

1 **Furfural, 5-HMF, acid-soluble lignin and sugar contents in *C. ladanifer***
2 **and *E. arborea* lignocellulosic biomass hydrolysates obtained from**
3 **microwave-assisted treatments in different solvents**

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15 **Abstract**

16 *Cistus ladanifer* L. and *Erica arborea* L. are the two most representative shrub species from
17 the Iberian Peninsula. With a view to their valorization, their biomass hydrolysate components,
18 obtained from microwave-assisted treatments with choline chloride/urea - HNO₃ 10 %, *N,N*-
19 dimethylacetamide/NaHCO₃ and *N,N*-dimethylacetamide/CH₃OK as solvents, have been
20 measured using a spectrophotometric method. Concentrations of furfural and 5-
21 (hydroxymethyl)furfural (5-HMF) in the filtrate have been determined after reduction with
22 NaBH₄. The production of total sugars, reducing sugars and non-reducing sugars contents has
23 also been assessed. The obtained results support the choice of MW-assisted choline
24 chloride/urea deep eutectic solvent in acid media as the preferred method (over the polar aprotic
25 solvent-based solvents) for the extraction of lignin, furfural, 5-HMF and sugars from *C.*
26 *ladanifer* and *E. arborea* biomass, attaining the best production yields for 60 min exposure
27 times. Another is the case if the aim of the treatments is to recovery sugars from both shrubs for

28 subsequent enzymatic saccharification: the very low 5-HMF contents resulting from the
29 dimethylacetamide systems (especially is association with CH₃OK) make them highly
30 advantageous as compared to the traditional method using NaOH.

31

32 **Keywords:** deep eutectic solvents; furan compounds; hydrolysis; microwave; polar aprotic
33 solvents; sugars.

34

35 **1. Introduction**

36 Lignin, interlaced with cellulose and hemicellulose, forms a complex crystal structure called
37 lignocellulose that provides support and protection to plant cells [1]. This matrix is difficult to
38 degrade, requiring treatments that break down its structure, hydrolyze the hemicellulose and
39 increase the exposed surface to favor the enzymatic hydrolysis of cellulose [2]. For this purpose,
40 different approaches may be used: physical processes, such as grinding or heating; chemical
41 methods, such as the addition of acids or bases; physical-chemical treatments, such as self-
42 hydrolysis or thermo-hydrolysis; and biological ones, such as the use of enzymes capable of
43 degrading lignin (ligninases or lignin-modifying enzymes, LMEs).

44 In conventional biomass treatments, thermochemical pretreatments are generally carried out
45 at high temperatures or high operating pressures in order to achieve high cellulose conversion.
46 An alternative to conventional heating is the application of microwave radiation [3-5], in which
47 the direct contact between the product and the electromagnetic field generated by the
48 microwaves results in a volumetric heating that causes an instantaneous temperature increase [6,
49 7], resulting in an acceleration of the process and higher yields under milder reaction conditions
50 with significant energy-savings [8].

51 The composition of the liquid phase of the treatments includes organic acids (mainly acetic
52 acid, formic acid and levulinic acid), furan derivatives (2-furfuraldehyde, furfural and 5-
53 (hydroxymethyl)-2-furaldehyde), and phenolic compounds (mainly coumaric acid,
54 syringaldehyde and vanillin) [9, 10]. Under acidic conditions, and especially at high

55 temperatures, furfural is readily produced from pentoses and 5-HMF is formed from hexoses.
56 Because both furfural and 5-HMF are formed from carbohydrates, they interfere with the
57 accuracy of sugar analysis of any biomass materials. Furthermore, both are harmful to the
58 fermentation of sugars if their concentrations exceed certain thresholds [11].

59 In the study presented herein, the suitability of various MW-assisted treatments in different
60 eco-friendly reaction media has been investigated with a view to breaking the intricate structure
61 of the lignocellulosic biomass obtained from two Mediterranean shrubs (viz. *C. ladanifer* and *E.*
62 *arborea*). One of the proposed treatments involves an innovative solvent category: the so-called
63 deep eutectic solvents (DESs), which consist of a hydrogen bond donor and a hydrogen bond
64 acceptor, associated with each other by means of hydrogen bond interactions, resulting in a
65 eutectic mixture with a melting temperature much lower than that of its constituents. DESs have
66 advantages over conventional ionic liquids (ILs), characterized by the formation of strong ionic
67 bonds, since the later are more expensive and toxic [12]. The other two assayed solvents have
68 been mixtures of a polar aprotic solvent (*N,N*-dimethylacetamide, DMAc) with weak and strong
69 bases (namely sodium bicarbonate (NaHCO_3) and potassium methoxide (CH_3OK),
70 respectively). Both categories of solvents can be used as environmentally friendly replacements
71 of conventional solvents and processes with a view to optimizing biorefineries, aiming at a
72 greener and more sustainable industry. The efficiencies of these three reaction media (a DES
73 mixture of choline chloride:urea and HNO_3 10 %, DMAc/ NaHCO_3 and DMAc/ CH_3OK) have
74 been compared in terms of lignin, furfural, 5-HMF and sugars extraction.

75

76 **2. Materials and methods**

77 **2.1. Samples and reagents**

78 The study was carried out on a plot located in the municipality of Ayoó de Vidriales (42° 07'
79 10" N, 6° 06' 59" W), in the province of Zamora, Castilla y Leon, Spain. The chosen area (>1.2
80 ha) is a mixed shrubland in which the dominant shrub species are *Erica arborea* L. subsp.
81 *angustifolius* (Daveau) Sennen & Pau, and *Cistus ladanifer* L. cultivar 'Spanish Lime'. Details

82 on the sampling procedure, analogous to that described by Ruiz-Peinado, *et al.* [13], have been
83 reported in a previous paper [14]. There were no size fractionation processes, which are
84 common in annual and perennial species. Selected samples corresponded to healthy individuals
85 and featured similar characteristics to the rest of the population.

86 Samples of biomass (mainly from the stem) were dried in a stove and crushed in a knife mill.
87 Their chemical composition (elemental analysis, summative constituent analysis, and moisture
88 content) can be found in Table 1:

89
90 [Table 1 here]
91

92 Furfural (CAS No. 98-01-1), 5-HMF (CAS No. 67-47-0), lignin (CAS No. 8068-05-1) and
93 D-(+)-glucose analytical standards (CAS No. 50-99-7) were purchased from Sigma-Aldrich
94 Quimica SL (Madrid, Spain). The standard solutions were prepared with deionized water.
95 Sodium borohydride (CAS No. 16940-66-2), 3-amino-5-nitrosalicylic acid (DNS, CAS No.
96 831-51-6), phenol (CAS No. 108-95-2), choline chloride (ChCl, CAS No. 67-48-1), urea (CAS
97 No. 57-13-6), titanium dioxide (CAS No. 13463-67-7), *N,N'*-dimethylacetamide (DMAc, CAS
98 No. 127-19-5), sodium bicarbonate (CAS No. 144-55-8), potassium methoxide (CAS No. 865-
99 33-8), sodium hydroxide (CAS No. 1310-73-2) and potassium sodium tartrate (CAS No. 6381-
100 59-5) were also supplied by Sigma Aldrich.

101

102 **2.2. Methods**

103 2.2.1. *Microwave-assisted deep eutectic solvent*

104 A deep eutectic solvent system based on choline chloride-urea (Figure 1) was assessed for
105 the hydrolysis of *C. ladanifer* and *E. arborea* biomass. Choline chloride/urea DES was prepared
106 by stirring the mixture of choline chloride and urea (mole ratio 1:2) at 80 °C until a
107 homogeneous colorless liquid was formed, which was then stored in a vacuum dryer.
108 Subsequently, biomass samples (200 mg) were treated by a mixture (1 cm³) of choline

109 chloride/urea and HNO₃ 10 %, with TiO₂ (20 mg) as a catalyst, in a microwave digestion system
110 –a Milestone (Soriso, BG, Italy) Ethos-One microwave oven equipped with a magnetic stirrer
111 system– at 120 °C for an “effective time” (isothermal treatment time) between 1 and of 60 min
112 (viz., 1, 5, 10, 20, 30, 40, 50 and 60 min), plus the heating and cooling ramps, which also
113 contributed to the thermal budget. The heating up to 120 °C started with a ramp set to 19
114 °C·min⁻¹ during the first 5 min, followed by a second ramp at a rate of 2.5 °C·min⁻¹ for 10 min.
115 The cooling down to room temperature took 25 minutes, at a rate of ~4.8 °C·min⁻¹. The DES
116 was finally removed by washing with water and was recovered by crystallization.

117

118

[Figure 1 here]

119

120 2.2.2. Microwave-assisted DMAc-sodium bicarbonate polar aprotic solvent

121 Alternatively to the DES-based method, 8 cm³ of a colorless, water-miscible, high boiling
122 liquid –viz. *N,N*'-dimethylacetamide (DMAc) with formula CH₃C(O)N(CH₃)₂– was used, in the
123 presence of 40 mg of sodium hydrogen carbonate (NaHCO₃), as a treatment agent for *C.*
124 *ladanifer* and *E. arborea* wooden samples (200 mg of biomass samples). The same procedure
125 explained above for the microwave-assisted DES treatment was followed for the polar aprotic
126 solvent-based treatment. DMAc was removed by washing with water and recovered by
127 distillation.

128

129 2.2.3. Microwave-assisted DMAc-potassium methoxide system

130 The third approach investigated for the hydrolytic treatment of *C. ladanifer* and *E. arborea*
131 biomass (200 mg) was based on a mixture of 8 cm³ of DMAc with 40 mg of potassium
132 methoxide (commonly used as a catalyst for transesterification in the production of biodiesel).
133 The solution was then treated as in the previously discussed methods.

134

135 2.2.4. Alkaline treatment

136 Solutions were prepared with 200 mg of each sample and 2 cm³ of NaOH (4 kg m⁻³), which
137 were stirred for 24 h. From these solutions, 0.3 cm³ of each sample were isolated and then
138 diluted to 25 cm³ (to keep the concentration within the spectrophotometer measurement range
139 and to avoid absorption flattening due to saturation). When necessary, HCl was used to keep a
140 neutral pH.

141

142 2.2.5. Acid-soluble lignin, furfural and 5-HMF contents

143 The acid-soluble lignin (ASL), furfural and 5-(hydroxymethyl)-furfural contents were
144 determined according to the methodology proposed by Chi, *et al.* [15], based on the
145 measurement of their respective maximum absorbance at 205 nm, 277 nm and 285 nm, and
146 which makes use of the effect of the reduction with borohydride on the furfural and 5-HMF
147 maxima mentioned above. For these latter two chemical species, their initial absorbance in the
148 UV-vis spectrum was measured and, after the addition of 30 mg of sodium borohydride to
149 eliminate the interference of furanic compounds (followed, after 5 min, by the addition of a
150 small amount of HCl) [16], absorbance measurements were repeated. Their associated
151 absorption maxima at 277 and 285 nm completely disappeared upon reduction with NaBH₄.
152 Therefore, the furfural and 5-HMF contents could be readily calculated from the absorbance
153 difference before and after reduction (ΔA_R) at their respective wavelengths.

154 All determinations were performed with three replications, except for the kinetic studies, and
155 all results are in average.

156

157 2.2.6. Sugars content

158 The quantification of reducing sugars was conducted according to Miller [17], using DNS as
159 the most specific reagent in a solution containing sodium hydroxide and potassium sodium
160 tartrate. The solution was prepared by mixing 0.8 g of NaOH, 15 g of sodium potassium tartrate
161 and 0.5 g of DNS, completing up to 50 cm³ with distilled water. To ensure the homogeneity of
162 the mixture, it was boiled for 5 minutes. It was then cooled with water and ice, 5 cm³ of water

163 were added to compensate for the evaporated volume, and it was allowed to rest for 15 min.
164 With this solution, which will be referred to as 'DNS', 1:1 mixtures with the samples or the
165 standard solutions to be analyzed were prepared (0.5 cm³ of DNS and 0.5 cm³ of either the
166 sample or the standard solution). The determination of reducing sugars in these mixtures was
167 conducted by measuring their absorbance at 540 nm.

168 The total sugars determination was carried out in agreement with the method proposed by
169 DuBois, *et al.* [18]. This method is usually called 'phenol-sulfuric acid method' because in the
170 preparation of the measuring solutions, 1-2 cm³ of sample, 1 cm³ of phenol (5 %) and 5 cm³ of
171 concentrated sulfuric acid (95.5 %) are mixed in the test tubes. The test tubes containing these
172 solutions were placed in a rack which was kept in a thermostatic bath, between 25 and 30 °C,
173 for 10-15 min. Glucose at various concentrations was used as a standard. The
174 spectrophotometric measurement of the total sugars was carried out at 490 nm, that is, at the
175 wavelength at which hexoses and their methylated derivatives exhibit their maximum
176 absorption. Non-reducing sugars content was calculated by difference between the total sugars
177 and the reducing sugars percentages.

178 All determinations were performed in triplicate biological replications, except for the kinetic
179 studies, and all results are in average.

180

181 2.2.7. Calibration curves

182 In order to obtain the calibration curves for each component under study (shown in Figure
183 2), dissolutions with different concentrations of the analytical standards used as a reference (*viz.*
184 furfural, lignin, 5-HMF and glucose) were prepared. Absorption values for increasing
185 concentrations of the analytical standards were plotted and data was fitted with a straight line, in
186 agreement with Beer's Law.

187 Apropos of ASL, furfural and 5-HMF, excellent linear relationships (Eq. 1-3) were obtained
188 at their three respective wavelengths (at $\lambda=280$ nm for ASL, at $\lambda=277$ nm for furfural and at
189 $\lambda=285$ nm for 5-HMF), with Pearson coefficients (R^2 values) above 0.95 in all cases.

190 $y_{ASL} = 109.11x + 0.0841; R^2 = 0.9802$ Eq. 1

191 $y_F = 270.76x + 0.2236; R^2 = 0.9534$ Eq. 2

192 $y_{HMF} = 10.56x + 0.017; R^2 = 0.9921$ Eq. 3

193 As regards the calibration curves for glucose (depicted in Figure 2b), the equation of the
194 calibration curve of total sugars (TS) (Eq. 4) was built by applying the methodology proposed
195 by DuBois, *et al.* [18], measuring the absorbance at 490 nm. On the other hand, the method by
196 Miller [17] was used for the calibration for reducing sugars (RS) (Eq. 5), measuring the
197 absorbance at 540 nm. R^2 values were close to 1. Eq. 6 for non-reducing sugars (NRS) is the
198 difference between the calibration curves of total and reducing sugars.

199 $y_{TS} = 5.0694x + 0.0525; R^2 = 0.9807$ Eq. 4

200 $y_{RS} = 17.867x + 0.0442; R^2 = 0.9946$ Eq. 5

201 $y_{NS} = y_{490} - y_{540} = -12.7976x + 0.0083$ Eq. 6

202

203 [Figure 2 here]

204

205 2.2.8. Kinetic studies

206 The processing of lignocellulosic biomass follows complex kinetic mechanisms involving
207 productive reactions (for instance, taking the case of furfural and 5-HMF production, the
208 conversion of cellulose to hexoses and of hemicellulose to pentoses, the generation of isomers
209 and/or intermediates and the subsequent production of furanic compounds) and parasitic
210 reactions (taking the same example, substrate fragmentation and/or reversion, furanic
211 compounds consumption by reactions with themselves and/or with reactive species present in
212 the reaction media, furanic compounds rehydration to yield levulinic and formic acids, etc.).
213 The overall mechanism is still subjected to debate, as noted by [19], and –to the best of the
214 authors' knowledge– there is no information available on the kinetic modelling of furanic
215 compounds generation from lignocellulose in ionic liquids or DES, only a few studies on 5-
216 HMF production from glucose in ILs. For practical reasons, given the variety of products
217 studied herein, simplifications to the above model are necessary for performing the kinetic

218 studies, and a single lumped reaction –without considering the individual reactions yielding
219 various products– has been chosen in this case, interpreting the processes on the basis of first-
220 order (or pseudo-first-order) kinetics. The aim of this (over)simplification was to gain basic
221 insight into the speed of the chemical reactions and yields for each of the solvents under study.

222

223 2.2.9. Statistical analyses

224 Data were subjected to analysis of variance (ANOVA). For post hoc comparison of means,
225 Tukey's multiple range test at 0.05 probability level ($p < 0.05$) was used. All tests were made
226 using IBM SPSS Statistics v.25 software.

227

228 3. Results and discussion

229 3.1. Furfural, 5-HMF and ASL

230 The highest values of ASL, furfural and 5-HMF were generally obtained after 60 min of
231 microwave-assisted treatment, both for *E. arborea* and *C. ladanifer*-derived biomass (see Table
232 2). As noted above, furfural and 5-HMF values, obtained by the difference in the absorption
233 values before and after the reduction with borohydride, were not influenced by the lignin
234 content.

235 According to Table 3, and as depicted in Figure 3, both for *E. arborea* and *C. ladanifer*, the
236 choline chloride/urea treatment was significantly more effective in the production of ASL,
237 furfural and 5-HMF than the treatment with DMAc/sodium bicarbonate, which –in turn–
238 showed better or similar performance than the DMAc/potassium methoxide alternative in
239 almost all cases (the latter only performed better in ASL production from *E. arborea*).

240 It is worth noting that after the choline chloride/urea treatment, *E. arborea* samples led to
241 higher contents in furan-derived products than those of *C. ladanifer*, although the differences
242 were not significant from a statistical point of view in all cases (see Table 3). However,
243 treatment times below 10 min showed a higher production of furfural and 5-HMF from *C.*
244 *ladanifer* than from *E. arborea*. As regards the lignin content in the liquid phase after the MW-

245 assisted treatments, it was significantly higher in *E. arborea* than in *C. ladanifer* in the three
246 media.

247

248 [Table 2 here]

249 [Table 3 here]

250 [Figure 3 here]

251

252 For the choline chloride/urea treated shrubs biomass, the values for soluble lignin content
253 (1.26-1.80 %), furfural content (2.33-2.74 %) and 5-HMF content (0.77-0.82 %) were in
254 agreement with those reported by Chi, *et al.* [15] for the acid hydrolysis of *Pinus taeda* L.
255 (ASL: 1.43 %; furfural: 2.02 %; and 5-HMF: 1.05 %). Da Silva *et al.* found furfural+5-HMF
256 contents ranging from 0.57 % for macauba shell and up to 7.28 % for native cellulose in one of
257 their works [20], and furfural and 5-HMF values of 5.25 % and 0.87 %, respectively, for native
258 cellulose in another study [21] (Table 4).

259 Non MW-assisted alkaline treatments (with NaOH), used for comparison purposes, gave
260 soluble lignin contents twice as high for *E. arborea* (2.25 %) as those for *C. ladanifer* (1.31 %),
261 and both were higher than those obtained for the other treatments. However, furfural contents
262 for the alkaline procedure were 0.40 % for *E. arborea* and 0.19 % for *C. ladanifer*, significantly
263 lower than those obtained in the MW-assisted treatments. 5-HMF contents (0.52 % and 0.47 %
264 for *E. arborea* and *C. ladanifer*, respectively) were similar to those obtained after 20 min of
265 MW-assisted DES treatment and higher than those obtained in the other two polar aprotic
266 solvent-based alternatives (Table 4).

267

268 [Table 4 here]

269

270 **3.2. Sugar content**

271

272 From the data summarized in Table 5, it may be observed that the concentration of reducing
273 sugars in the hydrolysates obtained from both species was low ($w_B = 0.23-0.44\%$), and that *E.*
274 *arborea* biomass led to significantly higher values than that from *C. ladanifer* for all treatment media
275 (see Table 3). On the other hand, the production of non-reducing sugars was high, close to that of
276 total sugars (provided that they were determined by subtracting the reducing sugars from the total
277 ones). In this case, significant differences were only found for the polar aprotic solvents, not for the
278 ChCl:urea DES (Table 3).

279 It may also be noted that the greatest increase in the production of total sugars –and therefore
280 in the production of non-reducing sugars– occurred for MW-treatment times ranging from 10 to
281 20 min, both for *C. ladanifer* and *E. arborea*. For reducing sugars this only occurred for the
282 DMAc-based treatments in the case of *C. ladanifer*. The greatest increase in the production of
283 reducing sugars for *E. arborea* took place between 5 and 10 min for all the treatments.

284 Both for *E. arborea* and *C. ladanifer* hydrolysates, the DES treatment was found to be
285 significantly more effective in terms of sugar production than the treatments based on the polar
286 aprotic solvent (Table 3), although it is worth noting the DMAc/CH₃OK solvent showed a similar
287 performance to the DES in the reducing sugars production. No significant differences were
288 observed between the results of the microwave-assisted DMAc-potassium methoxide and the
289 DMAc-sodium hydrogen carbonate systems for TS and NRS, only for RS (in which –as noted
290 above- DMAc/CH₃OK performed better).

291 Upon application of the choline chloride/urea treatment, *E. arborea* samples produced more
292 total sugars and non-reducing sugars than *C. ladanifer* ones, but the differences were not
293 significant. On the other hand, the reducing sugars content was significantly higher for the
294 former in the three media.

295 Upon alkaline treatment for 24 h (Table 4), the obtained total sugar values ($w_B = 4.63\%$ for
296 *E. arborea* and 5.64% for *C. ladanifer*) were similar to those obtained for a 10-20 min MW-
297 assisted treatment in choline ChCl/urea and higher than those in DMAc-based solvents.
298 Reducing sugars production ($w_B = 1.29\%$ for *E. arborea* and 1% for *C. ladanifer*) were three

299 times higher than those attained with the microwave treatments. Non-reducing sugars for the
300 NaOH treatment ($w_B = 3.34\%$ and 4.64% , respectively) would be similar to those obtained for
301 a 5 min treatment with choline ChCl/urea, for a 40-50 min treatment with DMAc/NaHCO₃ and
302 for a 50-60 min treatment with DMAc/CH₃OK in the case of *E. arborea*; and for a 10 min
303 treatment with choline ChCl/urea in the case of *C. ladanifer* ($w_B = 4.64\%$ was much higher than
304 the values resulting from the polar aprotic solvent-based treatments).

305 For comparison purposes, Table 6 shows the concentration of total sugars and reducing
306 sugars for corncob (twice higher) and bamboo (ten times higher) [22].

307

308 [Table 5 here]

309 [Table 6 here]

310

311 **3.3. Analysis of kinetic data**

312 The kinetic coefficients (k) calculated for the different treatments are reported in Table 7. It
313 may be observed that, in general terms, the highest constants agree with the highest rates of
314 production. That is, for the ChCl/urea treatment, in addition to the highest concentrations of
315 lignin and furfural, the highest kinetic constants were also obtained –both for *E. arborea* and *C.*
316 *ladanifer*–: k_{lignin} values of 0.296 and 0.175, respectively; and $k_{furfural}$ values of 0.319 and 0.065,
317 respectively. Another is the case of 5-HMF and total and reducing sugars, for which the highest
318 formation kinetics were obtained for the DMAc/CH₃OK solvent (k_{HMF} values of 0.488 for *E.*
319 *arborea* and 0.779 for *C. ladanifer*; k_{TS} values of 1.404 and 1.778, respectively; and k_{RS} values
320 of 0.435 and 0.952, respectively). The difference in the kinetic behavior between furfural and 5-
321 HMF has to be referred to the different percentages of pentose in the raw materials [23].

322

323 [Table 7 here]

324

325 **3.4. On treatment methods and mechanisms**

326 It is known that the use of oxidant acids (HNO_3) for pretreating lignocellulosic biomass
327 allows the disruption of the association between carbohydrates and lignin [20, 21]. On the other
328 hand, alkaline treatments (NaOH , CH_3OK) can also be used to remove lignin and thereby
329 increase the digestibility of cellulose. Compared to acid and hydrothermal processes, mild
330 alkaline pretreatments (NaHCO_3) lead to less solubilization of hemicelluloses and less
331 formation of inhibitory compounds, and they can be operated at lower temperatures [24].

332 Although the solvents under study have the ability to disrupt the hydrogen bond network of
333 biopolymers, their different mechanisms result in different efficiencies. Further, the lower
334 performance of DMAc-based systems can be explained by fact that they are disturbed by water
335 impurities [25].

336 In the DES system, ChCl may act as a bridge between the urea and the biomass biopolymers
337 units to, subsequently, weaken and break the specific linkages into the biopolymer (e.g., the
338 ether linkages between the phenylpropane units present in lignin, as reported by Alvarez-Vasco,
339 *et al.* [26]). Another possibility would be that, instead of ChCl and urea, the intermediate agents
340 were choline cation and $[\text{Cl}(\text{urea})_2]^-$ anion (Figure 1).

341 In the case of DMAc-based systems, the hydroxyl groups of lignocellulosic materials may
342 interact with a sodium- or potassium-DMAc macrocation via hydrogen bonding bridged by the
343 bicarbonate or methoxide anions (Figure 4). Sodium or potassium can interact with the carbonyl
344 oxygen via ion-dipole interaction [27], but for this interaction to take place no biopolymer
345 bound water can be present. On the contrary, such problem does not occur in the case of the
346 DES system: since water is linked to urea through hydrogen bonding, the deleterious water
347 effect is suppressed [28].

348

349

[Figure 4 here]

350

351 Regardless of the chosen method, acid-soluble lignin should be removed to increase
352 subsequent fermentation process. In agreement to Schwartz and Lawoko [29], a suitable and

353 economical approach would be to use Amberlite XAD-4 resin, which was shown to remove
354 90% of ASL. Subsequent fermentation of the resin-treated hydrolyzates gave ethanol yields as
355 high as 97% of theoretical and showed a marked increase in the fermentation rate.

356 The results of this study provide further evidence on the efficiency of microwave-assisted
357 DES treatment for biomass conversion, previously claimed by other authors: both strategies
358 exhibit a strong synergism, result in improvements in biomass digestibility and appear to require
359 much less energy to achieve a satisfactory treatment effectiveness within a very short period
360 [30]. As compared to common solvents used for biomass conversion, DESs clearly offer notable
361 advantages, apart from their low cost and low environmental impact, owing to their ability to
362 produce highly concentrated solutions of HMF or furfural [31]. Moreover, their high H-bond
363 accepting ability and polarity facilitates lignin degradation and/or extraction from wood fibers
364 [26]. As regards the concurrent use of microwave irradiation, it can maximize ionic
365 characteristics and increase molecular polarity of DES [32] and, thus, it can significantly
366 shorten the reaction time for DES treatment while achieving a similar or even higher degree of
367 effectiveness compared to DES pretreatment alone [33-35].

368

369 **4. Conclusions**

370 The results suggest that the deep eutectic solvent-based treatment offers an efficient, safe,
371 sustainable, and cost-effective alternative to conventional methods for the extraction of
372 bioactive compounds from *C. ladanifer* and *E. arborea* biomass. Samples of these shrubs may
373 be easily dissolved by a MW-assisted procedure in a ChCl/urea DES to give lignin, furfural, 5-
374 (hydroxymethyl)furfural and sugars with reasonable yields. Conversely, the DMAc/NaHCO₃
375 and DMAc/CH₃OK solvent exchange systems would be less appropriate due the disruptive
376 effect of water impurities. Nevertheless, if the aim of treating *C. ladanifer* and *E. arborea*
377 biomass is to recover sugars for subsequent enzymatic saccharification, the very low 5-HMF
378 contents attained with the dimethylacetamide systems (especially the CH₃OK one) make them
379 highly advantageous as compared to the traditional method using NaOH.

380 A peculiarity of the present work is that the operating conditions led to higher contents of
381 non-reducing sugars than of reducing sugars. This finding can be useful to modify cured phenol-
382 formaldehyde resins: whereas reduced sugars cannot be used to modify these resins, non-
383 reducing sugars can be used to replace a major portion of the adhesive resin. These non-
384 reducing sugars may also be advantageously used as a starting material in bioprocesses to
385 produce succinic acid (one of the chemical platforms suggested by the DOE), farnesene
386 (sesquiterpenes) and sucralose.

387

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394

395 **Declaration of interest**

396 The authors have no competing interests to declare.

397

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- 512

513 **Table 1.** Overall chemical composition of *E. arborea* and *C. ladanifer* [14, 36]. Values are given as an
 514 average of 25 repetitions, followed by the minimum and maximum values in brackets.

	<i>Erica arborea</i>	<i>Cistus ladanifer</i>
<i>Elemental analysis:</i>		
C (%)	51.0 (49.3-52.8)	47.8 (47.5-50.1)
H (%)	6.2 (6.0-6.4)	6.4 (6.0-6.8)
N (%)	1.0 (0.3-1.1)	0.8 (0.3-1.9)
O (by diff., %)	~41.8	~45.0
<i>Vegetal components:</i>		
Cellulose (%)	40.0 (37.3-41.1)	55.0 (54.9-55.7) [†]
Lignin (%)	39.5 (39.3-40.1)	25.3 (24.5-34.2)
Hemi-cellulose (%)	11.0 (9.7-13.8) [‡]	10.2 (10.1-10.9) [‡]
Extractive (%)	9.5 (5.7-11.0)	9.5 (9.4-9.6)
<i>Moisture (wt.%)</i>	26.0	26.8

515 [†] This cellulose content is higher than that of most woods, which is usually in the 35-50% range.

516 [‡] These hemicellulose contents are lower than those of most woods, which usually range from 20% to 30%.

517

518 **Table 2.** Mass fraction (w_B , in %) for lignin, furfural and 5-HMF in hydrolysates after MW-assisted deep
 519 eutectic solvent or polar aprotic solvent extraction. The tests were performed in triplicate, and
 520 standard deviations were <5 %, except in those cases in which the furan compounds yields were below
 521 1.5 % (in which the standard deviations were higher, up to 10 %).

Treatment	Time (min) [†]	<i>Erica arborea</i>			<i>Cistus ladanifer</i>		
		w_{lignin}	w_{furfural}	w_{5HMF}	w_{lignin}	w_{furfural}	w_{5HMF}
MW-assisted ChCl/urea DES extraction	1	0.52	1.00	0.25	0.48	1.05	0.23
	5	0.82	1.13	0.34	0.69	1.38	0.33
	10	1.25	1.30	0.39	0.93	1.45	0.36
	20	1.35	1.73	0.59	1.03	1.58	0.45
	30	1.63	2.59	0.65	1.22	1.94	0.49
	40	1.67	2.70	0.65	1.28	2.13	0.58
	50	1.79	2.69	0.75	1.40	2.26	0.63
MW-assisted DMAc/NaHCO ₃ extraction	1	0.33	0.97	0.22	0.42	0.92	0.20
	5	0.45	1.02	0.23	0.58	1.06	0.21
	10	0.47	1.08	0.24	0.59	1.16	0.24
	20	0.55	1.18	0.25	0.61	1.20	0.26
	30	0.69	1.26	0.27	0.62	1.23	0.28
	40	0.80	1.37	0.28	0.70	1.25	0.28
	50	0.85	1.43	0.33	0.79	1.29	0.29
MW-assisted DMAc/CH ₃ OK extraction	1	0.52	0.62	0.00	0.46	0.62	0.05
	5	0.69	0.93	0.02	0.64	0.80	0.05
	10	0.90	1.08	0.02	0.68	1.06	0.07
	20	0.98	1.43	0.06	0.73	1.29	0.09
	30	0.99	1.42	0.11	0.76	1.25	0.09
	40	1.04	1.37	0.12	0.77	1.16	0.10
	50	1.07	1.36	0.15	0.80	1.30	0.11
60	1.10	1.35	0.18	0.82	1.23	0.13	

522 [†] This time refers to the isothermal treatment time. It should be noticed that the heating and cooling ramps also
 523 contribute to the thermal budget (i.e., for $t=0$ min, there would be a non-zero production of lignin, furfural and 5-
 524 HMF due to heating and cooling ramps).

525 **Table 3.** Mass fractions (w_B , in %) for acid soluble lignin (ASL), furfural, 5-HMF, total sugars (TS),
 526 reducing sugars (RS) and non-reducing sugars (NRS) in the hydrolysates after a 60 min treatment for the
 527 MW-assisted ChCl/urea, DMAc/NaHCO₃ and DMAc/CH₃OK media.

Treatment	<i>Erica arborea</i>						<i>Cistus ladanifer</i>					
	ASL	Furfural	5-HMF	TS	RS	NRS	ASL	Furfural	5-HMF	TS	RS	NRS
ChCl:urea DES	1.80 aA	2.74 aA	0.82 aA	9.19 aA	0.41 aA	8.78 aA	1.26 aB	2.33 aB	0.77 aA	8.45 aA	0.33 aB	8.13 aA
DMAc/NaHCO ₃	0.90 bA	1.46 bA	0.34 bA	3.74 bA	0.34 bA	3.40 bA	0.78 bB	1.30 bB	0.29 bB	3.22 bB	0.23 bB	2.99 bB
DMAc/CH ₃ OK	1.10 cA	1.35 bA	0.18 cA	3.80 bA	0.44 aA	3.36 bA	0.82 bB	1.23 bA	0.13 cB	2.90 bB	0.36 aB	2.54 bB

528 * Means followed by the same lowercase letter within each column are not significantly different at p<0.05 by
 529 Tukey's test. Means of the same product (viz. ASL, furfural, 5-HMF, TS, RS or NRS) followed by the same
 530 uppercase letter for *E. arborea* and *C. ladanifer* are not significantly different at p<0.05 by Tukey's test. All values
 531 are presented as the average of three repetitions.
 532

533 **Table 4.** Comparative measurements of soluble lignin, furfural and 5-HMF in the hydrolysates (w_B , in %).
 534 Tests were performed in triplicate, and standard deviations were <10 % in all cases.

Component	Solvent	Shrubs		Native cellulose	Hardwoods	References
		<i>E. arborea</i>	<i>C. ladanifer</i>			
Lignin	ChCl/urea	0.52-1.80	0.48-1.4			
	DMAc/NaHCO ₃	0.33-0.90	0.42-0.79		1.43	Chi, <i>et al.</i> [15]
	DMAc/CH ₃ OK	0.52-1.10	0.46-0.82			
	NaOH	2.25	1.31			
Furfural	ChCl/urea	1.00-2.74	1.05-2.33	2.30-5.25		da Silva <i>et al.</i> [20]
	DMAc/NaHCO ₃	0.97-1.46	0.92-1.30			
	DMAc/CH ₃ OK	0.62-1.43	0.62-1.30			
	NaOH	0.40	0.19			
5-HMF	ChCl/urea	0.25-0.82	0.23-0.77	0.23-0.87		da Silva <i>et al.</i> [21]
	DMAc/NaHCO ₃	0.22-0.34	0.20-0.29			
	DMAc/CH ₃ OK	0.00-0.18	0.05-0.13			
	NaOH	0.52	0.47			

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537

538 **Table 5.** Total sugars (TS), reducing sugars (RS) and non-reducing sugars (NRS) mass fractions (w_B , in
 539 %) for the MW-assisted ChCl:urea, DMAc/NaHCO₃ and DMAc/CH₃OK treatments as a function of
 540 exposure times. Tests were performed in triplicate, and standard deviations were <5 %.

Treatment	Time (min) [†]	<i>Erica arborea</i>			<i>Cistus ladanifer</i>		
		w_{TS}	w_{RS}	w_{NRS}	w_{TS}	w_{RS}	w_{NRS}
MW-assisted ChCl:urea DES extraction	1	2.94	0.17	2.76	3.33	0.12	3.21
	5	3.54	0.20	3.34	3.97	0.14	3.84
	10	4.04	0.27	3.78	4.86	0.23	4.63
	20	6.45	0.28	6.17	6.36	0.23	6.13
	30	8.15	0.31	7.84	7.03	0.28	6.75
	40	8.44	0.35	8.09	8.06	0.29	7.77
	50	8.83	0.40	8.43	8.09	0.30	7.79
	60	9.19	0.41	8.78	8.45	0.33	8.13
MW-assisted DMAc/NaHCO ₃ extraction	1	0.45	0.17	0.29	0.39	0.11	0.29
	5	0.61	0.18	0.43	0.40	0.11	0.28
	10	0.75	0.22	0.53	0.57	0.12	0.45
	20	2.46	0.25	2.21	2.44	0.19	2.25
	30	3.29	0.28	3.01	2.44	0.19	2.25
	40	3.33	0.28	3.05	2.60	0.20	2.40
	50	3.68	0.30	3.37	2.70	0.23	2.47
	60	3.74	0.34	3.40	3.22	0.23	2.99
MW-assisted DMAc/CH ₃ OK extraction	1	0.28	0.16	0.11	0.12	0.12	0.00
	5	0.71	0.19	0.52	0.26	0.13	0.13
	10	1.05	0.28	0.77	0.63	0.16	0.47
	20	2.63	0.30	2.33	2.21	0.26	1.94
	30	3.27	0.36	2.91	2.39	0.28	2.11
	40	3.32	0.37	2.95	2.64	0.29	2.35
	50	3.48	0.42	3.06	2.82	0.33	2.49
	60	3.80	0.44	3.36	2.90	0.36	2.54

541 [†] This time refers to the isothermal treatment time. It should be noticed that the heating and cooling ramps also
 542 contribute to the thermal budget (i.e., for $t=0$ min, there would be a non-zero production of TS, RS and NRS due to
 543 heating and cooling ramps).
 544

545

546 **Table 6.** Comparison of the sugar mass fractions (w_B , in %) in the lignocellulosic biomass hydrolysates
 547 from *E. arborea* and *C. ladanifer* studied herein with values reported by other authors for corncob and
 548 bamboo.

Component	Solvent	Shrubs		Corncob	Bamboo	References
		<i>E. arborea</i>	<i>C. ladanifer</i>			
Total sugars (w_{TS})	ChCl/urea	2.94-9.19	3.33-8.45	18.6-20.9		Procentese, <i>et al.</i> [37]
	DMAc/NaHCO ₃	0.45-3.74	0.39-2.70			
	DMAc/CH ₃ OK	0.28-3.80	0.12-2.90			
	NaOH	4.63	5.64			
Reducing sugars (w_{RS})	ChCl/urea	0.17-0.41	0.12-0.33		3.4	Wu, <i>et al.</i> [38]
	DMAc/NaHCO ₃	0.17-0.34	0.11-0.23			
	DMAc/CH ₃ OK	0.16-0.44	0.12-0.36			
	NaOH	1.29	1.00			
Non-reducing sugars (w_{NRS})	ChCl/urea	2.76-8.75	3.21-8.13			
	DMAc/NaHCO ₃	0.29-3.40	0.28-2.99			
	DMAc/CH ₃ OK	0.11-3.36	0.00-2.54			
	NaOH	3.34	4.64			

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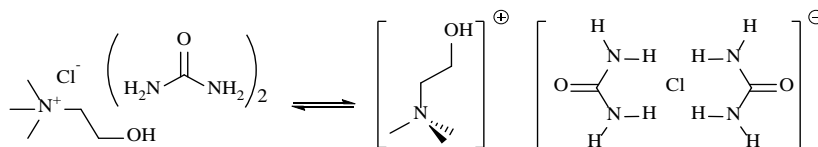
551 **Table 7.** Kinetic coefficients (k), correlation coefficients (r^2) and initial concentration of each sample (H_0)
 552 determined from the concentration as a function of time for lignin, furfural, 5-HMF, total sugars and
 553 reducing sugars production from the hydrolysis of *E. arborea* and *C. ladanifer* lignocellulosic biomass.

Component	Solvent	<i>E. arborea</i>			<i>C. ladanifer</i>			References
		k	r^2	H_0	k	r^2	H_0	
Soluble lignin	ChCl/urea	0.2959	0.9707	0.0320	0.1752	0.9479	0.0321	
	DMAc/NaHCO ₃	0.2118	0.8583	0.0321	0.0088	0.8646	0.0321	
	DMAc/CH ₃ OK	0.0348	0.9745	0.0321	0.0042	0.9831	0.0320	
Furfural	ChCl/urea	0.3192	0.8214	0.0320	0.0649	0.8900	0.0321	0.2712 (macauba pulp) [21]
	DMAc/NaHCO ₃	0.0011	0.8500	0.0321	0.0001	0.9905	0.0321	
	DMAc/CH ₃ OK	0.0433	0.8908	0.0321	0.0309	0.8782	0.0320	
5-HMF	ChCl/urea	0.3844	0.9025	0.0320	0.3296	0.8365	0.0321	0.2729 (macauba pulp), 0.0810 (macauba shell) [21]
	DMAc/NaHCO ₃	0.0025	0.6806	0.0321	0.0013	0.9240	0.0321	
	DMAc/CH ₃ OK	0.4883	0.8024	0.0321	0.7798	0.8367	0.0320	
Total sugars	ChCl/urea	0.3778	0.8704	0.0100	0.1605	0.9149	0.0100	
	DMAc/NaHCO ₃	1.4143	0.8309	0.0100	1.3890	0.8024	0.0100	
	DMAc/CH ₃ OK	1.4044	0.8928	0.0100	1.7780	0.8634	0.0100	
Reducing sugars	ChCl/urea	0.3005	0.8780	0.0667	0.5469	0.9137	0.0668	
	DMAc/NaHCO ₃	0.1600	0.8976	0.0668	0.6234	0.8339	0.0668	
	DMAc/CH ₃ OK	0.4351	0.9132	0.0668	0.9528	0.8690	0.0668	

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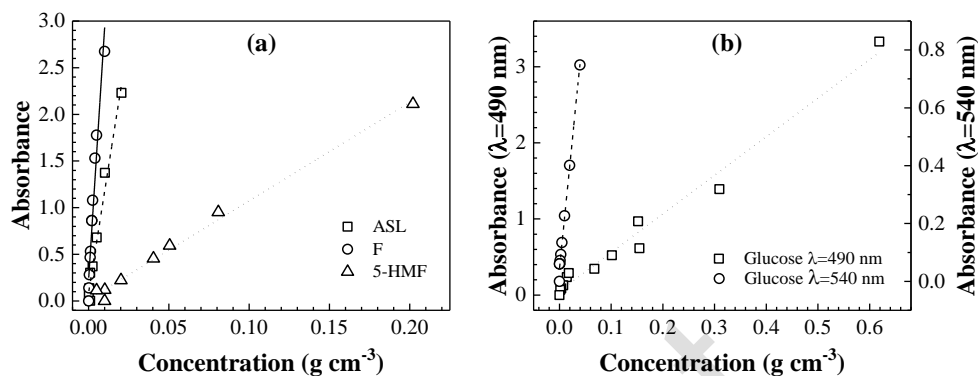
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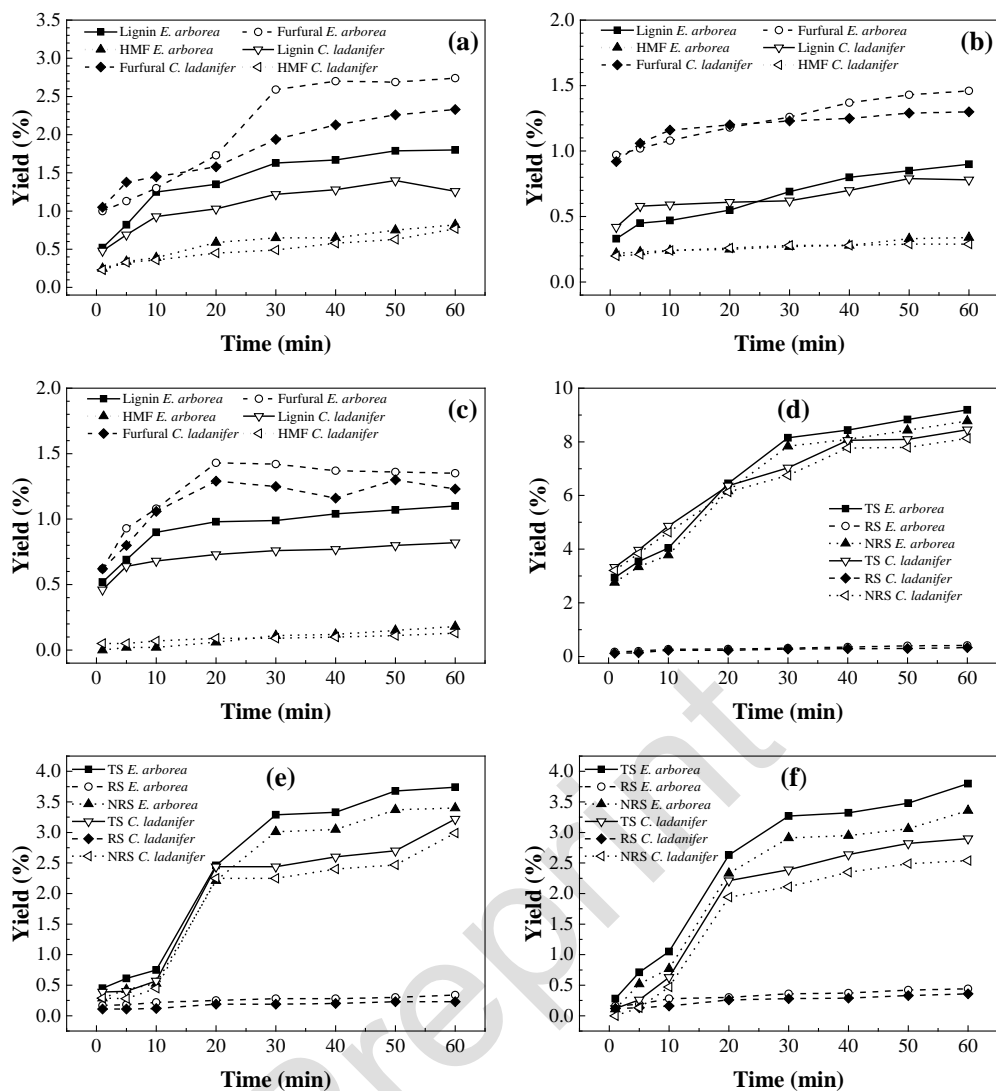
557 **Figure 1.** DES of ChCl and urea where a [choline]⁺ cation is energetically competitive with [Cl(urea)₂][−].

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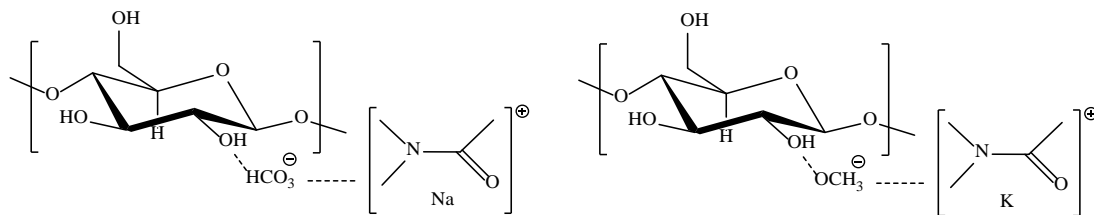
560 **Figure 2.** (a) Calibration curves for furfural (F), acid-soluble lignin (ASL) and 5-(hydroxymethyl)-
 561 furfural (5-HMF) concentrations. (b) Calibration curves for glucose concentration. Each data point was
 562 the mean of three determinations. Standard deviation bars were omitted for clarity.



563

564 **Figure 3.** Lignin, furfural and 5-HMF yields for the *E. arborea* and *C. ladanifer* lignocellulosic biomass
 565 hydrolysates after: (a) MW-assisted ChCl/urea extraction; (b) MW-assisted DMAc/NaHCO₃ extraction;
 566 and (c) MW-assisted DMAc/CH₃OK extraction. Total, reducing and non-reducing sugars in the
 567 hydrolysates after: (d) MW-assisted ChCl/urea treatment; (e) MW-assisted DMAc/NaHCO₃ treatment;
 568 and (f) MW-assisted DMAc/CH₃OK treatment.

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570

571 **Figure 4.** Proposed interaction between DMAc-NaHCO₃ and DMAc-CH₃OK solvents and sugar polymer