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

Paula Carrión-Prieto[a], Pablo Martín-Ramos[b,*], Salvador Hernández-Navarro[a], Luis F. Sánchez-Sastre[a], José L. Marcos-Robles[a] and Jesús Martín-Gil[a]

[a] Agriculture and Forestry Engineering Department, ETSIIAA, Universidad de Valladolid, Avda. Madrid 44, 34004, Palencia, Spain.

[b] Department of Agricultural and Environmental Sciences, EPS, Instituto de Investigación en Ciencias Ambientales de Aragón (IUCA), Universidad de Zaragoza, Carretera de Cuarte s/n, 22071, Huesca, Spain. Phone: +34 (974) 292668; Fax: +34 (974) 239302; E-mail: pmr@unizar.es

Abstract

Cistus ladanifer L. and *Erica arborea* L. are the two most representative shrub species from the Iberian Peninsula. With a view to their valorization, their biomass hydrolysate components, obtained from microwave-assisted treatments with choline chloride/urea - HNO_3 10 %, *N*,*N*-dimethylacetamide/$NaHCO_3$ and *N*,*N*-dimethylacetamide/CH_3OK as solvents, have been measured using a spectrophotometric method. Concentrations of furfural and 5-(hydroxymethyl)furfural (5-HMF) in the filtrate have been determined after reduction with $NaBH_4$. The production of total sugars, reducing sugars and non-reducing sugars contents has also been assessed. The obtained results support the choice of MW-assisted choline chloride/urea deep eutectic solvent in acid media as the preferred method (over the polar aprotic solvent-based solvents) for the extraction of lignin, furfural, 5-HMF and sugars from *C. ladanifer* and *E. arborea* biomass, attaining the best production yields for 60 min exposure times. Another is the case if the aim of the treatments is to recovery sugars from both shrubs for

subsequent enzymatic saccharification: the very low 5-HMF contents resulting from the dimetylacetamide systems (especially is association with CH_3OK) make them highly advantageous as compared to the traditional method using NaOH.

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

1. Introduction

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

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

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

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

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

2. Materials and methods

2.1. *Samples and reagents*

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

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

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

[Table 1 here]

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

2.2. *Methods*

2.2.1. *Microwave-assisted deep eutectic solvent*

A deep eutectic solvent system based on choline chloride-urea (Figure 1) was assessed for the hydrolysis of *C. ladanifer* and *E. arborea* biomass. Choline chloride/urea DES was prepared by stirring the mixture of choline chloride and urea (mole ratio 1:2) at 80 °C until a homogeneous colorless liquid was formed, which was then stored in a vacuum dryer. Subsequently, biomass samples (200 mg) were treated by a mixture (1 cm^3) of choline

chloride/urea and HNO_3 10 %, with TiO_2 (20 mg) as a catalyst, in a microwave digestion system –a Milestone (Sorisole, BG, Italy) Ethos-One microwave oven equipped with a magnetic stirrer system– at 120 ºC for an "effective time" (isothermal treatment time) between 1 and of 60 min (*viz.*, 1, 5, 10, 20, 30, 40, 50 and 60 min), plus the heating and cooling ramps, which also contributed to the thermal budget. The heating up to 120 ºC started with a ramp set to 19 $ºC \cdot min^{-1}$ during the first 5 min, followed by a second ramp at a rate of 2.5 $ºC \cdot min^{-1}$ for 10 min. The cooling down to room temperature took 25 minutes, at a rate of ~4.8 $ºC \cdot min^{-1}$. The DES was finally removed by washing with water and was recovered by crystallization.

[Figure 1 here]

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

Alternatively to the DES-based method, 8 cm^3 of a colorless, water-miscible, high boiling liquid –viz. *N,N´*-dimethylacetamide (DMAc) with formula $CH_3C(O)N(CH_3)_2$– was used, in the presence of 40 mg of sodium hydrogen carbonate ($NaHCO_3$), as a treatment agent for *C. ladanifer* and *E. arborea* wooden samples (200 mg of biomass samples). The same procedure explained above for the microwave-assisted DES treatment was followed for the polar aprotic solvent-based treatment. DMAc was removed by washing with water and recovered by distillation.

2.2.3. *Microwave-assisted DMAc-potassium methoxide system*

The third approach investigated for the hydrolytic treatment of *C. ladanifer* and *E. arborea* biomass (200 mg) was based on a mixture of 8 cm^3 of DMAc with 40 mg of potassium methoxide (commonly used as a catalyst for transesterification in the production of biodiesel). The solution was then treated as in the previously discussed methods.

2.2.4. *Alkaline treatment*

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

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

The acid-soluble lignin (ASL), furfural and 5-(hydroxymethyl)-furfural contents were determined according to the methodology proposed by Chi, *et al.* [15], based on the measurement of their respective maximum absorbance at 205 nm, 277 nm and 285 nm, and which makes use of the effect of the reduction with borohydride on the furfural and 5-HMF maxima mentioned above. For these latter two chemical species, their initial absorbance in the UV-vis spectrum was measured and, after the addition of 30 mg of sodium borohydride to eliminate the interference of furanic compounds (followed, after 5 min, by the addition of a small amount of HCl) [16], absorbance measurements were repeated. Their associated absorption maxima at 277 and 285 nm completely disappeared upon reduction with $NaBH_4$. Therefore, the furfural and 5-HMF contents could be readily calculated from the absorbance difference before and after reduction (ΔA_R) at their respective wavelengths.

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

2.2.6. *Sugars content*

The quantification of reducing sugars was conducted according to Miller [17], using DNS as the most specific reagent in a solution containing sodium hydroxide and potassium sodium tartrate. The solution was prepared by mixing 0.8 g of NaOH, 15 g of sodium potassium tartrate and 0.5 g of DNS, completing up to 50 cm^3 with distilled water. To ensure the homogeneity of the mixture, it was boiled for 5 minutes. It was then cooled with water and ice, 5 cm^3 of water

were added to compensate for the evaporated volume, and it was allowed to rest for 15 min. With this solution, which will be referred to as 'DNS', 1:1 mixtures with the samples or the standard solutions to be analyzed were prepared (0.5 cm^3 of DNS and 0.5 cm^3 of either the sample or the standard solution). The determination of reducing sugars in these mixtures was conducted by measuring their absorbance at 540 nm.

The total sugars determination was carried out in agreement with the method proposed by DuBois, *et al.* [18]. This method is usually called 'phenol-sulfuric acid method' because in the preparation of the measuring solutions, 1-2 cm^3 of sample, 1 cm^3 of phenol (5 %) and 5 cm^3 of concentrated sulfuric acid (95.5 %) are mixed in the test tubes. The test tubes containing these solutions were placed in a rack which was kept in a thermostatic bath, between 25 and 30 °C, for 10-15 min. Glucose at various concentrations was used as a standard. The spectrophotometric measurement of the total sugars was carried out at 490 nm, that is, at the wavelength at which hexoses and their methylated derivatives exhibit their maximum absorption. Non-reducing sugars content was calculated by difference between the total sugars and the reducing sugars percentages.

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

2.2.7. *Calibration curves*

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

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

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

$$y_F = 270.76x + 0.2236; R^2 = 0.9534 \quad \text{Eq. 2}$$

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

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

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

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

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

[Figure 2 here]

2.2.8. *Kinetic studies*

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

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

2.2.9. *Statistical analyses*

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

3. Results and discussion

3.1. *Furfural, 5-HMF and ASL*

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

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

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

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

[Table 2 here]

[Table 3 here]

[Figure 3 here]

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

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

[Table 4 here]

3.2. *Sugar content*

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

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

Both for *E. arborea* and *C. ladanifer* hydrolysates, the DES treatment was found to be significantly more effective in terms of sugar production than the treatments based on the polar aprotic solvent (Table 3), although it is worth noting the DMAc/CH_3OK solvent showed a similar performance to the DES in the reducing sugars production. No significant differences were observed between the results of the microwave-assisted DMAc-potassium methoxide and the DMAc-sodium hydrogen carbonate systems for TS and NRS, only for RS (in which –as noted above- DMAc/CH_3OK performed better).

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

Upon alkaline treatment for 24 h (Table 4), the obtained total sugar values (w_B = 4.63 % for *E. arborea* and 5.64 % for *C. ladanifer*) were similar to those obtained for a 10-20 min MW-assisted treatment in choline ChCl/urea and higher than those in DMAc-based solvents. 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/$NaHCO_3$ and
302 for a 50-60 min treatment with DMAc/CH_3OK 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/CH_3OK 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 $[Cl(urea)_2]^-$ 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

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

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

4. Conclusions

The results suggest that the deep eutectic solvent-based treatment offers an efficient, safe, sustainable, and cost-effective alternative to conventional methods for the extraction of bioactive compounds from *C. ladanifer* and *E. arborea* biomass. Samples of these shrubs may be easily dissolved by a MW-assisted procedure in a ChCl/urea DES to give lignin, furfural, 5-(hydroxymethyl)furfural and sugars with reasonable yields. Conversely, the DMAc/$NaHCO_3$ and DMAc/CH_3OK solvent exchange systems would be less appropriate due the disruptive effect of water impurities. Nevertheless, if the aim of treating *C. ladanifer* and E. *arborea* biomass is to recover sugars for subsequent enzymatic saccharification, the very low 5-HMF contents attained with the dimetylacetamide systems (especially the CH_3OK one) make them highly advantageous as compared to the traditional method using NaOH.

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

Acknowledgments

This work was supported by the European Union LIFE+ Programme under project "CO_2 Operation: Integrated agroforestry practices and nature conservation against climate change", ref. LIFE11 ENV/ES/000535. P.M.R. gratefully acknowledges the financial support of Santander Universidades through the “Becas Iberoamérica Jóvenes Profesores e Investigadores, España” scholarship program.

Declaration of interest

The authors have no competing interests to declare.

References

[1] C.A. Mooney, S.D. Mansfield, R.P. Beatson, J.N. Saddler, The effect of fiber characteristics on hydrolysis and cellulase accessibility to softwood substrates, Enzyme Microb. Technol. 25(8-9) (1999) 644-650.

[2] Y. Sun, J. Cheng, Hydrolysis of lignocellulosic materials for ethanol production : a review, Bioresour. Technol. 83(1) (2002) 1-11.

[3] Z. Hu, Z. Wen, Enhancing enzymatic digestibility of switchgrass by microwave-assisted alkali pretreatment, Biochem. Eng. J. 38(3) (2008) 369-378.

[4] D. Jackowiak, J.C. Frigon, T. Ribeiro, A. Pauss, S. Guiot, Enhancing solubilisation and methane production kinetic of switchgrass by microwave pretreatment, Bioresour. Technol. 102(3) (2011) 3535-3540.

[5] S.M. Nomanbhay, R. Hussain, K. Palanisamy, Microwave-assisted alkaline pretreatment and microwave-assisted enzymatic saccharification of oil palm empty fruit bunch fiber for enhanced fermentable sugar yield, Journal of Sustainable Bioenergy Systems 03(01) (2013) 7-17.

[6] A.d.l. Hoz, A. Diaz-Ortiz, A. Moreno, Microwaves in organic synthesis. Thermal and non-thermal microwave effects, Chem. Soc. Rev. 34 (2005) 164-178.

[7] D.R. Keshwani, J.J. Cheng, Microwave-based alkali pretreatment of switchgrass and coastal bermudagrass for bioethanol production, Biotechnol. Progr. 26(3) (2010) 644-652.

[8] A. Isaac, J. de Paula, C.M. Viana, A.B. Henriques, A. Malachias, L.A. Montoro, From nano- to micrometer scale: the role of microwave-assisted acid and alkali pretreatments in the sugarcane biomass structure, Biotechnol. Biofuels 11(1) (2018).

[9] A.K. Chandel, C. Es, R. Rudravaram, M.L. Narasu, V. Rao, P. Ravindra, Economics and environmental impact of bioethanol production technologies: an appraisal, Biotechnol. Mol. Biol. Rev. 2 (2007) 14-32.

[10] H. Lee, D.H. Cho, Y.H. Kim, S.J. Shin, S.B. Kim, S.O. Han, J. Lee, S.W. Kim, C. Park, Tolerance of *Saccharomyces cerevisiae* K35 to lignocellulose-derived inhibitory compounds, Biotechnol. Bioprocess Eng. 16(4) (2011) 755-760.

[11] A. Martinez, M.E. Rodriguez, S.W. York, J.F. Preston, L.O. Ingram, Use of UV absorbance to monitor furans in dilute acid hydrolysates of biomass, Biotechnol. Progr. 16(4) (2000) 637-641.

[12] J. García-Álvarez, Deep eutectic mixtures: Promising sustainable solvents for metal-catalysed and metal-mediated organic reactions, Eur. J. Inorg. Chem. 2015(31) (2015) 5147-5157.

[13] R. Ruiz-Peinado, G. Montero, M. Del Rio, Biomass models to estimate carbon stocks for hardwood tree species, Forest Syst. 21(1) (2012) 42-52.

[14] P. Carrión-Prieto, S. Hernández-Navarro, P. Martín-Ramos, L.F. Sánchez-Sastre, F. Garrido-Laurnaga, J.L. Marcos-Robles, J. Martín-Gil, Mediterranean shrublands as carbon sinks for climate change mitigation: new root-to-shoot ratios, Carbon Manag. 8(1) (2017) 1-11.

[15] C. Chi, Z. Zhang, H.-m. Chang, H. Jameel, Determination of furfural and hydroxymethylfurfural formed from biomass under acidic conditions, Journal of Wood Chemistry and Technology 29(November 2013) (2009) 265-276.

[16] S. Sun, Y. Song, P. Sun, J. Chen, Furfural, acid-soluble lignin and reducing sugar content in biomass hydrolysate by spectrophotometric method, Acta Energ. Sol. Sin. 34(2) (2013) 331-336.

[17] G.L. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar, Anal. Chem. 31(3) (1959) 426-428.

[18] M. DuBois, K.a. Gilles, J.K. Hamilton, P.a. Rebers, F. Smith, Colorimetric method for determination of sugars and related substances, Anal. Chem. 28(3) (1956) 350-356.

[19] L. Penín, S. Peleteiro, R. Yañez, J.C. Parajó, V. Santos, Kinetics of 5-hydroxymethylfurfural production from monosaccharides in media containing an ionic liquid and a solid acid catalyst, BioResources 12(4) (2017) 10.15376/biores.12.4.8402-8418.

[20] V. da Silva Lacerda, J.B. López-Sotelo, A. Correa-Guimarães, P. Martín-Ramos, S. Hernández-Navarro, M. Sánchez-Bascones, L.M. Navas-Gracia, Efficient microwave-assisted acid hydrolysis of lignocellulosic materials into total reducing sugars in ionic liquids, Cellul. Chem. Technol. 50(7-8) (2016) 761-770.

[21] V. da Silva Lacerda, J.B. López-Sotelo, A. Correa-Guimarães, S. Hernández-Navarro, M. Sánchez-Bascones, L.M. Navas-Gracia, P. Martín-Ramos, E. Pérez-Lebeña, J. Martín-Gil, A kinetic study on microwave-assisted conversion of cellulose and lignocellulosic waste into hydroxymethylfurfural/furfural, Bioresour. Technol. 180(0) (2015) 88-96.

[22] A. Martel, Transformation of *Symphytum officinale* L. and *Panicum virgatum* L. biomass to 5-Hydroxymethylfurfural for biofuel production, Faculty of Graduate Studies, Laurentian University, Sudbury, Ontario, Canada, 2016, p. 136.

[23] Y. Liu, J. Wang, M. Wolcott, Modeling the production of sugar and byproducts from acid bisulfite pretreatment and enzymatic hydrolysis of Douglas-fir, Bioresour. Technol. 224 (2017) 389-396.

[24] L.J. Jönsson, C. Martín, Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects, Bioresour. Technol. 199 (2016) 103-112.

[25] A. Potthast, T. Rosenau, R. Buchner, T. Röder, G. Ebner, H. Bruglachner, H. Sixta, P. Kosma, The cellulose solvent system N,N-dimethylacetamide/lithium chloride revisited: The effect of water on physicochemical properties and chemical stability, Cellulose 9(1) (2002) 41-53.

[26] C. Alvarez-Vasco, R. Ma, M. Quintero, M. Guo, S. Geleynse, K.K. Ramasamy, M. Wolcott, X. Zhang, Unique low-molecular-weight lignin with high purity extracted from wood by deep eutectic solvents (DES): a source of lignin for valorization, Green Chem. 18(19) (2016) 5133-5141.

[27] C.L. McCormick, P.A. Callais, B.H. Hutchinson, Solution studies of cellulose in lithium chloride and N,N-dimethylacetamide, Macromolecules 18(12) (1985) 2394-2401.

[28] C. Du, B. Zhao, X.-B. Chen, N. Birbilis, H. Yang, Effect of water presence on choline chloride-2urea ionic liquid and coating platings from the hydrated ionic liquid, Sci. Rep. 6 (2016) 29225-29225.

[29] T.J. Schwartz, M. Lawoko, Removal of acid-soluble lignin from biomass extracts using Amberlite XAD-4 resin, BioResources 5(4) (2010) 2337-2347.

[30] Z. Chen, C. Wan, Ultrafast fractionation of lignocellulosic biomass by microwave-assisted deep eutectic solvent pretreatment, Bioresour. Technol. 250 (2018) 532-537.

[31] K.D.O. Vigier, G. Chatel, F. Jérôme, Contribution of Deep Eutectic Solvents for Biomass Processing: Opportunities, Challenges, and Limitations, ChemCatChem 7(8) (2015) 1250-1260.

[32] Y. Liu, W. Chen, Q. Xia, B. Guo, Q. Wang, S. Liu, Y. Liu, J. Li, H. Yu, Efficient cleavage of lignin-carbohydrate complexes and ultrafast extraction of lignin oligomers from wood biomass by microwave-assisted treatment with deep eutectic solvent, ChemSusChem 10(8) (2017) 1692-1700.

[33] A. Aguilar-Reynosa, A. Romaní, R. Ma. Rodríguez-Jasso, C.N. Aguilar, G. Garrote, H.A. Ruiz, Microwave heating processing as alternative of pretreatment in second-generation biorefinery: An overview, Energy Convers. Manage. 136 (2017) 50-65.

[34] K. Ninomiya, T. Yamauchi, C. Ogino, N. Shimizu, K. Takahashi, Microwave pretreatment of lignocellulosic material in cholinium ionic liquid for efficient enzymatic saccharification, Biochem. Eng. J. 90 (2014) 90-95.

[35] R.D. Singh, K. Bhuyan, J. Banerjee, J. Muir, A. Arora, Hydrothermal and microwave assisted alkali pretreatment for fractionation of arecanut husk, Ind. Crop. Prod. 102 (2017) 65-74.

[36] P. Carrión-Prieto, P. Martín-Ramos, S. Hernández-Navarro, L.F. Sánchez-Sastre, J.L. Marcos-Robles, J. Martín-Gil, Valorization of *Cistus ladanifer* and *Erica arborea* shrubs for fuel: Wood and bark thermal characterization, Maderas: Cienc. Tecnol. 19(4) (2017) 443-454.

[37] A. Procentese, E. Johnson, V. Orr, A. Garruto Campanile, J.A. Wood, A. Marzocchella, L. Rehmann, Deep eutectic solvent pretreatment and subsequent saccharification of corncob, Bioresour. Technol. 192 (2015) 31-36.

[38] Y. Wu, C. Zhang, Y. Liu, Z. Fu, B. Dai, D. Yin, Biomass char sulfonic acids (BC-SO3H)-catalyzed hydrolysis of bamboo under microwave irradiation, BioResources 7(4) (2012) 5950-5959.

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

	Erica arborea	*Cistus ladanifer*
Elemental analysis:		
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
Vegetal components:		
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)
Moisture (wt.%)	26.0	26.8

[†] This cellulose content is higher than that of most woods, which is usually in the 35-50% range.
[‡] These hemicellulose contents are lower than those of most woods, which usually range from 20% to 30%.

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

Treatment	Time (min)[†]	*Erica arborea*			*Cistus ladanifer*		
		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
	60	1.80	2.74	0.82	1.26	2.33	0.77
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
	60	0.90	1.46	0.34	0.78	1.30	0.29
MW-assisted DMAc/CH_3OK 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

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

Table 3. Mass fractions (w_B, in %) for acid soluble lignin (ASL), furfural, 5-HMF, total sugars (TS), reducing sugars (RS) and non-reducing sugars (NRS) in the hydrolysates after a 60 min treatment for the MW-assisted ChCl/urea, DMAc/$NaHCO_3$ and DMAc/CH_3OK media.

Treatment	*Erica arborea*						*Cistus ladanifer*					
	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_3OK	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

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

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

Component	Solvent	Shrubs		Native cellulose	Hardwoods	References
		E. arborea	*C. ladanifer*			
Lignin	ChCl/urea	0.52-1.80	0.48-1.4		1.43	Chi, *et al.* [15]
	DMAc/$NaHCO_3$	0.33-0.90	0.42-0.79			
	DMAc/CH_3OK	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 *et al.* [20]
	DMAc/$NaHCO_3$	0.97-1.46	0.92-1-30			
	DMAc/CH_3OK	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 *et al.* [21]
	DMAc/$NaHCO_3$	0.22-0.34	0.20-0.29			
	DMAc/CH_3OK	0.00-0.18	0.05-0.13			
	NaOH	0.52	0.47			

Table 5. Total sugars (TS), reducing sugars (RS) and non-reducing sugars (NRS) mass fractions (w_B, in %) for the MW-assisted ChCl/urea, DMAc/$NaHCO_3$ and DMAc/CH_3OK treatments as a function of exposure times. Tests were performed in triplicate, and standard deviations were <5 %.

Treatment	Time (min)†	*Erica arborea*			*Cistus ladanifer*		
		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_3OK 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

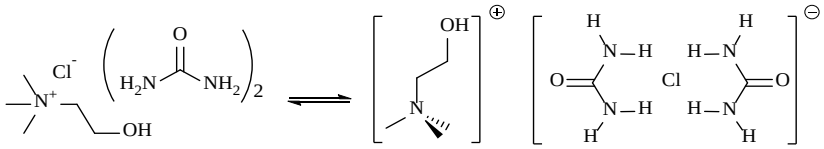
† This time refers to the isothermal treatment time. It should be noticed that the heating and cooling ramps also contribute to the thermal budget (i.e., for t=0 min, there would be a non-zero production of TS, RS and NRS due to heating and cooling ramps).

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

Component	Solvent	Shrubs		Corncob	Bamboo	References
		E. arborea	*C. ladanifer*			
Total sugars (w_{TS})	ChCl/urea	2.94-9.19	3.33-8.45	18.6-20.9		Procentese, *et al.* [37]
	DMAc/$NaHCO_3$	0.45-3.74	0.39-2.70			
	DMAc/CH_3OK	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, *et al.* [38]
	DMAc/$NaHCO_3$	0.17-0.34	0.11-0.23			
	DMAc/CH_3OK	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_3OK	0.11-3.36	0.00-2.54			
	NaOH	3.34	4.64			

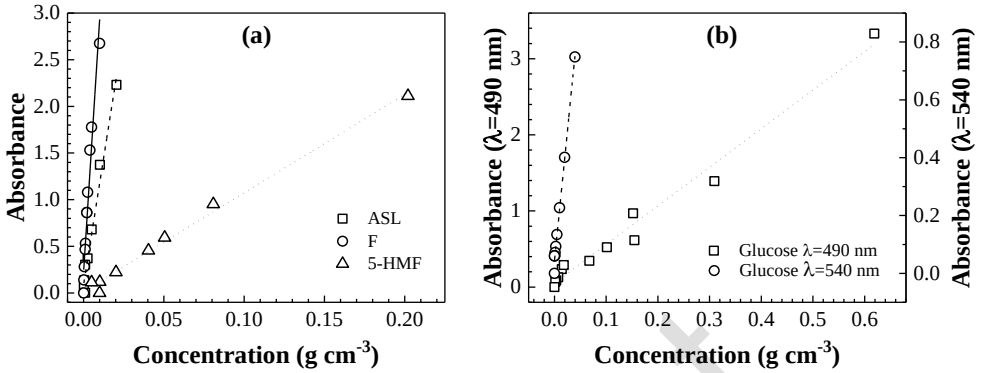
Table 7. Kinetic coefficients (k), correlation coefficients (r^2) and initial concentration of each sample (H_o) determined from the concentration as a function of time for lignin, furfural, 5-HMF, total sugars and reducing sugars production from the hydrolysis of *E. arborea* and *C. ladanifer* lignocellulosic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

Component	Solvent	*E. arborea*			*C. ladanifer*			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_3OK	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_3OK	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_3OK	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_3OK	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_3OK	0.4351	0.9132	0.0668	0.9528	0.8690	0.0668	

556 

557 **Figure 1.** DES of ChCl and urea where a $[choline]^+$ cation is energetically competitive with $[Cl(urea)_2]^-$.

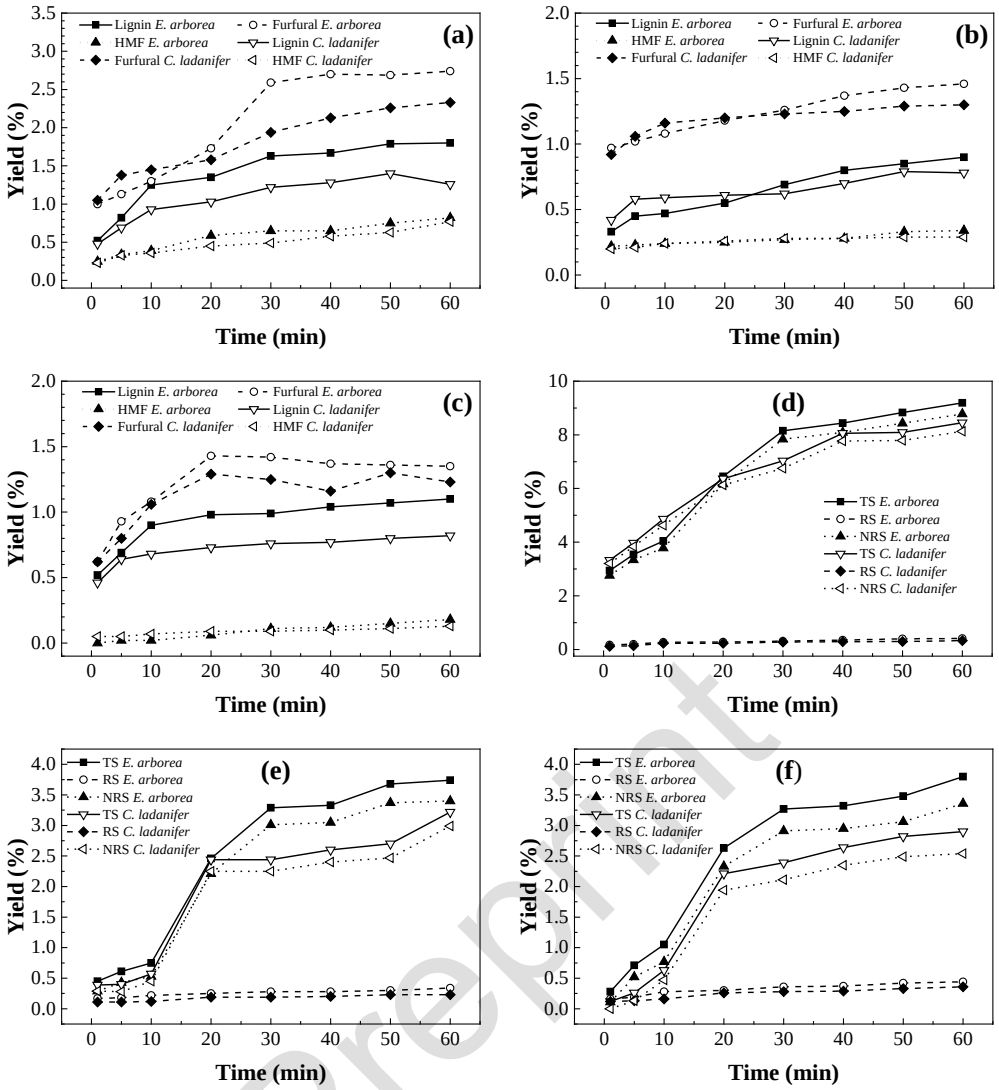
558

559 

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

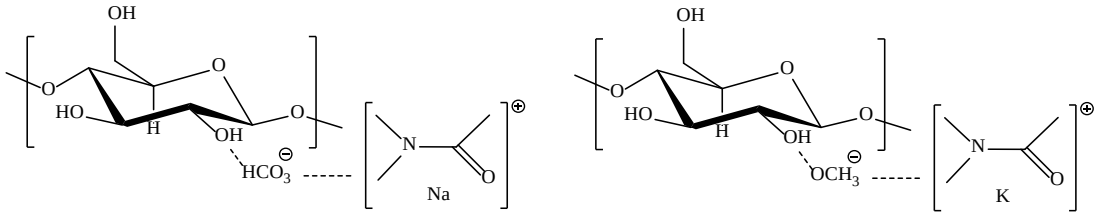
562 the mean of three determinations. Standard deviation bars were omitted for clarity.



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/$NaHCO_3$ extraction;
566 and (*c*) MW-assisted DMAc/CH_3OK extraction. Total, reducing and non-reducing sugars in the
567 hydrolysates after: (*d*) MW-assisted ChCl/urea treatment; (*e*) MW-assisted DMAc/$NaHCO_3$ treatment;
568 and (*f*) MW-assisted DMAc/CH_3OK treatment.

569



570

571 **Figure 4.** Proposed interaction between DMAc-$NaHCO_3$ and DMAc-CH_3OK solvents and sugar polymer