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Lignin Recovery from Cocoa Bean Shell Using Microwave-Assisted Extraction and Deep Eutectic Solvents

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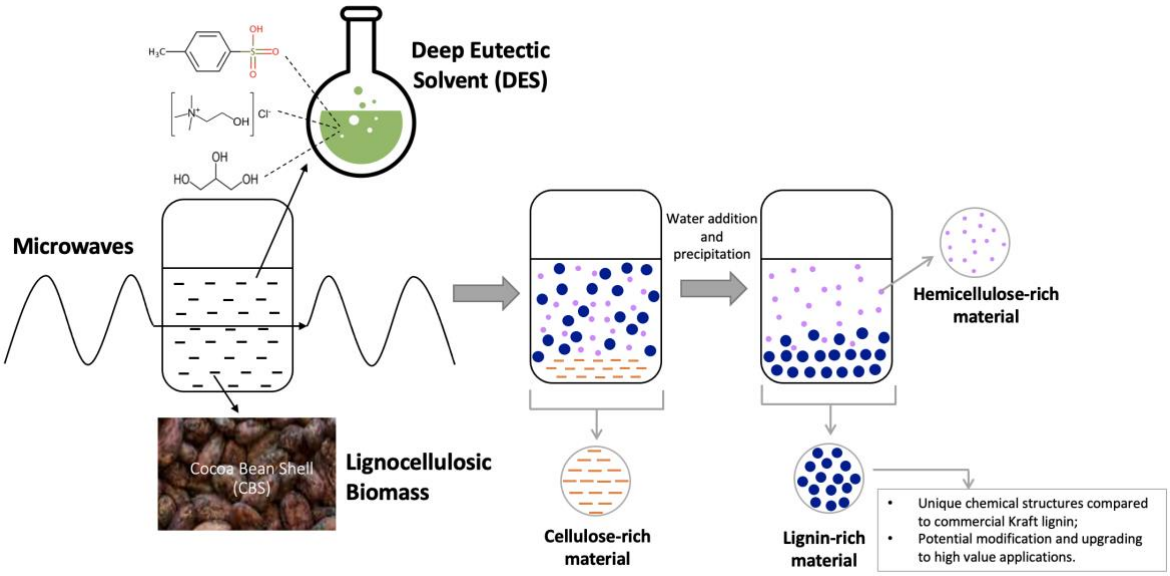
Abstract:

Lignin is the second most abundant natural polymer after cellulose, and valorisation of lignin-rich streams has attracted increasing attention recently. This paper presents a novel and sustainable method to recover lignin from Cocoa Bean Shells (CBS) using Deep Eutectic Solvents (DES) and microwaves. A DES containing p-toluenesulfonic acid, choline chloride and glycerol (2:1:1 molar ratio) was selected based on its dielectric properties. Under 200 W microwave power, the optimum yield of 95.5% lignin was achieved at 130 ºC and 30 mins. DES-extracted lignin exhibited unique structural characteristics including larger particle sizes (242.5 µm D50 size), structural diversity (410.4 µm D90-D10 size) and H/G sub-unit ratio (71.9%) compared with commercial Kraft lignin (77.2 µm, 157.9 µm and 0.1% respectively), indicating the potential of DES in the modification and upgrading of lignin for novel value-added products.

Graphical abstract:



Keywords:

Lignin; Deep eutectic Solvents; Waste valorisation; Microwave-assisted extraction; Dielectric properties

1. Introduction

Lignin is the most abundant naturally occurring aromatic polymer and the second most abundant polymer after cellulose. It is mainly found in the secondary cell wall of most terrestrial plants in the approximate range of 15 to 40% of dry weight (Korányi et al., 2020). Lignin is comprised of three basic phenylpropanoid monomers, namely p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) sub-units, derived from p-coumaryl, coniferyl, and sinapyl alcoholic monolignols respectively, and with different numbers of methoxy groups (none, one, and two) connected to the aromatic rings. The structure and composition of lignin varies greatly by origin of the biomass; for example, softwood derived lignin is constituted of approximately 90-95% G-units, hardwood derived lignin contains 25-50% of G- and 50-75% of S-units (Korányi et al., 2020), while lignin in herbaceous biomass has relatively large quantity of H-units compared to woody biomasses (Wang et al., 2021).

Lignin occurs widely as a co-product in multiple industrial processes, with global annual production estimated to reach 225 million tons by 2030 (Haq et al., 2020). However, the current valorisation rate of lignin is less than 2%, and most lignin is discarded as waste or utilised in low-value applications such as combustion (Korányi et al., 2020). The pulp and paper industry is a major lignin producer; cellulose from lignocellulosic biomass is utilised to make paper and lignin remains in the large volume of wastewater causing huge environmental problems such as aquatic toxicity (Haq et al., 2020). Another example is the production of second-generation bioethanol from biomass fermentation, which requires pre-removal of large quantities of lignin present in the biomass, to avoid yield reduction (Haq et al., 2020). Due to the presence of large quantities of lignin in industry, as well as the urgency to achieve a more circular economy and find new renewable resources to replace fossil fuels, researchers have been working to develop lignin into practically and commercially relevant precursors for a new range of value-added chemicals. Emerging research has demonstrated the potential of lignin in several novel and value-added applications in civil construction, food, packaging, environmental and pharmaceutical realms, including bioplastics, bioadhesives, bioflocculants/heavy metal adsorbent, and hydrogels with antimicrobial and antioxidant properties etc. (Ge & Li, 2018; Rico-García et al., 2020; Xia et al., 2021).

To utilise the versatility of lignin as a feedstock and to promote lignin-based novel applications, there has been increasing interest in developing variable techniques for lignin recovery from biomass. Lignin is commonly extracted using strong acids, alkalis and toxic organic solvents at elevated temperatures and pressures or using expensive enzymes for prolonged processing times (see supplementary materials). Pitfalls associated with these techniques are fragmented lignin with lower purity, poor environmental credentials or low commercial practicability (Ning et al., 2021). Therefore, for the advancement of novel lignin applications, there is an increasing need for novel lignin recovery methods.

The use of Deep Eutectic Solvents (DES) in biomass extraction has attracted considerable attention in recent years. DES are commonly defined as systems composed of a mixture of at least two components in proper molar ratios, comprising a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD) self-associated to form a new eutectic phase characterised by a melting point lower than that of each individual component (Chen et al., 2020). DES have recently emerged as sustainable alternatives to water and organic solvents because of their advantages such as low volatility, non-flammability, high dissolution ability and tailorability (Perna et al., 2020). DES also have several advantages over their predecessor ionic liquids (IL) because typical DES can be prepared from relatively common low cost and environmentally friendly components from renewable sources (e.g., choline chloride (ChCl), glycerol (Gly), carbohydrates, urea, polyalcohols, lactic acid, amino acids and vitamins) and therefore exhibit low toxicity and high biodegradability (Perna et al., 2020). Additionally, DES are suitable for large-scale applications because the components themselves can be in solid phase for easier shipping and can be prepared on site by mixing of the components with moderate heating (Tomé et al., 2018).

DES are found to be particularly promising in lignin recovery (Chen et al., 2020). Firstly, DES have good lignin solubility and can achieve similar or even higher extraction yield at relatively low temperatures. Despite the extraction times reported for lignin vary from 30 minutes to 6 hours irrespective of the type of solvent used, a typical DES extraction range is 80-150 °C (Kohli et al., 2020) compared to 160-200 °C using alkali (to produce commercial Kraft lignin) (Chio et al., 2019) or organic solvents (Liu et al., 2020). Additionally, lignin obtained from DES extraction has a higher yield and unique structural properties such as high molecular weight, preserved β-O-4 ether linkage and high content of phenolic hydroxyl groups (Chen et al., 2019; Provost et al., 2022; Xia et al., 2018), indicating the lignin extracts are less degraded and closer to the natural molecule.

Apart from the investigations on the use of different solvents, novel and sustainable heating methods have also been explored, among which microwave heating has attracted the most interest. Rather than relying on indirect conventional heating mechanisms by conduction, convention and radiation which usually causes uneven heating, microwaves are electromagnetic waves that can penetrate the material and heat the molecules directly through dipolar polarisation and ionic conduction heating mechanisms, resulting in uniform heating throughout the bulk material without the need for direct contact (Foong et al., 2022) and providing benefits such as reduced extract times and minimising degradation of bio-active extracts (Mao et al., 2021). Microwave also offers unique selective heating mechanism by heating different components in a heterogeneous system at different rates determined by the dielectric properties, and it is thought that this can lead to a range of microwave-enhanced mass transfer effects (Taqi et al., 2020), potentially including disruption of cells within the biomass resulting in higher extraction yields and the ability to treat recalcitrant materials (Mao et al., 2019). During microwave-assisted biomass extraction, the volumetric and selective heating also allow heat to be generated within the core of the

biomass before conventionally lost to the solvent, which leads to the same mass and energy transfer direction within the material and thus accelerated extraction rates (Foong et al., 2020). Additionally, microwaves can be powered by carbon-free electrical energy rather than traditional fossil fuel heat sources as in conventional heating technology, potentially supporting urgent global environmental targets such as the net-zero carbon commitment (Mao et al., 2021).

Several researchers have investigated the potential of combining the use of DES and Microwave-Assisted Extraction (MAE). For example, Chen and Wan (2018) used a DES (choline chloride : lactic acid (ChCl:LA)) and microwave irradiation at 800 W for only 45 seconds to remove 65%, 72% and 80% lignin from *Miscanthus*, Switchgrass and Corn Stover, respectively, while retaining most of cellulose in the biomasses. Similarly, using a DES (choline chloride : oxalic acid (ChCl:OA)) and 110 °C, Liu et al. (2017) were able to achieve a similar extraction performance of lignin from Poplar wood and reduce the lignin extraction time from 9 hours using conventional heating to 3 mins using microwave at 800 W. Although those literatures have shown the potential of microwave heating to accelerate DES-lignin extraction, little is known about how different DES interact with microwaves, and how this could enhance processing through accelerated extraction times and selective heating effects. Other aspects also need further considerations when investigating the advantages and disadvantages of DES and MAE. Firstly, González-Rivera et al. (2020) has shown that DES can be heated much faster under microwaves than their individual components and traditional solvents, which makes it theoretically promising for MAE of lignin. However, the heating ability of DES under microwave is often not quantified by dielectric properties, nor the difference between different DES systems. Secondly, one of the major disadvantages of DES over traditional solvents is their high viscosity, causing the heat and mass transfer limitation during conventional heating (Patil & Rathod, 2023). However, this can be overcome by microwaves, which is evident in the faster heating rate in MAE. Additionally, DESs (especially carboxylic acid−ChCl-based DESs) are found thermally unstable, and their degradation will take place at high temperatures or prolonged processing times (Anuchi et al., 2022). However, when combined with microwave, the ability of DES for lignin extraction can be reinforced as MAE can usually achieve the same extraction performance in lower temperatures or reduced the extraction time, minimising thermal degradation of the DES and extracts (Mao et al., 2019).

Cocoa Bean Shells (CBS) are a major by-product of the chocolate industry, and were selected as the focus of this study. The world production of cocoa beans approached 5.8 million tonnes in 2020 (FAOSTAT, 2020), of which CBS constitutes 10%-17%, producing a sizable volume of feedstock for potential valorisation. The chemical composition of CBS mainly constitutes dietary fibres (i.e. cellulose, hemicellulose, lignin and pectin), carbohydrates, protein, methylxantines (i.e. caffeine, theobromine, and theophylline), fats, a large spectrum of phenolic compounds, minerals and vitamins (see supplementary materials). CBS is traditionally considered as ‘waste’, being left to rot at the cocoa

plantation, leading to soil and water contamination (Picchioni et al., 2020), or used in low-value applications such as fertiliser or animal feed (Lu et al., 2018). However, there is increasing research effort to valorise CBS into a range of new products, with application for example in the pharmaceutical, functional food, cosmetic and biofuel industries (Lu et al., 2018). Different from the recovery and valorisation of woody biomass that primarily focuses on the lignocellulosic fraction, the efforts on CBS reported in literature concern largely the bioactive components including polyphenols, methylxanthines and fats (de Souza Vandenberghe et al., 2022; Hernández-Hernández et al., 2018). However, to-date little is known about the recovery of lignin despite that CBS contains 25-35% lignin (Panak Balentić et al., 2018). The present work is the first study focusing on lignin recovery from CBS using DES and microwaves.

The aim of this study was to investigate how DES and microwave influence lignin extraction in terms of the quality and structure of lignin recovered from CBS. Specifically, the present research provides novelty with respect to:

1) To determine the susceptibility of a range of DES to microwave heating through dielectric property measurements and use this to select a suitable DES for the study of lignin extraction from CBS. To the best of authors' knowledge, this is the first time DES dielectric properties have been reported.
2) To investigate the effect of various extraction conditions including extraction temperature, time, and solid-liquid ratio on the lignin yield and quality and achieve extraction optimisation in a comprehensive and systematic manner.
3) To compare the chemical structure of extracts with commercial Kraft lignin by complete characterisation performed by Laser Scattering Particle Sizer, Fourier Transform Infra-Red (FTIR) Spectrometry and Pyrolysis-Gas Chromatograph-Mass Spectrometer (Pyrolysis-GC/MS), with special attention informing potential novel and value-added applications of the DES-extracted lignin products.

2. Materials and methods

2.1 Materials

Cocoa Bean Shell (CBS) material was obtained from Mondelēz (Mondelēz International, UK) and milled to a fine powder (mean diameter of 473 μm) using a hammer mill (24 series Circ-u-flow hammer mill, Schutte, USA).

Choline chloride (ChCl), Glycerol (Gly), Ethylene Glycol (EG), p-Toluenesulfonic acid (PTSA), Citric Acid (CA), Betaine (Bet), Acetic Acid Glacial, Acetyl Bromide, Hydroxylamine Hydrochloride,

Sodium Hydroxide and commercial Kraft lignin (KL, ≥ 95% w/w of dry basis) were purchased from Sigma Aldrich, UK. Ethanol (absolute) was purchased from Scientific Laboratory Supplies, UK. Deionised (D.I.) water was made by Milli-Q Plus water purification system (Merck KGaA, Germany).

2.2 Preparation of Deep Eutectic Solvents (DES)

Five DES previously reported for lignin extraction were prepared using the respective chemical components in appropriate molar ratios listed in Table 1. The mixtures were heated to 50 °C using a water bath for 30 mins with constant stirring at 600 rpm, until transparent and homogeneous solvents were formed.

2.3 Dielectric property measurement

Dielectric property measurements were performed following the method from Mao et al. (2021) with some modifications using an 85070E Dielectric Probe Kit (Agilent, USA). The dielectric loss factor data was taken at 2.47 GHz, which is within 20 MHz of the MAE equipment used. Prepared DES were heated on a hot plate from 20-80 °C with measurements taken at 10 °C intervals. The probe was immersed into the sample solution. After each measurement, the probe was cleaned with D.I. water and wiped to dry.

2.4 Microwave-Assisted Extraction (MAE) of lignin

Figure 1 shows a schematic of the extraction method and the downstream processes. MAE of lignin was carried out using a Monowave 200 microwave (Anton Parr, UK) at 2.45 GHz. The CBS and the DES were combined at 5% (1 grams CBS + 20 grams DES) or 10% (2 grams CBS + 20 grams DES) solid-liquid ratio and loaded into the Monowave reaction tubes. The samples were heated to the set temperature (70, 90, 110 and 130 °C) and held at this temperature for the required length of time (5-60 min). A maximum incident power of 200 W was applied. Stirrer speed was set at 600 rpm. After the hold time, compressed air was applied to cool the sample down to 70°C before the Monowave system can be opened and samples can be taken out for down-streaming processes. The temperature and power profile of MAE are also shown in Figure 1.

After the microwave treatment, 20 mL ethanol was added to aid transfer of the treated mixture into a centrifuge tube. The solid cellulose-rich material (CRM) was removed by centrifugation at 3900 rpm for 20 mins. An equal 20 mL volume of D.I. water was added to the supernatant and stored in a 4 °C fridge overnight. The lignin-rich material (LRM) was precipitated out and separated by centrifugation

at 3900 rpm for 1 hour. The hemicellulose-rich material (HRM) remained in the liquid phase. The LRM was dialysed to remove associated DES using dialysis tubes (cellulose membrane, typical molecular weight cut-off = 14,000 Da, Sigma Aldrich, UK) against D.I. water for 5 days. The LRM was then freeze-dried using a LyoDry freeze dryer (Mechatech Systems, UK) for yield calculations and further analytical characterisation. All experiments were performed in triplicate.

2.5 Acetyl Bromide Soluble Lignin (ABSL) Assay

The content of lignin in the raw CBS and LRM, also referred to as the lignin purity, was determined by the Acetyl Bromide Soluble Lignin (ABSL) Assay. 100 mg of raw CBS or freeze-dried LRM was dissolved in 4.0 mL of 25% acetyl bromide in glacial acetic acid. The mixture was held in a water bath at 50 °C for 2 hours then cooled down in an ice bath. The volume of the mixture was made up to 16 mL with glacial acetic acid and left to settle for 30 mins before centrifugation at 3900 rpm for 20 mins. An aliquot (0.5 mL) of this solution was mixed with 2.5 mL of glacial acetic acid, 1.5 mL of 0.3 M NaOH, 0.5 mL of 0.5 M hydroxylamine hydrochloride solution, and further diluted with 5.0 mL of glacial acetic acid to a final volume of 10.0 mL. Solutions were measured with an UV-Vis spectrophotometer (Cecil Instruments 1000 Series, UK) at the wavelength of 280 nm. Glacial acetic acid was used as the reference. The lignin standard solution was made using commercial Kraft lignin (KL) in a concentration range of 0-0.1 mg/mL and was treated the same way as above. The purity of LRM (% on dry basis), referring to the content of lignin in LRM extracts, was calculated by:

$$\%\ lignin\ purity\ in\ LRM = \frac{Dry\ weight\ of\ lignin\ in\ LRM\ by\ ABSL}{Dry\ weight\ of\ LRM} \times 100\% \quad (1)$$

The results and error bars of LRM lignin purity were determined from 4 repeats ((2 freeze-dried samples) × (duplicate measurements for each sample in UV–Vis), such that the error from both the extraction method and ABSL method were incorporated into the error bars).

2.6 Extraction yield calculation

The yield of LRM was presented as the percentage content of LRM of dry basis per gram of raw CBS and the lignin yield was presented as the percentage content of lignin on a dry basis per gram total lignin content in raw CBS.

The yield of LRM (% of dry basis) per gram of raw CBS was calculated by:

$$\%\ yield\ of\ LRM = \frac{Dry\ weight\ of\ LRM}{Dry\ weight\ of\ CBS} \times 100\% \quad (2)$$

The yield of LRM (% of dry basis) as the percentage of lignin extracted from the total lignin content in CBS, was calculated by:

$$\% \text{ yield of } lignin = \frac{Dry\ weight\ of\ lignin \text{ in LRM } by\ ABSL}{Dry\ weight\ of\ total\ lignin\ in\ CBS\ by\ ABSL} \times 100\% \quad (3)$$

$$\text{Dry weight of lignin in LRM by ABSL} = \text{Dry weight of LRM} \times \text{\% lignin purity of LRM} \quad (4)$$

Where, the total lignin content in CBS was found as 27.5% ± 1.6% using the ABSL method.

The lignin yield results and their error bars were calculated based on the average and standard deviation of triplicated runs.

2.7 Particle sizing measurements

Particle size measurements were conducted in a laser scattering particle size distribution analyser (Partica LA-960, Horiba, Japan) with a red solid-state 5 mW laser diode (650 nm) channel and a blue solid-state 3 mW LED channel. All LRM extracts were prepared at 1 mg/mL in D.I. water, following the method developed by Zwilling et al. (2021) and Matsakas et al. (2018) with some modifications. LRM suspension was loaded into the 10 mL Horiba Fraction Cell. According to Adamcyk et al. (2021), the refractive index of the particles was set as 1.53 and the imaginary refractive index as 0.1. A stirrer bar was placed in the cell to ensure mixing of the sample solution during the measurements. Three values (in µm) were reported: D50, the median, the diameter where half of the particle sizing population lies below this value; D10, the diameter where 10% of the population lies below; and D90, the diameter where 90% of the distribution lies below. Particle sizing results were determined by triplicate.

2.8 FTIR analysis

The chemical structures of KL and LRM were analysed with a Fourier Transform Infrared (FTIR) spectroscopy (Cary 630, Agilent, USA). The spectra were taken in the wavenumber range 4000 to 650 cm^{-1} with a resolution of 4 cm^{-1}. The FTIR spectra presented were the average of triplicates. The assignments of functional groups in the FTIR spectrum are referenced from Lu et al. (2017) and Sathawong et al. (2018) (see supplementary materials).

2.9 Pyrolysis-GC-MS

Pyrolysis was performed in a platinum filament pyrolysis instrument (CDS Analytical Pyroprobe 5000, USA) coupled to a Gas Chromatograph - Mass Spectrometer (GC–MS, Agilent 5977 series, USA) with a single quadrupole mass selective detector (MSD), using a fused silica capillary column (DB-1701, 60 m × 0.32 mm I.D., 1 µm stationary phase thickness). Around 500 µg freeze-dried LRM or KL sample was placed into a quartz tube (25 mm Length × 1.9 mm I.D.) and placed into the pyrolysis chamber. The pyrolysis was carried out at 550 °C for 10 s. Pyrolysis chamber was purged with helium as carrier

gas with a constant flow of 1.1 ml/min to transfer the pyrolysis products into the GC column. Injection was performed in split mode with a ratio of 75:1. The GC oven was heated from 45 ºC to 260 ºC at 6 °C/min and held at 260 ºC for 20 mins. The MS was operated by the electron impact ionization mode at 70 eV and mass scan range m/z from 40 to 400.

Compounds were identified by comparing their peak retention time shown on the mass spectrum with the National Institute of Standards and Technology (NIST) library, version 14. The area of each resulting peak was integrated and then converted into relative abundance (RA, in %) by dividing the peak area by the total area, as shown below. The total peak area was considered as 100%.

$$RA\ of\ each\ indentified\ compound = \frac{Peak\ area\ of\ the\ compound}{Total\ peak\ area} \times 100\% \quad (5)$$

The peak of p-hydroxyphenyl (H) and guaiacyl (G) sub-unit were the sum of peak areas of p-hydroxyphenyl or guaiacyl types of derivatives respectively, the classification of which were based on Van Erven et al. (2017) and Nunes et al. (2010) and shown in Table 2. The p-hydroxyphenyl/guaiacyl (H/G) ratio was calculated by dividing the sum of peak areas from p-hydroxyphenyl units by the sum of peak areas of guaiacyl derivatives. All results were calculated based on triplicates.

3. Results and discussion

3.1 Selection of DES based on dielectric property measurement

To help understand the response of DES under microwave field, the dielectric loss factors of 5 DES that were previously reported for lignin extraction and D.I. water were measured, and the values are shown in Figure 2. Firstly, all five measured DES shown higher dielectric loss factor at elevated temperature from 20-80 °C, which is opposite to D.I water, which showed decreased dielectric loss due to the reduction in water's viscosity and dipole rotation mechanism with increasing temperature. This may be because all the five measured DES have components that contain ions (PTSA and CA) and their viscosities decrease at higher temperatures, both of which lead to freer ion movements and higher ionic conduction mechanisms. Secondly, the components that made up the DES affected their dielectric properties. The DES contained PTSA has shown higher dielectric properties than other DES. This could be because PTSA is a strong acid that can provide complete disassociation of its hydroxyl and carboxylic acid groups when dissolved and thus have higher ionic conduction mechanisms than a weak acid such as CA. The high dielectric property of PTSA DES may be linked to its lignin extraction ability as several studies have reported positive influence of using PTSA in lignin extraction. For example, Chen et al. (2019) found that the DES made of PTSA as the HBD can remove up to 82% of lignin while retaining most of the cellulose in the switchgrass; additionally the lignin recovered by PTSA-ChCl DES with a small amount of Gly or EG added was kept more intact during the extraction. The addition of

PTSA to other solvents, though not as DES, was also found to help increase the yield of lignin. For example, Ouyang et al. (2018) found out that even a small addition of PTSA into the methanol-water solvent can effectively double the lignin extraction yield from 30% to 64% for oak sawdust. Thirdly, the different molar ratio of the same components in the DES also affects their dielectric properties. For DES consisting of CA-ChCl-EG, the DES with a higher molar ratio of CA had higher dielectric properties, which may again be due to its higher amount of ions and thus ionic conduction.

Overall, PTSA-ChCl-Gly (2:1:1) had the highest dielectric loss factor (from 3.4 at 20 °C to 12.0 at 80 °C) compared with other DES and D.I. water above 40 °C and can be assumed to be heated the fastest under microwave heating. Therefore, this DES was selected as the study DES for lignin recovery and used to investigate the effect of different processing conditions on lignin extraction performance in terms of extract yield and structure.

3.2 Lignin extraction yield

The influence of different operating parameters, including temperature (70-130 °C), time (5-60 mins), solid-liquid ratio (5% and 10%) and the use of selected PTSA-ChCl-Gly (2:1:1) DES on the LRM yield was calculated and shown in Figure 3.

The LRM yield results are shown in Figure 3A. Firstly, a higher extraction temperature achieved a higher LRM yield. The LRM yield increased from negligible at 70 °C, to 4.6% for 5% solid-liquid ratio at 90 ^{o}C, and tripled every 20 ^{o}C thereafter, up to a maximum of 36.9% at 130 °C and 5% solid-liquid ratio. This may be because higher temperature leads to better cleavage of lignin from the cell wall matrix and promotes its solubility following release from the cell wall matrix. Although the mechanism of lignin cleavage in DES remains elusive, some researchers have proposed that in a DES rich in chloride ions (Cl^-) and under acidic conditions, it is due to the possible interaction of H-bonds from the DES and hydroxyl in polysaccharides from the lignocellulose, which enables fractionation of lignin-polysaccharide complex (Chen et al., 2020; Xia et al., 2018). Xia et al. (2018) reported the same effect of temperature on lignin extraction, where the lignin yield increased from 15.34% to 20.37% when temperature increased from 110 to 130 ^{o}C using a ChCl : glycerol : $AlCl_3 \cdot 6H_2O$ DES, which was thought to be corresponding to the increased H-bond-accepting ability of the DES and thus better fractionation of lignin-polysaccharide complex and facilitation of lignin extraction at elevated temperature. The results also indicate that lignin is a very recalcitrant material and a high temperature of 130 ^{o}C is essential for an efficient extraction when the study DES is used.

Secondly, the LRM yield was higher at a solid-liquid ratio of 5% compared to 10%, increasing from 3.6% to 4.6%, 10.5% to 13.8% and 26.4% to 36.9% at 90, 110 and 130 °C respectively. The likely

reason for this well-known effect is that in the sample with a higher solid-liquid ratio, the concentration of lignin in the solvent is higher for the same extraction yield. This leads to a lower concentration gradient of dissolved lignin between the biomass cells and the solvent compared with the samples with a lower solid-liquid ratio. Concentration is a major contributor to chemical potential, which governs mass transfer. For this reason, a lower percentage of lignin present in the CBS at a high solid-liquid ratio could be solubilised and extracted into the liquid and thus, a lower LRM yield.

Additionally, the optimum yield was achieved for all extraction temperatures and solid-liquid ratios at 30 mins. The optimum condition of LRM yield was found as 130 ^{o}C, 30 mins and 5% solid-liquid ratio. At this condition, the LRM yield reached 36.9%, corresponding to 95.5% (see Figure 3B) of the total lignin content of the studied CBS material. The total lignin content of the CBS was 27.5 ± 1.6% (ABSL method), which is consistent with the value of 26% reported in literature (Chung et al., 2003; Panak Balentić et al., 2018)), demonstrating a high level of extraction achieved. Furthermore, the LRM yield started to decrease after the optimum condition, the results of which is consistent with the literature (Kohli et al., 2020). Although the reason for the decrease in yield remains unclear, one proposed explanation is the polymerisation and depolymerisation of pseudo-lignin, which has a structure resembling lignin produced by cell wall polysaccharides and without significant contributions from lignin in the biomass (Sannigrahi et al., 2011).

Another characteristic of DES is that it is not selective for lignin alone but would also contribute to the extraction of other cell wall components such as hemicellulose and cellulose, although the effect on different components is not at the same extent. Hemicellulose fraction dissolved in DES can be further depolymerised into oligomers and monomers, while cellulose is often more recalcitrant and cannot be easily dissolved or depolymerised in DES (Chen et al., 2020). The work of Chen et al. (2019) has also reflected this and shown 30% lignin extraction alongside 31% xylan (the main constituent in hemicellulose) and 1% cellulose when the same DES was used. Referring to these, it is highly possible that our LRM extracts also contain hemicellulose, and this will be further discussed in Section 3.3.

3.3 Extracts structural information

3.3.1 Particle sizing

The particle sizing (D10, D50 and D90 values in μm) of the Kraft lignin standard and LRM extracts were found and presented in Figure 4. D50, the median, represents the diameter where half of the particle sizing population lies below this value; D10 is the diameter where 10% of the population lies below; and D90 is the diameter where 90% of the distribution lies below. Firstly, when comparing the extracts obtained at the same optimum conditions of 30 mins and 5% solid-liquid ratio, lower extraction

temperatures resulted in larger particle sizes. LRM at 90 ºC was able to achieve a higher median particle size (D50) of 382.5 ± 18.5 µm than LRM at 110 ºC and 130 ºC, the D50 of which were 254.2 ± 16.5 and 242.5 ± 13.8 µm respectively. This may suggest that higher temperature can lead to more severe biomass treatment and cause more molecular depolymerisation. The similar effect of temperature was reported in Shen et al. (2020), where the molecular weight of the lignin extracts obtained from Eucalyptus sawdust by a DES (Choline Chloride: Lactic Acid) decreased from 12,930 to 2,070 g/mol when extraction temperature increased from 60 to 140 ºC.

Secondly, a shorter extraction time produced higher extract particle sizes. When comparing extracts at 130 ºC and 5% solid-liquid ratio, the shortest extraction time of 10 mins yielded extracts with the largest D50 size of 279.1 ± 15.7 µm; however, the median sizes reduced to approximately 242.5 ± 13.8 µm for LRM obtained after 30 mins extraction time. Additionally, although the median size of LRM after 30 mins stayed the same, their D10 and D90 sizes decreased from 91.0 ± 11.7 and 501.4 ± 21.9 µm to 58.7 ± 11.8 and 356.7 ± 26.1 µm respectively from 30 mins to 60 mins extraction time, again suggesting that a higher extract particle size can also result from shorter processing time. Furthermore, a shorter extraction time is associated with a larger difference between D90 and D10 values (showing as D90 - D10 in Figure 4) indicating a wider particle size distribution (which can be correlated to polydispersity) and thus inhomogeneous extracts. This result is in accordance with the work of Provost et al. (2022), where the softwood lignin extracts obtained at 60 ºC presented the broadest distribution with a polydispersity index value (PDI) of 1.53, yet showed a narrower distribution with PDI between 1.03 and 1.31 when the extraction temperature increased to 80 ºC with the same DES (Choline Chloride: Lactic Acid) used. These results have shown that shorter extraction times have the same effect as lower temperature as discussed earlier. This causes less molecular depolymerisation to occur and thus results in the recovery of lignin with larger extract particle sizes.

3.3.2 FTIR spectra

The FTIR spectra of the LRMs were studied and compared with the Kraft lignin standard (see supplementary materials). Generally speaking, all LRMs presented similar trends of peaks though some peaks were more intense than others comparing extracts obtained at different extracting conditions. While the Kraft lignin standard had different spectra than the LRMs, indicating the structure difference between the LRM extracted from CBS by DES and the Kraft lignin standards commercially produced from wood materials by alkali Kraft extraction. Specifically, there are several peaks at certain wavenumbers in the 3200 – 600 cm^{-1} wavenumber range indicating interesting structures of the extracts and the results in this range are discussed below in detail.

Firstly, both the commercial Kraft lignin and the extracted LRMs contained components other than lignin, although the type and amount of the components varied. The Kraft lignin did not show a peak at 1732 cm^{-1}, which would have indicated the presence of hemicellulose as a peak at this wavenumber can be attributed to the C=O stretching from hemicellulose. Meanwhile, all tested LRMs had this peak, suggesting the presence of hemicellulose in the extracts. LRM yielded at higher temperature showed less transmittance at this peak compared to lower temperature implying that the level of hemicellulose in the extracts decreased with increasing extraction temperature. The extract obtained at 130 °C has shown high lignin purity and low hemicellulose content, indicating successful fractionation of the biomass at this temperature. Another peak at wavenumber 812 cm^{-1} representing the C-H stretching in the glucose ring from cellulose was clearly shown in the Kraft lignin standard. However, only the LRM extracted at the highest temperature of 130 ºC had some but a lower amount of cellulose content than the Kraft lignin standard; the LRM extracted at lower temperatures contained negligible cellulose content. This difference can be attributed to the varied production methods; Kraft lignin is commercially produced by treating woody lignocellulosic biomass at higher temperature, in the range of 150–170 °C, than applied in this study and in the presence of sodium hydroxide (NaOH) and sodium sulfide (Na_2S) (Chio et al., 2019). The presented results indicate that the extracting temperature had a significant influence on the purity of the lignin product, and that a low temperature may lead to high hemicellulose content and a temperature above 130 °C may yield high cellulose contents, irrespective of the extraction solvents.

Secondly, the structure of the lignin in the commercial sample and the extracted LRMs was different. The peak at 1507 cm^{-1} represents the aromatic skeletal stretching in the aromatic ring in lignin and the result suggested that the Kraft lignin included more aromatic ring content than the LRMs. Lignin G-units are identified by a peak at 1208 cm^{-1} presented in the spectrum for the Kraft lignin but absent in the LRM spectra. The lignin sub-unit structure difference between the commercial and the extracted material was further studied by Pyrolysis-GC-MS, see Section 3.3.3. Furthermore, absorbances at 2923 cm^{-1} and 2852 cm^{-1} are attributed to C-H stretching in alkyl chains adjacent to an aromatic centre, and CH variation in methoxyl groups respectively in the lignin side chains. Both the Kraft lignin and the LRMs show peaks at these two wavenumbers suggesting the lignin in all samples was at least partially methylated, which has previously been observed (Provost et al., 2022). However, these peaks were higher for the LRMs implying a greater degree of methylation compared to the Kraft lignin. When comparing LRM extracted at different temperatures, LRM at higher temperatures had gradually reduced absorption peaks of the bonds at their respective corresponding location mentioned above compared to the LRM at lower temperature, indicating that some methoxyls may be degraded from the aromatic ring during the thermal process at higher temperatures (Liu et al., 2014).

3.3.3 Pyrolysis-GC-MS

The Pyrolysis-GC-MS was performed to determine the abundance of different sub-units in the Kraft lignin and LRM samples. The relative abundance (RA, in %) of p-hydroxyphenyl (H) and guaiacyl (G) sub-unit were calculated by dividing the sum of peak areas of p-hydroxyphenyl or guaiacyl types of derivatives from the GC-MS spectra (see supplementary materials) by the total area that is considered as 100%. The p-hydroxyphenyl/guaiacyl (H/G) ratio was calculated by dividing the sum of peak areas from p-hydroxyphenyl units by the sum of peak areas of guaiacyl derivatives. Results are shown in percentage ratios of dry weight in Figure 5.

Kraft lignin contained primarily G-unit of 67.1% with 8.4% H-unit; while LRM obtained in this work had a negligible amount of G-units but high H-units percentage varying from 15.4% to 45.4%. This made the H/G sub-unit ratio in LRM reached much higher values ranging from 36.9% to 71.9%, compared to a low ratio of 0.1% in Kraft lignin. This is because the sources of the Kraft lignin and LRM were different and thus the structure and composition of lignin vary greatly among the origin of the different biomass. Kraft lignin is produced from coniferous (soft) wood, which explains why the primary unit is G-unit (Korányi et al., 2020). LRM were extracted from Cocoa Bean Shells (CBS), which had similar lignin structure as herbaceous biomass, and therefore has a relatively high H-unit content compared to wood lignin (Wang et al., 2021). Similar results were reported by Happs et al. (2021) for Kraft lignin and Provost et al. (2022) for herbaceous biomass (Brewer's Spent Grain) using DES.

Extraction conditions also have great influence on the sub-unit contents in the LRM. Firstly, with extraction temperatures increased from 90 to 130 ºC, an increased fraction of H-units from 15.4% to 45.4% was recovered from lignin, showing that H-units extraction is favored at higher extraction temperatures. It also worth noting that S-units were not determined in both Kraft lignin and LRM in this study, the same phenomenon was also reported in Provost et al. (2022) when using NMR to characterise the strcuture of their lignin extracts extarcted using choline chloride: lactic acid DES (ChCl:LA) at 60 ºC and 80 ºC. The possible reason, as proposed by Alvarez-Vasco et al. (2016), might be that H- and G-units from the lignin products extracted by DES between 80 to 140 °C are more prone to determinations than their S-units, suggesting the extraction of S-units would require a higher extracting temperature during DES treatment. Additionally, when comparing LRM with different extraction times, the optimum H-unit content and therefore the highest H/G ratio of 71.9% was both achieved at conditions of 30 mins and 130 ºC, which was also the condition for optimum lignin yield.

3.4 Implications and future work

This paper presents a novel and environmentally-friendly method to valorise food industry waste; specifically, Deep Eutectic Solvents (DES) are combined with Microwave-Assisted Extraction (MAE) to extract lignin from Cocoa Bean Shells (CBS). The dielectric properties of 5 commonly reported DES were determined and connected with their responses under microwave heating. All tested DES had increasing dielectric properties with temperature, which is attributed to the decreased viscosities and increased ionic conduction mechanism. Among the tested DES, PTSA-based DES, especially PTSA-ChCl-Gly (in 2:1:1molar ratio), showed the highest dielectric properties and thus was selected as the study solvent in this work. To the best of the authors' knowledge, this is the first attempt to quantify the susceptibility of a range of DES to MAE. However, further work is required to a). determine the dielectric properties of a wider range of DES so that solvent screening can be achieved; b) compare the extraction performance of different DES under MAE in terms of the extract yield and quality and connect this with their dielectric properties, in order to provide insights to develop better extraction processes when combining the use of DES and MAE; c) understand the chemistry and synthesis of DES, proposing an explanation on the mechanisms influencing their dielectric properties.

The effect of various extraction conditions including extraction temperature (70-130 °C), time (5-60 mins), and solid-liquid ratio (5 and 10%) on the lignin yield and structure were investigated. Lignin product yield was favoured at higher extraction temperatures and lower solid-liquid ratio, with the optimum yield of 95.5% lignin from the CBS recovered at 130 ºC and 30 mins. The lignin extraction time and temperature ranges applied here are considerably lower than those reported for other solvents in literature, where 160-200 °C and more than 2 hours are often required to obtain a lignin yield above 60% of the total lignin content in the studied biomass when alkali or organic solvents are used (Chio et al., 2019). Though the process optimisation has been performed for this study, to achieve a more comprehensive understanding on the potentials of MAE and DES, more experimental work needs to be done to systematically compare a) the use of microwave and conventional heating to elucidate any unique mass transfer effects caused by microwave selective heating that could be exploited to improve the extraction process, b) the use of different DES, alkali (as in Kraft processing) and organic solvents, c) the difference between lignin extracted from CBS and other lignin-rich secondary biomasses such as food, agricultural and forestry co-products (e.g. woody material such as soft and hardwood, other herbaceous biomasses such as wheat straw and brewer's spent grain). Additionally, the fundamental mechanism of the decrease in yield after optimum condition is not studied in this manuscript and further work is required to elucidate this.

The physicochemical structures of DES-extracted lignin are characterised and compared with commercial Kraft lignin (KL). Firstly, DES-extracted lignin exhibited larger particle sizes (242.5 µm D50 size) and structural diversity (410.4 µm D90-D10 size, for lignin extracted at 130 oC and 30 mins) compared with KL (77.2 µm and 157.9 µm respectively). Lower extraction temperature and shorter

time can also be beneficial in producing more “intact” lignin fragments suitable for higher value applications. Considering this characterisation finding, MAE could be a preferable technology because it can offer faster heating and thus reduce the extraction time or achieve similar yield at lower temperatures compared to conventional extractions (Mao et al., 2021). Secondly, FTIR characterisation suggested a higher degree of methylation in the DES-extracted lignin than KL and lignin obtained at lower temperatures. It has been reported that a higher degree of methylation in the lignin structure is related to better antioxidant capacity (Sumerskii et al., 2017), suggesting the potential of the DES lignin in this study compared to commercial Kraft lignin for the application as antioxidant. Finally, DES-extracted lignin presented different content values of sub-units than KL. Lignin obtained in this study showed a high content of H-unit (45.4%) and low G-unit (0.6%), resulting a much higher H/G sub-unit ratio (71.9%, all above data was for lignin extracted at 130 ^{o}C and 30 mins) compared with KL (0.1%). The difference in the sub-unit results may be due to the feedstock type as well as the different extraction conditions applied, more work is needed to understand the reaction mechanism and sub-unit decomposition during the extraction. Nevertheless, those unique characteristics make DES-extracted lignin promising for certain novel applications. For example, Kalami et al. (2017) found that lignin containing more H-unit was preferable for use as phenol replacement in phenolic adhesive formulation, thus showing a great potential to form a 100% lignin-based bio-adhesive. Other possible applications of the DES-extracted lignin product are biofilms with low wettability (Souza et al., 2022) and thermoplastic material with favorable mechanical properties (Saito et al., 2012).

In conclusion, this work demonstrates differences in the unique structure of lignin obtained from different biomass sources, solvents and extraction conditions, suggesting promise for certain novel and value-added applications that commercial Kraft lignin is not suitable for. However, the ability of the lignin products targeting the specific application was not studied and not within the scope of this work. With the success in the work presented in this study, future work is required to determine the functionality of the lignin extracts for a wide range of novel applications, including but not limited to bioemulsifiers, bio-based resins, biocomposites, bioplastics etc.

4. Conclusions

An efficient lignin extraction method from CBS was established combining DES and MAE. PTSA-ChCl-Gly (2:1:1) was selected due to its higher dielectric properties than other previously reported DES. The optimum yield of 95.5% lignin was achieved at 130 ^{o}C and 30 mins, demonstrating efficient extraction achieved at a temperature and time considerably lower than reported for alkali- and organic-solvent-based methods. Structural characterisations revealed that lignin obtained in this study had larger particle sizes and structural diversity, a higher degree of methylation and higher H/G sub-unit ratio compared to commercial Kraft lignin, showing great potential for novel value-added applications.

E-supplementary data for this work can be found in e-version of this paper online.

References

1. Adamcyk, J., Beisl, S., Amini, S., Jung, T., Zikeli, F., Labidi, J., Friedl, A. 2021. Production and Properties of Lignin Nanoparticles from Ethanol Organosolv Liquors—Influence of Origin and Pretreatment Conditions. *Polymers*, **13**(3), 384.
2. Anuchi, S.O., Campbell, K.L.S., Hallett, J.P. 2022. Effective pretreatment of lignin-rich coconut wastes using a low-cost ionic liquid. *Scientific reports*, **12**(1), 1-11.
3. Chen, Z., Jacoby, W.A., Wan, C. 2019. Ternary deep eutectic solvents for effective biomass deconstruction at high solids and low enzyme loadings. *Bioresource technology*, **279**, 281-286.
4. Chen, Z., Ragauskas, A., Wan, C. 2020. Lignin extraction and upgrading using deep eutectic solvents. *Industrial Crops and Products*, **147**, 112241.
5. Chen, Z., Wan, C. 2018. Ultrafast fractionation of lignocellulosic biomass by microwave-assisted deep eutectic solvent pretreatment. *Bioresource technology*, **250**, 532-537.
6. Chio, C., Sain, M., Qin, W. 2019. Lignin utilization: a review of lignin depolymerization from various aspects. *Renewable and Sustainable Energy Reviews*, **107**, 232-249.
7. Chung, B.Y., Iiyama, K., Han, K.W. 2003. Compositional Characterization Of Cacao (Theobroma cacao L.) Hull. *Journal of Applied Biological Chemistry*, **46**(1), 12-16.
8. de Souza Vandenberghe, L.P., Valladares-Diestra, K.K., Bittencourt, G.A., de Mello, A.F.M., Vásquez, Z.S., de Oliveira, P.Z., de Melo Pereira, G.V., Soccol, C.R. 2022. Added-value biomolecules' production from cocoa pod husks: A review. *Bioresource Technology*, **344**, 126252.
9. FAOSTAT. 2020. in: *Food and Agriculture Organisation of the United Nations, Online database from: http://www.fao.org/faostat/en/#data/qc*.
10. Foong, S.Y., Chan, Y.H., Lock, S.S.M., Chin, B.L.F., Yiin, C.L., Cheah, K.W., Loy, A.C.M., Yek, P.N.Y., Chong, W.W.F., Lam, S.S. 2022. Microwave processing of oil palm wastes for bioenergy production and circular economy: Recent advancements, challenges, and future prospects. *Bioresource Technology*, 128478.
11. Foong, S.Y., Liew, R.K., Yang, Y., Cheng, Y.W., Yek, P.N.Y., Mahari, W.A.W., Lee, X.Y., Han, C.S., Vo, D.-V.N., Van Le, Q. 2020. Valorization of biomass waste to engineered activated biochar by microwave pyrolysis: Progress, challenges, and future directions. *Chemical Engineering Journal*, **389**, 124401.
12. Ge, Y., Li, Z. 2018. Application of lignin and its derivatives in adsorption of heavy metal ions in water: a review. *ACS Sustainable Chemistry & Engineering*, **6**(5), 7181-7192.
13. González-Rivera, J., Husanu, E., Mero, A., Ferrari, C., Duce, C., Tinè, M.R., D'Andrea, F., Pomelli, C.S., Guazzelli, L. 2020. Insights into microwave heating response and thermal decomposition behavior of deep eutectic solvents. *Journal of Molecular Liquids*, **300**, 112357.

14. Happs, R.M., Addison, B., Doeppke, C., Donohoe, B.S., Davis, M.F., Harman-Ware, A.E. 2021. Comparison of methodologies used to determine aromatic lignin unit ratios in lignocellulosic biomass. *Biotechnology for biofuels*, **14**(1), 1-16.
15. Haq, I., Mazumder, P., Kalamdhad, A.S. 2020. Recent advances in removal of lignin from paper industry wastewater and its industrial applications–A review. *Bioresource Technology*, **312**, 123636.
16. Hernández-Hernández, C., Viera-Alcaide, I., Morales-Sillero, A.M., Fernández-Bolaños, J., Rodríguez-Gutiérrez, G. 2018. Bioactive compounds in Mexican genotypes of cocoa cotyledon and husk. *Food chemistry*, **240**, 831-839.
17. Kalami, S., Arefmanesh, M., Master, E., Nejad, M. 2017. Replacing 100% of phenol in phenolic adhesive formulations with lignin. *Journal of Applied Polymer Science*, **134**(30), 45124.
18. Kohli, K., Katuwal, S., Biswas, A., Sharma, B.K. 2020. Effective delignification of lignocellulosic biomass by microwave assisted deep eutectic solvents. *Bioresource technology*, **303**, 122897.
19. Korányi, T.I., Fridrich, B., Pineda, A., Barta, K. 2020. Development of 'Lignin-First'approaches for the valorization of lignocellulosic biomass. *Molecules*, **25**(12), 2815.
20. Liu, L.-Y., Patankar, S.C., Chandra, R.P., Sathitsuksanoh, N., Saddler, J.N., Renneckar, S. 2020. Valorization of bark using ethanol–water organosolv treatment: isolation and characterization of crude lignin. *ACS Sustainable Chemistry & Engineering*, **8**(12), 4745-4754.
21. Liu, Y., Chen, W., Xia, Q., Guo, B., Wang, Q., Liu, S., Liu, Y., Li, J., Yu, H. 2017. Efficient cleavage of lignin–carbohydrate complexes and ultrafast extraction of lignin oligomers from wood biomass by microwave‐assisted treatment with deep eutectic solvent. *ChemSusChem*, **10**(8), 1692-1700.
22. Liu, Y., Hu, T., Wu, Z., Zeng, G., Huang, D., Shen, Y., He, X., Lai, M., He, Y. 2014. Study on biodegradation process of lignin by FTIR and DSC. *Environmental Science and Pollution Research*, **21**(24), 14004-14013.
23. Lu, F., Rodriguez-Garcia, J., Van Damme, I., Westwood, N.J., Shaw, L., Robinson, J.S., Warren, G., Chatzifragkou, A., Mason, S.M., Gomez, L. 2018. Valorisation strategies for cocoa pod husk and its fractions. *Current Opinion in Green and Sustainable Chemistry*, **14**, 80-88.
24. Lu, Y., Lu, Y.-C., Hu, H.-Q., Xie, F.-J., Wei, X.-Y., Fan, X. 2017. Structural characterization of lignin and its degradation products with spectroscopic methods. *Journal of Spectroscopy*, **2017**.
25. Mao, Y., Lei, R., Ryan, J., Rodriguez, F.A., Rastall, B., Chatzifragkou, A., Winkworth-smith, C., Harding, S.E., Ibbett, R., Binner, E. 2019. Understanding the Influence of Processing Conditions on the Extraction of Rhamnogalacturonan-I "Hairy" Pectin from Sugar Beet Pulp. *Food Chemistry: X*, 100026.

26. Mao, Y., Robinson, J., Binner, E. 2021. Understanding heat and mass transfer processes during microwave-assisted and conventional solvent extraction. *Chemical Engineering Science*, **233**, 116418.
27. Matsakas, L., Karnaouri, A., Cwirzen, A., Rova, U., Christakopoulos, P. 2018. Formation of lignin nanoparticles by combining organosolv pretreatment of birch biomass and homogenization processes. *Molecules*, **23**(7), 1822.
28. Ning, P., Yang, G., Hu, L., Sun, J., Shi, L., Zhou, Y., Wang, Z., Yang, J. 2021. Recent advances in the valorization of plant biomass. *Biotechnology for Biofuels*, **14**(1), 1-22.
29. Nunes, C.A., Lima, C.F., Barbosa, L.C., Colodette, J.L., Gouveia, A., Silvério, F.O. 2010. Determination of Eucalyptus spp lignin S/G ratio: A comparison between methods. *Bioresource technology*, **101**(11), 4056-4061.
30. Ouyang, X., Huang, X., Hendriks, B.M., Boot, M.D., Hensen, E.J. 2018. Coupling organosolv fractionation and reductive depolymerization of woody biomass in a two-step catalytic process. *Green Chemistry*, **20**(10), 2308-2319.
31. Panak Balentić, J., Ačkar, Đ., Jokić, S., Jozinović, A., Babić, J., Miličević, B., Šubarić, D., Pavlović, N. 2018. Cocoa shell: A by-product with great potential for wide application. *Molecules*, **23**(6), 1404.
32. Patil, S.S., Rathod, V.K. 2023. Extraction and purification of curcuminoids from Curcuma longa using microwave assisted deep eutectic solvent based system and cost estimation. *Process Biochemistry*, **126**, 61-71.
33. Perna, F.M., Vitale, P., Capriati, V. 2020. Deep eutectic solvents and their applications as green solvents. *Current Opinion in Green and Sustainable Chemistry*, **21**, 27-33.
34. Picchioni, F., Warren, G.P., Lambert, S., Balcombe, K., Robinson, J.S., Srinivasan, C., D. Gomez, L., Faas, L., Westwood, N.J., Chatzifragkou, A. 2020. Valorisation of natural resources and the need for economic and sustainability assessment: The case of cocoa pod husk in Indonesia. *Sustainability*, **12**(21), 8962.
35. Provost, V., Dumarcay, S., Ziegler-Devin, I., Boltoeva, M., Trébouet, D., Villain-Gambier, M. 2022. Deep eutectic solvent pretreatment of biomass: Influence of hydrogen bond donor and temperature on lignin extraction with high β-O-4 content. *Bioresource Technology*, **349**, 126837.
36. Rico-García, D., Ruiz-Rubio, L., Pérez-Alvarez, L., Hernández-Olmos, S.L., Guerrero-Ramírez, G.L., Vilas-Vilela, J.L. 2020. Lignin-based hydrogels: Synthesis and applications. *Polymers*, **12**(1), 81.
37. Saito, T., Brown, R.H., Hunt, M.A., Pickel, D.L., Pickel, J.M., Messman, J.M., Baker, F.S., Keller, M., Naskar, A.K. 2012. Turning renewable resources into value-added polymer: development of lignin-based thermoplastic. *Green Chemistry*, **14**(12), 3295-3303.
38. Sannigrahi, P., Kim, D.H., Jung, S., Ragauskas, A. 2011. Pseudo-lignin and pretreatment chemistry. *Energy & Environmental Science*, **4**(4), 1306-1310.

39. Sathawong, S., Sridach, W., Techato, K.-a. 2018. Lignin: Isolation and preparing the lignin based hydrogel. *Journal of environmental chemical engineering*, **6**(5), 5879-5888.
40. Shen, X.-J., Chen, T., Wang, H.-M., Mei, Q., Yue, F., Sun, S., Wen, J.-L., Yuan, T.-Q., Sun, R.-C. 2020. Structural and morphological transformations of lignin macromolecules during bio-based deep eutectic solvent (DES) pretreatment. *ACS Sustainable Chemistry & Engineering*, **8**(5), 2130-2137.
41. Souza, L.O., Santos, I.A., de Carvalho Tavares, I.M., Sampaio, I.C.F., Dias, M.C., Tonoli, G.H.D., de Carvalho, E.E.N., de Barros Vilas Boas, E.V., Irfan, M., Bilal, M. 2022. Procurement and characterization of biodegradable films made from blends of eucalyptus, pine and cocoa bean shell nanocelluloses. *Waste and Biomass Valorization*, 1-13.
42. Sumerskii, I., Zweckmair, T., Hettegger, H., Zinovyev, G., Bacher, M., Rosenau, T., Potthast, A. 2017. A fast track for the accurate determination of methoxyl and ethoxyl groups in lignin. *RSC advances*, **7**(37), 22974-22982.
43. Taqi, A., Farcot, E., Robinson, J.P., Binner, E.R. 2020. Understanding microwave heating in biomass-solvent systems. *Chemical Engineering Journal*, **393**, 124741.
44. Tomé, L.I., Baião, V., da Silva, W., Brett, C.M. 2018. Deep eutectic solvents for the production and application of new materials. *Applied Materials Today*, **10**, 30-50.
45. Van Erven, G., de Visser, R., Merkx, D.W., Strolenberg, W., de Gijsel, P., Gruppen, H., Kabel, M.A. 2017. Quantification of lignin and its structural features in plant biomass using 13C lignin as internal standard for pyrolysis-GC-SIM-MS. *Analytical chemistry*, **89**(20), 10907-10916.
46. Wang, T.-P., Li, H., Yuan, J.-M., Li, W.-X., Li, K., Huang, Y.-B., Xiao, L.-P., Lu, Q. 2021. Structures and pyrolytic characteristics of organosolv lignins from typical softwood, hardwood and herbaceous biomass. *Industrial Crops and Products*, **171**, 113912.
47. Xia, Q., Chen, C., Yao, Y., Li, J., He, S., Zhou, Y., Li, T., Pan, X., Yao, Y., Hu, L. 2021. A strong, biodegradable and recyclable lignocellulosic bioplastic. *Nature Sustainability*, **4**(7), 627-635.
48. Xia, Q., Liu, Y., Meng, J., Cheng, W., Chen, W., Liu, S., Liu, Y., Li, J., Yu, H. 2018. Multiple hydrogen bond coordination in three-constituent deep eutectic solvents enhances lignin fractionation from biomass. *Green chemistry*, **20**(12), 2711-2721.
49. Zwilling, J.D., Jiang, X., Zambrano, F., Venditti, R.A., Jameel, H., Velev, O.D., Rojas, O.J., Gonzalez, R. 2021. Understanding lignin micro-and nanoparticle nucleation and growth in aqueous suspensions by solvent fractionation. *Green Chemistry*, **23**(2), 1001-1012.

	DES components	Respective molar ratio
1	PTSA-ChCl-EG	2:1:1
2	PTSA-ChCl-Gly	2:1:1
3	CA-ChCl-EG	2:1:1
4	CA-ChCl-EG	1:2:2
5	CA-Gly	1:4

Table 1: Five common DES studied in this work and their chemical components molar ratios.

#	Compound	Retention time (min)	Molecular weight (g/mol)	Main fragments m/z	Corresponding structural features
1	Phenol	25.91	94	94.0 | 66.1 | 65.1 | 95.1 | 40.1	H
2	o-Guaiacol	26.89	124	109.1 | 124.1 | 81.1 | 53.1 | 125.1	G
3	Phenol, 2-methyl-	27.23	108	108.1, 107.1, 79.1, 77.1, 90.0	H
4	Methylguaiacol	27.47	138	138.0 | 123.0 | 95.1 | 77.1 | 68.0	G
5	Phenol, 2,5-dimethyl-	27.86	122	122.1 | 107.1 | 121.0 | 77.1 | 91.0	H
6	Phenol, 4-methyl-	28.10	108	107.1 | 108.1 | 77.1 | 79.1 | 90.1	H
7	Phenol, 4-methoxy-3-methyl-	28.69	138	123.0 | 138.1 | 77.1 | 95.1 | 67.1	G
8	Phenol, 2,4-dimethyl-	29.34	122	122.1 | 107.0 | 121.1 | 77.0 | 91.1	H
9	Phenol, 2-methoxy-4-methyl-	29.49	138	138.1 | 123.0 | 95.1 | 67.1 | 77.1	G
10	Phenol, 2-ethyl-	30.29	122	107.0 | 122.1 | 77.1 | 108.1 | 121.1	H
11	Phenol, 5-methoxy-2,3-dimethyl-	31.01	152	152.1 | 137.1 | 121.0 | 109.1 | 91.1	G
12	Phenol, 3-ethyl-5-methyl-	31.38	136	121.1 | 136.1 | 77.1 | 91.1 | 151.0	H
13	Phenol, 4-ethyl-2-methoxy-	31.50	152	137.1 | 152.1 | 122.0 | 138.1 | 91.1	G
14	2-Methoxy-4-vinylphenol	32.86	164	150.1 | 135.1 | 107.1 | 77.1 | 151.1	G
15	Eugenol	33.38	164	164.1 | 149.1 | 131.0 | 103.1 | 77.1	G
16	Syringol	44.94	154	154.1 | 139.0 | 111.0 | 93.1 | 96.0	S
17	Phenol, 2-methoxy-4-(1-propenyl)-	34.62	164	164.1 | 149.1 | 77.1 | 103.1 | 131.0	G
18	trans-Isoeugenol	35.76	164	164.1 | 149.1 | 131.1 | 103.1 | 77.1	G
19	Vanillin	36.46	152	151.0 | 152.0 | 123.0 | 81.1 | 109.0	G
20	Guaiacylacetone	39.11	180	137.1 | 180.1 | 122.0 | 138.1 | 43.1	G

Table 2: Compounds identified in Pyrolysis-GC-MS and their corresponding structural features.

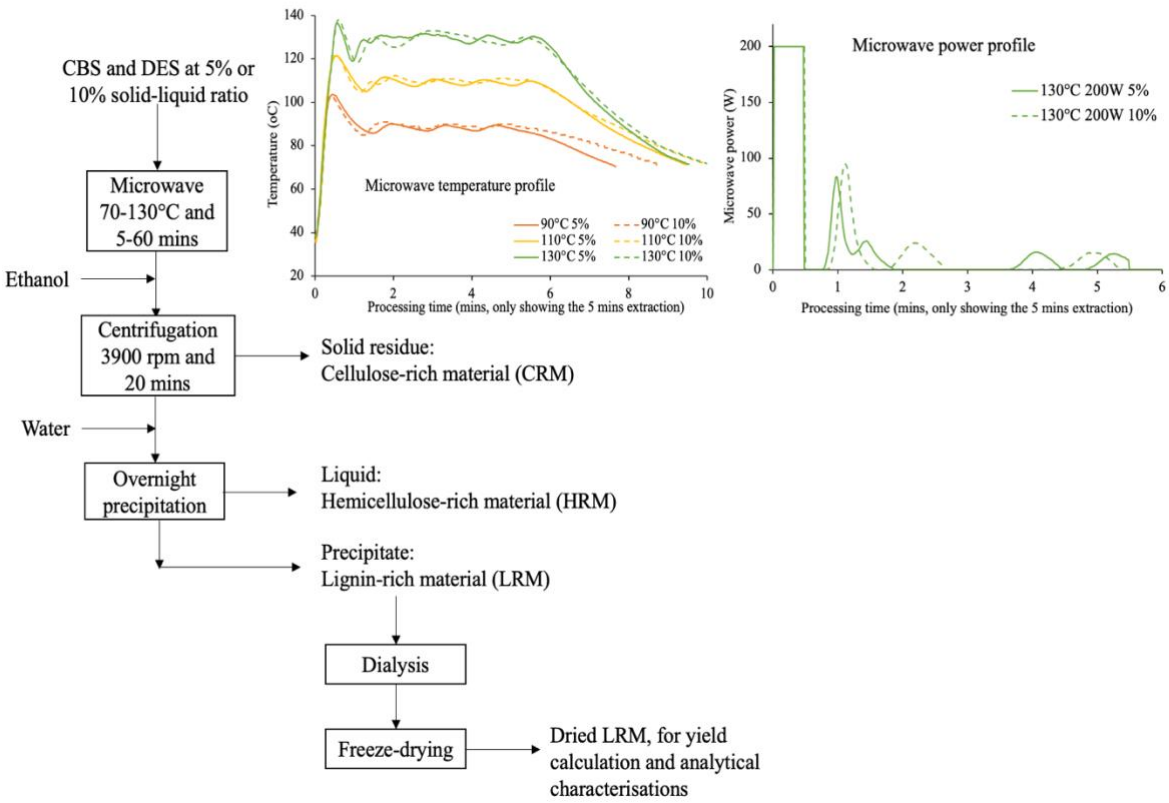


Figure 1: Schematic of the microwave-assisted extraction of lignin and the downstream processing. The microwave temperature and power profile of MAE at 90, 110 and 130°C temperature, 5% and 10% solid-liquid ratio and 5 mins hold time are also shown.

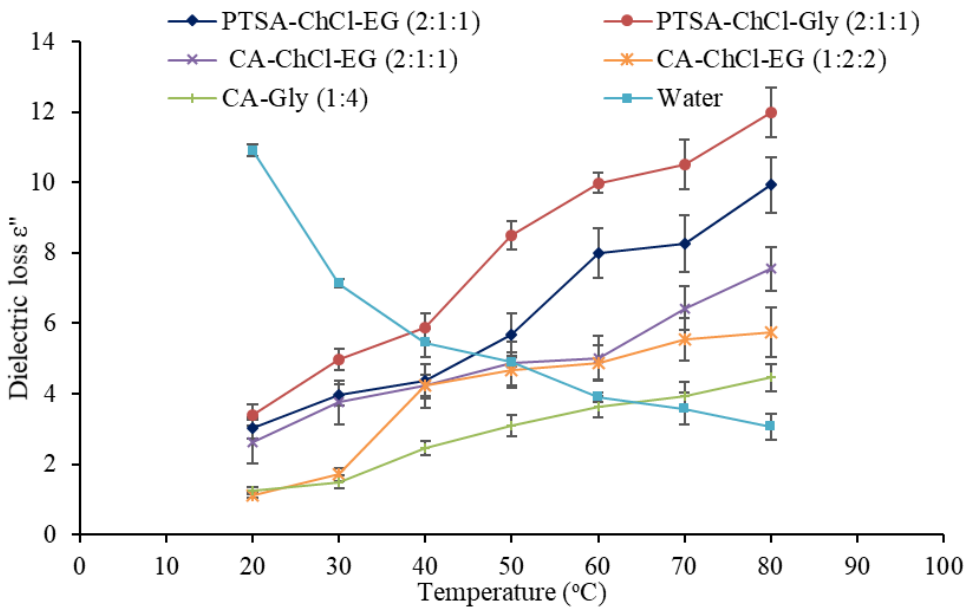


Figure 2: Dielectric loss factors of five DES and D.I. water between 20 and 80 °C and at 2.47 GHz. ChCl stands for Choline chloride, Gly for Glycerol, EG for Ethylene Glycol, PTSA for p-Toluenesulfonic acid, and CA for Citric Acid.

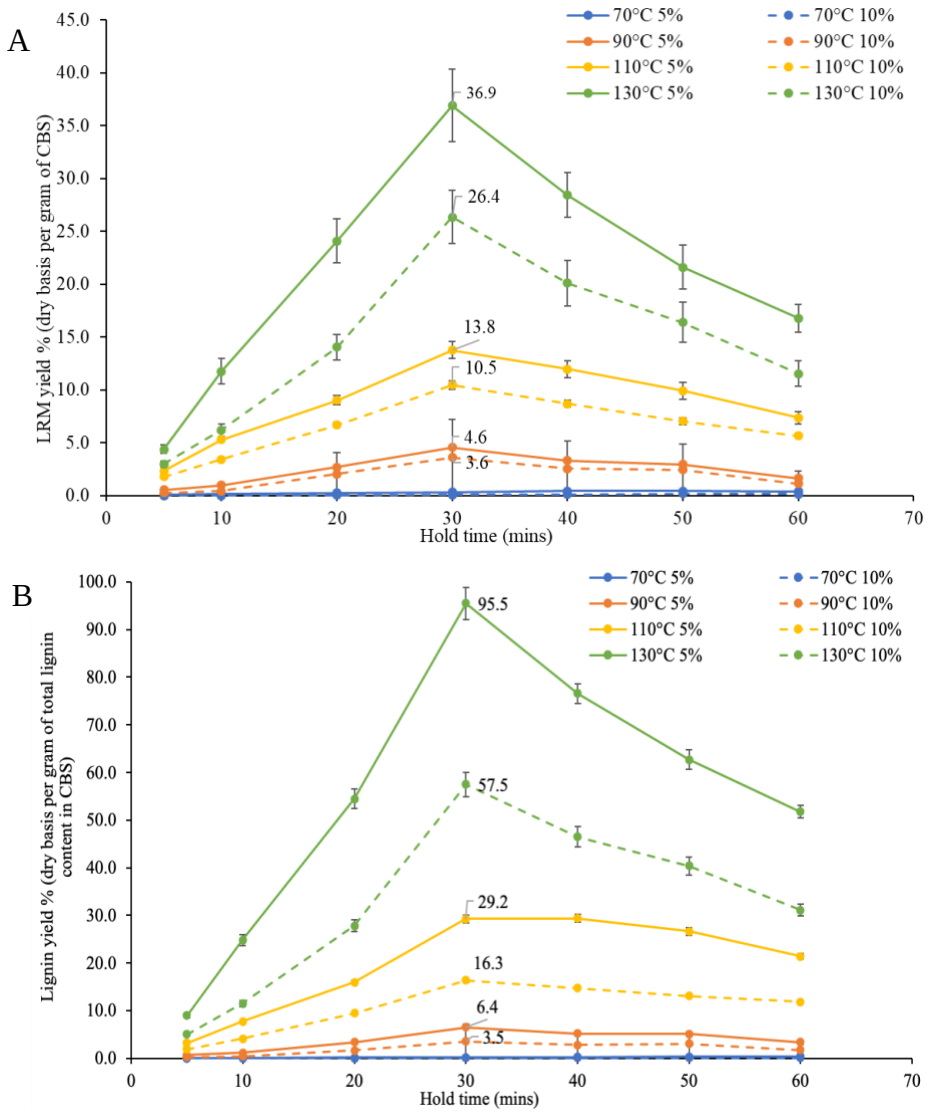


Figure 3: (A) LRM yield as % content of dry basis per gram of raw CBS and **(B)** Lignin yield as % content of dry basis per gram total lignin content in raw CBS, for extractions at 70-130 °C, 5-60 mins, 5 and 10% solid-liquid ratio.

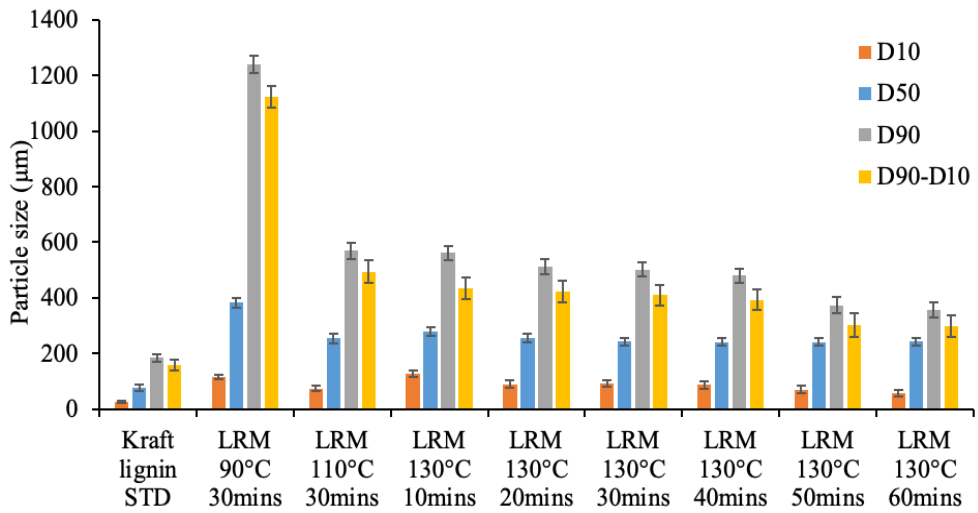


Figure 4: Particle sizing of Kraft lignin STD and LRMs extracted at 90 to 130 °C, 10 to 60 mins and 5% solid to liquid ratio.

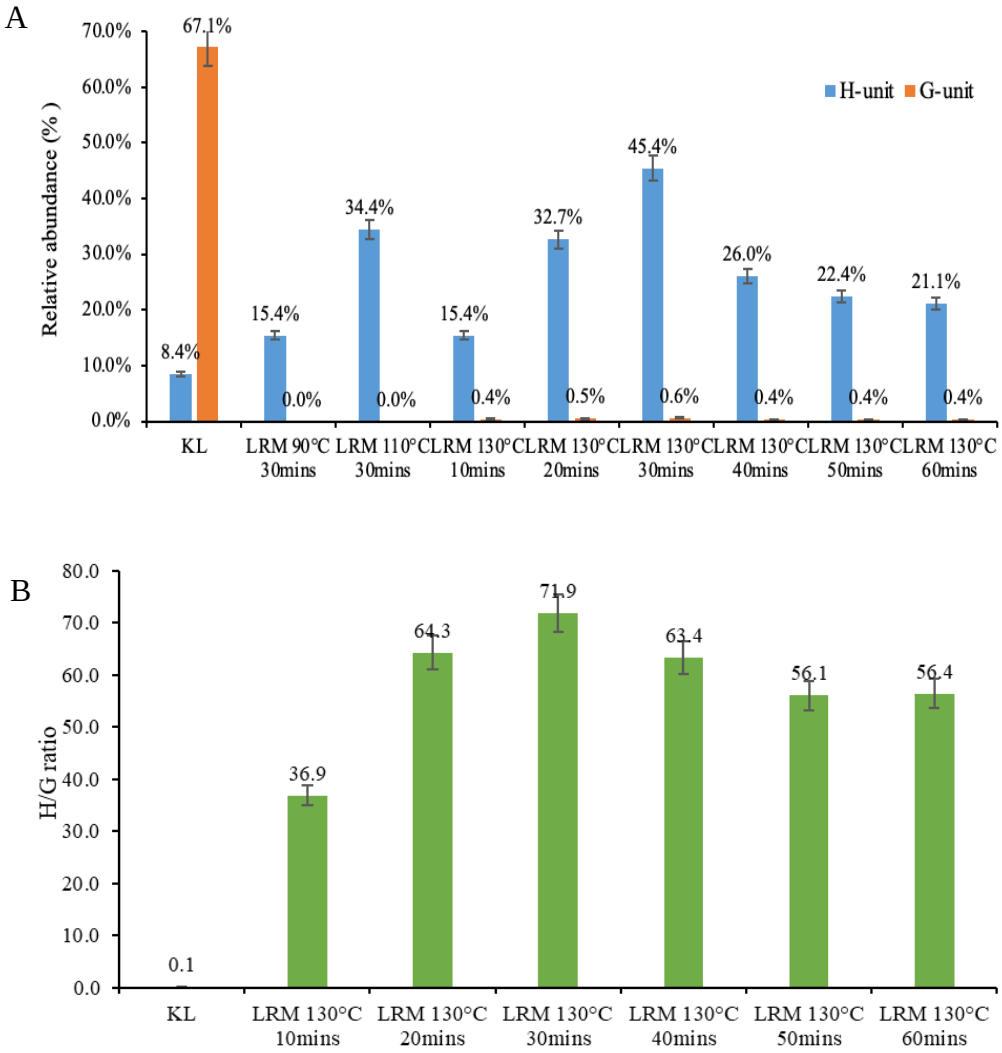


Figure 5: (A) Relative abundance of H- and G-unit in % and **(B)** H/G ratio in % for Kraft lignin STD and LRM extracted at 90-130 ºC, 60 mins and 5% solid-liquid ratio.