and straw and their isolated MWLs.

		Sugarcane Bagasse		Sugarcane Straw	
	Compounds	CW	MWL	CW	MWL
1	phenol	2.4	2.7	3.1	3.0
2	guaiacol	2.5	2.2	3.9	8.0
3	3-methylphenol	1.0	0.7	1.1	0.8
4	4-methylphenol	2.4	4.2	3.1	4.4
5	4-methylguaiacol	2.3	3.0	4.0	8.9
6	4-ethylphenol	1.4	3.7	2.1	2.9
7	4-ethylguaiacol	0.8	1.0	1.8	2.8
8	4-vinylguaiacol	10.7	3.3	17.0	8.7
9	4-vinylphenol	51.8	41.7	43.8	30.0
10	eugenol	0.4	0.3	0.8	0.8

11	syringol	3.0	4.2	2.1	4.3
12	*cis*-isoeugenol	0.3	0.3	0.5	0.7
13	*trans* 4-propenylphenol	0.5	1.0	0.5	0.8
14	*trans*-isoeugenol	1.7	1.5	3.1	3.0
15	4-methylsyringol	2.3	5.1	1.7	3.5
16	vanillin	2.3	1.9	2.6	2.5
17	propynylguaiacol	0.2	0.4	0.2	0.3
18	propynylguaiacol	0.3	0.5	0.3	0.6
19	4-ethylsyringol	0.5	1.2	0.3	0.9
20	vanillic acid methyl ester	0.0	0.3	0.0	0.4
21	acetovanillone	0.5	0.9	0.8	1.3
22	4-hydroxybenzaldehyde	0.0	1.3	0.0	0.3
23	4-vinylsyringol	2.9	4.0	1.9	2.5
24	guaiacylacetone	0.2	0.2	0.5	0.8
25	4-allylsyringol	0.8	1.1	0.6	0.7
26	*cis*-4-propenylsyringol	0.5	0.8	0.3	0.6
27	propinylsyringol	0.4	0.3	0.4	0.0
28	propinylsyringol	0.4	0.3	0.2	0.0
29	*trans*-4-propenylsyringol	4.8	3.2	2.0	2.1
30	syringaldehyde	0.6	3.2	0.0	1.3
31	syringic acid methyl ester	0.1	0.3	0.1	0.1
32	acetosyringone	0.6	1.8	0.4	1.4
33	syringylacetone	0.6	1.2	0.4	0.9
34	propiosyringone	0.2	0.4	0.1	0.3
35	syringyl vinyl ketone	0.2	0.3	0.2	0.1
36	*trans*-coniferaldehyde	0.2	1.3	0.3	0.2
37	*trans*-sinapaldehyde	0.3	0.3	0.0	0.0
	S/G ratio[a]	1.3	1.6	0.5	0.5

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

p-Hydroxycinnamates form linkages with polysaccharides and/or lignin and are particularly abundant in grasses [17,46-49]. As their pyrolysis products can be conflated with those from lignins, care must be exercised when estimating the lignin composition by pyrolysis. In this sense, 4-vinylphenol and 4-vinylguaiacol cannot be used for the estimation of the lignin H:G:S composition in grasses, as these products primarily arise not from the lignin structural units but from *p*-hydroxycinnamates. However, and as already performed for other grasses, an estimation of the S/G ratio of the lignins in sugarcane bagasse and straw and in their isolated MWLs was accomplished by ignoring 4-vinylguaiacol (and the analogous 4-vinylsyringol). Analysis revealed strong differences in the composition of both lignins, with sugarcane bagasse lignin being S-rich (S/G ratio of 1.3–1.6) and sugarcane straw lignin being G-rich (S/G ratio of 0.5) (**Table 2**). This is fairly readily explained by the average maturity of the tissues in each as mature material is always richer in S-units; bagasse is mainly derived from the mature stems, whereas the straw includes the leaves and other immature parts of the plant.

The presence of *p*-hydroxycinnamates in the whole cell-walls, as well as in the isolated MWLs, was confirmed by pyrolysis in the presence of tetramethylammonium hydroxide (TMAH), a reagent that prevents decarboxylation and liberates diagnostic methyl esters from the esterified components [44, 50]. **Figure 2** shows the Py/TMAH chromatograms of the whole cell-walls of sugarcane bagasse and straw, and of their respective MWLs. The identities of the compounds released and their relative molar abundances are listed in **Table 3**. Py/TMAH induces cleavage of alkyl-aryl ether bonds in lignin and releases products similar to those obtained upon CuO alkaline degradation, including methylated derivatives of hydroxybenzaldehydes (**4**, **11**, **17**), hydroxyacetophenones (**14**, **20**) and hydroxybenzoic acids (**7**, **13**, **21**), among others [44,50-52]. As seen in **Figure 2**, Py/TMAH of the whole cell-walls of sugarcane bagasse and straw released high amounts (43.8% and 38.4% of the total peak areas in bagasse and straw) of the fully methylated derivative of *p*-coumaric acid, the *trans*-3-(4-methoxyphenyl)-propenoic acid methyl ester (**19**), as well as significant amounts (8.5% and 13% of total peak areas in bagasse and straw) of the fully methylated derivative of ferulic acid, the *trans*-3-(3,4-dimethoxyphenyl)-propenoic acid methyl ester (**26**). These Py/TMAH products clearly indicate that the high amounts of 4-vinylphenol and 4-vinylguaiacol released upon Py-GC/MS of the whole cell-wall and MWL samples arise mainly from *p*-coumarate and ferulate esters in the wall, and not from the core lignin itself. However, Py/TMAH of the respective MWLs released higher relative amounts of *p*-coumarate derivatives (68.7% and 54.8% in the MWLs from bagasse and straw), and considerably lower amounts of the ferulate derivatives (2.3% and 4.5% in the MWLs from bagasse and straw). The relative abundance of *p*-hydroxycinnamates (*p*-coumarate/ferulate ratio) in sugarcane bagasse and straw and in their isolated MWLs, estimated by Py/TMAH (**Table 3**), revealed additional features. *p*-Coumarates and ferulates were found in important amounts in the whole cell-walls of sugarcane bagasse and straw, with a *p*-coumarate/ferulate ratio of 5.2 and 3.0, respectively. However, this ratio sharply increases to 31.1 and 12.8 in the MWLs from bagasse and straw, respectively, indicating that in sugarcane bagasse and straw, ferulates are mostly attached to the carbohydrates in the cell-wall whereas *p*-coumarates are primarily attached to the lignin polymer, as also occur in other grasses [17]. Studies on different plants, including other grasses, have indicated that *p*-coumarate acylates the γ-OH of lignin side-chains, and predominantly on S-units, whereas ferulates acylate cell-wall polysaccharides and participate in both polysaccharide-polysaccharide and lignin-polysaccharide cross-coupling reactions [17, 34, 36, 40, 46, 48, 49, 53].

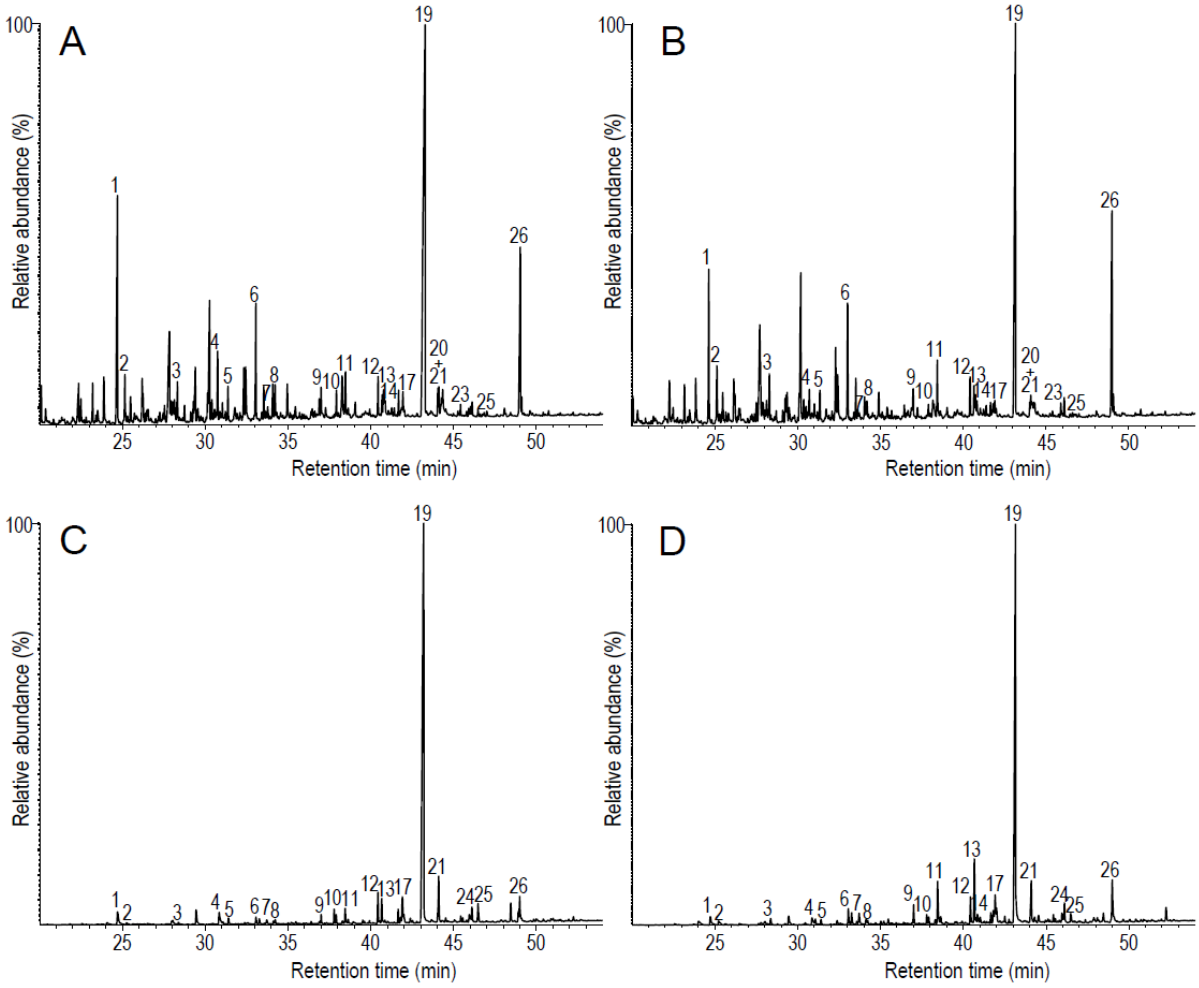

Figure 2. Py-TMAH-GC/MS chromatograms of the whole cell-walls of sugarcane bagasse (A) and straw (B), and of the MWLs from sugarcane bagasse (C) and straw (D). The identities and relative abundances of the released compounds are listed in Table 3.

Table 3. Identity and relative molar abundances of the compounds released after Py/TMAH of whole cell-walls of sugarcane (*Saccharum spp.*) bagasse and straw and their isolated MWLs.

Label	Compound	Bagasse CW	Bagasse MWL	Straw CW	Straw MWL
1	4-methoxybenzeneethylene	18.0	3.0	12.3	2.5
2	1,2-dimethoxybenzene	2.7	0.4	4.8	1.3
3	3,4-dimethoxytoluene	1.9	0.3	2.9	1.0
4	4-methoxybenzaldehyde	3.8	2.9	2.3	2.0
5	1,2,3-trimethoxybenzene	1.4	0.6	1.7	0.5
6	3,4-dimethoxybenzeneethylene	5.6	0.7	7.6	1.9
7	4-Methoxybenzoic acid methyl ester	0.3	0.5	0.6	1.7
8	3,4,5-trimethoxytoluene	1.6	0.4	1.3	0.4
9	1,2-dimethoxy-4-propenylbenzene	1.3	0.8	1.8	1.8
10	3,4,5-trimethoxybenzeneethylene	1.0	0.7	0.7	0.6
11	3,4-dimethoxybenzaldehyde	2.1	1.7	3.8	5.2

12	*cis* 3-(4-methoxyphenyl)-propenoic acid methyl ester	1.5	2.7	2.1	2.5
13	3,4-dimethoxybenzoic acid methyl ester	0.8	1.9	1.4	5.4
14	3,4-dimethoxyacetophenone	0.6	0.4	0.8	1.0
15	1-(3,4,5-trimethoxyphenyl)-1-propene	0.9	0.9	0.6	0.8
16	*cis* 1-(3,4-dimethoxyphenyl)-2-methoxyethylene	0.1	0.5	0.3	1.1
17	3,4,5-trimethoxybenzaldehyde	1.0	3.0	0.7	2.9
18	*trans* 1-(3,4-dimethoxyphenyl)-2-methoxyethylene	0.2	0.6	0.4	1.2
19	*trans* 3-(4-methoxyphenyl)-propenoic acid methyl ester	43.8	68.7	38.4	54.8
20	3,4,5-trimethoxyacetophenone	0.9	0.6	0.5	1.5
21	3,4,5-trimethoxybenzoic acid methyl ester	0.8	3.1	0.9	2.6
22	3,4,5-trimethoxyphenyl-2-propanone	0.4	0.4	0.2	0.6
23	*cis* 3-(3,4-dimethoxyphenyl)-propenoic acid methyl ester	0.3	0.0	0.7	0.0
24	*cis* 1-(3,4,5-trimethoxyphenyl)-2-methoxyethylene	0.2	1.2	0.1	1.5
25	*trans*1-(3,4,5-trimethoxyphenyl)-2-methoxyethylene	0.2	1.7	0.1	0.9
26	*trans* 3-(3,4-dimethoxyphenyl)-propenoic acid methyl ester	8.5	2.3	13.0	4.5
	p-Coumarate/Ferulate ratio[a]	5.2	31.1	3.0	12.8

[a] Relative abundance of *p*-coumarates (peaks 12 and 19) relative to ferulates (peaks 23 and 26)

3.3 Lignin structural units and inter-unit linkages in sugarcane bagasse and straw as elucidated by 2D-NMR

The whole cell-walls of sugarcane bagasse and straw were analyzed *in situ* by 2D-NMR, according to the method previously described [28,29], and the spectra were compared with those of the isolated MWLs. The oxygenated aliphatic side-chain (δ_C/δ_H 48-90/2.5-5.8) and the aromatic/unsaturated (δ_C/δ_H 90-155/5.5-8.0) regions of the HSQC spectra of the whole cell-walls from sugarcane bagasse and straw, and their isolated MWLs, are shown in **Figures 3** and **4**. Carbohydrate signals from xylans (X_2, X_3, X_4, X_5), including signals from acetylated xylan moieties (X'_2 and X'_3), were predominant in the aliphatic side-chain region of the spectra of the whole cell-walls, partially overlapping with lignin signals. The spectra of the isolated MWLs, however, presented mostly lignin signals that matched those observed in the HSQC spectra of their respective whole cell-walls but that are better resolved. The main lignin and carbohydrate cross-signals assigned in the HSQC spectra are listed in **Table 4** and the main substructures present are shown in **Figure 5**.

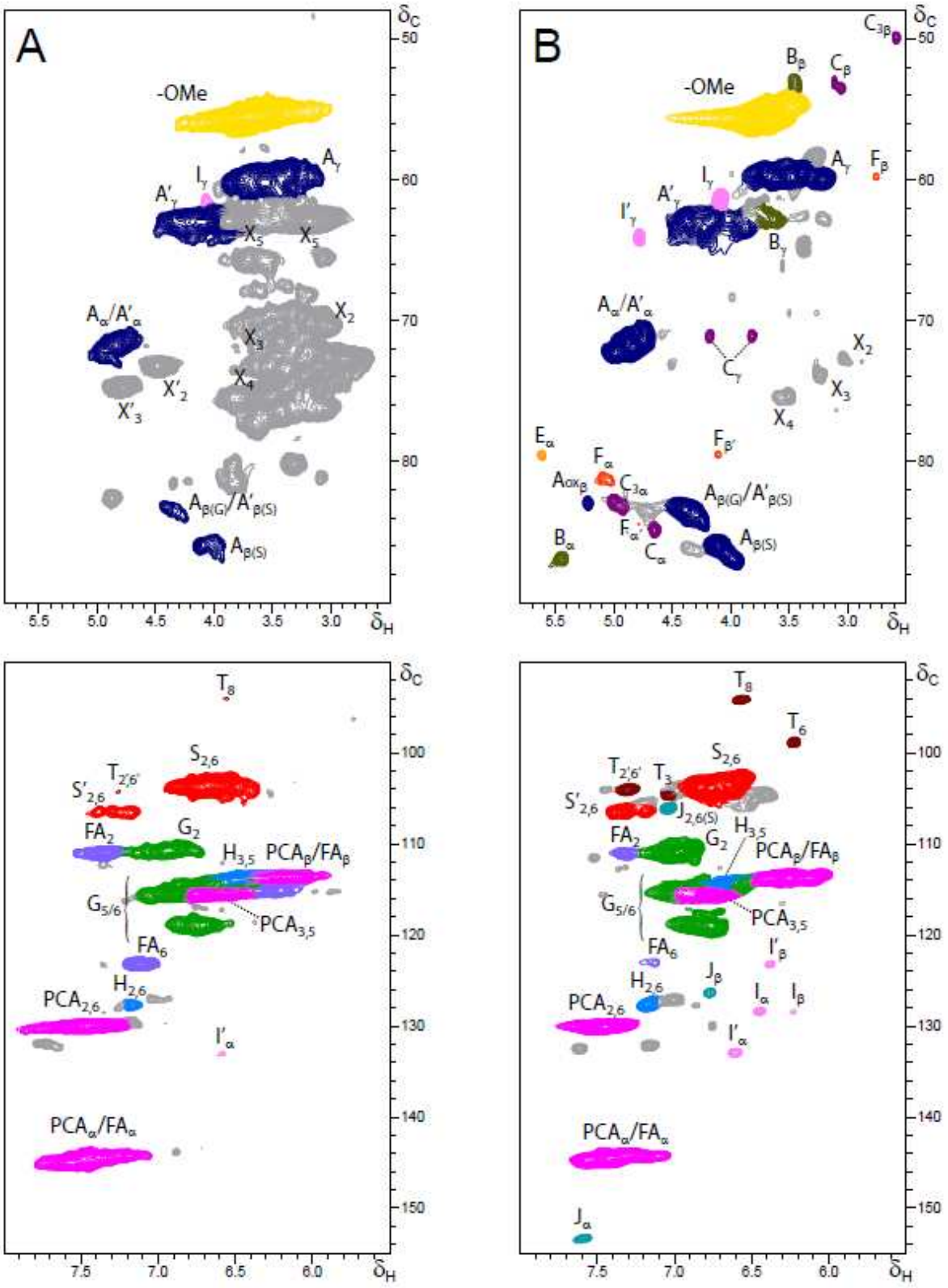

Figure 3. Side-chain (δ_C/δ_H 48-90/2.5-5.8, top) and aromatic (δ_C/δ_H 90-155/5.5-8.0, bottom) regions from the 2D HSQC NMR spectra of the whole cell-walls from sugarcane bagasse (A) and its isolated MWL (B). See Table 4 for signal assignments and Figure 5 for the main lignin structures identified.

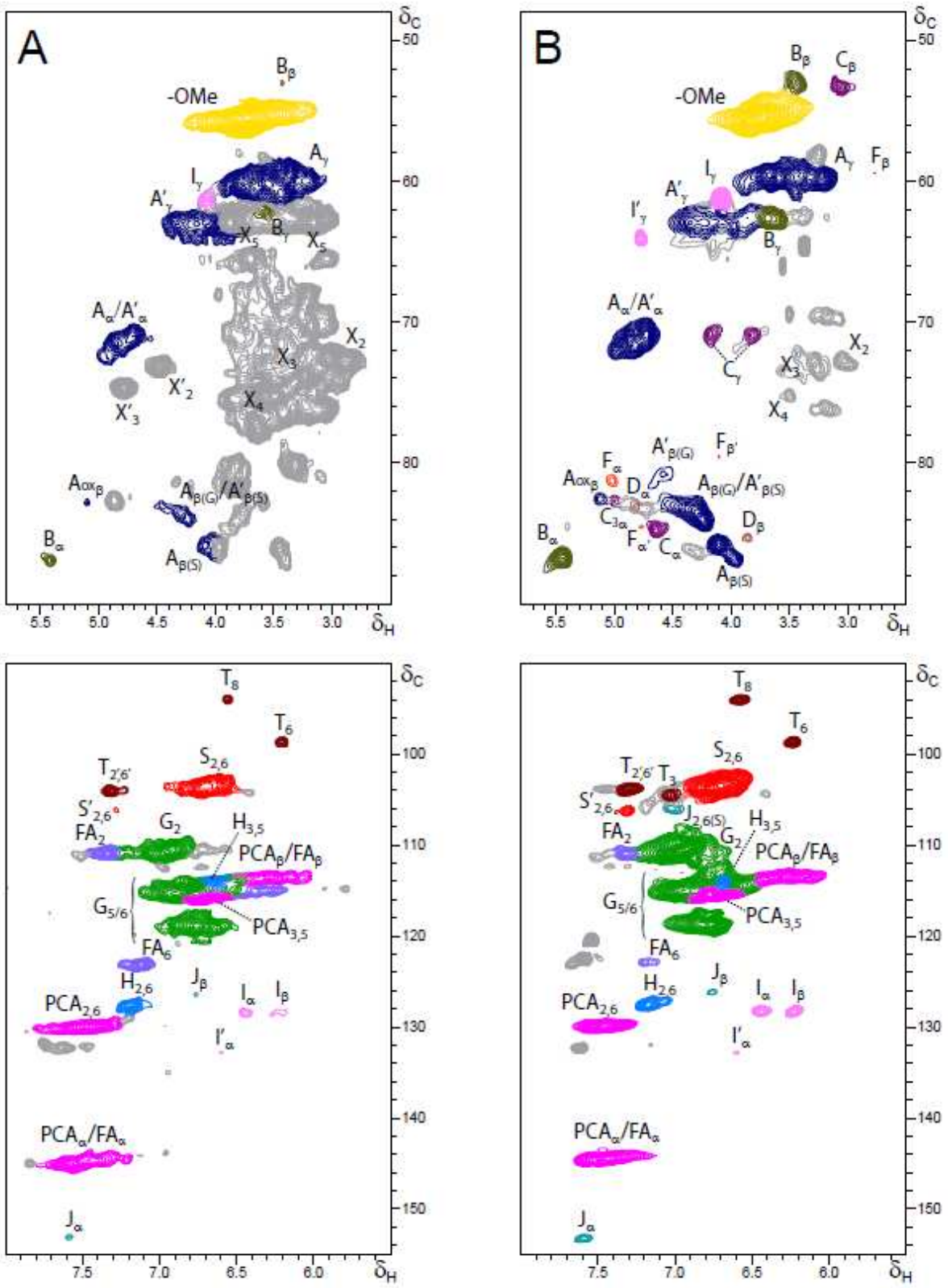

Figure 4. Side-chain (δ_C/δ_H 48-90/2.50-5.80, top) and aromatic (δ_C/δ_H 90-155/5.50-8.00, bottom) regions from the 2D HSQC NMR spectra of the whole cell-walls from sugarcane straw (A) and its isolated MWL (B). See Table 4 for signal assignments and Figure 5 for the main lignin structures identified.

Table 4. Assignments of ^{13}C–^{1}H correlation signals in the 2D HSQC spectra from the whole cell-walls of sugarcane (*Saccharum spp.*) bagasse and straw and their isolated MWLs in DMSO-d_6.

Label	δ_C/δ_H (ppm)	Assignment
Lignin cross-peak signals		
$C_{3\beta}$	49.8/2.59	C_β/H_β in γ-acylated β–β´ tetrahydrofuran substructures (**C_3**)
B_β	53.6/3.47	C_β/H_β in β–5´ phenylcoumaran substructures (**B**)
C_β	53.6/3.07	C_β/H_β in β–β´ resinol substructures (**C**)
-OCH_3	55.6/3.73	C/H in methoxyls
A_γ	59.4 /3.40 and 3.72	C_γ/H_γ in γ-hydroxylated β–*O*–4´ substructures (**A**)
F_β	59.7/2.76	C_β/H_β in spirodienone substructures (**F**)
I_γ	61.3/4.09	C_γ/H_γ in cinnamyl alcohol end-groups (**I**)
B_γ	62.6/3.68	C_γ/H_γ in β–5´ phenylcoumaran substructures (**B**)
A'_γ	62.7/3.83-4.30	C_γ/H_γ in γ-acylated β–*O*–4´ substructures (**A'**)
I'_γ	64.0/4.79	C_γ/H_γ in γ-acylated cinnamyl alcohol end-groups (**I'**)
C_γ	71.0/3.83 and 4.19	C_γ/H_γ in β–β´ resinol substructures (**C**)
A_α/A'_α	71.8/4.87	C_α/H_α in β–*O*–4´ substructures (**A, A'**)
$F_{\beta'}$	79.5/4.11	$C_\beta/H_{\beta'}$ in spirodienone substructures (**F**)
E_α	79.5/5.50	C_α/H_α in α,β-diaryl ether substructures (**E**)
$A'_{\beta(G)}$	80.8/4.58	C_β/H_β in γ-acylated β–*O*–4´ substructures linked to a G unit (**A'**)
F_α	81.2/5.10	C_α/H_α in spirodienone substructures (**F**)
$C_{3\alpha}$	82.8/5.00	C_α/H_α in γ-acylated β–β´ tetrahydrofuran structures (**C_3**)
$A_{ox\beta}$	82.8/5.23	C_β/H_β in α-oxidized β–*O*–4´ substructures (**A_{ox}**)
$A'_{\beta(S)}$	83.0/4.33	C_β/H_β in γ-acylated β–*O*–4´ substructures linked to a S unit (**A'**)
D_α	83.4/4.82	C_α/H_α in 5-5´ dibenzodioxocin substructures (**D**)
$A_{\beta(G)}$	83.6/4.29	C_β/H_β in β–*O*–4´ substructures linked to a G unit (**A**)
C_α	84.8/4.67	C_α/H_α in β–β´ resinol substructures (**C**)
$F_{\alpha'}$	84.9/4.88	$C_{\alpha'}/H_{\alpha'}$ in spirodienone substructures (**F**)
D_β	85.4/3.86	C_β/H_β in 5-5´ dibenzodioxocin substructures (**D**)
$A_{\beta(S)}$	85.9/4.12	C_β/H_β in β–*O*–4´ substructures linked to a S unit (**A**)
B_α	86.9/5.47	C_α/H_α in phenylcoumaran substructures (**B**)
T_8	94.0/6.56	C_8/H_8 in tricin units (**T**)
T_6	98.7/6.22	C_6/H_6 in tricin units (**T**)
$S_{2,6}$	103.8/6.69	C_2/H_2 and C_6/H_6 in etherified syringyl units (**S**)
$T_{2',6'}$	103.9/7.30	$C_{2'}/H_{2'}$ and $C_{6'}/H_{6'}$ in tricin units (**T**)
T_3	104.5/7.03	C_3/H_3 in tricin units (**T**)
$S'_{2,6}$	106.1/7.32 and 106.4 /7.19	C_2/H_2 and C_6/H_6 in α-oxidized syringyl units (**S'**)
$J_{2,6(S)}$	106.8/7.06	C_2/H_2 and C_6/H_6 in cinnamyl aldehyde end-groups in S units (**J**)
G_2	110.9/7.00	C_2/H_2 in guaiacyl units (**G**)
FA_2	111.0/7.32	C_2/H_2 in ferulic acid units (**FA**)
PCA_β and FA_β	113.5/6.27	C_β/H_β in *p*-coumarate (**PCA**) and ferulate (**FA**)
$H_{3,5}$	114.5/6.62	C_3/H_3 and C_5/H_5 in *p*-hydroxyphenyl units (**H**)
G_5/G_6	114.9/6.72 and 6.94 118.7/6.77	C_5/H_5 and C_6/H_6 in guaiacyl units (**G**)
$PCA_{3,5}$	115.5/6.77	C_3/H_3 and C_5/H_5 in *p*-coumarate (**PCA**)
I'_β	123.2/6.38	C_β/H_β in γ-acylated cinnamyl alcohol end-groups (**I'**)
FA_6	123.3/7.10	C_6/H_6 in ferulate (**FA**)
J_β	126.3/6.76	C_β/H_β in cinnamyl aldehyde end-groups (**J**)
$H_{2,6}$	128.0/7.23	C_2/H_2 and C_6/H_6 in *p*-hydroxyphenyl units (**H**)
I_β	128.4/6.23	C_β/H_β in cinnamyl alcohol end-groups (**I**)
I_α	128.4/6.44	C_α/H_α in cinnamyl alcohol end-groups (**I**)
$PCA_{2,6}$	130.0/7.46	C_2/H_2 and C_6/H_6 in *p*-coumarate (**PCA**)
I'_α	132.9/6.60	C_α/H_α in γ-acylated cinnamyl alcohol end-groups (**I'**)
PCA_α and FA_α	144.4/7.41	C_α/H_α in *p*-coumarate (**PCA**) and ferulate (**FA**)
J_α	153.4/7.61	C_α/H_α in cinnamyl aldehyde end-groups (**J**)
Polysaccharide cross-peak signals		
X_5	63.2/3.26 and 3.95	C_5/H_5 in β-D-xylopyranoside
X_2	72.9/3.14	C_2/H_2 in β-D-xylopyranoside
X'_2	73.5/4.61	C_2/H_2 in 2-*O*-acetyl-β-D-xylopyranoside
X_3	74.1/3.32	C_3/H_3 in β-D-xylopyranoside
X'_3	74.9/4.91	C_3/H_3 in 3-*O*-acetyl-β-D-xylopyranoside
X_4	75.6/3.63	C_4/H_4 in β-D-xylopyranoside

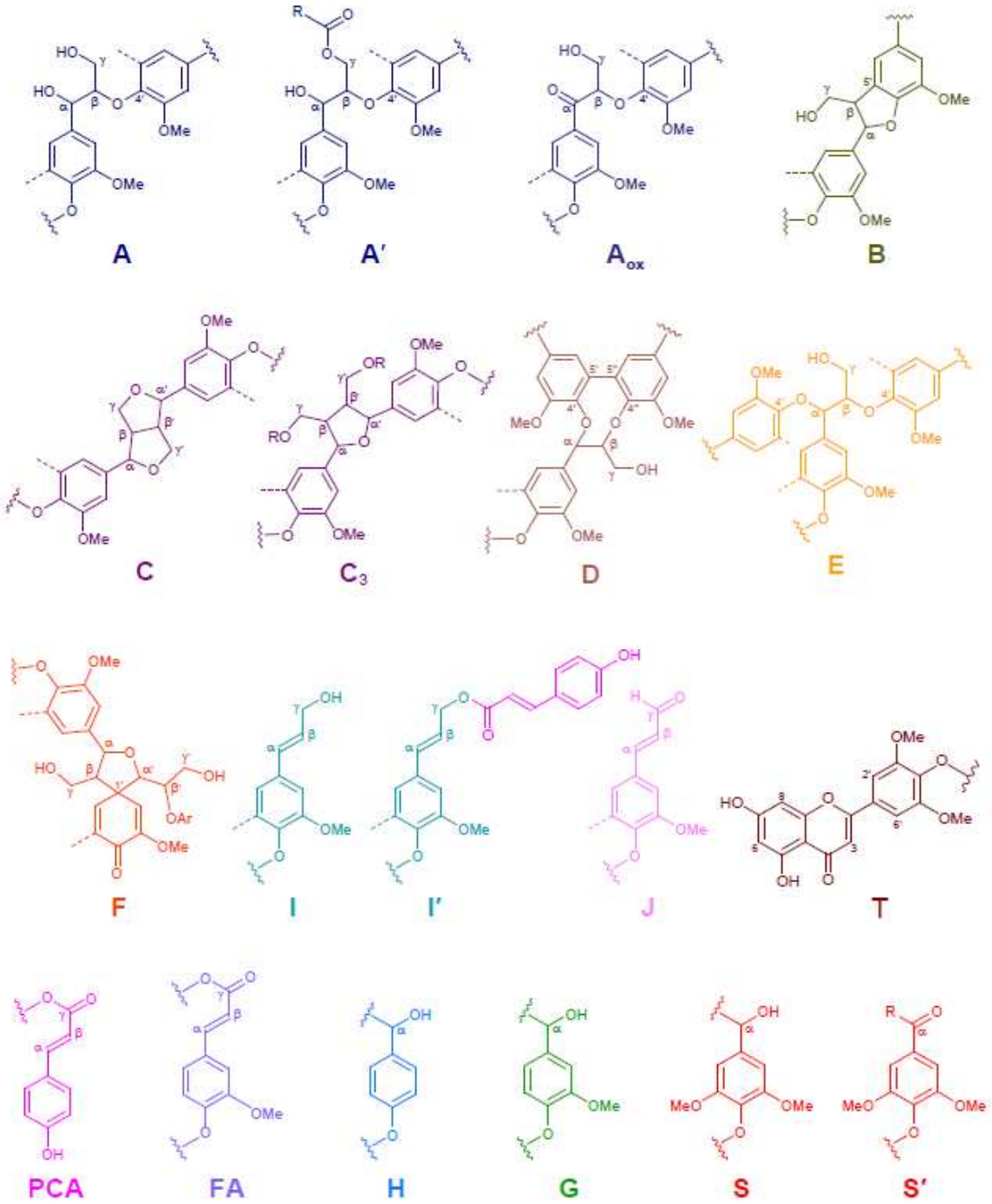

Figure 5. Main structures present in the lignins of sugarcane bagasse and straw as identified in the NMR spectra of Figures 3 and 4: (A) β–*O*–4′-structures; (A') β-*O*-4′-structures with acylated (by acetates or *p*-coumarates) γ-OH; (A_{ox}) C_α-oxidized β–*O*–4′ structures; (B) phenylcoumaran structures formed by β–5′-coupling; (C) resinol structures formed by β–β′-coupling; (C_3) tetrahydrofuran structures formed by β–β′-coupling of monolignols acylated at the γ-carbon; (D) dibenzodioxocin structures formed by 5–5′-

followed by 4–O–β-coupling; (E) α,β-diaryl ether structures; (F) spirodienone structures formed by β–1´-coupling; (I) *p*-hydroxycinnamyl alcohol end-groups; (I') *p*-hydroxycinnamyl alcohol end-groups acylated at the γ-OH; (J) cinnamaldehyde end-groups; (T) tricin end-groups; (PCA) *p*-coumarate moieties; (FA) ferulate moieties; (H) *p*-hydroxyphenyl units; (G) guaiacyl units; (S) syringyl units; (S') oxidized syringyl units bearing a carbonyl group at Cα. The structures are colored to match the assigned contours in the NMR spectra in Figures 3 and 4.

The side-chain region of the spectra provided important information about the different interunit linkages present in the lignins from sugarcane bagasse and straw. β−*O*−4′ Alkyl-aryl ether substructures (**A)** were the most prominent interunit linkages observed in this region of the spectra, together with other condensed substructures as well as cinnamyl end-groups. Interestingly, the spectra of the whole cell-walls and of their corresponding MWLs showed the occurrence of intense signals in the range δ_C/δ_H 62.7/3.83−4.30 assigned to the C_γ/H_γ correlations of γ-acylated units (**A′**), together with the presence of signals from normal γ-OH in β−*O*−4′ units (**A**) and other substructures (at δ_C/δ_H 59.4/3.40 and 3.72). The HSQC spectra therefore revealed that the lignins from sugarcane bagasse and straw are extensively acylated at the γ-position of the lignin side-chain, as also occurs in the lignins of other grasses. An estimation of the extent of γ-acylation of the lignin side-chains was performed by integration of the signals corresponding to the hydroxylated versus the acylated C_γ/H_γ correlations in the HSQC spectra of the isolated MWLs (where the signals from carbohydrates do not interfere), and ranged from 42% in the bagasse lignin to 36% in the straw lignin (**Table 5**). Other authors also found that the bagasse lignin was acylated at the γ–carbon, although to a lower extent (only 10.6%) [20]. The spectra showed other prominent signals corresponding to the β−*O*−4′ alkyl-aryl ether linkages. The C_α/H_α correlations in β−*O*−4′ substructures (**A**, **A′**) were observed at δ_C/δ_H 71.8/4.87, whereas the C_β/H_β correlations were observed at δ_C/δ_H 85.9/4.12 in normal γ-OH β−*O*−4′ substructures linked to a S-unit but shifted to δ_C/δ_H 83.0/4.33 in γ-acylated β−*O*−4′ substructures (**A′**), which overlaps with the C_β/H_β correlations of normal γ-OH β−*O*−4′ substructures linked to a G-unit at δ_C/δ_H 83.6/4.29. The C_β/H_β correlations of γ-acylated β−*O*−4′ substructures linked to a G-units shifted to δ_C/δ_H 80.8/4.58, and were clearly visible in the HSQC spectrum of the MWL from sugarcane straw, indicating an important γ-acylation extent of G-lignin units in this particular lignin, but was absent in the HSQC of the MWL from sugarcane bagasse.

Table 5. Structural characteristics (lignin inter-unit linkage types, end-groups, γ-acylation, aromatic units, and S/G Ratio, cinnamate content and *p*-coumarate/ferulate ratio, and tricin content) from integration of $^{13}C/^{1}H$ correlation peaks in the HSQC spectra from the whole cell-walls of sugarcane (*Saccharum spp.*) bagasse and straw and their isolated MWLs.

	Sugarcane Bagasse CW	Sugarcane Bagasse MWL	Sugarcane Straw CW	Sugarcane Straw MWL
Lignin inter-unit linkages (%)				
β–*O*–4´ aryl ethers (**A/A'**)	-	80	-	75

α-oxidized β–*O*–4´ aryl ethers (**A$_{ox}$**)	-	3	-	0
Phenylcoumarans (**B**)	-	6	-	15
Resinols (**C**)	-	2	-	3
Tetrahydrofurans (**C$_3$**)	-	4	-	1
Dibenzodioxocins (**D**)	-	0	-	3
α,β-diaryl ethers (**E**)	-	2	-	0
Spirodienones (**F**)	-	3	-	3
Lignin end-groups[a]				
Cinnamyl alcohol end-groups (**I**)	-	3	-	7
γ-acylated cinnamyl alcohol end-groups (**I'**)	-	2	-	3
Cinnamaldehyde end-groups (**J**)	-	4	-	2
Lignin side-chain γ-acylation (%)	-	42	-	36
Lignin aromatic units[b]				
H (%)	3	2	5	4
G (%)	37	38	66	68
S (%)	60	60	29	28
S/G ratio	1.6	1.6	0.4	0.4
p-Hydroxycinnamates[c]				
p-Coumarate (%)	68	48	35	21
Ferulate (%)	26	4	18	5
p-Coumarate/ferulate ratio	2.6	12.0	1.9	4.2
Tricin[c]	2	2	4	4

[a]Expressed as a fraction of the total lignin inter-unit linkage types **A**-**F**
[b]Molar percentages (H+G+S=100)
[c]*p*-Coumarate, ferulate and tricin molar contents as percentages of lignin content (H+G+S)

Signals from other lignin substructures were also observed in the HSQC spectra, and were particularly more evident in the HSQC of the isolated MWLs. Signals for phenylcoumarans (**B**) were found in the spectra, with their C_α/H_α and C_β/H_β correlations being observed at δ_C/δ_H 86.9/5.47 and 53.6/3.47, whereas the C_γ/H_γ correlations overlapped with other C_γ/H_γ signals around δ_C/δ_H 62.6/3.68. Signals for resinols (**C**) were also observed, with their C_α/H_α, C_β/H_β and the double C_γ/H_γ correlations at δ_C/δ_H 84.8/4.67, 53.6/3.07 and 71.0/3.83 and 4.19. In addition, signals for a β−β′ coupled tetrahydrofuran substructure (**C$_3$**), arising from β−β′ coupling of two γ-acylated sinapyl alcohols, were also observed in the HSQC spectrum of bagasse MWL, and in lower intensities in the spectrum of straw MWL, with their characteristic C_α/H_α and C_β/H_β correlations at δ_C/δ_H 82.8/5.00 and 49.8/2.59. This tetrahydrofuran structure has also been observed in the lignins from other grasses [36,54]. Signals for dibenzodioxocins (**D**) were observed only in the MWL from sugarcane straw, a lignin more rich in G-units, with their C_α/H_α and C_β/H_β correlations at δ_C/δ_H 83.4/4.82 and 85.4/3.86, but were absent in the HSQC spectrum of the MWL from sugarcane bagasse. Signals for α,β-diaryl ethers (**E**) could also be detected in the HSQC of the MWL from

bagasse, although in low intensities, as revealed by the C_α/H_α correlation at δ_C/δ_H 79.5/5.50, but were absent in the MWL from sugarcane straw. Finally, signals corresponding to spirodienones (**F**) could also be observed in the spectra of both MWLs, their C_α/H_α, $C_{\alpha'}/H_{\alpha'}$, C_β/H_β and $C_{\beta'}/H_{\beta'}$ correlations being at δ_C/δ_H 81.2/5.10, 84.9/4.88, 59.7/2.76 and 79.5/4.11. Other signals in the side-chain region of the HSQC spectra corresponded to the C_γ/H_γ correlations assigned to cinnamyl alcohol end-groups (**I**) at δ_C/δ_H 61.3/4.09 and to γ-acylated cinnamyl alcohol end-groups (**I′**) at δ_C/δ_H 64.0/4.79, as well as to the C_β/H_β correlations of α-keto- β−*O*−4′ alkyl-aryl ethers (**A_{ox}**) at δ_C/δ_H 82.8/5.23.

In the aromatic/unsaturated region of the HSQC spectra the main correlation signals corresponding to the aromatic rings and the unsaturated side-chains of the different lignin and *p*-hydroxycinnamate units. The lignin S-units showed an intense signal for the $C_{2,6}/H_{2,6}$ correlation at δ_C/δ_H 103.8/6.69. Signals corresponding to $C_{2,6}/H_{2,6}$ correlations in Cα-oxidized S-lignin units (**S′**) were observed at δ_C/δ_H 106.1/7.32 and 106.4/7.19. The G-lignin units showed different correlations for C_2/H_2 (δ_C/δ_H 110.9/7.00), and for mixed C_5/H_5 and C_6/H_6 (δ_C/δ_H 114.9/6.72 and 6.94, and 118.7/6.77). Signals for the $C_{2,6}/H_{2,6}$ and $C_{3,5}/H_{3,5}$ correlations of H-lignin units at δ_C/δ_H 128.0/7.23 and 114.5/6.62, the latest overlapping with other lignin signals, were also detected in the HSQC spectra, although in lower amounts. Prominent signals corresponding to *p*-coumarate structures (**PCA**) were observed in the spectra of the whole cell-walls and of their isolated MWLs. Cross-signals corresponding to the $C_{2,6}/H_{2,6}$ and $C_{3,5}/H_{3,5}$ correlations of the aromatic ring were observed at δ_C/δ_H 130.0/7.46 and 115.5/6.77, whereas signals for the C_α/H_α and C_β/H_β correlations were observed at δ_C/δ_H 144.4/7.41 and 113.5/6.27. Signals corresponding to the C_2/H_2 and C_6/H_6 correlations of ferulate moieties (**FA**) were also observed at δ_C/δ_H 111.0/7.32 and 123.3/7.10 in the spectra. The correlations corresponding to the unsaturated C_α/H_α and C_β/H_β overlapped with those of the *p*-coumarates.

In this region of the HSQC spectra, it was also possible to distinguish the two distinctive and characteristic signals at δ_C/δ_H 94.0/6.56 and 98.7/6.22 corresponding to the C_8/H_8 and C_6/H_6 correlations of tricin (**T**) [35]. The HSQC spectra also show the C_3/H_3 correlation of tricin at δ_C/δ_H 104.5/7.03 while the correlations for $C_{2'}/H_{2'}$ and $C_{6'}/H_{6'}$ are observed at δ_C/δ_H 103.9/7.30. Tricin is a flavone that is widely found and is apparently incorporated into the lignin structure in grasses [35, 45, 55-57] and that also occur in the lignin of other monocotyledons, such as coconut coir [58].

Other signals in aromatic/unsaturated region of the HSQC spectra are from cinnamyl alcohol end-groups (**I**), with their C_α/H_α and C_β/H_β correlations observed at δ_C/δ_H 128.4/6.44 and 128.4/6.23, and cinnamaldehyde end-groups (**J**), with the C_α/H_α and C_β/H_β correlations observed at δ_C/δ_H 153.4/7.61 and 126.3/6.76. The relative content of the cinnamaldehyde end-groups was estimated by comparison of the intensities of the C_α/H_α correlations in cinnamyl alcohols (**I**) and aldehydes (**J**). In addition, a signal for the $C_{2,6}/H_{2,6}$ correlation of sinapaldehyde end-groups (**$J_{(S)}$**), was also observed at δ_C/δ_H 106.8/7.06, however, no aromatic signals for coniferaldehyde end-groups were detected in the spectra. Signals for γ-acylated cinnamyl alcohol end-groups (**I′**) were also detected in this region of the spectra, with the characteristic C_α/H_α and C_β/H_β correlations observed at δ_C/δ_H 132.9/6.60 and 123.2/6.38.

These signals were particularly evident in the HSQC spectrum of the MWL from sugarcane bagasse, as corresponds to its higher extent of γ-acylation, but were present in lower amounts in the HSQC of the MWL from sugarcane straw.

Some authors [Zeng et al. 2014] have also reported the occurrence of *p*-hydroxybenzoates in the lignin from sugarcane bagasse based on the presence of a signal in the HSQC spectra at δ_C/δ_H 132.4/7.63, which is also present in the HSQC of our sugarcane samples. However, a close comparison with the HSQC spectra of other lignins from our own collection with a proven occurrence of *p*-hydroxybenzoates indicates that this assignment was not correct as the $C_{2,6}/H_{2,6}$ correlation signal for *p*-hydroxybenzoates that should appear around δ_C/δ_H 131.2/7.68 [58] does not match with that signal. The absence of other known correlations in the HMBC spectra also rejects this signal as belonging to *p*-hydroxybenzoates. *p*-Hydroxybenzoates have been widely found acylating the γ-alcohol of the lignin in palms and *Populus* species (willow, aspen, poplar) [54,58-63] but have never been found in grasses, and therefore, in addition to our not observing methylated *p*-hydroxybenzoate in the Py/TMAH analysis, we can dismiss the occurrence of *p*-hydroxybenzoates in the lignins from sugarcane bagasse and straw.

The relative abundances of the main lignin interunit linkages and cinnamyl end-groups, as well as the percentage of γ-acylation, the molar abundances of the different lignin units (H, G, and S), *p*-coumarates, ferulates, and tricin in the lignins from sugarcane bagasse and straw, estimated from volume integration of contours in the HSQC spectra, are shown in **Table 5**. The relative abundances of the main lignin interunit linkages were determined only in the HSQC spectra of the isolated MWLs. The data confirmed the completely different composition of the lignins from sugarcane bagasse and straw noted above from the Py-GC/MS data. The H:G:S composition of the lignin from sugarcane bagasse (2:38:60) was quite different to that from the sugarcane straw (4:68:28). The S/G ratios obtained by 2D-NMR closely matched those determined by Py-GC/MS, indicating a predominance of S-lignin units in the lignin from sugarcane bagasse (S/G ratio of 1.6) and a predominance of G-lignin units in the lignin from sugarcane straw (S/G ratio of 0.4). This data also confirms that the H-units content in the lignins from both is quite low (<5%, **Table 5**), and that the high abundance of *p*-hydroxyphenyl compounds observed upon Py-GC/MS was due to pyrolytic degradation of *p*-coumarates. *p*-Coumarates are highly abundant in the whole cell-walls of sugarcane bagasse and straw, as well as in their isolated MWLs, whereas ferulates are present in much lower abundance in the isolated MWLs than in the respective whole cell-walls, as already observed by Py-GC/MS and Py/TMAH. This is because, as already indicated above, ferulates are primarily attached to the polysaccharides whereas *p*-coumarates are predominantly attached to the lignin. This information, together with the high extent of acylation of the γ-OH observed in these lignins (42% and 36% of lignin acylation in bagasse and straw), seems to corroborate that *p*-coumarates mostly acylate the γ-hydroxyl of the lignin side-chain, as has been observed in other lignins [17,34-36,40,46, 49,53]. Tricin was found to be present in both lignins, being more abundant in the lignin from sugarcane straw. Tricin, as other flavones, is a metabolic hybrid as it is derived from a combination of the shikimate-derived phenylpropanoid and the acetate/malonate-derived polyketide pathways,

and appears to act as a lignin monomer in grasses [35,57]. Tricin cannot couple with another tricin, so it can only undergo cross-coupling with monolignols and therefore must be present at the start of a lignin chain; it therefore acts as a nucleation or initiation site for lignification [35,57]. The incorporation of tricin into the lignin polymer through 4–*O*–β coupling reactions has been suggested [35].

The differences in composition between the lignins from sugarcane bagasse and straw are also responsible for the differences in the relative abundances of the various interunit linkages between them. Thus, the lignin from sugarcane bagasse is mostly made up of β–*O*–4′ alkyl-aryl ether units (accounting for 83% of all the interunit linkage types), followed by minor amounts of phenylcoumarans (6%) and other condensed units. The lignin from sugarcane straw, on the other hand, has lower amounts of β–*O*–4′ alkyl-aryl ether units (75% of all the interunit linkages) and has higher amounts of phenylcoumarans (15%) and dibenzodioxocins (3%), as is consistent with a lignin enriched in G-units. Minor amounts of α,β-diaryl ethers (2%) were also found in the lignin from sugarcane bagasse, but were absent in the lignin of sugarcane straw. Other condensed substructures, such as resinols and spirodienones, are present in minor amounts in both lignins. In particular, there are strikingly low proportions of resinol structures, which account for only 2% of all interunit linkages in the lignin from sugarcane bagasse and 3% in the lignin from sugarcane straw. This low abundance of resinols is related to the high extent of γ-acylation of the lignin side-chains, as also observed in other highly acylated lignins [33-36,57]. During lignification, if the γ-OH of a monolignol is acylated, the formation of the normal resinol structures cannot occur because a free γ-OH is needed to rearomatize the intermediate quinone methide (following the radical dehydrodimerization step). Instead, new tetrahydrofuran structures are formed from either β−β′ homocoupling of two acylated monolignols or cross-coupling of a monolignol with a γ-acylated monolignol [54,62,64]. Interestingly, significant amounts of a tetrahydrofuran structure (**C**$_3$) formed by β−β′ homocoupling of two γ-acylated monolignols were found in these lignins, being especially abundant in the lignin from bagasse (4% of all interunit linkages), as is consistent with its higher degree of γ-acylation (42% of all side-chains).

3.4. Details of lignin acylation by *p*-coumarate (and more minor levels of acetate) from 2D-NMR and DFRC

p-Coumarates in grasses are always present acylating the γ-OH of the lignin side-chain [17,34-36, 40,46,49,53]. In order to demonstrate that the *p*-coumarates present in the lignins from sugarcane bagasse and straw are of the same type, we performed 2D-NMR HMBC experiments, correlating protons with carbons separated by 2- or 3-bonds. **Figure 6** shows the sections of the HMBC spectra of the MWLs isolated from sugarcane bagasse and straw for the correlations of the carbonyl carbons of the groups acylating the lignin γ-OH. The correlations of the carbonyl carbon at δ_C 166.0 with the α- and β-protons of *p*-coumarate esters at δ_H 7.41 and 6.27 confirm that they belong to *p*-coumarate esters. The correlations of this carbonyl carbon with protons in the range δ_H 4.0-4.8 ppm conclusively demonstrate that *p*-coumarate groups are acylating the γ-position of the lignin side-chains in sugarcane bagasse

and straw, as usually occurs in grasses. The HMBC spectra, however, did not show any correlation (at δ_C 169.8) for acetate groups acylating the lignin γ-OH, despite acetates being widely found acylating the γ-OH in the lignins of many plants, including other grasses [34-36,41,42,65]. Apparently, the extent of γ-acetylation of these lignins, if any, is below the detection limit of the HMBC experiment.

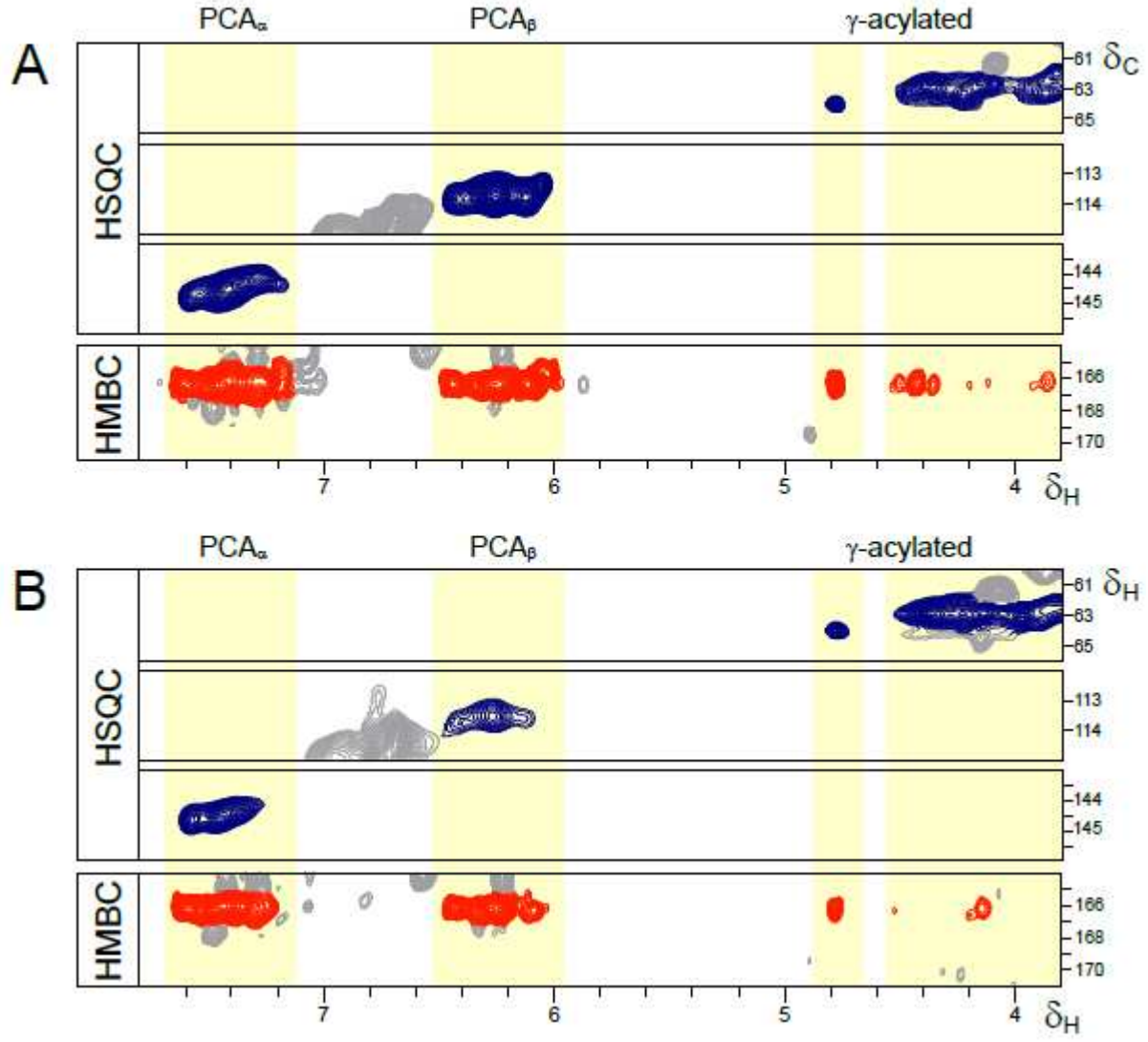

Figure 6. Sections of the HMBC spectra (δ_C/δ_H 164−171/3.8−7.8) of the MWLs from sugarcane bagasse and straw showing the main correlations for the carbonyl carbons of *p*-coumarates (PCA) acylating the γ-OH of the lignin side-chains. Appropriate sections of the HSQC spectra showing the C_γ/H_γ correlations of the acylated lignin (δ_C 60−66) and the C_α/H_α and C_β/H_β correlations of *p*-coumarates (δ_C 112−115 and 142−147, respectively), are also depicted. Note the absence of signals for the carbonyl carbons from acetates acylating the γ-position of the lignin side-chains (at δ_C 169.8).

Additional information regarding the nature of the acylation of the γ-OH was obtained from DFRC, a degradation method that cleaves α- and β-ether bonds but leaves γ-esters intact [37-40]. The chromatograms of the DFRC degradation products of the MWLs from

sugarcane bagasse and straw are depicted in **Figure 7**. The DFRC released the *cis*- and *trans*-isomers of the *p*-hydroxyphenyl (*t*H), guaiacyl (*c*G and *t*G) and syringyl (*c*S and *t*S) lignin monomers (as their acetylated derivatives) arising from normal, non-γ-*p*-coumaroylated β-ethers in lignin. In addition, the DFRC chromatograms revealed the presence of important peaks corresponding to the *cis*- and *trans*-isomers of S-lignin units acylated with both saturated (*c*S$_{pC}$ and *t*S$_{pC}$) and unsaturated (*c*S$_{pC´}$ and *t*S$_{pC´}$) *p*-coumarates, that were particularly abundant in the MWL from sugarcane bagasse, together with minor amounts of the guaiacyl *p*-coumarate conjugates (*c*G$_{pC}$ and *t*G$_{pC}$). The release of these compounds confirmed that *p*-coumarate groups are attached to the γ-OH of these lignins, and predominantly on S units, as usually occurs in grasses [17,34-36,40,46,49,53]. The original DFRC degradation method, however, cannot be used to assess the occurrence of natively acetylated lignin units because the degradation products are acetylated during the degradation procedure, but with an appropriate modification of the original protocol by substituting acetylating reagents with propionylating ones (DFRC´) it is possible to obtain information about the existence of native acetates in lignin [41,42]. The DFRC´ analyses using this modified protocol revealed the presence of minor amounts of γ-acetylated G- and S-lignin units, providing evidence that native acetylation of the γ-OH also occurred in these lignins, although to a low extent.

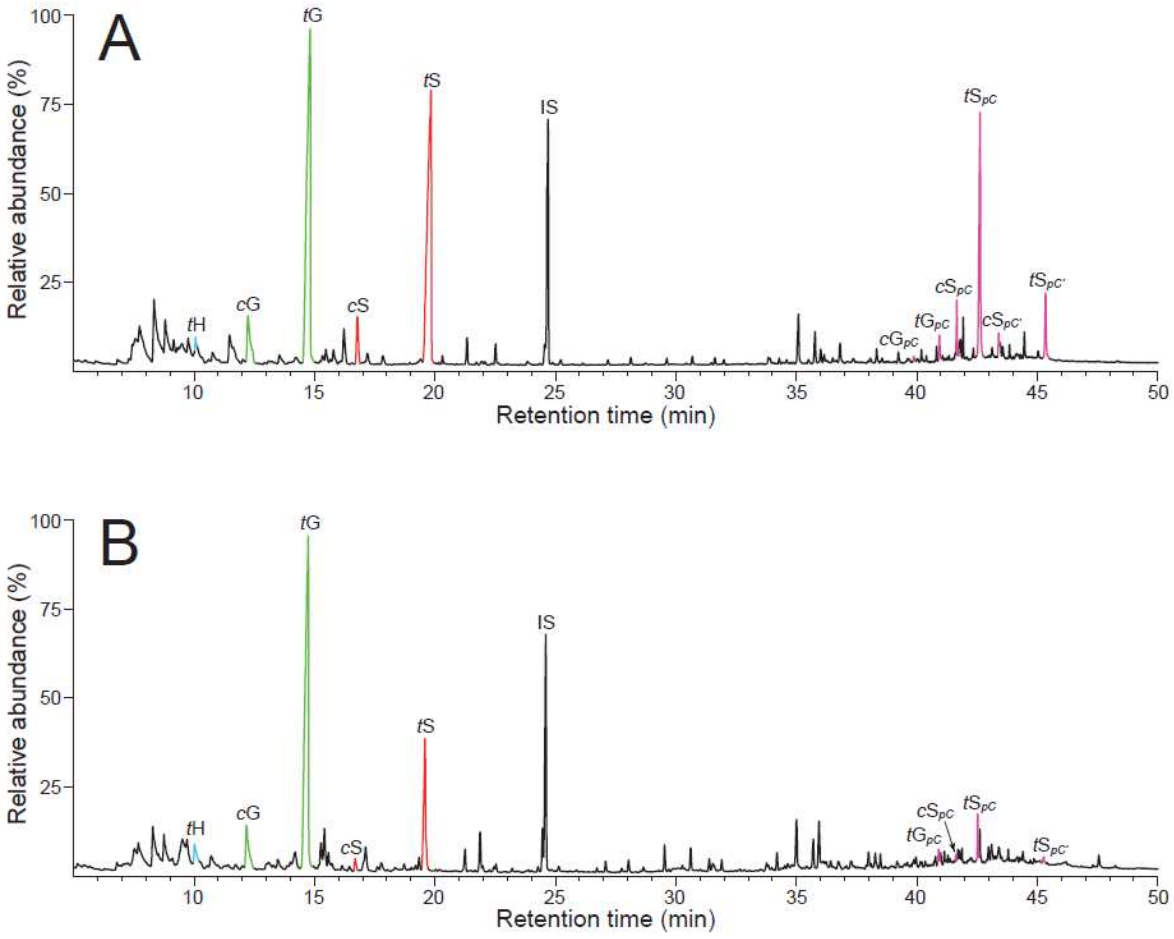

Figure 7. Chromatograms of the DFRC degradation products from the MWLs isolated from sugarcane bagasse (A) and straw (B) showing the presence of sinapyl (and minor

coniferyl) units acylated by *p*-coumarate moieties. *t*H, *c*G, *t*G, *c*S and *t*S are the normal *cis*- and *trans*-*p*-hydroxyphenyl (H), coniferyl (G) and sinapyl (S) alcohol monomers (as their acetate derivatives). cG_{pC}, tG_{pC}, cS_{pC} and tS_{pC} are the *cis*- and *trans*-coniferyl and sinapyl dihydro-*p*-coumarates (as their acetate derivatives). $cS_{pC'}$ and $tS_{pC'}$ are the *cis*- and *trans*-sinapyl *p*-coumarates (as their acetate derivatives). IS: internal standard (*n*-tetracosane).

The results from the DFRC and DFRC´ analyses of the MWLs from sugarcane bagasse and straw, namely the molar yields of the released monomers (H, G, G_{ac}, G_{pC}, S, S_{ac}, S_{pC}), as well as the percentages of naturally acetylated guaiacyl ($\%G_{ac}$) and syringyl ($\%S_{ac}$) and *p*-coumaroylated guaiacyl ($\%G_{pc}$) and syringyl ($\%S_{pc}$) lignin units, are presented in **Table 6** (the values for S_{pC} include the sum of both the saturated and the unsaturated *p*-coumarate analogs). The data indicate that a high extent of γ-acylation occurs in the lignin of sugarcane bagasse, with *p*-coumarate being the main group acylating this lignin, and predominantly on S-units. However, only minor amounts of γ-*p*-coumaroylated guaiacyl and syringyl conjugates were released in the DFRC from the MWL from sugarcane straw, despite the important amounts of *p*-coumarates present in this lignin (21% with respect to the total lignin aromatic units, as estimated by 2D-NMR in **Table 5**) and the fact that they acylate the γ-OH, as seen by HMBC. Similar results were also observed in the lignin from wheat straw [35], where only traces of γ-*p*-coumaroylated syringyl conjugates were released, despite the occurrence of significant amounts of *p*-coumarates being attached to the γ-OH. This finding seems to indicate that, as also occurred in the lignin from wheat straw, *p*-coumarate groups are mostly attached to the γ-OH in condensed lignin substructures that are not amenable to DFRC analysis, a slightly troubling observation that requires further study. This assumption is also supported by the presence of an intense signal for γ-acylated β−*O*−4′ substructures linked to G-units in the HSQC spectrum of the MWL from sugarcane straw that indicates an important γ-acylation extent of G-units in this lignin. On the other hand, the DFRC´ data also revealed a minor extent of γ-acylation of these lignins with acetate groups, but which takes place predominantly on G units (3.5% and 3.3% of G-lignin units were acetylated in the lignins from bagasse and straw) rather than on S units (only 0.6% and 1.9%). Low levels of lignin acetylation, with a preference for G-units, have also been found in other grasses, such as bamboo, wheat straw or the pith of elephant grass [35,36,42]. In contrast, in the lignin of most plants, γ-acetylation occurs predominantly on S-units [34,42,65]. This fact seems to indicate that as yet unknown acetyl transferases with a higher affinity towards coniferyl alcohol than towards sinapyl alcohol, contrary to what occurs in most plants, are probably involved in monolignol acetylation in grasses.

Table 6. Abundance (μmol/g of lignin) of the monomers obtained from DFRC degradation of the MWLs from sugarcane (*Saccharum spp.*) bagasse and straw, and relative percentages of the different acylated (acetylated and *p*-coumaroylated) lignin monomers.

	H	G	G_{ac}	G_{pC}	S	S_{ac}	S_{pC}[a]	$\%G_{ac}$[b]	$\%G_{pC}$[c]	$\%S_{ac}$[d]	$\%S_{pC}$[e]
MWL Bagasse	2	128	5	2	98	1	30	3.4	1.7	0.5	23.4

MWL Straw	4	112	4	1	24	1	3	3.3	1.1	1.7	10.6

[a] includes the sum of sinapyl *p*-coumarates (S_{pC}) and sinapyl dihydro-*p*-coumarates ($S_{pC'}$).
[b] $\%G_{ac}$ is the percentage of acetylated G units (G_{ac}) with respect to the total G units (G, G_{ac}, G_{pC}).
[c] $\%G_{pC}$ is the percentage of *p*-coumaroylated G units (G_{pc}) with respect to the total G units (G, G_{ac}, G_{pC}).
[d] $\%S_{ac}$ is the percentage of acetylated S units (S_{ac}) with respect to the total S units (S, S_{ac}, S_{pC}).
[e] $\%S_{pC}$ is the percentage of total *p*-coumaroylated S units (including sinapyl *p*-coumarates, S_{pC}, and sinapyl dihydro-*p*-coumarates, $S_{pC'}$), with respect to the total S units (S, S_{ac}, S_{pC}).

3.5. Diversity of β–β (resinol and tetrahydrofuran) structures in the lignins from sugarcane bagasse and straw

The HSQC spectra of the lignins from sugarcane bagasse and straw clearly show the presence of significant amounts of a tetrahydrofuran structure (**C3**) arising from β–β coupling of two γ-acylated monolignols. As *p*-coumarates are by far the main group acylating the sinapyl alcohol monomer for lignification in sugarcane bagasse and straw, as seen above, it is clear that the tetrahydrofuran structure **C3**, identified in the HSQC spectra, is formed from the β–β′-coupling of two sinapyl *p*-coumarate monomers, and will bear two *p*-coumarate groups in its structure, as in **Figure 8**. A similar structure has been found in the lignins from other grasses, such as maize and elephant grass [36,54], and analogous structures bearing acetates or *p*-hydroxybenzoates as acyl groups have also been identified in the lignins from other plants [54,62,63,66].

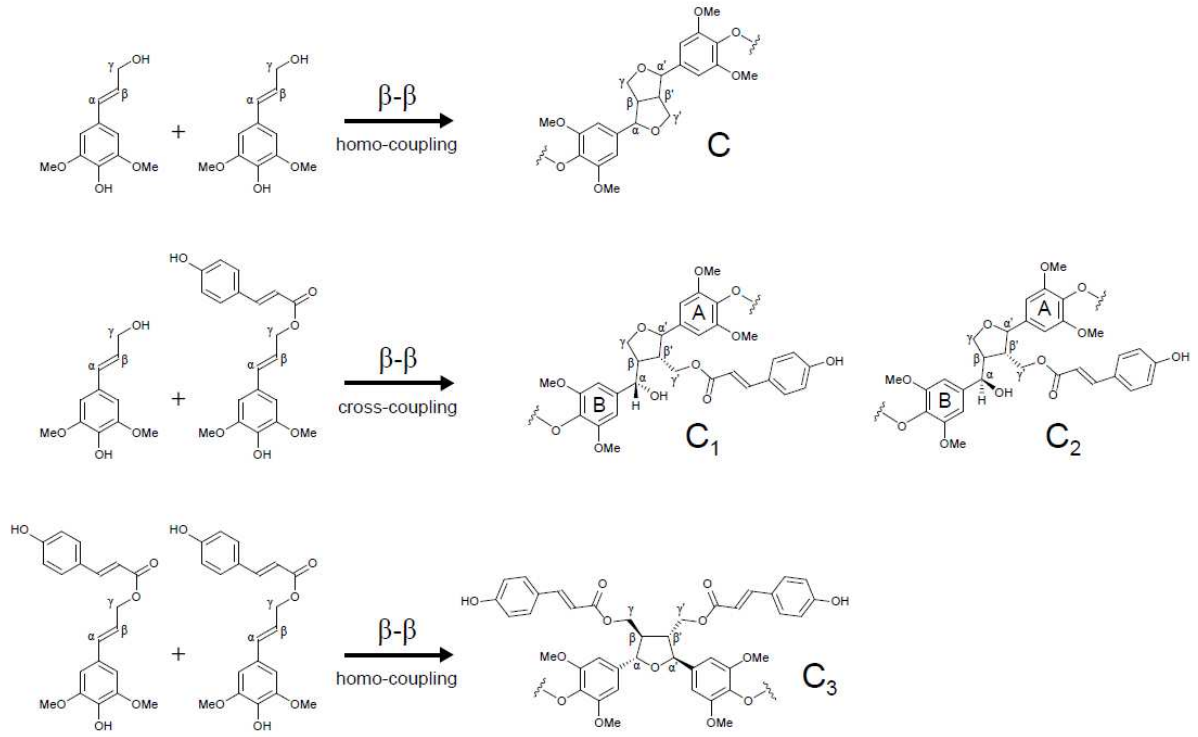

Figure 8. Resinol (C) and tetrahydrofuran (C_1, C_2 and C_3) structures produced from β–β´-homo- and cross-coupling of sinapyl alcohol and sinapyl *p*-coumarate. (Adapted from Lu and Ralph, 2005).

Besides tetrahydrofuran structure **C3**, the β–β coupling and cross-coupling of sinapyl alcohol and γ-*p*-coumaroylated sinapyl alcohol can form non-symmetrical tetrahydrofurans **C1** and **C2**, as detailed in **Figure 8**. A wide variety of tetrahydrofuran structures arising from β–β coupling and cross-coupling of normal γ-OH and γ-acylated (with acetates, *p*-coumarates and *p*-hydroxybenzoates) monolignols, have been synthetized in past years and their different correlation signals assigned in the HSQC spectra [54,62,63,66]. In those papers, the different synthetic structures were acetylated to improve the spectral properties. Therefore, in order to have detailed information of the different β–β structures present in the lignins of sugarcane bagasse and straw, the MWLs were acetylated for subsequent HSQC analyses and the correlation signals were compared with those previously published [54,62,63,66]. **Figure 9** shows the 2D-NMR HSQC spectra of the (acetylated) MWLs from sugarcane bagasse and straw, with the C_α/H_α and C_β/H_β and C_γ/H_γ correlations of the different resinol and tetrahydrofuran structures arising from the β–β coupling and cross-coupling of sinapyl alcohol and sinapyl *p*-coumarate. The signals observed in the HSQC spectra closely match those previously reported for synthesized model compounds and related lignins and provide compelling evidence for the occurrence of tetrahydrofuran structures arising from the β–β homo- and cross-coupling of sinapyl alcohol and sinapyl *p*-coumarate, together with the presence of traditional β–β resinol structures (**C**). These structures include those arising from cross-coupling of sinapyl *p*-coumarate with sinapyl alcohol (**C1** and **C2**) as well as from the homo-coupling of two sinapyl *p*-coumarates (**C3**), and are more prominent in the HSQC spectrum of the MWL from sugarcane bagasse. The occurrence of the tetrahydrofuran structures **C1-C3** in these lignins conclusively demonstrates that the sinapyl *p*-coumarate conjugate acts as a monomer participating in coupling and cross-coupling reactions in the lignification of sugarcane, and therefore implicates the presence of corresponding *p*-coumaroyl transferases in this plant. In fact, the *p*-coumaroyl monolignol transferase involved in the *p*-coumaroylation of monolignols has been discovered in grasses [49], and a candidate gene has been recently identified [67] and its action validated in *Brachypodium distachyon*, a model grass [68].

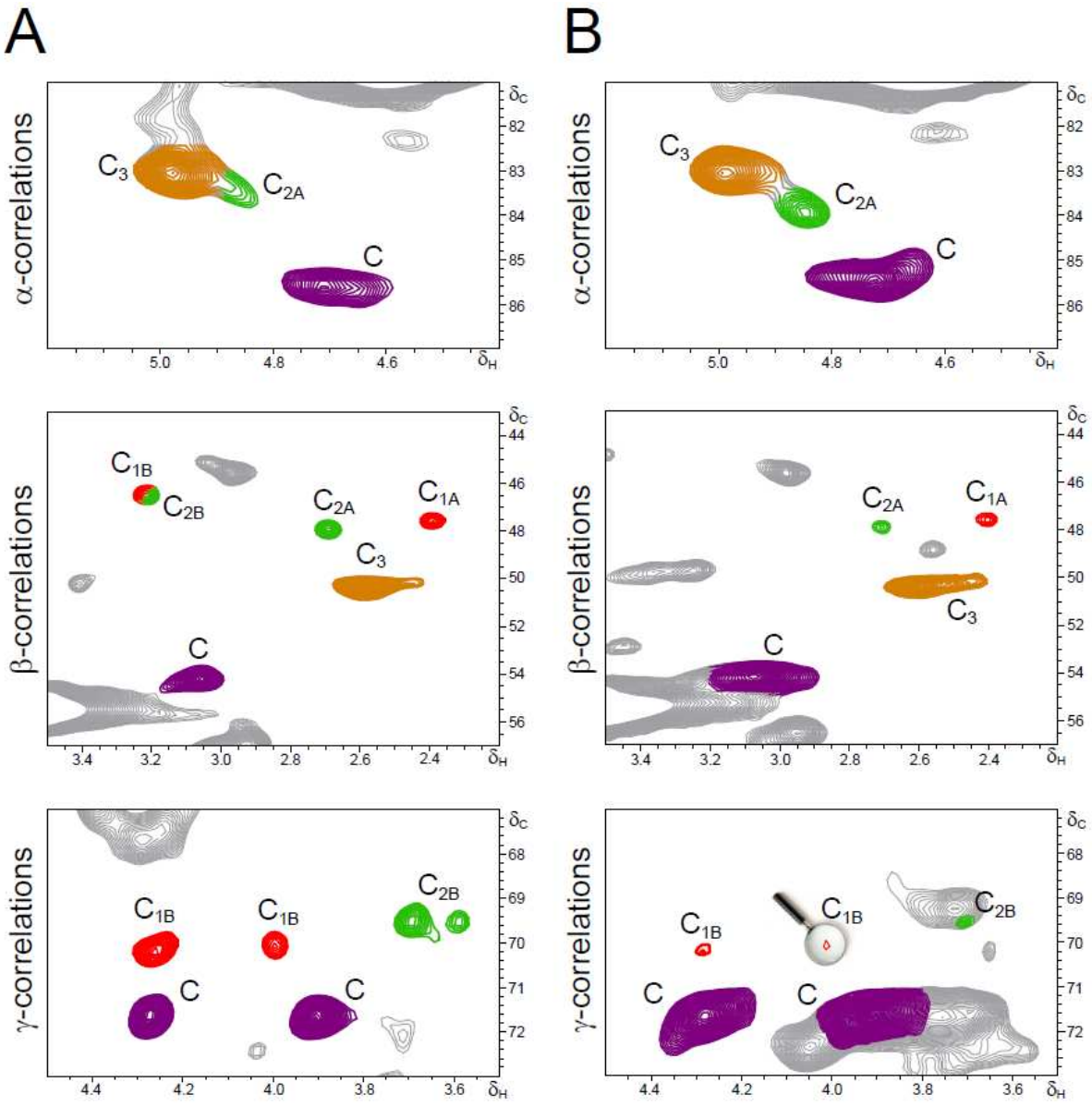

Figure 9. 2D-NMR HSQC of the (acetylated) MWLs from sugarcane bagasse and straw showing the C_α/H_α and C_β/H_β and C_γ/H_γ correlations of the different β–β resinol (C) and tetrahydrofuran (C_1-C_3) structures arising from the coupling and cross-coupling of sinapyl alcohol and sinapyl *p*-coumarate, as depicted in Figure 8. Signals were assigned from previously published literature [Lu and Ralph, 2005, 2008].

4. Conclusions

Detailed structural analysis of the lignins from sugarcane bagasse and straw indicated that they have very different H-G-S compositions. Whereas the lignin from sugarcane bagasse is S-rich (H:G:S of 2:38:60), the lignin from sugarcane straw is G-rich (H:G:S of 4:68:28). The differences in composition are also reflected in differences in the relative abundances of the various interunit linkages in both lignins. Thus, the lignin from bagasse is mostly made up by β–*O*–4′ alkyl-aryl ether units (accounting for 83% of all the interunit

linkage types), followed by minor amounts of phenylcoumarans (6%) and other condensed units. The lignin from straw, on the other hand, has lower amounts of β–*O*–4′ alkyl-aryl ether units (75% of all the interunit linkages) and has higher amounts of phenylcoumarans (15%) and dibenzodioxocins (3%), as consistent with a lignin enriched in G-units. Other condensed substructures, such as resinols and spirodienones are present in minor amounts in both lignins. Both lignins are extensively acylated at the side-chain γ-OH predominantly with *p*-coumarate, and to a minor extent, with acetate. Although *p*-coumarate groups are preferentially attached to S-units in both lignins, acetates are attached preferentially to G-units. In addition, whereas *p*-coumarate groups in the lignin from bagasse are mostly attached to the lignin γ-OH in β-ether units, *p*-coumarate groups in the lignin from sugarcane straw are mostly attached to the lignin γ-OH in condensed lignin substructures. Various tetrahydrofuran structures arising from β–β coupling and cross-coupling of sinapyl alcohol and sinapyl *p*-coumarate have also been shown to occur in the structure of these lignins, indicating that sinapyl *p*-coumarate acts as a monomer participating in lignification reactions. Finally, the flavone tricin was also found to be incorporated into the lignins from sugarcane bagasse and straw, as also occurs in the lignins from other grasses. The structural differences in the lignins of the two sugarcane residues, bagasse from the stems after expelling the sugar, and straw from the residual plant (including leaves), suggests that the bagasse material will pretreat more easily (because of its higher syringyl content) than the straw and indicates that methods will need to be optimized for each. In addition, the two residues contain, in addition to the polysaccharides that may be saccharified and fermented to liquid biofuels and lignins that might be valorized, significant levels of *p*-coumarate, ferulate, and tricin that might be valuable to extract as commodity chemicals. Optimizing the utilization of all of the plant biomass that is sustainably available will improve biorefinery operations and help to offer fuels and chemicals with a reduced carbon footprint compared to fossil-derived products.

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Lipophilic phytochemicals in sugarcane bagasse and straw

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ABSTRACT

The composition of lipophilic phytochemicals in sugarcane bagasse and straw, the two major residues of sugarcane processing, was investigated in detail by gas chromatography and mass spectrometry. The composition of the lipid extracts from sugarcane bagasse and straw were completely different from each other. While the extracts of sugarcane bagasse were dominated by *n*-aldehydes (ca. 48% of all identified lipids) and *n*-fatty alcohols (ca. 23%) with lower amounts of *n*-fatty acids (10%) and steroid ketones (14%), the extracts from sugarcane straw were strongly dominated by *n*-fatty acids (accounting for ca. 60% of all identified compounds) with significant amounts of steroid compounds, particularly sterols (10%) and steroid ketones (14%). Tocopherols and triterpenols were also found, being particularly abundant among the extractives of sugarcane straw. Sugarcane bagasse and straw can thus be viewed as promising feedstocks for obtaining highly valuable phytochemicals of nutraceutical or pharmaceutical interest.

Keywords: Sugarcane, bagasse, straw, aldehydes, alcohols, fatty acids, sterols

1. Introduction

Sugarcane (*Saccharum spp.*) is a perennial monocot plant belonging to the grass family (Poaceae). Sugarcane is an important economic plant as it is the main feedstock for the production of sugar as well as ethanol, with Brazil leading the world production [Goldemberg, 2008, Santos et al., 2012; de Souza et al., 2014]. The plant originated from Asia but it is well adapted to most tropical and subtropical climates, where it is one of the most important bioenergy crops. Two main types of wastes are produced from sugarcane during the production of sugar, the fibrous residue after extraction of the juice (named bagasse), and the left over harvest residues (straw). The wastes from the sugarcane agro-industry are produced in large quantities, about 280 million tons of bagasse and straw per year [Ortiz and Oliveira, 2014], and they are likely to increase in the near future as this crop expands and new industrial plants are implemented. Currently, sugarcane residues are mostly burned for the production of heat and electricity at the sugar factory. However, these residues can also be used as feedstocks for the production of other high-value products in the context of the lignocellulosic biorefinery.

Sugarcane bagasse and straw are lignocellulosic materials basically composed of cellulose, hemicelluloses and lignin. These residues also contain significant amounts of lipids (ca. 1-2% by weight) that can be extracted to produce high-value products. Natural waxes have a wide range of industrial applications in cosmetics, personal care products, polishes and coatings. On the other hand, these lipids, even when present in low amounts in the raw material, may play an important role during the industrial processing of lignocellulosic materials, such as pulp and paper manufacture, since they are at the origin of the so-called pitch deposits (Back and Allen, 2000). Lipids include different classes of compounds (i.e. alkanes, fatty alcohols, fatty acids, free and conjugated sterols, terpenoids and triglycerides),

which have different behavior during pulping and bleaching (Back and Allen, 2000; Gutiérrez and del Río 2003; Marques et al., 2010).

The composition of lipids of sugarcane plants has been the subject of several investigations and several compounds, including triterpenoids and steroids (Ohmoto et al., 1970; Deshmane and Dev, 1971; Feng et al., 2014a,2014b), or aliphatic components such as long-chain alcohols (polycosanol) and aldehydes (Purcell et al., 2005; Asikin et al., 2012) have been reported. On the other hand, the lipid composition of sugarcane waxes recovered from different sugarcane mill by-products such as the filter cake from sugar refinery or from fermentation wastes and vinasses from alcohol distilleries and rum factories, have also been well documented (Nuissier et al., 2002; Georges et al., 2006; de Lucas et al., 2007). However, studies concerning the detailed composition of lipids in the two major solid wastes generated by the sugar and alcohol industry, sugarcane bagasse and straw, have not been performed so far. Therefore, in this paper we performed a comprehensive characterization of the lipophilic compounds present in sugarcane bagasse and straw by the use of gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) using short- and medium-length high temperature capillary columns, respectively, with thin films, which enables the elution and analysis of a wide range of compounds from fatty acids to intact high molecular weight lipids such as sterol glycosides (Gutiérrez et al., 1998). This study can provide significant further knowledge about the composition of lipids in these sugarcane residues, which can be regarded as valuable sources of active phytochemicals.

2. Materials and methods

2.1. Samples

Sugarcane bagasse and straw were collected at harvesting age from high performance sugarcane plantations and were supplied by a mid-size ethanol mill located in the São Pedro dos Ferros county, MG, Brazil. The processing of sugarcane involved two steps. The first was the crushing of sugarcane stalks in a semi-industrial chipper; then the fragments were processed by milling to extract the sugarcane juice. The resulting residue (bagasse) was used for the subsequent characterization studies. Straw, heterogeneous material composed of leaves and tops of sugarcane, was crushed in the same equipment used to grind the stalks in order to have a better homogenization of the material. The samples were subsequently air-dried and knife milled (Janke and Kunkel, Analysenmühle), and the lipophilic compounds extracted with acetone for 8 h in a Soxhlet apparatus. The acetone extracts were evaporated to dryness, and resuspended in chloroform for chromatographic analysis of the lipid fraction. The acetone-extracted sample was then extracted with hot water (100 mL, 3 h at 100 ºC) to determine the water soluble material. Klason lignin content was estimated as the residue after sulphuric acid hydrolysis of the pre-extracted material, corrected for ash and protein content, according to the TAPPI method T222 om-88 (Tappi, 2004). The acid-soluble lignin was determined, after the insoluble lignin was filtered off (Duran filter crucible 4; nominal pore size max. 10−16 μm), by UV-spectroscopic determination at 205 nm wavelength using 110 L

cm^{-1} g^{-1} as the extinction coefficient. Holocellulose was isolated from the pre-extracted fibers by delignification for 4 h using the acid chlorite method (Browning, 1967). The α-cellulose content was determined by removing the hemicelluloses from the holocellulose by alkali extraction (Browning, 1967). Ash content was estimated as the residue after 6 h of heating at 575 ºC according to the TAPPI method T211 om-02 (Tappi, 2004). Three replicates were used for each sample.

2.2. GC and GC-MS analyses

The lipophilic extractives (1 mg) were silylated with 0.250 mL bis(trimethylsilyl)trifluoroacetamide (BSTFA, from Supelco) in the presence of 0.050 mL pyridine at 70 ºC for 2h before GC and GC-MS analyses. An HP 5890 gas chromatograph (Hewlett Packard, Hoofddorp, Netherlands) equipped with a split-splitless injector and a flame ionization detector (FID) was used for GC analyses. The injector and the detector temperatures were set at 300 ºC and 350 ºC respectively. Samples were injected in the splitless mode at a concentration of 7.5 mg/mL. Helium was used as the carrier gas. The capillary column used was a high temperature, polyimide coated fused silica tubing DB5-HT (5 m x 0.25 mm I.D., 0.1 µm film thickness; J&W Scientific). The oven was temperature-programmed from 100 ºC (1 min) to 350 ºC (3 min) at 15 ºC min^{-1}. Peaks were quantified by area, and a mixture of different standards such as octadecane (Sigma-Aldrich, 99%), palmitic acid (Sigma-Aldrich, 99%), oleic acid (Sigma-Aldrich, ≥99%), linoleic acid (Sigma-Aldrich, ≥99%), hexadecanol (Sigma-Aldrich, ≥97%), sitosterol (Calbiochem, 98%), stigmasterol (Sigma-Aldrich, 95%), sitosteryl 3β-d-glucopyranoside (Matreya LLC, a mixture of three steryl glucosides of 98% purity, of which 56% correspond to sitosteryl glucoside), cholestan-3-one (Sigma-Aldrich, 99%) and cholesta-3,5-diene (Sigma-Aldrich, ≥93%), with a concentration range between 0.1 and 1 mg/mL, was used to elaborate calibration curves. The correlation coefficient was higher than 0.99 in all the cases. Quantification was obtained using response factors of the same or similar compounds (in the case of triterpenols and alkylresorcinols, they were quantified against sitosterol). The data from the three replicates were averaged. In all cases, the standard deviations from replicates were below 10% of the mean values. The total amounts of the different lipid families were determined by adding up the amounts of their constituent compounds.

The GC-MS analysis were performed on a Varian Star 3400 gas chromatograph (Varian, Walnut Creek, CA) coupled with an ion-trap detector (Varian Saturn 400) equipped with a high-temperature capillary column (DB-5HT, 15 m × 0.25 mm i.d., 0.1 µm film thickness; J&W Scientific). Helium was used as carrier gas at a rate of 2 mL/min. The samples, at concentration of 2.5 mg/mL, were injected with an autoinjector (Varian 8200) directly onto the column using a SPI (septum-equipped programmable injector) system. The temperature of the injector during the injection was 60 ºC, and 0.1 min after injection was programmed to 380 ºC at a rate of 200 ºC min^{-1} and held for 10 min. The oven was heated from 120 ºC (1 min) to 380 ºC (5 min) at 10 ºC min^{-1}. The temperature of the transfer line was set at 300 ºC. Compounds were identified by comparing their retention times and mass

spectra with authentic standards, except for alkylresorcinols and triterpenols, which were tentatively identified by comparing their mass spectra with those reported in the literature. Single ion chromatographic profiles were used to estimate compound abundances when two peaks partially overlapped.

3. Results and discussion

3.1. Composition of the main constituents of sugarcane bagasse and straw

The abundance of the main constituents (water soluble material, acetone extractives, Klason lignin, acid-soluble lignin, holocellulose, α-cellulose, and ash) of the samples of sugarcane bagasse and straw selected for this study are shown in **Table 1**. The composition is in the range of values previously published for similar samples, except for the much higher content of extractives reported in previous papers [Rocha et al., 2015]. In this study, the lipid compounds (acetone extractives) accounted for 0.8% in sugarcane bagasse and 1.3% in sugarcane straw. The extractives content reported by other authors displayed a wide dispersion; thus, Rocha et al. (2015) reported extractives content in sugarcane bagasse in the range from 2.3-10.5% and Benjamin et al., (2014) in the range from 4.3-9.9%. In sugarcane straw, the extractives content was reported to be 5.9% (Oliveira et al., 2014). However, these high values are mostly due to the solvents used for their determination, usually ethanol (95%) that may also extract low-molecular-weight carbohydrates, salts, polyphenols and other water-soluble compounds.

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

	Sugarcane bagasse[a]	Sugarcane straw[a]
Water-soluble material	1.4 ± 0.2	2.2 ± 0.2
Acetone extractives	0.8 ± 0.1	1.3 ± 0.1
Klason lignin[b]	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

[a] Average of three replicates
[b] Corrected for proteins and ash

3.2. Lipid composition of sugarcane bagasse and straw

The detailed analysis of the chemical composition of the lipidic compounds in sugarcane bagasse and straw was accomplished by GC and GC-MS (after preparation of the

trimethylsilyl (TMS)-ether derivatives) according to the method previously described (Gutiérrez et al., 1998). The chromatographic conditions used for the analyses, by using short- and medium-length high-temperature capillary columns with thin films, allow for the elution of a wide range of compounds, from low-molecular-weight free fatty acids and sterols to intact high molecular weight lipids, such as sterol glycosides, that are usually biased in more standard GC conditions.

The GC-MS chromatograms of the lipids (as their TMS-ether derivatives) extracted from sugarcane bagasse and straw are shown **Figure 1**. A large number of compounds were identified among the acetone extractives, which were subsequently quantified. The identities and abundances (mg/Kg, dry, ash-free basis) of the main lipid compounds identified in the acetone extracts of sugarcane bagasse and straw are listed in **Table 2**. Structures representative of the main classes of lipids identified in sugarcane bagasse and straw, including aliphatic and steroid/triterpenoid compounds, are shown in **Figure 2** and **Figure 3**. The analyses indicated that the predominant lipids identified among the acetone extracts of sugarcane bagasse and straw were series of long-chain fatty acids, alcohols, aldehydes, as well as steroids (sterols, sterol glycosides, steroid hydrocarbons and ketones), triterpenols, tocopherols, and lower amounts of *n*-alkylresorcinols and high molecular weight esters.

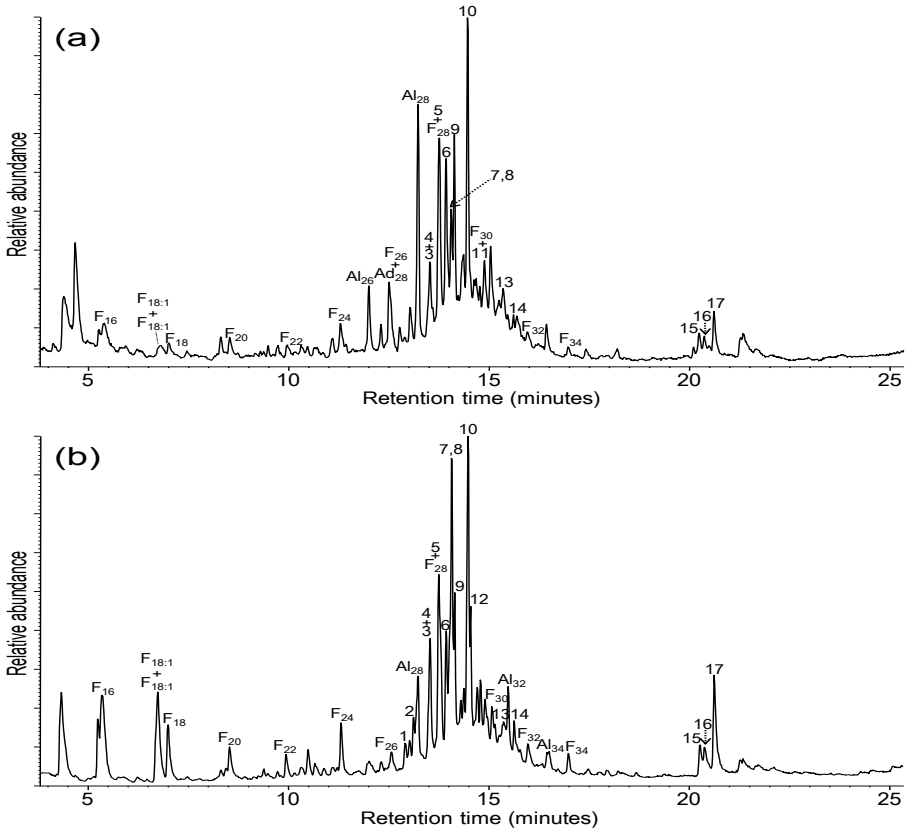

Figure 1. GC-MS chromatograms of the lipid extracts (as TMS-ether derivatives) from (**a**) sugarcane bagasse, and (**b**) sugarcane straw. F(n): *n*-fatty acid series; Ad(n): *n*-aldehyde series; Al(n): *n*-fatty alcohol series. Other compounds reflected are: 1: α-tocoferol;

2: taraxerol methyl ether; 3: campesterol; 4: campestanol; 5: stigmasterol; 6: ergost-4-en-3-one; 7: sitosterol; 8: stigmastanol; 9: stigmasta-4,22-dien-3-one; 10: stigmast-4-en-3-one; 11: ergostane-3,6-dione; 12: isoarborinol; 13: stigmastane-3,6-dione; 14: 7-oxo-sitosterol; 15: campesteryl-β-d-glucopyranoside; 16: stigmasteryl-β-d-glucopyranoside; 17: sitosteryl-β-d-glucopyranoside.

Table 2. Composition and abundance (mg/Kg fiber, dry, ash-free) of main lipids identified in the acetone extracts of sugarcane bagasse and straw.

Compound	**Bagasse**	**Straw**
***n*-Fatty acids**	**140**	**1210**
n-hexadecanoic acid	14	246
n-heptadecanoic acid	0	11
cis,*cis*-octadeca-9,12-dienoic acid	1	86
cis-octadec-9-enoic acid	2	210
n-octadecanoic acid	5	160
n-nonadecanoic acid	0	2
n-eicosanoic acid	8	75
n-heneicosanoic acid	0	5
n-docosanoic acid	4	50
n-tricosanoic acid	2	16
n-tetracosanoic acid	10	90
n-pentacosanoic acid	1	14
n-hexacosanoic acid	12	35
n-heptacosanoic acid	1	8
n-octacosanoic acid	60	160
n-nonacosanoic acid	tr	2
n-triacontanoic acid	15	20
n-dotriacontanoic acid	3	12
n-tetratriacontanoic acid	2	8
***n*-Aldehydes**	**700**	**40**
n-docosanal	5	tr
n-tetracosanal	7	1
n-hexacosanal	58	4
n-octacosanal	480	30
n-triacontanal	150	5
***n*-Fatty alcohols**	**330**	**85**
n-tetracosanol	12	4
n-hexacosanol	62	10
n-octacosanol	194	48
n-triacontanol	42	12
n-dotriacontanol	12	7
n-tetratriacontanol	8	4

Tocopherols	**10**	**70**
α-tocopherol	10	45
γ-tocopherol	0	25
***n*-Alkylresorcinols**	**0**	**5**
5-*n*-nonadecylresorcinol	0	1
5-*n*-heneicosylresorcinol	0	3
5-*n*-tricosylresorcinol	0	1
Triterpenols	**2**	**50**
taraxerol methyl ether	0	12
arundoin	0	10
isoarborinol	2	28
Sterols	**40**	**215**
campesterol	9	25
campestanol	7	10
stigmasterol	6	30
sitosterol	12	100
stigmastanol	4	42
7-oxo-sitosterol	2	8
Steroid hydrocarbons	**24**	**46**
ergostatriene	tr	12
stigmasta-3,5,22-triene	24	34
Steroid ketones	**210**	**285**
ergost-4-en-3-one	50	36
ergostane-3,6-dione	15	20
stigmasta-4,22-dien-3-one	46	52
stigmasta-3,5-dien-7-one	1	2
stigmast-4-en-3-one	78	130
stigmastane-3,6-dione	20	45
Sterol glycosides	**8**	**30**
campesteryl-β-D-glucopyranoside	2	1
stigmasteryl-β-D-glucopyranoside	1	4
sitosteryl-β-D-glucopyranoside	5	25
High molecular weight esters	**5**	**8**
esters C_{42}	1	2
esters C_{44}	3	4
esters C_{46}	1	2

tr; trace amounts

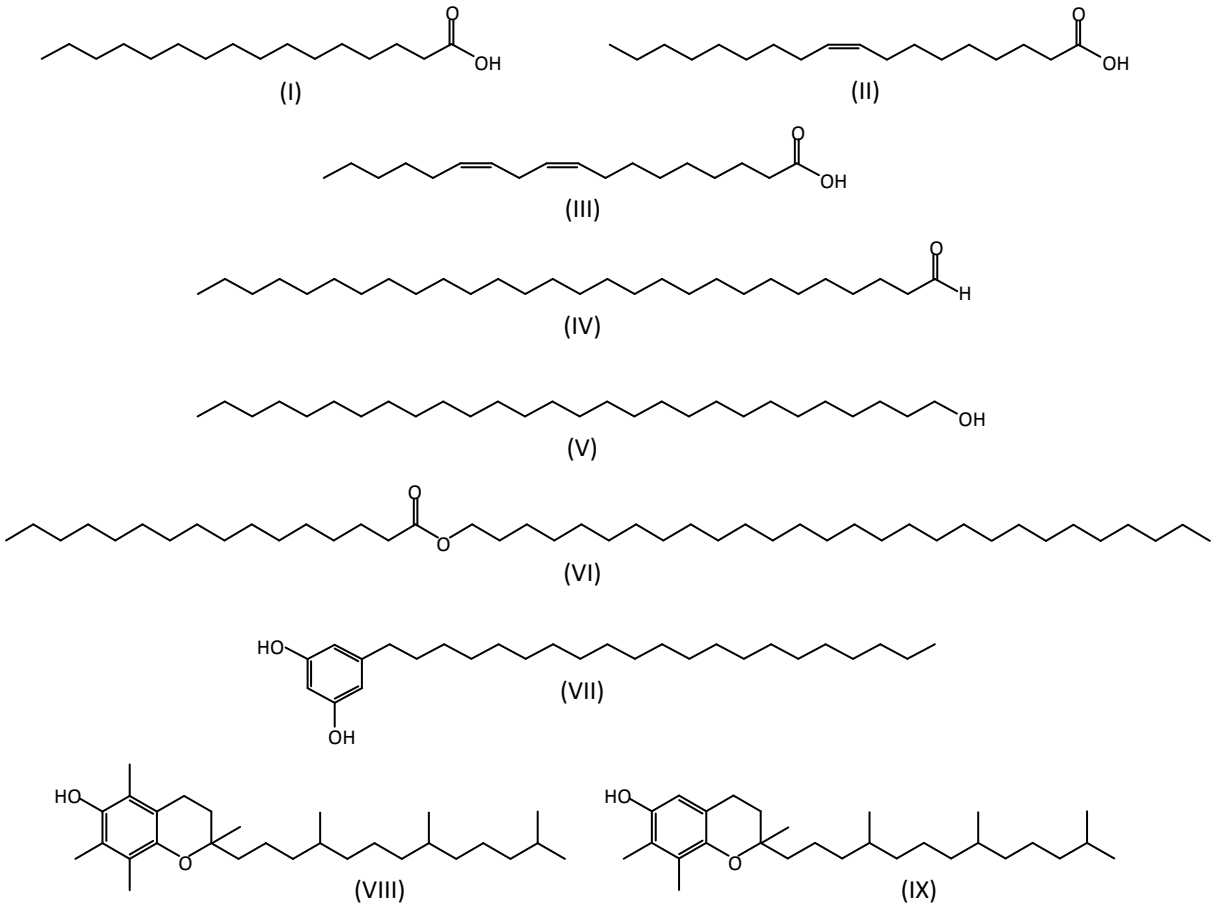

Figure 2. Structures representative of the main aliphatic lipophilic compounds identified in the extracts of sugarcane bagasse and straw and referred in the text. (**I**) hexadecanoic (palmitic) acid; (**II**) *cis*-octadec-9-enoic (oleic) acid; (**III**) *cis*,*cis*-octadeca-9,12-dienoic (linoleic) acid; (**IV**) *n*-octacosanal; (**V**) *n*-octacosanol; (**VI**) hexadecanoic acid octacosyl ester; (**VII**) 5-*n*-heneicosylresorcinol; (**VIII**) α-tocopherol; (**IX**) γ-tocopherol.

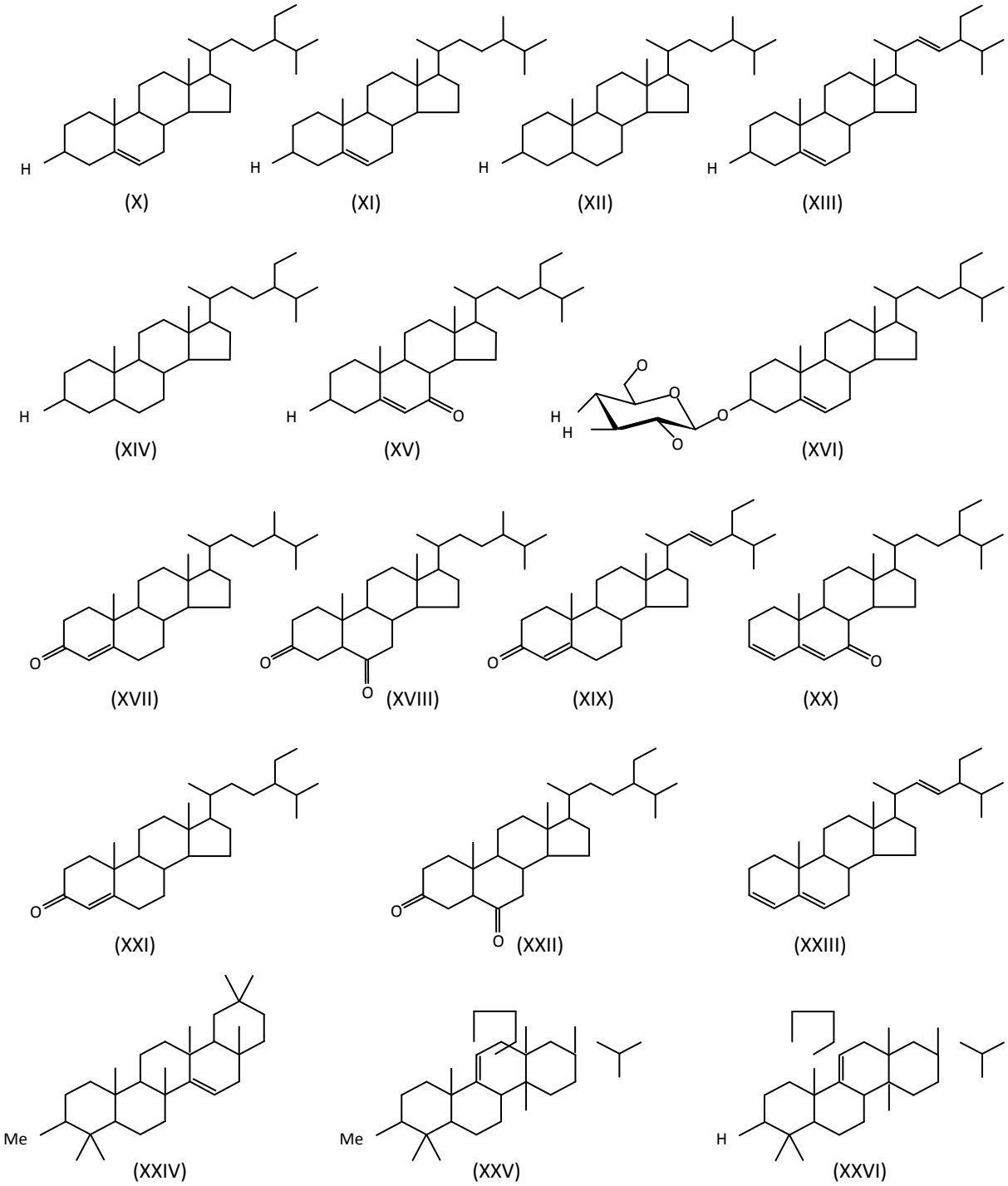

Figure 3. Structures of the main steroid and triterpenoid compounds identified in the extracts of sugarcane bagasse and straw and referred in the text. (**X**) sitosterol; (**XI**) campesterol; (**XII**) campestanol; (**XIII**) stigmasterol; (**XIV**) stigmastanol; (**XV**) 7-oxositosterol; (**XVI**) sitosteryl 3β-d-glucopyranoside; (**XVII**) ergost-4-en-3-one; (**XVIII**) ergostane-3,6-dione; (**XIX**) stigmasta-4,22-dien-3-one; (**XX**) stigmast-3,5-dien-7-one; (**XXI**) stigmast-4-en-3-one; (**XXII**) stigmastane-3,6-dione; (**XXIII**) stigmasta-3,5,22-triene; (**XXIV**) taraxerol methyl ether (crusgallin); (**XXV**) arundoin; (**XXVI**) isoarborinol.

The analyses revealed that the composition of the lipid extracts from sugarcane bagasse and straw were completely different from each other. **Figure 4** shows the percentages of the main lipid classes identified in the acetone extracts of sugarcane bagasse and straw. While the acetone extracts of sugarcane bagasse were dominated by *n*-aldehydes (ca. 48% of all identified lipids) and *n*-fatty alcohols (ca. 23%), together with significant amounts of *n*-fatty acids (10%) and steroid ketones (14%), the acetone extracts from sugarcane straw were largely dominated by *n*-fatty acids (accounting for ca. 60% of all identified compounds) with significant amounts of steroid compounds, particularly free sterols (10%) and steroid ketones (14%).

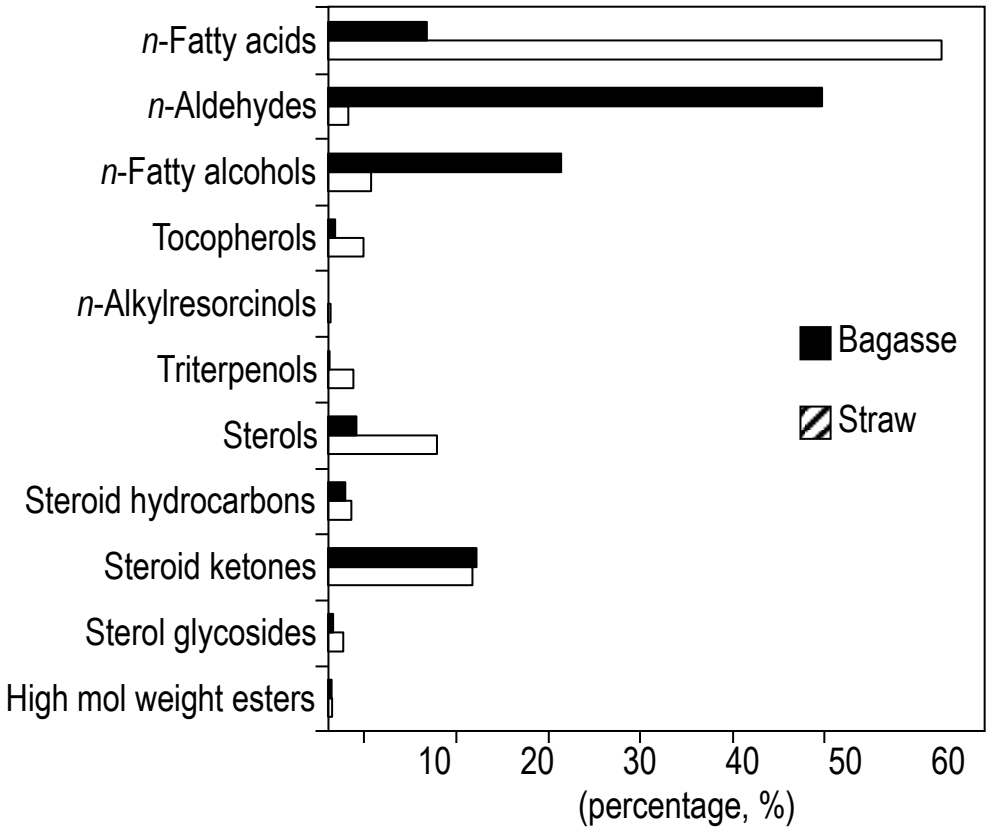

Figure 4. Percentages of the main lipid classes identified in the acetone extracts of sugarcane bagasse and straw.

3.2.1. Aliphatic series

The main aliphatic series identified among the lipophilic extractives of sugarcane bagasse and straw were series of long-chain fatty acids, alcohols and aldehydes. The distributions of these aliphatic series in the extracts of sugarcane bagasse and straw are represented in the histograms of **Figure 5**. It is clear that while the distribution of long-chain alcohols and aldehydes are similar in both sugarcane residues, the distribution of fatty acids in bagasse and straw was quite different.

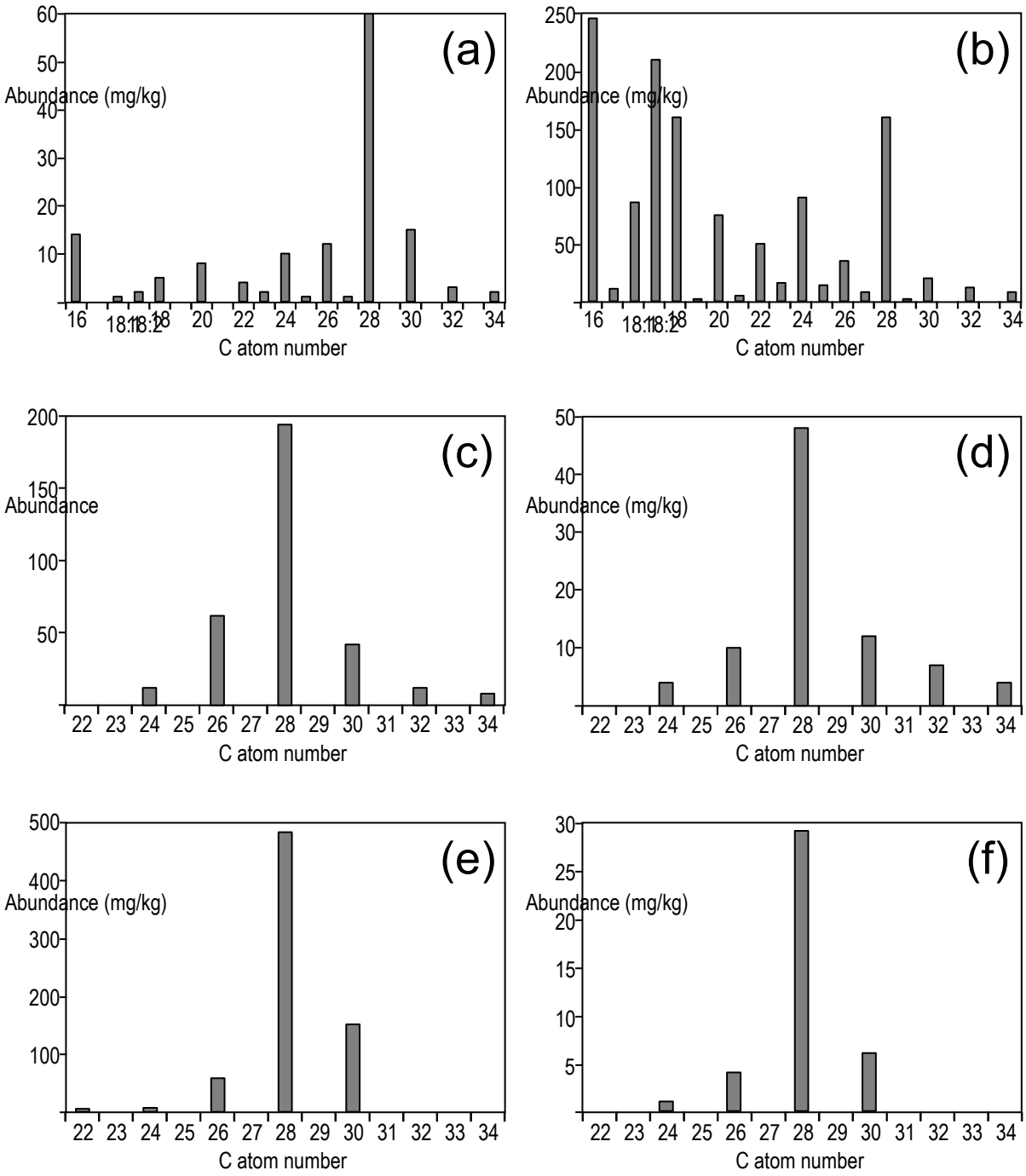

Figure 5. Distribution of the main aliphatic series identified in the extracts of sugarcane bagasse and straw: series of *n*-fatty acids in bagasse (a) and in straw (b); series of *n*-fatty alcohols in bagasse (c) and in straw (d); series of *n*-aldehydes in bagasse (e) and in straw (f). The histograms are scaled up to the abundance of the major compound in the series.

n-Fatty acids were the most abundant family of lipids identified in sugarcane straw, accounting for 1210 mg/kg, but were only present in minor amounts (140 mg/kg) in sugarcane bagasse. In both sugarcane residues, the series of free fatty acids were found in the range from hexadecanoic acid (C_{16}, palmitic acid, **I**) to tetratriacontanoic acid (C_{34}), with a strong predominance of the even carbon atom number homologues, although the distribution of the fatty acid series was different in sugarcane bagasse and straw. The series of fatty acids

in sugarcane bagasse presented a unimodal distribution with a maximum for *n*-octacosanoic acid (C_{28}) that accounts for 60 mg/kg, whereas the fatty acid series in sugarcane straw presented a bimodal distribution, with a maximum for *n*-hexadecanoic acid (246 mg/kg) and a second maximum for octacosanoic acid (160 mg/kg), and with the occurrence of important amounts of the unsaturated *cis*-octadec-9-enoic (oleic acid, **II**, 210 mg/kg) and *cis,cis*-octadeca-9,12-dienoic (linoleic acid, **III**, 86 mg/kg) acids, as well as the saturated octadecanoic acid (stearic acid, 160 mg/kg). Unsaturated fatty acids, however, were present in almost negligible amounts in sugarcane bagasse.

The series of *n*-aldehydes was identified in high amounts among the acetone extractives of sugarcane bagasse, accounting for 700 mg/kg, but was present in far lower amounts in the extracts of sugarcane straw (only 40 mg/kg). In both cases, the series were found in the range from *n*-docosanal (C_{22}) to *n*-triacontanal (C_{30}), with the exclusive occurrence of the even carbon atom numbered homologs, and with *n*-octacosanal (C_{28}, **IV**) being the most predominant compound in the series, accounting for up to 480 mg/kg in sugarcane bagasse and only 30 mg/Kg in sugarcane straw. Other papers have also identified the series of aldehydes, in the range from hexacosanal (C_{26}) to triacontanal (C_{30}), with octacosanal (C_{28}) being the most abundant, in different sugarcane cultivars (Asikin et al., 2012).

The series of fatty alcohols were predominant among the lipophilic extractives of sugarcane bagasse (330 mg/kg), but were present in minor amounts in the extracts of sugarcane straw (85 mg/Kg). In both cases, the series of fatty alcohols ranged from *n*-tetracosanol (C_{24}) to *n*-tetratriacontanol (C_{34}), with the exclusive presence of the even carbon atom number homologs and with *n*-octacosanol (C_{28}, **V**) as the most prominent compound in the series, accounting for 194 mg/kg in sugarcane bagasse and 48 mg/Kg in sugarcane straw. Other papers have also identified a series of fatty alcohols, in the range from docosanol (C_{22}) to triacontanol (C_{30}), with octacosanol (C_{28}) being the most abundant, in different sugarcane cultivars (Asikin et al., 2012) as well as in sugarcane filter muds (de Lucas et al., 2007). This group of long-chain alcohols is commonly referred as to polycosanol, a product with nutraceutical and pharmaceutical properties including reduction of platelet aggregation, reduction of low-density lipoprotein levels in blood, inhibition of cholesterol synthesis and prevention of atherosclerosis development (Asikin et al., 2012; Arruzazabala etal., 2002; Noa et al., 2005; Singh et al., 2006). Interestingly, the distribution of the long-chain alcohol series parallels that of aldehydes, as usually occurs in the plant kingdom and observed in other plants (Gutiérez et al., 2003; del Río et al., 2013) suggesting that aldehydes are intermediates in the biosynthesis of alcohols from fatty acids (Tulloch, 1976; Bianchi, 1995).

A series of high molecular weight esters also occurred in the extracts of sugarcane bagasse and straw although in minor amounts (5 mg/kg in bagasse and 8 mg/kg in straw). The high molecular weight esters were found in the range from C_{42} to C_{46} with the exclusive occurrence of the even atom carbon number homologues, and the C_{44} analogs being the most abundant ones in both sugarcane residues. A close examination of the mass spectra of the ester peaks indicates that each chromatographic peak is a mixture of several long-chain fatty acids esterified to different long-chain fatty alcohols, with a strong predominance of

octacosanol. The C_{44} ester was then predominantly constituted by hexadecanoic acid octacosanyl ester (**VI**).

3.2.2. Alkylresorcinols

A series of 5-*n*-alkylresorcinols could be identified among the lipids of sugarcane straw, although in low amounts (5 mg/Kg), but were completely absent among the lipids of sugarcane bagasse. The identification of 5-*n*-alkylresorcinols was achieved from their mass spectra that show a base peak due to the McLafferty rearrangement at *m/z* 268, characteristic of these molecules (Zarnowski et al., 2002; Gunenc et al., 2013; Prinsen et al., 2014). The 5-*n*-alkylresorcinols ranged from 5-*n*-nonadecyl (C_{19}) to 5-*n*-tricosylresorcinol (C_{23}), with the sole occurrence of the odd carbon atom-numbered homologues, and with 5-*n*-heneicosylresorcinol (C_{21}, **VII**) being the most abundant one (3 mg/kg). Alkylresorcinols have been widely reported in grasses (Zarnowski et al., 2002; del Río et al., 2013; Prinsen et al., 2014).

3.2.3. Tocopherols

Tocopherols (α-tocopherol, **VIII**, and γ-tocopherol, **IX**) were identified in significant amounts among the lipophilic extractives of sugarcane straw, accounting for 70 mg/Kg, and with a predominance of α-tocopherol. However, only α-tocopherol could be identified in the extracts of sugarcane bagasse, although in lower amounts (10 mg/Kg), whereas no traces of γ-tocopherol could be detected. The knowledge about the content and composition of tocopherols in grasses and forages is still limited (Kalaç et al., 2012) and to our knowledge this is the only report on the composition of tocopherols in sugarcane residues. Among all tocopherols, α-tocopherol has the highest biological activity and is the most abundant vitamin E form in nature.

3.2.4. Steroid and triterpenoid compounds

Different families of steroid compounds were identified among the lipids of sugarcane bagasse and straw, including free sterols, sterol glycosides, steroid ketones and steroid hydrocarbons. Free sterols were prominent in sugarcane straw (accounting for 215 mg/Kg) but occurred only in minor amounts in sugarcane bagasse (40 mg/Kg). Among the free sterols identified, sitosterol (**X**) was the most abundant in both samples, accounting for 12 mg/Kg in bagasse and 100 mg/Kg in straw. Other sterols identified were campesterol (**XI**), campestanol (**XII**), stigmasterol (**XIII**), stigmastanol (**XIV**) and 7-oxositosterol (**XV**). Sitosterol and stigmasterol have already been reported as being the major sterols in different parts of different sugarcane cultivars, including stalks and leaves (Deshmane and Dev, 1971; Feng et al., 2014a, 2014b). However, other sterols found in minor amounts among the extracts of sugarcane wax, such as fucosterol, gramisterol, stigmast-7-en-3-ol, stigmasta-4,6,22-trien-3α-ol, or cholest-8(14)-en-3β-ol acetate (Feng et al., 2014b), were not detected among the acetone extracts of sugarcane bagasse and straw. Sterols conjugated with carbohydrates forming sterol glycosides were also identified among the lipophilic extractives in sugarcane bagasse and straw by comparison with the mass spectra and relative retention times of authentic standards (Gutiérrez and del Río, 2001), although in lower amounts (8 mg/LKg in

bagasse and 30 mg/Kg in straw). Sitosteryl 3β-d-glucopyranoside (**XVI**) was the most predominant sterol glycoside in bagasse and straw (5 mg/kg n bagasse and 25 mg/Kg in straw) with lower amounts of campesteryl and stigmasteryl 3β-d-glucopyranosides, as usually observed in other grasses (Prinsen et al. 2012, 2014; del Río et al., 2013a,2013b). As far as we know, and although steroils have been widely reported among the lipophilic comounds in sugarcane plants, this is the first report of the occurrence of sterol glycosides in sugarcane plant products. Different steroid ketones, namely ergost-4-en-3-one (**XVII**), ergostane-3,6-dione (**XVIII**), stigmasta-4,22-dien-3-one (**XIX**), stigmast-3,5-dien-7-one (**XX**), stigmast-4-en-3-one (**XXI**) and stigmastane-3,6-dione (**XXII**) were also identified among the lipids of sugarcane bagasse 210 mg/Kg) and sugarcane straw (285 mg/Kg). Similar steroid ketones were identified as components of sugarcane wax (Georges et al., 2006). Steroid hydrocarbons were also found, albeit in low amounts (24 mg/kg in bagasse and 46 mg/Kg in straw), and included ergostatriene and stigmasta-3,5,22-triene (**XXIII**), the latest being the most abundant one. The important amounts of steroid compounds present in these sugarcane residues, and particularly the high content of free sterols and steroid ketones in sugarcane straw, makes this waste material an interesting and potential source for obtaining valuable phytosterols.

Finally, several triterpenoid compounds could also be identified in significant amounts in the acetone extracts from sugarcane straw (accounting for 50 mg/Kg), including taraxerol methyl ether (crusgallin, **XXIV**, 12 mg/Kg), arundoin (**XXV**, 10 mg/Kg) and isoarborinol (**XXVI**, 28 mg/Kg). However, only minor amounts of isoarborinol could be detected among the lipophilic extractives of sugarcane bagasse. All of these triterpenoids have already been reported to occur in the culms and blades of sugarcane plant (Ohmoto et al., 1970; Deshmane and Dev, 1971).

4. Conclusions

We report here for the first time a comprehensive description of the chemical composition of lipophilic phytochemicals in sugarcane bagasse and straw. The lipophilic compounds were extracted with acetone and subsequently analyzed by GC and GC/MS. The analyses indicated that the composition of lipophilic extractives is very different in sugarcane bagasse and straw. The acetone extracts from sugarcane bagasse were dominated by *n*-aldehydes (ca. 48% of all identified lipids) and *n*-fatty alcohols (ca. 23%) with lower amounts of *n*-fatty acids (10%) and steroid ketones (14%), whereas the acetone extracts from sugarcane straw were strongly dominated by *n*-fatty acids (accounting for ca. 60% of all identified compounds) with significant amounts of steroid compounds, particularly sterols (10%) and steroid ketones (14%). Significant amounts of tocopherols and triterpenols were also found, being particularly abundant among the extractives of sugarcane straw. This information opens up new possibilities for a more complete industrial utilization of these residues. Due to the large amounts produced annually, sugarcane bagasse and straw can be viewed as low-cost and promising sources of highly valuable phytochemicals that can be of use in the cosmetic, food or pharmaceutical industry.

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Structural Characterization of the Lignins from Sugarcane 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.

I. INTRODUCTION

Sugarcane 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 lignocellulosic materials composed of cellulose (ca. 41%), hemicelluloses (ca. 39%) and lignin (19-21%), with lower amounts of extractives (2.4-4.8%) and ash (ca. 5%). Therefore, they are attractive feedstocks to produce second–generation bioethanol and other value-added products in the context of the so-called lignocellulosic biorefinery. The conversion of lignocellulosic biomass to bioethanol involves saccharification of carbohydrates to fermentable reducing sugars via hydrolysis 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. 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.

II. EXPERIMENTAL

Samples

Sugarcane bagasse and straw were supplied by the University of Viçosa (MG, Brasil). Klason lignin content was estimated as the residue after sulphuric acid hydrolysis according to the TAPPI method T222 om-8. The acid-soluble 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 [1]. The final yields ranged from 5-15%. Cellulolytic lignin (CEL) preparations were isolated by enzymatically saccharifying polysaccharides as described [2]. 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).

III. 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 [1,2], 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**.

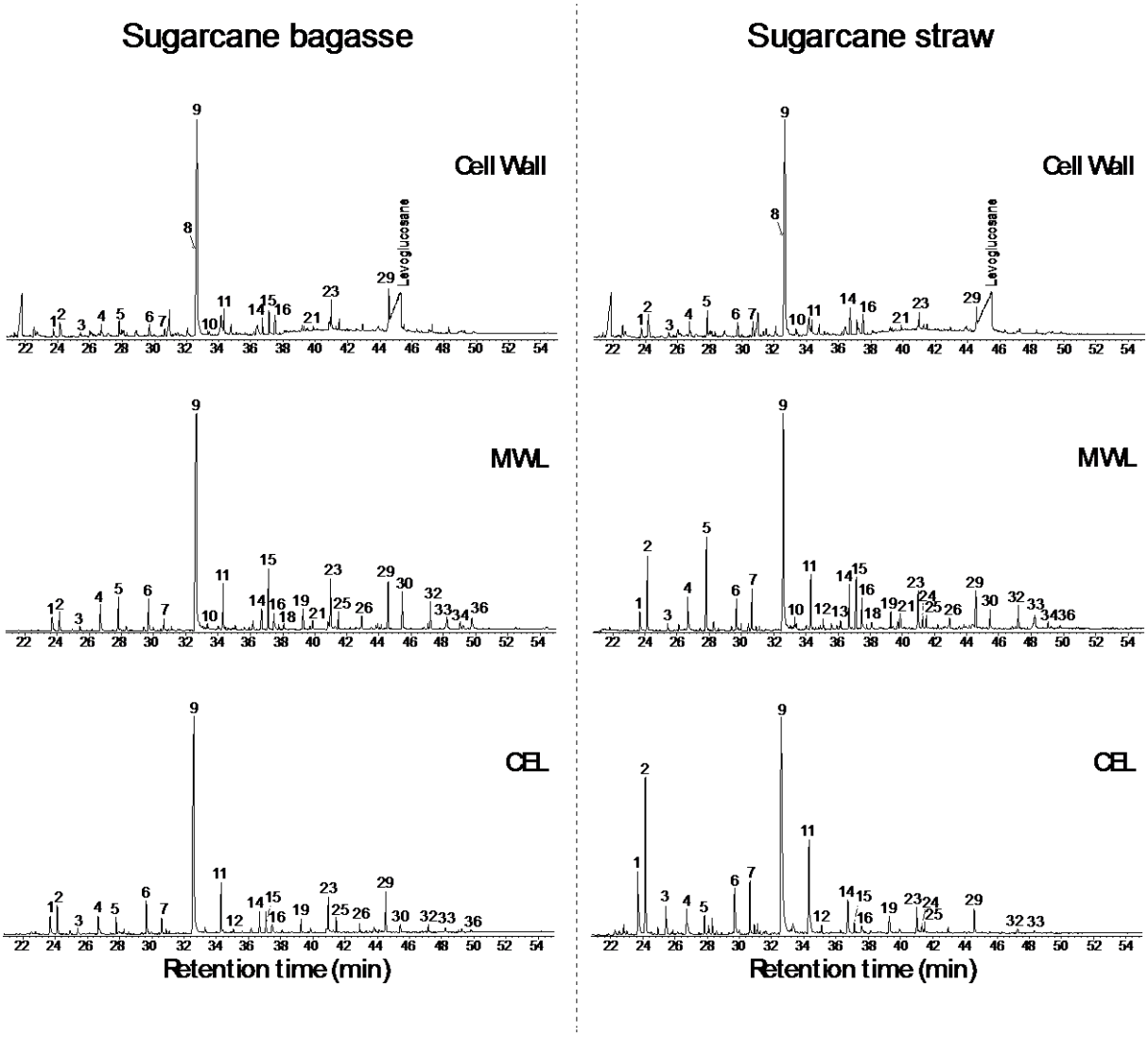

Figure 1. Py-GC/MS of sugarcane bagasse and straw (whole cell-walls), and their isolated MWL and CEL lignin preparations. The identities and relative abundances of the released compounds are listed in Table 2.

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 [3–6]. 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 [5,6].

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 spectra indicated that *p*-coumarates are acylating the γ-position of the lignin side-chains, as also occurs in other grasses [5–7]. The spectra also revealed that the flavone tricin was incorporated into the structure of these lignins, as also occurred in other grasses [5,6].

IV. 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 found to be incorporated into these lignins, as also occurs in other grasses.

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7. CONCLUSÕES GERAIS

- ✓ A combinação das técnicas analíticas Pi-CG/EM, 2D-NMR e DFRC se mostrou muito útil para resolver a estrutura das ligninas. As análises das amostras MWL proporcionaram espectros intensos e bem resolvidos de 2D-NMR fornecendo informações suficientes. A presença de carboidratos diminui a sensibilidade nas três técnicas. As análises de Pi-CG/EM oferece mais vantagens pois é uma técnica que não requer pré-tratamento da amostra e é muito mais rápida porém é um método semi-quantitativo. As análises de DFRC se aplicam preferencialmente com as amostras MWL.
- ✓ Análise das ligninas da palha e do bagaço de cana indicaram que estas ligninas são H-G-S com algumas quantidades associadas ao ácido p-coumárico (acilados na posição γ da cadeia lateral da lignina) e ácido ferúlico (associadas aos carboidratos). As ligninas do bagaço estudado apresentam uma maior proporção de unidades S (H:G:S razão molar de 2:38:60) enquanto as ligninas da palha são mais enriquecidas em unidades G-lignina (4:68:28).
- ✓ As principais ligações entre as unidades presentes na lignina do bagaço são do tipo β–*O*–4´ subestruturas aril-alquil-éter (cerca de 83% das ligações medidas no RMN) seguidas por β-5´ (fenilcumarano, 6%) e outras subestruturas condensadas. A lignina a partir da palha tem menor quantidade de ligações β–*O*–4′ aril-alquil-éter (75%), mas os níveis relativamente mais altos de fenilcumarano (β-5´, 15%) e dibenzodioxocina (5-5/4-O β, 3%), correspondendo com uma lignina enriquecida de unidades G.
- ✓ A maior quantidade de unidades de lignina siringila e ligações β–*O*–4′ faz com que o bagaço da cana apresente uma estrutura mais reativa, sendo esta mais fácil de ser dissolvida durante o processo de polpação Kraft ou um processo de deslignificação para produção de biocombustível, quando comparado com a palha (com maior quantidade de estruturas condensadas).
- ✓ Os dois resíduos da indústria de açúcar e álcool contêm polissacarídeos que podem ser sacarificados e fermentados em biocombustíveis, ligninas que podem ser valorizadas e contém níveis significativos de p-coumarato, ácido ferúlico e tricina que poderiam ser extraídos e vendidos como produtos químicos.
- ✓ As análises revelaram que a composição de extraíveis lipofílicos no bagaço e palha são muito diferente. No conteúdo de extraíveis lipofílicos do bagaço de cana predominou *n*-aldeídos (48%) e *n*-álcoois graxos (23%) com menor quantidade de *n*-ácidos graxos (10%) e cetonas esteroides (14 %), já na palha os extratos foram fortemente dominados por *n*-ácidos graxos (60%) com quantidades significativas de compostos esteroides, particularmente esteróis (10%) e esteroides cetonas (14%). Quantidades significantes de tocoferóis e triterpenos foram encontradas, sendo particularmente abundante entre os extrativos da palha da cana.
- ✓ A informação divulgada aqui abre novas oportunidades para a utilização completa destes resíduos da indústria a partir de uma perspectiva de biorrefinaria. Devido às grandes quantidades de bagaço de cana e palha produzidos anualmente, eles podem ser vistos como biomassa de baixo custo e fontes altamente promissoras de

fitoquímicos valiosos que podem ser de uso na indústria de cosméticos, alimentos ou farmacêutica.