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1 **Manuscript submitted to *Bioresource Technology***

2

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

4

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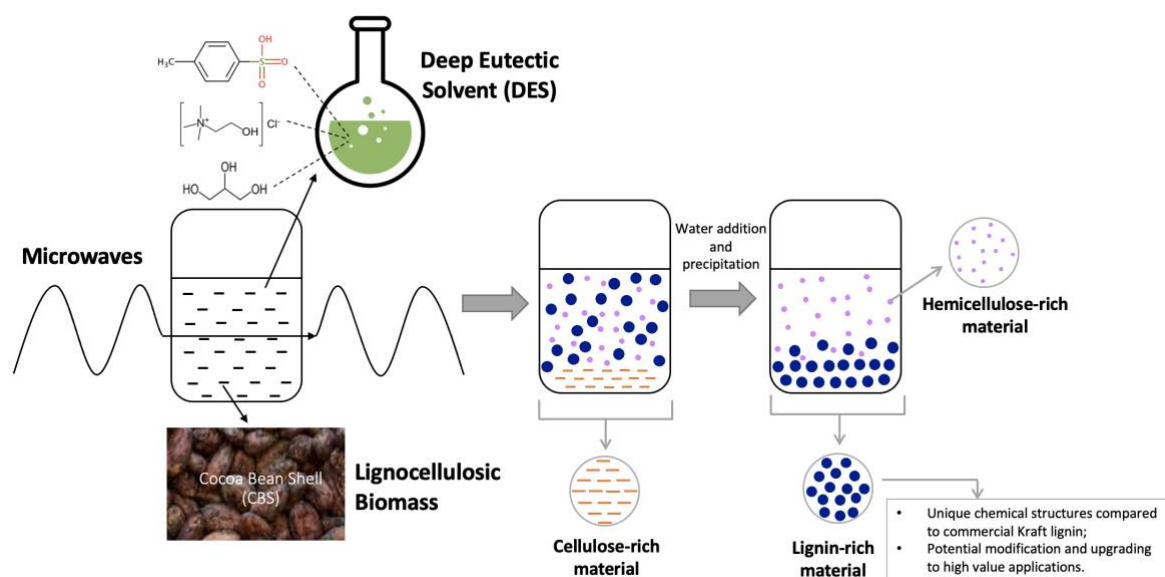
27 **Abstract:**

28

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

39

40 **Graphical abstract:**



41

42

43 **Keywords:**

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

46

47 1. Introduction

48
49 Lignin is the most abundant naturally occurring aromatic polymer and the second most abundant
50 polymer after cellulose. It is mainly found in the secondary cell wall of most terrestrial plants in the
51 approximate range of 15 to 40% of dry weight (Korányi et al., 2020). Lignin is comprised of three basic
52 phenylpropanoid monomers, namely p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) sub-units,
53 derived from p-coumaryl, coniferyl, and sinapyl alcoholic monolignols respectively, and with different
54 numbers of methoxy groups (none, one, and two) connected to the aromatic rings. The structure and
55 composition of lignin varies greatly by origin of the biomass; for example, softwood derived lignin is
56 constituted of approximately 90-95% G-units, hardwood derived lignin contains 25-50% of G- and 50-
57 75% of S-units (Korányi et al., 2020), while lignin in herbaceous biomass has relatively large quantity
58 of H-units compared to woody biomasses (Wang et al., 2021).

59
60 Lignin occurs widely as a co-product in multiple industrial processes, with global annual production
61 estimated to reach 225 million tons by 2030 (Haq et al., 2020). However, the current valorisation rate
62 of lignin is less than 2%, and most lignin is discarded as waste or utilised in low-value applications such
63 as combustion (Korányi et al., 2020). The pulp and paper industry is a major lignin producer; cellulose
64 from lignocellulosic biomass is utilised to make paper and lignin remains in the large volume of
65 wastewater causing huge environmental problems such as aquatic toxicity (Haq et al., 2020). Another
66 example is the production of second-generation bioethanol from biomass fermentation, which requires
67 pre-removal of large quantities of lignin present in the biomass, to avoid yield reduction (Haq et al.,
68 2020). Due to the presence of large quantities of lignin in industry, as well as the urgency to achieve a
69 more circular economy and find new renewable resources to replace fossil fuels, researchers have been
70 working to develop lignin into practically and commercially relevant precursors for a new range of
71 value-added chemicals. Emerging research has demonstrated the potential of lignin in several novel and
72 value-added applications in civil construction, food, packaging, environmental and pharmaceutical
73 realms, including bioplastics, bioadhesives, bioflocculants/heavy metal adsorbent, and hydrogels with
74 antimicrobial and antioxidant properties etc. (Ge & Li, 2018; Rico-García et al., 2020; Xia et al., 2021).

75
76 To utilise the versatility of lignin as a feedstock and to promote lignin-based novel applications, there
77 has been increasing interest in developing variable techniques for lignin recovery from biomass. Lignin
78 is commonly extracted using strong acids, alkalis and toxic organic solvents at elevated temperatures
79 and pressures or using expensive enzymes for prolonged processing times (see supplementary
80 materials). Pitfalls associated with these techniques are fragmented lignin with lower purity, poor
81 environmental credentials or low commercial practicability (Ning et al., 2021). Therefore, for the
82 advancement of novel lignin applications, there is an increasing need for novel lignin recovery methods.

83

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

97

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

107

108 Apart from the investigations on the use of different solvents, novel and sustainable heating methods
109 have also been explored, among which microwave heating has attracted the most interest. Rather than
110 relying on indirect conventional heating mechanisms by conduction, convection and radiation which
111 usually causes uneven heating, microwaves are electromagnetic waves that can penetrate the material
112 and heat the molecules directly through dipolar polarisation and ionic conduction heating mechanisms,
113 resulting in uniform heating throughout the bulk material without the need for direct contact (Foong et
114 al., 2022) and providing benefits such as reduced extract times and minimising degradation of bio-active
115 extracts (Mao et al., 2021). Microwave also offers unique selective heating mechanism by heating
116 different components in a heterogeneous system at different rates determined by the dielectric properties,
117 and it is thought that this can lead to a range of microwave-enhanced mass transfer effects (Taqi et al.,
118 2020), potentially including disruption of cells within the biomass resulting in higher extraction yields
119 and the ability to treat recalcitrant materials (Mao et al., 2019). During microwave-assisted biomass
120 extraction, the volumetric and selective heating also allow heat to be generated within the core of the

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

126
127 Several researchers have investigated the potential of combining the use of DES and Microwave-
128 Assisted Extraction (MAE). For example, Chen and Wan (2018) used a DES (choline chloride : lactic
129 acid (ChCl:LA)) and microwave irradiation at 800 W for only 45 seconds to remove 65%, 72% and 80%
130 lignin from *Miscanthus*, Switchgrass and Corn Stover, respectively, while retaining most of cellulose
131 in the biomasses. Similarly, using a DES (choline chloride : oxalic acid (ChCl:OA)) and 110 °C, Liu et
132 al. (2017) were able to achieve a similar extraction performance of lignin from Poplar wood and reduce
133 the lignin extraction time from 9 hours using conventional heating to 3 mins using microwave at 800
134 W. Although those literatures have shown the potential of microwave heating to accelerate DES-lignin
135 extraction, little is known about how different DES interact with microwaves, and how this could
136 enhance processing through accelerated extraction times and selective heating effects. Other aspects
137 also need further considerations when investigating the advantages and disadvantages of DES and MAE.
138 Firstly, González-Rivera et al. (2020) has shown that DES can be heated much faster under microwaves
139 than their individual components and traditional solvents, which makes it theoretically promising for
140 MAE of lignin. However, the heating ability of DES under microwave is often not quantified by
141 dielectric properties, nor the difference between different DES systems. Secondly, one of the major
142 disadvantages of DES over traditional solvents is their high viscosity, causing the heat and mass transfer
143 limitation during conventional heating (Patil & Rathod, 2023). However, this can be overcome by
144 microwaves, which is evident in the faster heating rate in MAE. Additionally, DESs (especially
145 carboxylic acid–ChCl-based DESs) are found thermally unstable, and their degradation will take place
146 at high temperatures or prolonged processing times (Anuchi et al., 2022). However, when combined
147 with microwave, the ability of DES for lignin extraction can be reinforced as MAE can usually achieve
148 the same extraction performance in lower temperatures or reduced the extraction time, minimising
149 thermal degradation of the DES and extracts (Mao et al., 2019).

150
151 Cocoa Bean Shells (CBS) are a major by-product of the chocolate industry, and were selected as the
152 focus of this study. The world production of cocoa beans approached 5.8 million tonnes in 2020
153 (FAOSTAT, 2020), of which CBS constitutes 10%-17%, producing a sizable volume of feedstock for
154 potential valorisation. The chemical composition of CBS mainly constitutes dietary fibres (i.e. cellulose,
155 hemicellulose, lignin and pectin), carbohydrates, protein, methylxantines (i.e. caffeine, theobromine,
156 and theophylline), fats, a large spectrum of phenolic compounds, minerals and vitamins (see
157 [supplementary materials](#)). CBS is traditionally considered as ‘waste’, being left to rot at the cocoa

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

168

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

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

183

184 **2. Materials and methods**

185

186 **2.1 Materials**

187

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

191

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

194 Sodium Hydroxide and commercial Kraft lignin (KL, $\geq 95\%$ w/w of dry basis) were purchased from
195 Sigma Aldrich, UK. Ethanol (absolute) was purchased from Scientific Laboratory Supplies, UK.
196 Deionised (D.I.) water was made by Milli-Q Plus water purification system (Merck KGaA, Germany).
197

198 **2.2 Preparation of Deep Eutectic Solvents (DES)**

199
200 Five DES previously reported for lignin extraction were prepared using the respective chemical
201 components in appropriate molar ratios listed in Table 1. The mixtures were heated to 50 °C using a
202 water bath for 30 mins with constant stirring at 600 rpm, until transparent and homogeneous solvents
203 were formed.
204

205 **2.3 Dielectric property measurement**

206
207 Dielectric property measurements were performed following the method from Mao et al. (2021) with
208 some modifications using an 85070E Dielectric Probe Kit (Agilent, USA). The dielectric loss factor
209 data was taken at 2.47 GHz, which is within 20 MHz of the MAE equipment used. Prepared DES were
210 heated on a hot plate from 20-80 °C with measurements taken at 10 °C intervals. The probe was
211 immersed into the sample solution. After each measurement, the probe was cleaned with D.I. water and
212 wiped to dry.
213

214 **2.4 Microwave-Assisted Extraction (MAE) of lignin**

215
216 Figure 1 shows a schematic of the extraction method and the downstream processes. MAE of lignin
217 was carried out using a Monowave 200 microwave (Anton Parr, UK) at 2.45 GHz. The CBS and the
218 DES were combined at 5% (1 grams CBS + 20 grams DES) or 10% (2 grams CBS + 20 grams DES)
219 solid-liquid ratio and loaded into the Monowave reaction tubes. The samples were heated to the set
220 temperature (70, 90, 110 and 130 °C) and held at this temperature for the required length of time (5-
221 60 min). A maximum incident power of 200 W was applied. Stirrer speed was set at 600 rpm. After the
222 hold time, compressed air was applied to cool the sample down to 70°C before the Monowave system
223 can be opened and samples can be taken out for down-streaming processes. The temperature and power
224 profile of MAE are also shown in Figure 1.
225

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

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

235

236 **2.5 Acetyl Bromide Soluble Lignin (ABSL) Assay**

237

238 The content of lignin in the raw CBS and LRM, also referred to as the lignin purity, was determined by
239 the Acetyl Bromide Soluble Lignin (ABSL) Assay. 100 mg of raw CBS or freeze-dried LRM was
240 dissolved in 4.0 mL of 25% acetyl bromide in glacial acetic acid. The mixture was held in a water bath
241 at 50 °C for 2 hours then cooled down in an ice bath. The volume of the mixture was made up to 16 mL
242 with glacial acetic acid and left to settle for 30 mins before centrifugation at 3900 rpm for 20 mins. An
243 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,
244 0.5 mL of 0.5 M hydroxylamine hydrochloride solution, and further diluted with 5.0 mL of glacial
245 acetic acid to a final volume of 10.0 mL. Solutions were measured with an UV-Vis spectrophotometer
246 (Cecil Instruments 1000 Series, UK) at the wavelength of 280 nm. Glacial acetic acid was used as the
247 reference. The lignin standard solution was made using commercial Kraft lignin (KL) in a concentration
248 range of 0-0.1 mg/mL and was treated the same way as above. The purity of LRM (% on dry basis),
249 referring to the content of lignin in LRM extracts, was calculated by:

$$250 \quad \% \text{ lignin purity in LRM} = \frac{\text{Dry weight of lignin in LRM by ABSL}}{\text{Dry weight of LRM}} \times 100\% \quad (1)$$

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

254

255 **2.6 Extraction yield calculation**

256

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

260

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

$$262 \quad \% \text{ yield of LRM} = \frac{\text{Dry weight of LRM}}{\text{Dry weight of CBS}} \times 100\% \quad (2)$$

263

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

266 % yield of *lignin* = $\frac{\text{Dry weight of lignin in LRM by ABSL}}{\text{Dry weight of total lignin in CBS by ABSL}} \times 100\%$ (3)

267 Dry weight of lignin in LRM by ABSL = Dry weight of LRM \times % lignin purity of LRM (4)

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

269

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

272

273 **2.7 Particle sizing measurements**

274

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

285

286 **2.8 FTIR analysis**

287

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

293

294 **2.9 Pyrolysis-GC-MS**

295

296 Pyrolysis was performed in a platinum filament pyrolysis instrument (CDS Analytical Pyroprobe 5000,
297 USA) coupled to a Gas Chromatograph - Mass Spectrometer (GC-MS, Agilent 5977 series, USA) with
298 a single quadrupole mass selective detector (MSD), using a fused silica capillary column (DB-1701, 60
299 $\text{m} \times 0.32 \text{ mm I.D.}$, 1 μm stationary phase thickness). Around 500 μg freeze-dried LRM or KL sample
300 was placed into a quartz tube (25 mm Length \times 1.9 mm I.D.) and placed into the pyrolysis chamber.
301 The pyrolysis was carried out at 550 $^{\circ}\text{C}$ for 10 s. Pyrolysis chamber was purged with helium as carrier

302 gas with a constant flow of 1.1 ml/min to transfer the pyrolysis products into the GC column. Injection
303 was performed in split mode with a ratio of 75:1. The GC oven was heated from 45 °C to 260 °C at
304 6 °C/min and held at 260 °C for 20 mins. The MS was operated by the electron impact ionization mode
305 at 70 eV and mass scan range m/z from 40 to 400.

306
307 Compounds were identified by comparing their peak retention time shown on the mass spectrum with
308 the National Institute of Standards and Technology (NIST) library, version 14. The area of each
309 resulting peak was integrated and then converted into relative abundance (RA, in %) by dividing the
310 peak area by the total area, as shown below. The total peak area was considered as 100%.

$$311 \text{ RA of each indentified compound} = \frac{\text{Peak area of the compound}}{\text{Total peak area}} \times 100\% \quad (5)$$

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

317

318 **3. Results and discussion**

319

320 **3.1 Selection of DES based on dielectric property measurement**

321

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

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

344

345 Overall, PTSA-ChCl-Gly (2:1:1) had the highest dielectric loss factor (from 3.4 at 20 °C to 12.0 at
346 80 °C) compared with other DES and D.I. water above 40 °C and can be assumed to be heated the
347 fastest under microwave heating. Therefore, this DES was selected as the study DES for lignin recovery
348 and used to investigate the effect of different processing conditions on lignin extraction performance in
349 terms of extract yield and structure.

350

351 **3.2 Lignin extraction yield**

352

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

356

357 The LRM yield results are shown in Figure 3A. Firstly, a higher extraction temperature achieved a
358 higher LRM yield. The LRM yield increased from negligible at 70 °C, to 4.6% for 5% solid-liquid ratio
359 at 90 °C, and tripled every 20 °C thereafter, up to a maximum of 36.9% at 130 °C and 5% solid-liquid
360 ratio. This may be because higher temperature leads to better cleavage of lignin from the cell wall matrix
361 and promotes its solubility following release from the cell wall matrix. Although the mechanism of
362 lignin cleavage in DES remains elusive, some researchers have proposed that in a DES rich in chloride
363 ions (Cl⁻) and under acidic conditions, it is due to the possible interaction of H-bonds from the DES and
364 hydroxyl in polysaccharides from the lignocellulose, which enables fractionation of lignin-
365 polysaccharide complex (Chen et al., 2020; Xia et al., 2018). Xia et al. (2018) reported the same effect
366 of temperature on lignin extraction, where the lignin yield increased from 15.34% to 20.37% when
367 temperature increased from 110 to 130 °C using a ChCl : glycerol : AlCl₃·6H₂O DES, which was
368 thought to be corresponding to the increased H-bond-accepting ability of the DES and thus better
369 fractionation of lignin-polysaccharide complex and facilitation of lignin extraction at elevated
370 temperature. The results also indicate that lignin is a very recalcitrant material and a high temperature
371 of 130 °C is essential for an efficient extraction when the study DES is used.

372

373 Secondly, the LRM yield was higher at a solid-liquid ratio of 5% compared to 10%, increasing from
374 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

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

381
382 Additionally, the optimum yield was achieved for all extraction temperatures and solid-liquid ratios at
383 30 mins. The optimum condition of LRM yield was found as 130 °C, 30 mins and 5% solid-liquid ratio.
384 At this condition, the LRM yield reached 36.9%, corresponding to 95.5% (see Figure 3B) of the total
385 lignin content of the studied CBS material. The total lignin content of the CBS was $27.5 \pm 1.6\%$ (ABSL
386 method), which is consistent with the value of 26% reported in literature (Chung et al., 2003; Panak
387 Balentić et al., 2018)), demonstrating a high level of extraction achieved. Furthermore, the LRM yield
388 started to decrease after the optimum condition, the results of which is consistent with the literature
389 (Kohli et al., 2020). Although the reason for the decrease in yield remains unclear, one proposed
390 explanation is the polymerisation and depolymerisation of pseudo-lignin, which has a structure
391 resembling lignin produced by cell wall polysaccharides and without significant contributions from
392 lignin in the biomass (Sannigrahi et al., 2011).

393
394 Another characteristic of DES is that it is not selective for lignin alone but would also contribute to the
395 extraction of other cell wall components such as hemicellulose and cellulose, although the effect on
396 different components is not at the same extent. Hemicellulose fraction dissolved in DES can be further
397 depolymerised into oligomers and monomers, while cellulose is often more recalcitrant and cannot be
398 easily dissolved or depolymerised in DES (Chen et al., 2020). The work of Chen et al. (2019) has also
399 reflected this and shown 30% lignin extraction alongside 31% xylan (the main constituent in
400 hemicellulose) and 1% cellulose when the same DES was used. Referring to these, it is highly possible
401 that our LRM extracts also contain hemicellulose, and this will be further discussed in Section 3.3.

402

403 **3.3 Extracts structural information**

404

405 **3.3.1 Particle sizing**

406

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

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

419
420 Secondly, a shorter extraction time produced higher extract particle sizes. When comparing extracts at
421 130 °C and 5% solid-liquid ratio, the shortest extraction time of 10 mins yielded extracts with the largest
422 D50 size of $279.1 \pm 15.7 \mu\text{m}$; however, the median sizes reduced to approximately $242.5 \pm 13.8 \mu\text{m}$ for
423 LRM obtained after 30 mins extraction time. Additionally, although the median size of LRM after 30
424 mins stayed the same, their D10 and D90 sizes decreased from 91.0 ± 11.7 and $501.4 \pm 21.9 \mu\text{m}$ to 58.7
425 ± 11.8 and $356.7 \pm 26.1 \mu\text{m}$ respectively from 30 mins to 60 mins extraction time, again suggesting that
426 a higher extract particle size can also result from shorter processing time. Furthermore, a shorter
427 extraction time is associated with a larger difference between D90 and D10 values (showing as D90 -
428 D10 in Figure 4) indicating a wider particle size distribution (which can be correlated to polydispersity)
429 and thus inhomogeneous extracts. This result is in accordance with the work of Provost et al. (2022),
430 where the softwood lignin extracts obtained at 60 °C presented the broadest distribution with a
431 polydispersity index value (PDI) of 1.53, yet showed a narrower distribution with PDI between 1.03
432 and 1.31 when the extraction temperature increased to 80 °C with the same DES (Choline Chloride:
433 Lactic Acid) used. These results have shown that shorter extraction times have the same effect as lower
434 temperature as discussed earlier. This causes less molecular depolymerisation to occur and thus results
435 in the recovery of lignin with larger extract particle sizes.

436

437 **3.3.2 FTIR spectra**

438

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

447

448 Firstly, both the commercial Kraft lignin and the extracted LRMs contained components other than
449 lignin, although the type and amount of the components varied. The Kraft lignin did not show a peak at
450 1732 cm^{-1} , which would have indicated the presence of hemicellulose as a peak at this wavenumber
451 can be attributed to the C=O stretching from hemicellulose. Meanwhile, all tested LRMs had this peak,
452 suggesting the presence of hemicellulose in the extracts. LRM yielded at higher temperature showed
453 less transmittance at this peak compared to lower temperature implying that the level of hemicellulose
454 in the extracts decreased with increasing extraction temperature. The extract obtained at $130\text{ }^{\circ}\text{C}$ has
455 shown high lignin purity and low hemicellulose content, indicating successful fractionation of the
456 biomass at this temperature. Another peak at wavenumber 812 cm^{-1} representing the C-H stretching in
457 the glucose ring from cellulose was clearly shown in the Kraft lignin standard. However, only the LRM
458 extracted at the highest temperature of $130\text{ }^{\circ}\text{C}$ had some but a lower amount of cellulose content than
459 the Kraft lignin standard; the LRM extracted at lower temperatures contained negligible cellulose
460 content. This difference can be attributed to the varied production methods; Kraft lignin is commercially
461 produced by treating woody lignocellulosic biomass at higher temperature, in the range of $150\text{--}170\text{ }^{\circ}\text{C}$,
462 than applied in this study and in the presence of sodium hydroxide (NaOH) and sodium sulfide (Na_2S)
463 (Chio et al., 2019). The presented results indicate that the extracting temperature had a significant
464 influence on the purity of the lignin product, and that a low temperature may lead to high hemicellulose
465 content and a temperature above $130\text{ }^{\circ}\text{C}$ may yield high cellulose contents, irrespective of the extraction
466 solvents.

467
468 Secondly, the structure of the lignin in the commercial sample and the extracted LRMs was different.
469 The peak at 1507 cm^{-1} represents the aromatic skeletal stretching in the aromatic ring in lignin and the
470 result suggested that the Kraft lignin included more aromatic ring content than the LRMs. Lignin G-
471 units are identified by a peak at 1208 cm^{-1} presented in the spectrum for the Kraft lignin but absent in
472 the LRM spectra. The lignin sub-unit structure difference between the commercial and the extracted
473 material was further studied by Pyrolysis-GC-MS, see Section 3.3.3. Furthermore, absorbances at
474 2923 cm^{-1} and 2852 cm^{-1} are attributed to C-H stretching in alkyl chains adjacent to an aromatic centre,
475 and CH variation in methoxyl groups respectively in the lignin side chains. Both the Kraft lignin and
476 the LRMs show peaks at these two wavenumbers suggesting the lignin in all samples was at least
477 partially methylated, which has previously been observed (Provost et al., 2022). However, these peaks
478 were higher for the LRMs implying a greater degree of methylation compared to the Kraft lignin. When
479 comparing LRM extracted at different temperatures, LRM at higher temperatures had gradually reduced
480 absorption peaks of the bonds at their respective corresponding location mentioned above compared to
481 the LRM at lower temperature, indicating that some methoxyls may be degraded from the aromatic ring
482 during the thermal process at higher temperatures (Liu et al., 2014).

483

484 3.3.3 Pyrolysis-GC-MS

485

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

493

494 Kraft lignin contained primarily G-unit of 67.1% with 8.4% H-unit; while LRM obtained in this work
495 had a negligible amount of G-units but high H-units percentage varying from 15.4% to 45.4%. This
496 made the H/G sub-unit ratio in LRM reached much higher values ranging from 36.9% to 71.9%,
497 compared to a low ratio of 0.1% in Kraft lignin. This is because the sources of the Kraft lignin and LRM
498 were different and thus the structure and composition of lignin vary greatly among the origin of the
499 different biomass. Kraft lignin is produced from coniferous (soft) wood, which explains why the
500 primary unit is G-unit (Korányi et al., 2020). LRM were extracted from Cocoa Bean Shells (CBS),
501 which had similar lignin structure as herbaceous biomass, and therefore has a relatively high H-unit
502 content compared to wood lignin (Wang et al., 2021). Similar results were reported by Happs et al.
503 (2021) for Kraft lignin and Provost et al. (2022) for herbaceous biomass (Brewer's Spent Grain) using
504 DES.

505

506 Extraction conditions also have great influence on the sub-unit contents in the LRM. Firstly, with
507 extraction temperatures increased from 90 to 130 °C, an increased fraction of H-units from 15.4% to
508 45.4% was recovered from lignin, showing that H-units extraction is favored at higher extraction
509 temperatures. It also worth noting that S-units were not determined in both Kraft lignin and LRM in
510 this study, the same phenomenon was also reported in Provost et al. (2022) when using NMR to
511 characterise the structure of their lignin extracts extracted using choline chloride: lactic acid DES
512 (ChCl:LA) at 60 °C and 80 °C. The possible reason, as proposed by Alvarez-Vasco et al. (2016), might
513 be that H- and G-units from the lignin products extracted by DES between 80 to 140 °C are more prone
514 to determinations than their S-units, suggesting the extraction of S-units would require a higher
515 extracting temperature during DES treatment. Additionally, when comparing LRM with different
516 extraction times, the optimum H-unit content and therefore the highest H/G ratio of 71.9% was both
517 achieved at conditions of 30 mins and 130 °C, which was also the condition for optimum lignin yield.

518

519 3.4 Implications and future work

520

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

535
536 The effect of various extraction conditions including extraction temperature (70-130 °C), time (5-60
537 mins), and solid-liquid ratio (5 and 10%) on the lignin yield and structure were investigated. Lignin
538 product yield was favoured at higher extraction temperatures and lower solid-liquid ratio, with the
539 optimum yield of 95.5% lignin from the CBS recovered at 130 °C and 30 mins. The lignin extraction
540 time and temperature ranges applied here are considerably lower than those reported for other solvents
541 in literature, where 160-200 °C and more than 2 hours are often required to obtain a lignin yield above
542 60% of the total lignin content in the studied biomass when alkali or organic solvents are used (Chio et
543 al., 2019). Though the process optimisation has been performed for this study, to achieve a more
544 comprehensive understanding on the potentials of MAE and DES, more experimental work needs to be
545 done to systematically compare a) the use of microwave and conventional heating to elucidate any
546 unique mass transfer effects caused by microwave selective heating that could be exploited to improve
547 the extraction process, b) the use of different DES, alkali (as in Kraft processing) and organic solvents,
548 c) the difference between lignin extracted from CBS and other lignin-rich secondary biomasses such as
549 food, agricultural and forestry co-products (e.g. woody material such as soft and hardwood, other
550 herbaceous biomasses such as wheat straw and brewer's spent grain). Additionally, the fundamental
551 mechanism of the decrease in yield after optimum condition is not studied in this manuscript and further
552 work is required to elucidate this.

553
554 The physicochemical structures of DES-extracted lignin are characterised and compared with
555 commercial Kraft lignin (KL). Firstly, DES-extracted lignin exhibited larger particle sizes (242.5 µm
556 D50 size) and structural diversity (410.4 µm D90-D10 size, for lignin extracted at 130 °C and 30 mins)
557 compared with KL (77.2 µm and 157.9 µm respectively). Lower extraction temperature and shorter

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

577

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

585

586 **4. Conclusions**

587

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

595

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

597

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	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

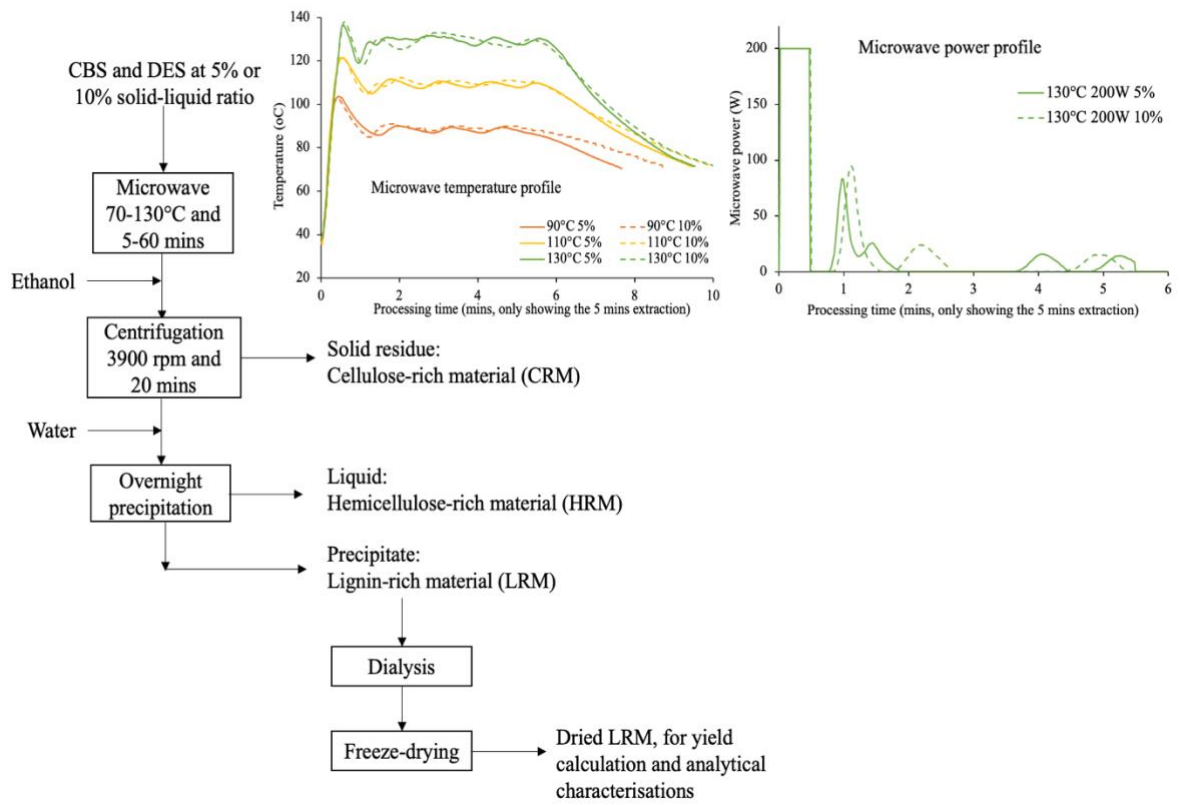
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Table 1: Five common DES studied in this work and their chemical components molar ratios.

#	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

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



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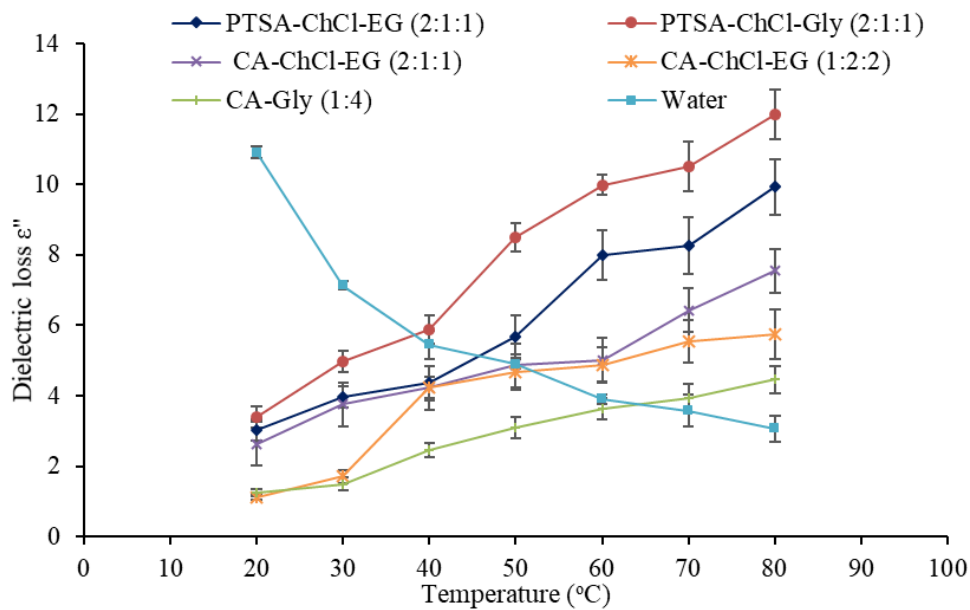
Figure 1: Schematic of the microwave-assisted extraction of lignin and the downstream processing.

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The microwave temperature and power profile of MAE at 90, 110 and 130°C temperature, 5% and

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10% solid-liquid ratio and 5 mins hold time are also shown.



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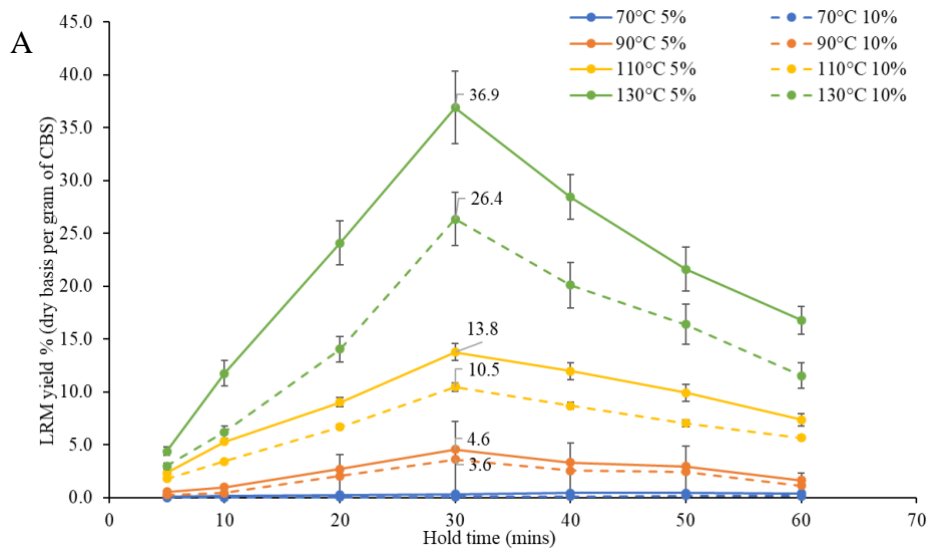
745 **Figure 2:** Dielectric loss factors of five DES and D.I. water between 20 and 80 °C and at 2.47 GHz.

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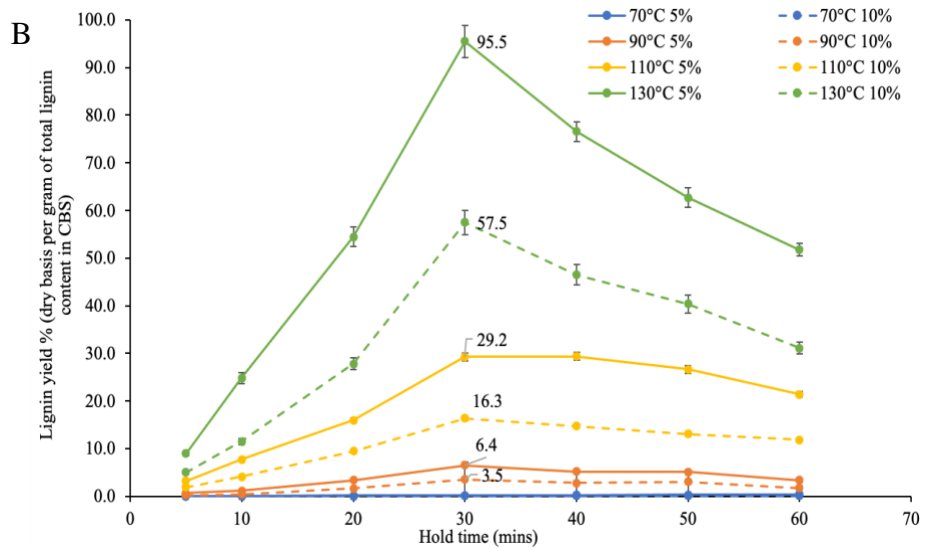
ChCl stands for Choline chloride, Gly for Glycerol, EG for Ethylene Glycol, PTSA for p-

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Toluenesulfonic acid, and CA for Citric Acid.

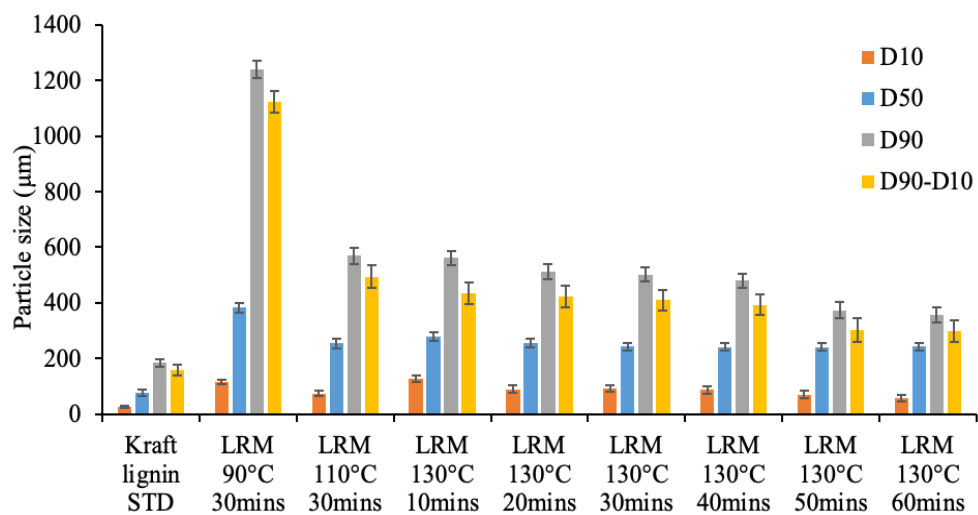


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750 **Figure 3:** (A) LRM yield as % content of dry basis per gram of raw CBS and (B) Lignin yield as %
 751 content of dry basis per gram total lignin content in raw CBS, for extractions at 70-130 °C, 5-60 mins,
 752 5 and 10% solid-liquid ratio.



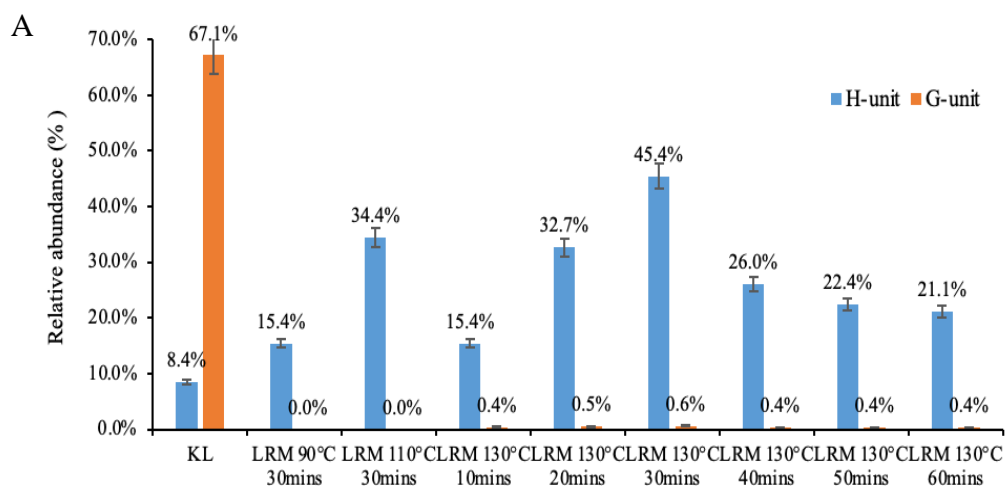
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754 **Figure 4:** Particle sizing of Kraft lignin STD and LRMs extracted at 90 to 130 °C, 10 to 60 mins and

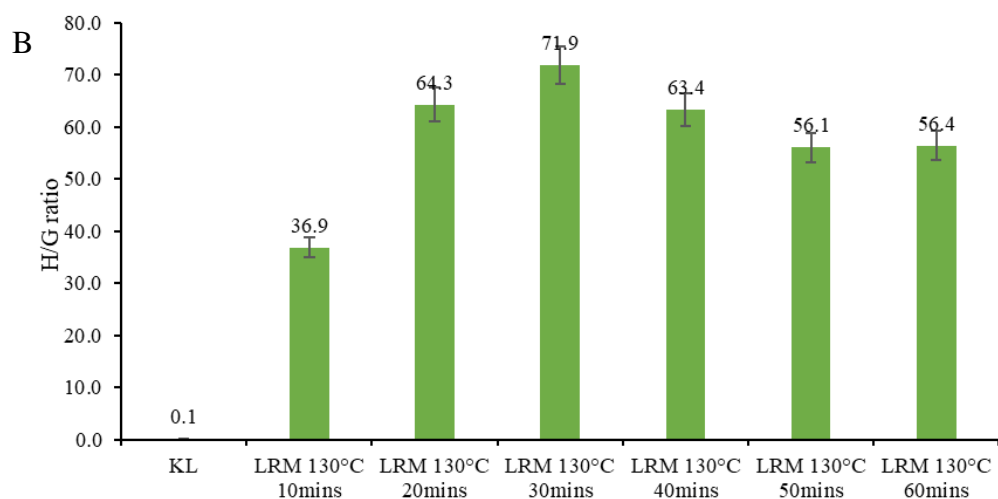
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5% solid to liquid ratio.

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759 **Figure 5: (A)** Relative abundance of H- and G-unit in % and **(B)** H/G ratio in % for Kraft lignin STD

760 and LRM extracted at 90-130 °C, 60 mins and 5% solid-liquid ratio.