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Organosolv Pretreatment of Cocoa Pod Husks: Isolation, Analysis and Use of Lignin from an Abundant Waste Product

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Cocoa pod husk, biorefinery, waste product, organosolv, lignin, flame-retardant, organophosphorus, acetylation

Cocoa pod husks (CPHs) represent an under-utilised component of the chocolate manufacturing process. Whilst industry’s current focus is understandably on the cocoa beans, the husks make up to 75 weight percent of the fruit. Previous studies have been dominated by the carbohydrate polymers present in CPHs, but this work highlights the presence of the biopolymer lignin in this biomass. An optimised organosolv lignin isolation protocol was developed, delivering significant practical improvements. This new protocol may also prove useful for agricultural waste-derived biomasses in general. NMR analysis of the high quality lignin led to an improved structural understanding, with evidence provided to support deacetylation of the lignin occurring during the optimised pretreatment. Chemical transformation, using a tosylation, azidation, copper-catalysed click protocol, delivered a modified lignin oligomer with an organophosphorous motif attached. Thermogravimetric analysis was used to demonstrate the oligomers potential as a flame-retardant. Preliminary analysis of the other product streams isolated from the CPHs was also carried out.

Introduction

Biorefining, the process by which renewable biomass feedstocks are converted into marketable products in an integrated manner, remains a challenge.1 In several biorefinery designs, a pretreatment process is used to simplify the starting biomass by addressing both its recalcitrant nature and inherent complexity (Figure 1).2-8 Whilst a wide range of approaches to biomass pretreatment exist,9-14 the use of mild organosolv pretreatments15-20 often delivers high quality intact lignin as well as cellulose, hemicellulose-derived and other fractions. Consideration of possible uses for an intact organosolv lignin illustrates the flexibility inherent in using a pretreatment strategy. Potential lignin applications range from alternative lignin depolymerisation protocols that give different aromatic monomers,21,22 to a number of approaches to building on the existing lignin template.22-25 For example, studies have described the preparation of flame retardant materials from lignin.26,27 These recent reports have inspired us to describe our complementary studies on the incorporation of 9,10-dihydro-9-oxa-10-phosphaphenanthrene-10-oxide (DOPO) **1** into lignin. DOPO **1** is an organophosphorus molecule known to be important in fire management strategies28 (Figure 1 and Scheme 1).

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**Figure 1** Summary of the fractions obtained during cocoa pod husk processing using the optimised butanosolv pretreatment developed in this report. Potential applications of the isolated fractions are proposed (*e.g.* the conversion of the lignin to a potential flame retardant material).

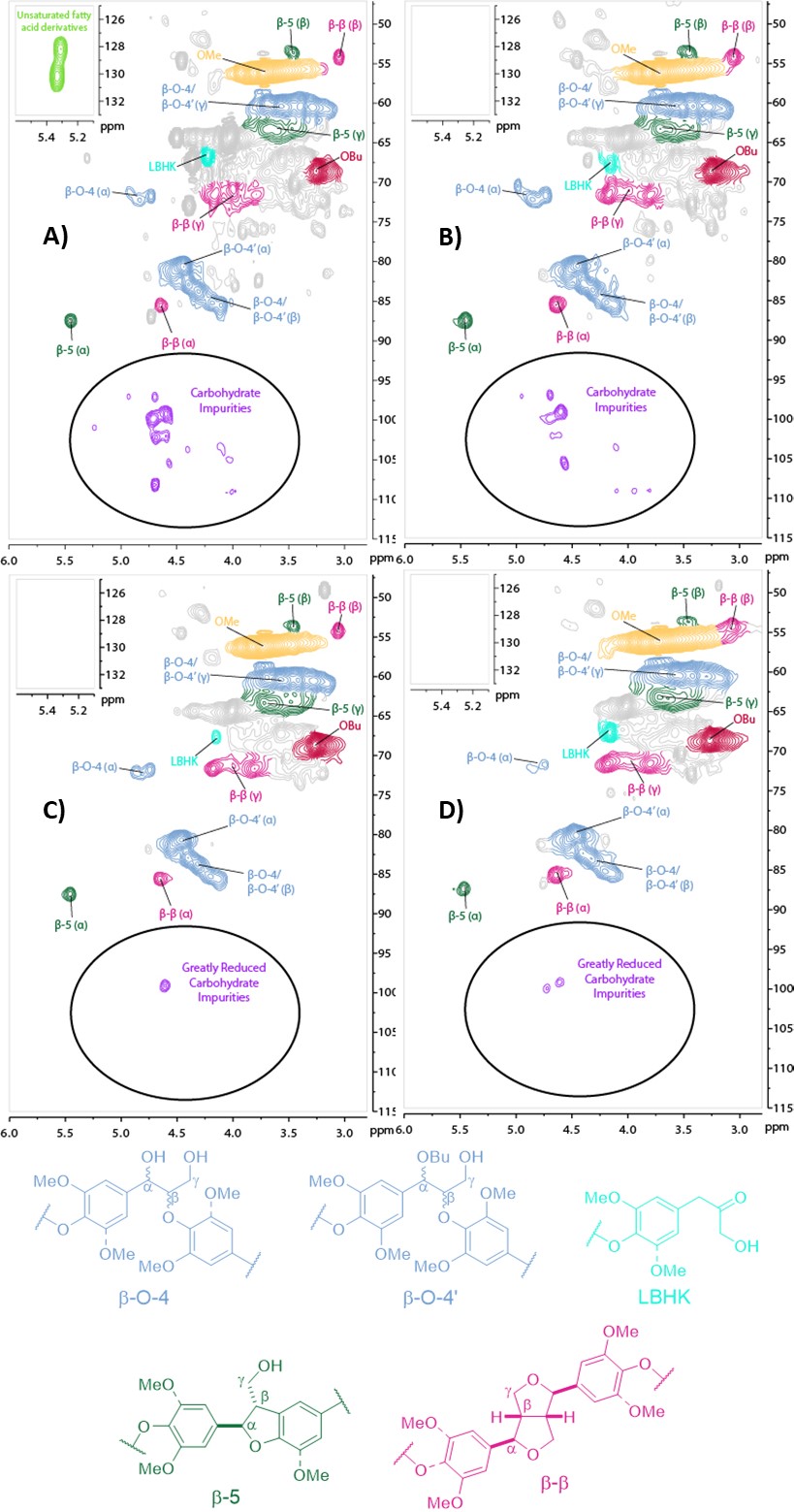
Another part of the biorefinery challenge is its application to less mainstream biomass sources. Whilst many studies use soft or hardwoods, researchers continue to explore less common biomass. Recent reports have described, for example, the processing of coffee husks,29 rice husks,30 and pomegranate peel31 but the list of possible starting materials continues to increase.7 Here the focus is on an understudied waste product from the chocolate industry – the cocoa pod husk (CPH). The total global harvest of cocoa beans for the 2021/22 growing season was 4.8 million tonnes with this estimated to increase slightly for the 2022/23 growing season.32 As the CPH represents the bulk (70-75%) of the fruit produced by the *Theobroma cacao* tree, this means that more than 20 million tonnes of cocoa pod husks are produced p.a. worldwide. The husk is usually left on the farm to biodegrade. However, the value of this practice is contested as it may have an overall negative impact by allowing proliferation of the “*cacao* disease trilogy”.33,34 Some cocoa farmers are considering preparing and selling CPH for alternative uses if collection is feasible and the price is sufficient.35 In terms of the current technology associated with CPH biorefining, reports have focused on the analysis and application of carbohydrate components36,37, particularly studies on CPH pectin.38-42 Here, whilst a number of fractions from the biomass are considered, we focus instead on the CPH lignin component. Limited precedent for the isolation of lignin from CPH is available which is surprising given that current estimates of lignin content place it at around 20 percent of the weight of the husk.43-46

Following a preliminary comparison of potential methods for the efficient pretreatment of CPH (Figures S1-S3), an optimised protocol for the butanosolv pretreatment of CPH was developed. This protocol delivered a high quality lignin that was characterised in detail providing novel insights into its structure and purity. In addition, the isolated lignin was used to prepare a potential flame-retardant material. Preliminary characterisation of the six other product streams (Figure 1) is also included. Whilst it remains unclear what the optimal way to process CPHs is, our approach delivered a high quality lignin and a number of other defined product streams for further study.

Results and Discussion

Optimised Isolation of Butanosolv Lignin from CPH

A mild butanosolv pretreatment for use with soft or hardwoods typically involves the heating of a suspension of the biomass in 95% butanol/5% aqueous hydrochloric acid at reflux for six hours. This literature protocol47 was applied to CPH biomass to obtain a butanosolv CPH lignin. A sample of this lignin was analysed by HSQC NMR48,49 prior to the final purification step to assess impurity levels. Signals corresponding to unsaturated fatty acid derivatives,50 likely based on linoleic acid,51 and aryl-ring containing small molecules were the major contaminants (*c.f.* Figures 2A/2B and S4A/S4B for analysis of lignin prior to and post-purification). Washing the starting biomass with ethanol before butanosolv pretreatment reduced the number of impurities and resulted in a lignin with improved purity (*c.f.* Figures 2B/S4B and S4E/F). The ethanol pre-wash also resolved practical challenges associated with precipitation of the lignin and this step was therefore incorporated into an “optimised pretreatment” method.



**Figure 2** HSQC NMR (700 MHz, DMSO-d6) analysis of Cocoa Pod Husk (CPH) lignin obtained by: butanosolv pretreatment using a literature method47 A) before the final purification step; B) after purification by reprecipitation using organic solvents; C) after purification using an alternative caustic soda purification method52. D) Analysis of the CPH lignin obtained using the optimised butanosolv pretreatment developed in this work for comparison. The relevant structures that correspond to interunit linkages are shown. The aromatic regions of the HSQC NMR spectra are shown in Figure S4.

Carbohydrates are known impurities in butanosolv lignins and recent studies52 have shown that caustic soda treatment of walnut shell butanosolv lignin decreased carbohydrate contamination. A modified caustic soda treatment was applied to the lignin to assess its impact on lignin purity. The lignin was recovered in good yield and had a lower carbohydrate content as expected52 (Figure 2C/S4C) whilst maintaining high levels of β-O-4 content. No de-butoxylation of the lignin was observed, in contrast to the previous report.52 The addition of this step did provide a higher purity lignin, however, alternatives were also considered.

The efficient aqueous extraction of one of the major carbohydrates in CPH, pectin, has been reported.38-44 In our study, hot water extraction of CPH prior to butanosolv pretreatment was found to decrease the carbohydrate content in the isolated lignin (*c.f.* Figures 2B and S4G). Pectin and a second fraction that contained fermentable sugars (referred to here as PESF) were also obtained. By combining (i) the ethanol pre-wash with (ii) the hot aqueous extraction and (iii) butanosolv pretreatment, a CPH lignin was obtained in 3.7 wt% that retained a high β-O-4 content and had low carbohydrate and fatty acid contaminants (Figure 2D, Table S1). In addition, all the practical challenges encountered on applying the literature pretreatment47 to CPH were addressed. For example, the lignin from the optimised pretreatment was a fine powder easily isolated by filtration (Figure S5). DOSY NMR analysis demonstrated that the diffusivity and estimated MW of the lignin obtained did not vary as the changes were made53 (Figure S6, Tables S2-S3). The MW values were within error (*e.g.* MW = 3700±900 Da for the lignin from the optimised pretreatment). The optimised pretreatment was suitable for lignin generation from CPH biomass and seems likely to be applicable to a range of non-woody biomasses.

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**Figure 3** A) HSQC NMR (700 MHz, DMSO-d6) analysis of acetylated β-O-4 model **3a**. Signal assignments are numbered and colour-coded. Relevant HMBC correlations are indicated by arrows; B) expansion of (i) the alkyl region shown in A (Insert 1), and corresponding signals in the HMBC NMR (middle) and (ii) the modified γ-primary alcohol region (A, Insert 2); C) expansion of a region of the HSQC NMR (700 MHz, DMSO-d6) analysis of CPH lignin isolated using the literature pretreatment47 showing (i) alkyl region (top) with signals corresponding to the methyl group of acetylated β-O-4 linkages indicated (yellow), (ii) the corresponding signals in band selective HMBC NMR analysis (middle) and (iii) expansion of modified γ-primary alcohol region (bottom) with signals unambiguously assigned as corresponding to acetylated β-O-4 linkages indicated (light blue); D) expansion of HSQC NMR (700 MHz, DMSO-d6) analysis of CPH lignin from the optimised pretreatment showing (i) alkyl region (top) with no signals corresponding to acetylated β-O-4 linkages, (ii) the corresponding absence of signals in the HMBC NMR analysis (middle) and (iii) expansion of modified γ-primary alcohol region (bottom) with no signals corresponding unambiguously to acetylated β-O-4 linkages. Lignin from both the literature47 and optimised protocols show no signals corresponding to acetylation at terminal phenolic oxygen positions. Regions in light grey are unassigned or cannot be assigned to a single structural feature in the lignin (see Figures S7-S9 for a more detailed discussion). Signals in the region 1H 4.55-3.85/13C 65.0-62.5 are known to correspond to cinnamate, *p*-coumarate54,55 and *p*-hydroxybenzoate esters.56,57

Further analysis of the lignin

In addition to increasing the purity of the lignin, use of the optimised pretreatment also altered the CPH lignin’s structure. Comparison of HSQC NMR spectra found that signals at 1H: 4.34/13C: 63.7 ppm and 1H: 1.96/13C: 20.7 ppm were present only in the lignin obtained from the literature pretreatment47 (Figure 3C/D). This observation was rationalised based on the presence or absence of an acetylated β-O-4 unit (acetylated on the primary γ-hydroxy group, Generalised structure **2**, Scheme 1) in the lignin. Novel model compounds **3a**-**c** were prepared (via **4a**-**c** and **5a**-**c**, Scheme 1) and comparison of their NMR spectra with those of the lignin confirmed that **2** was only present in the lignin obtained using the literature pretreatment47 and NOT the optimised pretreatment (Figures 3A/3B for **3a** and Figures S7-S9). This fortuitous difference in lignin structure helps the lignin modification studies discussed below, as more β-O-4 γ-hydroxyls are available when the optimised pretreatment is used. The controlled removal of acetyl and other ester groups from the β-O-4 γ-hydroxy position in lignins has been explored previously (*e.g.* triethylamine-catalysed de-esterification on Birch bark biomass58, and de-acetylation of Poplar biomass using deep eutectic solvents59), although not with CPH lignin.



**Scheme 1**: Synthesis of β-O-4 model compounds. Reagents and conditions: a) 9:1 BuOH/4M HCl, reflux, 20 min; b) 10.0 eq. Ac2O, Pyr, rt, 8 h; c) 3.0 eq. TsCl, 3.0 eq. NEt3, 0.5 eq. DMAP, DCM, rt, 18 h; d) 5.0 eq. NaN3, DMF, 50 °C, 18 h; e) 1.1 eq. 7, 1.1 eq. sodium ascorbate, 0.3 eq. CuSO4.5H2O, MeOH, rt, 12 h f) i) 1.1 eq. NCS, Toluene, rt, 18 h; ii) 1.1 eq. NEt3, 1.1 eq. propargyl alcohol, DCM, rt, 18 h.

Modification of CPH lignin

The use of lignin as a template for novel oligomers/materials synthesis is increasingly reported,for example the use of lignin as a scaffold in a variety of resins.60-63 In this case, CPH Lignin from the optimised pretreatment was modified (Scheme 2) to give first a tosylated lignin (Lignin-Ts, *c.f.* Figures 4A and 4B and S10A and S4D) and then an azidated lignin (Lignin-N3, Figure 4D and S10B). The reactions were monitored using HSQC NMR including comparison with the spectra of the synthesised model compounds. For example, the conversion of the lignin to Lignin-Ts was confirmed using model compounds **8a** and **8b** (Figure 4B and Scheme 1 for structures). Lignin-N3 (*c.f.* **9a** and **9b**, Figure 4D) was then reacted in a Cu-Catalysed Alkyne-Azide Cycloaddition (CuAAC) reaction with the previously used 1-nitro-4-(prop-2-yn-1-yloxy)benzene (Figures S10D and S10E)60 confirming that CPH lignin was a viable substrate.



**Scheme 2**: Synthetic procedure used to convert CPH lignin to DOPO modified lignin. Reagents and conditions: a) 2.25 wt eq. TsCl, Pyr, rt, 18 h; b) 5 wt eq. NaN3, DMF, 50 °C, 18 h; c) 0.5 wt eq. **10**, 0.5 wt eq. sodium ascorbate, 0.03 wt eq. CuSO4.5H2O, 5:1 DMF/H2O, rt, 18 h.

Lignin-N3 was then reacted with DOPO-derivative **10** (Schemes 1 and 2). DOPO **1** and its derivatives are incorporated into polymers to introduce flame-retardant properties via char formation, with their use offering a greener alternative to halogenated flame-retardants.64 The preparation of Lignin-DOPO was confirmed by IR (Figure 4C), HSQC NMR (Figure 4E and S10C, *c.f.* **11a** and **11b**) and 31P NMR analysis (Figure 4F). The broad signal in the 31P NMR spectrum (10.4-9.8 ppm) was consistent with the attachment of the DOPO unit onto the lignin with additional broadness resulting from modification of G and S-containing β-O-4 units.

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**Figure 4** Analysis of modified Lignins. HSQC NMR (700 MHz, DMSO-d6) analysis of linkage region of: A) starting CPH lignin with signals corresponding to the CH2 protons of the unmodified β-O-4 γ-alcohols highlighted (blue), overlaid with NMR analysis of model compounds **5a** (black) and **5b** (red). See Schemes 1 and 2 for all chemical structures; B) Lignin-Ts with signals corresponding to the CH2 protons of the tosyl modified β-O-4 γ-alcohols highlighted (pink), overlaid with model compounds **8a** (black) and **8b** (red); C) FTIR spectra of CPH butanosolv lignin (blue), Lignin-Ts (pink), Lignin-N3 (green) and Lignin-DOPO (orange). Highlighted region shows appearance of azide stretching frequency at 2100 cm-1 in Lignin-N3 and disappearance in Lignin-DOPO indicating CuAAC click reaction was successful; D) Lignin-N3 with signals corresponding to the CH2 protons of the azide modified β-O-4 γ-alcohols highlighted (green), overlaid with model compounds **9a** (black) and **9b** (red); E) Lignin-DOPO with signals corresponding to CH2 protons of the DOPO-triazole modified β-O-4 γ-alcohols highlighted (orange), overlaid with model compounds **11a** (black) and **11b** (red). Signal at 1H 4.40/13C 64.4 ppm in **11a** corresponds to the O-CH2-triazole signal in a minor stereoisomer not observed in the lignin; F) 31P NMR spectra (202 MHz, DMSO-d6) of **10**, **11a**-**c** and Lignin-DOPO. The pale pink signal in B, D and E corresponds to the additional methoxy group at the 4-position of model compounds that is not present in the lignins. The aromatic regions of CPH lignin, Lignin-Ts, Lignin-N3, and Lignin-DOPO are shown in Figure S10.

Thermogravimetric analysis (TGA) was carried out to assess the flame-retardant properties of CPH Lignin-DOPO. The TGA curves of lignin and Lignin-DOPO (Figure 5) showed similar expected mass losses (41.2 wt% and 41.1 wt%) during stage I pyrolysis between 200-400°C where alkyl C-O bonds in interunit ether linkages are cleaved.65,66 During stage II pyrolysis above 400°C, O-Me bonds are first broken giving increased phenolic content followed by cleavage and rearrangement of aromatic C-O and C-C bonds, ultimately leading to gasification.65,66 For lignin, the stage II pyrolysis led to a large mass loss (40.9 wt%), giving a total loss of 82.1 wt% at 950°C. This sample underwent further mass loss as it cooled leading to effectively no residual material (Figure 5A, insert). Lignin-DOPO behaved differently in the stage II pyrolysis with a smaller mass loss (25.6 wt%) observed. This gave a lower overall mass loss of 66.7 wt% at 950°C.

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**Figure 5** TGA (black) and DTG (blue, dashed) curves (N2, 10 °C/min) obtained A) CPH lignin and B) Lignin-DOPO. The percentage weight loss of each sample during stage I and stage II pyrolysis is given by the red lines.65,66 The residues recovered after TGA are shown (insert); CPH lignin gave little to no residue whilst Lignin-DOPO gave a pellet of char.

The temperature at which the greatest rate of mass loss occurred, Tmax, was obtained from DTG curves66 and was comparable for the lignin and Lignin-DOPO (353°C and 337°C respectively), occurring during stage I pyrolysis. Attachment of the DOPO-derivative **10** presumably promoted char formation on the surface of the sample during stage II pyrolysis to inhibit further decomposition. A char was recovered following TGA of Lignin-DOPO (Figure 5B, insert) supportive of potential flame-retardant properties that are not inherent to the lignin itself. Comparison with the TGA and DTG curves of a control lignin, prepared by reaction with an alternative alkyne that did not contain a phosphorus-based unit (Figure S11), highlighted the importance of the organophosphorus DOPO motif in the flame-retardant properties. Whilst these preliminary results were encouraging, future work will focus on scaled-up synthesis and testing of CPH Lignin-DOPO.

Analysis of the Additional Fractions obtained using the Optimised Pretreatment

In the final stage of this study, more detailed analysis of several of the other fractions generated in the optimised pretreatment of CPH was carried out (Figures 6A and S12). In brief, the powder X-ray diffraction pattern of the CPH cellulose pulp was compared to that of a commercial sample of cellulose I (Figure 6B).67 This technique, FTIR studies (Figure 6C) and acetyl bromide derivatisation (which solubilised the pulp to enable assessment of potential lignin content by solution-state 2D HSQC NMR68, Figure S13), indicated that the cellulose formed was lignin-free and of type I.

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**Figure 6** A) Summary of fractions produced from the optimised pretreatment and the observed mass balance. PESF = Pectin Ethanol Soluble Fraction; B) PXRD pattern of the cellulose pulp (black) and a commercial sample of cellulose I (red); C) FTIR spectra of the cellulose pulp (black) and a commercial sample of cellulose I (red); D) solid-state 13C CP/MAS NMR spectrum of pectin obtained from the optimised pretreatment; E) FTIR spectra of pectin (black) and a commercial sample of polygalacturonic acid (red) used as a model for a homogalactan pectin.

The monosaccharide composition of the pectin was determined (HPAEC analysis, Figure S14 and Table S4) with galacturonic acid being the major component, as expected.44 FTIR analysis of the pectin showed a signal at 1608 cm-1 (Figure 6E, black) assigned to carboxylate (COO-) groups.69 This requires the presence of metal counter ions (possibly K+ given the large amount present in CPH,35 Table S5). In contrast, carboxylic acid (COOH) functional groups were present in a commercial sample of a model pectin, polygalacturonic acid (Figure 6E, red, peak at 1732 cm-1). The pectin was also analysed using solid-state 13C NMR allowing calculation of the degree of methylation (DM) using the method of Zhu *et. al.*70 (Figure 6D). The pectin that was extracted here using hot water was a low-methoxy pectin in contrast to previous reports that used different extraction conditions.43 The signal at 21.2 ppm (Figure 6D) was consistent with pectin acetylation70 (Figures S15-S16 and Table S6). The second fraction obtained during pectin removal (PESF) was surprisingly abundant (17.1 wt%, Figure 6A). Its monosaccharide composition was determined (Figure S14 and Table S4) with glucose being most abundant. NMR analysis indicated that PESF was a complex mixture of oligo- and polysaccharides, with both α-glucose and α-mannose units present (Figure S17).

Despite the ethanol pre-wash, fatty acid derivatives were also found in the filtrate obtained during the final lignin purification step (Figure S18). However, the major component (73%) of the filtrate was a sample of lower MW lignin (Table S3) which was isolated by column chromatography If taken into account, this extra lignin increased the total lignin yield from 3.7 wt% to 5.4 wt%. Recovery of two batches of lignin with very different MWs showed that the CPH lignin was fractionated in the final purification step, and could likely be fractionated further.71,72

Conclusions

Many challenges remain as biorefinery development continues. Here a high quality lignin was isolated from cocoa pod husks (CPHs), a waste product connected with chocolate production. No previous studies have reported details of this lignin’s structure and reactivity. Reasonable yields of lignin were obtained, although significantly less lignin was isolated than expected based on literature reports.35,46 This discrepancy may reflect an over-estimation of the isolable lignin content in CPH. The isolated lignin was in a deacetylated form and this facilitated its modification at the γ-position of the β-O-4 unit. Use of novel model compounds confirmed the structure of the modified lignin and thermogravimetric analysis highlighted interesting potential flame-retardant properties. The optimised pretreatment also led to the formation of 6 other potential product streams, several of which were studied. Whilst detailed LCA, techno-economic and agronomic analysis of the reported process is outside the scope of this report, a recently reported study discusses several key aspects of developing a conceptual novel value chain from CPH (which could lead to a number of products, such as modified lignin or lignin-derived aromatics, ethanol, food ingredients, etc).33 This report assessed (i) the economic viability of CPH valorisation from a farmer’s perspective and (ii) the consequences on soil quality of diverting CPH from its role as a natural fertilizer through an agronomic trial.

Based on preliminary work reported here, future application of the high quality CPH lignin will focus on the production of novel flame-retardant materials (lignin-DOPO). This application for lignin is of current interest and this work provides complementary methodology to recent alternative approaches.26,27

Experimental Section

Materials

The cocoa pod husk biomass material was provided Mars Wrigley Confectionery from the Mars Cocoa Research Station in Indonesia. Cocoa pod husk (CPH) biomass was received as three different clone batches that had been freeze dried and were frozen upon arrival. The individual clone batches were defrosted just prior to milling and were milled using a Retsch SM 300 SM mill equipped with a 1 mm screen. The batches were combined and thoroughly mixed to give a homogenous CPH biomass. The CPH was stored frozen and defrosted just prior to use in pretreatment protocols. Commercially available compounds were purchased and used as received unless otherwise stated in the SI.

Methods

Full description of the pretreatment methods are given in the General Methods section of the SI. **Optimised butanosolv pretreatment for CPH:** CPH biomass was suspended in ethanol (10 mL/g) and stirred at room temperature for 18 h. The suspension was then filtered and the recovered CPH pulp was suspended in fresh ethanol (10 mL/g) and stirred for further 4 hours. The suspension was filtered and the CPH pulp dried *in vacuo* at 60 °C for 24 hours. The dried CPH pulp was then suspended in water (25 mL/g) and heated at reflux for 4 hours. The suspension was cooled to room temperature, centrifuged at 5500 rpm at 4 °C for 1 hour and strained through cheesecloth, washed with fresh water (3 x 10 mL/g) and squeezed until dry. The CPH pulp was dried *in vacuo* at 60 °C for 24 hours then butanosolv pretreatment was carried out according to a literature procedure.47 Tosylation and azidation reactions were carried out according to a literature procedure.47 For the CuAAC click reactions, based on a literature procedure,47 azidated butanosolv lignin (1 wt eq.), novel DOPO alkyne derivative **11** (0.5 wt eq.), sodium ascorbate (0.5 wt eq.) and CuSO4.5H2O (0.03 wt eq.) were stirred in DMF/water (5:1, 10 mL/g of lignin) at room temperature for 24 hours. The solution was added dropwise to 0.1 M HCl (10 v/v eq.) and the resulting precipitate isolated by filtration, washed with water (30 mL/g) and dried under vacuum at 60 °C for 24 hours. The crude lignin was purified by column chromatography on silica gel (30 g/g) eluting with DCM/hexane (0-100%), MeOH/DCM (0-10%) then 100% acetone. See SI for full details for the synthesis and analytical characterisation of model compounds.

ASSOCIATED CONTENT

Supplementary figures mentioned throughout the text, including additional NMR spectra, synthetic procedures for model compounds, characterization of novel compounds and associated spectra, and full lignin HSQC NMR spectra (PDF)

Crystallographic data of **10** (CIF)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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EaSI-CAT? Anyone else funding? James PhD?

NOTES

This manuscript is dedicated to the late Prof. Simon McQueen-Mason. The authors declare no conflict of interest.

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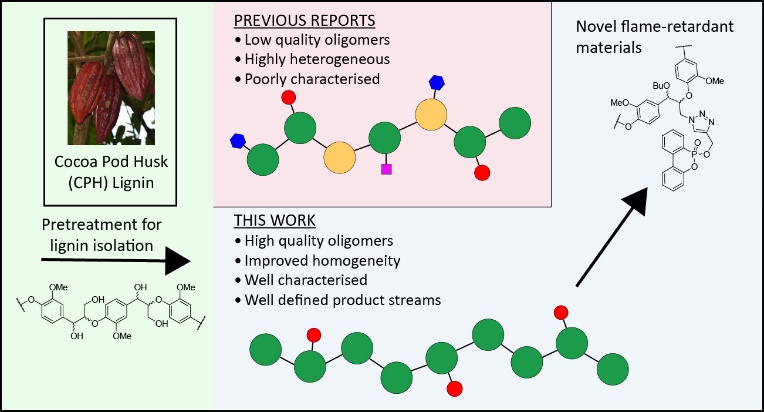
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ABBREVIATIONS

DM, degree of methylation; FTIR, Fourier transform infrared; HMBC, heteronuclear multiple bond correlation spectroscopy; HSQC, heteronuclear single quantum coherence spectroscopy; MW, molecular weight; NMR, nuclear magnetic resonance; PESF, pectin ethanol soluble sugar fraction; PXRD, powder X-ray diffraction.

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Lignin derived from cocoa pod husk waste from chocolate production was isolated, characterised and modified to produce novel flame-retardant materials.