EFFICIENT MICROWAVE-ASSISTED ACID HYDROLYSIS OF LIGNOCELLULOSIC MATERIALS INTO TOTAL REDUCING SUGARS IN IONIC LIQUIDS

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Different types of lignocellulosic materials (carnauba leaves, macauba shell and pine nut shell) and native cellulose have been studied for the production of total reducing sugars (TRS) through microwave-assisted acid-catalyzed hydrolysis in ionic liquids (ILs). Four reaction media have been assessed: two deep eutectic solvents (DES), choline chloride-oxalic acid (ChCl/ox) and choline chloride-urea (ChCl/urea), and two conventional ionic liquids, tetraethylammonium chloride (TEAC) and tetraethylammonium bromide (TEAB). Five acids (H_2SO_4, HCl, HNO_3, $H_3PO_4$and*p*-toluensulfonic acid) have been evaluated in varying concentrations (5-30%) and time intervals (0-60 min), at different temperatures (100-140°C). Significant TRS production yields (as high as 83.7% in ChCl/ox for carnauba leaves) have been attained for both DES in combination with HNO_3 10%, at 120°C for 30 min, with the additional advantage of low furfural and HMF by-products generation.

Keywords: acid hydrolysis, deep eutectic solvents, lignocellulosic materials, microwave, total reducing sugars

INTRODUCTION

Lignocellulosic biomass is a complex biopolymer that is primarily composed of cellulose, hemicellulose, and lignin: it consists of cellulose fibrils kept together by a matrix of lignin and hemicellulose,[1] which provides mechanical protection and acts as a natural barrier against microbial degradation. The study of lignocellulosic materials as a source of liquid fuels has gained importance in recent years due to the need to find an environmentally sustainable source that can replace fossil fuels. The aforementioned carbohydrates, present in high amounts in lignocellulosic biomass,[2] are susceptible of conversion into soluble sugars, either in a direct manner through acidic hydrolysis or indirectly by a two-step process involving pre-treatment and enzymatic hydrolysis.[3]

The hydrolysis process of lignocellulosic materials for sugar production is particularly challenging, given the number of factors that can influence it. While the inherent properties of the substrate (namely neutralization capacity, ratio between easily hydrolysable hemicellulose and cellulose, degree of polymerization of cellulose, configuration of the cellulose chains, and how cellulose is associated with other polymeric structures (lignin, pectin, hemicellulose, proteins, etc.)) can be regarded as immutable, it is necessary to finely tune other parameters in order to improve the hydrolysis efficiency. The dependence on the type and concentration of the acid, the liquid to solid ratio, the reaction time and temperature, the diluent and the type of reactor, for example, are amongst the most significant ones.[4] Cellulose is composed of two glucose molecules linked by 1,4-β-glucosidic bonds. Due to these bonds, there is a strong tendency to form crystals, which are completely insoluble in water

and in most organic solvents.[5] Several studies have shown that the dissolution of lignocellulosic biomass using ionic liquids promotes rupture, releasing carbohydrates.[6] Ionic liquids are solvents with low melting points, wide range of fluid temperature, high polarity, high thermal and chemical stability, non-flammability, negligible vapor pressure and good solvation properties.[7,8] Several of these ionic liquids have been studied in the literature, confirming their positive effect on the pretreatment of lignocellulosic biomass, such as bagasse,[9] wood,[10] or olive kernels.[11]

The aim of this research is to determine the best conditions for the hydrolysis of different lignocellulosic waste materials and to compare the results with those obtained for native cellulose. In this study, three agricultural products have been assayed for the production of total reducing sugars: carnauba leaves, a by-product of carnauba wax extraction; macauba shell, a by-product of oil extraction for biofuels; and pine nut shell, which is used for the production of biogas.[12,13] Tests have been conducted to assess the suitability of diverse ionic liquids as reaction media and the effect of the different acids, concentrations, reaction times and temperature choices on the efficiency of the microwave-assisted hydrolysis. The kinetics of the hydrolysis process has also been studied, so as to explain the variations in TRS production versus time.

EXPERIMENTAL

Raw materials and reagents

Carnauba palm leaves (*Copernicia prunifera*) from Ceará (Brazil), macauba palm endocarp (*Acrocomia aculeata*) from Minas Gerais (Brazil) and European stone pine nut shell (*Pinus pinea*) from Valladolid (Spain) were used as lignocellulosic raw materials. Commercial native cellulose (Merck) was used for comparison purposes. Choline chloride (ChCl), urea, oxalic acid, tetraethylammonium chloride (TEAC), tetraethylammonium bromide (TEAB), H_2SO_4 98%, HCl 34%, HNO_3 50%, H_3PO_4 65%, *p*-toluenesulfonic acid 98% (TsOH) and glucose were purchased from Panreac. Titanium dioxide (TiO_2, anatase variety) was supplied by Sigma Aldrich. 5-hydroxymethyl-2-furaldehyde 98% (HMF) and 2-furfural 98% were purchased from Alfa Aesar. Choline/chloride:urea (1:1)[14] and choline chloride/oxalic acid (1.5:1)[15] were used as DES. The lignocellulosic materials were ground using a Retsch ZM 100 ultra-centrifugal mill and sieved to a particle size <0.250 mm.

Characterization of raw lignocellulosic waste

The insoluble lignin content was calculated according to ANSI/ASTM standard.[16] Holocellulose (hemicellulose+cellulose) was obtained after delignification of the sample and its content was determined using the technique described by Browning.[17] Cellulose was determined according to ANSI/ASTM procedure.[18] Hemicellulose content was calculated by subtraction of the cellulose content from that of holocellulose.

The different biomass waste materials were characterized by X-ray powder diffraction (XRD) in a Bruker D8 Advance Bragg Brentano diffractometer, in reflection geometry. Fourier transform infrared (FTIR) spectra were registered with a Thermo Nicolet 380 FT-IR apparatus equipped with a Smart Orbit Diamond ATR system. The biomass crystallinity index (CrI) was calculated using to the peak intensity method.[19]

Microwave-assisted acid-catalyzed hydrolysis procedure

The acid-catalyzed hydrolysis was carried out in a Milestone Ethos-One microwave. 100 mg of substrate, 20 mg of catalyst (TiO_2), 5 mL of DES/IL and 5 mL of acid were mixed in a 10 mL reaction vessel. The mixture was heated under microwave radiation for 30 minutes and, subsequently, it was rapidly cooled down to room temperature (RT).

To assess the effect of different acids, five acids (H_2SO_4 (5%), HCl (6%), HNO_3 (10%), H_3PO_4(10%) and TsOH (5%)) were investigated, using ChCl/urea DES as a reaction medium and keeping concentration, temperature and reaction time constant. Upon selection of the most suitable acid, the acid concentration effect on the hydrolysis was studied by varying the concentration in the 5-15% range, under the same conditions mentioned above. To study the impact of the different DES/ILs, ChCl/Ox, ChCl/Urea, TEAC and TEAB were used, in combination with HNO_3 at temperatures in the 100-120°C range. The effect of temperature was studied at 120°C, 130°C and 140°C for the best combination of the previously studied parameters, varying the reaction time from 5 to 60 min. Finally, for the most efficient combination, the kinetics of thehydrolysis process was fitted to Saeman's (1945) model.[20]

All determinations were performed in triplicate biological replicates and all reported results are average values. One-way ANOVA analysis was conducted, and Tukey grouping (Tukey's HSD test for an alpha value of 0.05) was used to notice the significance for comparisons among the means.

Analysis

The total amount of reducing carbohydrates was determined using the standard DNS procedure proposed by Miller.[21] The DNS reagent was prepared according to IUPAC procedure: 1.0 mL of carbohydrate solution was added to 3.0 mL of DNS reagent, at 100 °C for 10 min. Upon cooling, the concentration was measured using a Hitachi U-2001 spectrophotometer at 490 nm, with distilled water as a

blank. Glucose was used for the calibration curves. The TRS yield was obtained with the following equation:

$$[\mathrm{TRS}] = \frac{\text{Concentration of TRS } (\mathrm{g \cdot L^{-1}})}{\text{Initial biomass concentration } (\mathrm{g \cdot L^{-1}})} \times 100 \quad (1)$$

The determination of furfural and HMF contents was conducted according to the method by Chi *et al*.,[22] measuring the absorbance of the samples at 277 nm and at 285 nm, for furfural and HMF, respectively. The concentrations of furfural and HMF were also determined with a HPLC spectrophotometer equipped with a UV detector and a Waters ODS-EP C18 reversed-phase column (5 µm, 250 mm × 4.6 mm). During this process, the column temperature was kept constant at 30°C. The mobile phase was water-acetonitrile 15:85 v/v, at a flow rate of 0.5 mL min^{-1}. The volume of each injection was 10 mL, using acetonitrile as an eluent. The UV detection was conducted at 280 nm for both HMF and furfural.

RESULTS AND DISCUSSION

Characterization of the raw lignocellulosic biomass

The raw lignocellulosic materials were analyzed according to their content of cellulose, hemicellulose and lignin, and their crystallinity indexes. The CrI of the different lignocellulosic materials was measured by two different techniques, XRD and FTIR (Figure 1). The results for the analysis of the components and the crystalline index are summarized in Table 1.

As expected, while carnauba leaves presented higher values of cellulose and hemicellulose than that of lignin, macauba and pine nut shell were characterized by the higher lignin content.

With regard to the crystallinity of the bulk materials, the CrI-XRD was determined by the ratio of the maximum intensity at 22.5º and the minimum intensity corresponding to the amorphous area at 18º in the powder diffractograms. The CrI-XRD values (Table 1) indicated that carnauba leaves were the most amorphous material (34.36%) and thus the easiest to attack, while macauba and pine nut shell showed higher crystallinity indexes (42.26 and 42.00%, respectively). Consequently, we could infer that although the latter two had lower contents of cellulose, their cellulosewas actually more crystalline than that of carnauba leaves.

In order to gain further insight on the specific degree of crystallinity of cellulose, useful information can also be obtained from FTIR data. Since the band at 1430 cm^{-1} is associated with the amount of crystalline structure of cellulose and the band at 898 cm^{-1} is assigned to the amorphous region of cellulose, their ratio – the lateral order index (LOI) – is indicative of the manner in which cellulose is arranged in the biomass. It should be noted that carnauba leaves had the highest associated LOI value (Table 1), which would imply that their cellulose content is more ordered than those of macauba and pine nut shell. Nevertheless, this apparent contradiction may be in fact related to the ATR technique, which –in spite of the automatic correction conducted with OMNIC® software – introduces distortion of the relative intensities of the bands,[23] and the CrI-XRD should be regarded as more reliable.

On the other hand, the ratio of the absorbance bands at 3400 and 1320 cm^{-1} can be used to study samples of cellulose according to the hydrogen bond strength (HBI), considering that the band at 3400 $cm^{-1}$is due to OH vibrations[24] and the band at 1320 cm^{-1} is associated with symmetrical C-H deformation modes in cellulose.[25] The HBI is closely related with the crystal system and the degree of intermolecular regularity, which in this case was higher for pine nut shell than for the other two samples.

Table 1
Components and crystallinity index for the lignocellulosic materials under study

Properties	Carnauba leaves	Macauba shell	Pine nut shell
Components analysis			
Cellulose (%)	33.9-41.1	27.6-31.8	29.5-32.4
Hemicellulose (%)	28.5-30.7	26.8-32.3	28.5-30.3
Lignin (%)	10.6-15.3	33.5-36.7	37.6-40.1
Crystallinity index (from XRD data)			
CrI-XRD (%)	34.36	42.26	42.00
Crystallinity index (from FTIR data)			
LOI	1.037	0.790	0.728
HBI	1.002	1.008	1.196

TCI: total crystalline index; LOI: lateral order index; HBI: hydrogen bond intensity

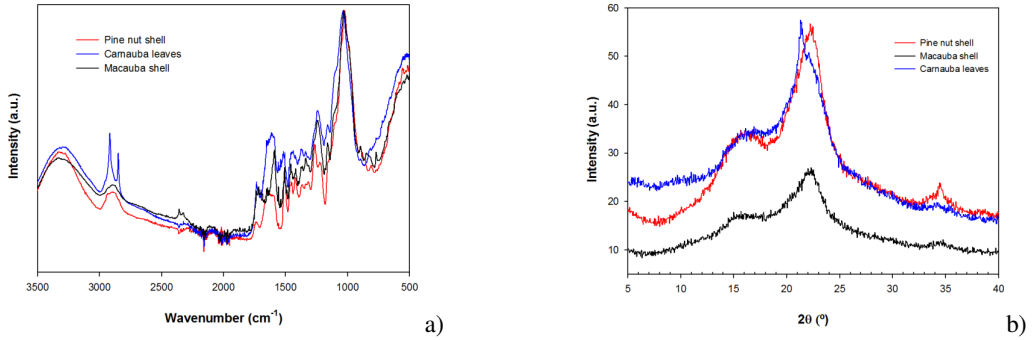

Figure 1: FTIR spectra (a) and XRD patterns (b) for the lignocellulosic materials under study

Table 2
Total reducing sugars (TRS) (%) and HMF/Furfural (HMF+Furf) (mg·L^{-1}) yields attained by microwave-assisted acid-catalyzed hydrolysis (120 °C, 30 min, ChCl/urea reaction medium)

Acids	Carnauba leaves		Macauba shell		Pine nut shell		Native cellulose	
	TRS	HMF+Furf	TRS	HMF+Furf	TRS	HMF+Furf	TRS	HMF+Furf
HNO_3 10%	62.9	0.00	48.3	5.66	59.9	0.00	63.6	72.80
H_3PO_4 10%	45.2	66.36	40.3	73.28	53.0	45.59	65.8	52.33
HCl 6%	38.2	2.80	39.6	3.39	33.7	0.42	37.7	15.39
H_2SO_4 5%	42.9	8.02	34.9	20.11	27.3	0.00	52.5	34.06
TsOH 5%	53.3	77.18	41.5	49.76	32.0	0.00	31.6	0.00

Assessment of the effect of acid type

The effect of the type of acid on the microwave-assisted hydrolysis of native cellulose, carnauba leaves, macauba shell and pine nut shell was investigated, due to the importance of the acid as a catalyst for the reaction.[26] Five acids were assayed, namely nitric, sulfuric, phosphoric, hydrochloric and *p*-toluensulfonic acid. According to Table 2, it could be easily concluded that all acids promoted the formation of reducing sugars, HNO_3 10% being the one with the highest associated TRS yields for all the lignocellulosic materials under study. However, in the case of native cellulose, an increased production of sugars was obtained when using H_3PO_4 10%, in agreement with Li *et al.*,[27] who attained higher TRS yields for H_3PO_4 (54%) acid when compared to HNO_3 (50%), using cellulose as a substrate and 1-butyl-3-methylimidazolium chloride as the reaction medium. Nevertheless, it is worth noticing that the hydrolysis with H_3PO_4led to the isomerization of glucose to fructose, and thus generated the highest HMF and furfural concentrations (ranging from 45.59 to 73.28 mg·L^{-1}) of all the acids under study. HMF and furfural are not desirable by-products, considering that they affect the fermentation process, which is the following step in bioethanol production.

Conversely, when the hydrolysis was catalyzed by HNO_3 acid, the formation of fermentation inhibitors (HMF and furfural) was very low (Table 2), a behavior that has also been reported by Kim *et al.*[28] This spares an additional detoxification step aimed at eliminating these by-products. Furthermore, the use of HNO_3 serves as a nitrogen source for the fermentation of yeast.[29]

When HCl 6% was assayed as a catalyst, TRS values were very low, ranging from 29.7 to 39.6% (2.97 to 3.96 g·L^{-1}), with slightly better results for macauba shell. These values differed from those reported for *Chlorella* algae biomass by Zhou *et al.*,[30] who obtained yields of 90% for hydrolysis conducted at 105°C for 3 hours using HCl (7%).

H_2SO_4 5% acid, which is widely used as a hydrolysis catalyst, showed intermediate TRS production values, ranging from 27.3 to 52.5% (2.73 to 5.25 g·L^{-1}). These results were lower than those reported by Saleh *et al.*,[11] who obtained a TRS yield of 89.70% at 195°C for 5 min, in the particular case of olive kernels. Slightly higher yields (58%) than those reported herein were also attained by Yunus *et al.*[31] for empty fruit bunch by using an ultrasound pretreatment and conducting the hydrolysis at 140°C for 200 min.

With regard to TsOH 5%, it promoted the formation of TRS with yields ranging from 31.6 to 53.3% (3.16 to 5.33 g·L^{-1}), but also promoted

the formation of undesirable products (HMF and furfural) for carnauba leaves and macauba shell (77.18 and 49.76 $mg \cdot L^{-1}$, respectively). These values were slightly higher than those reported by Amarasekara *et al*.,[32] who obtained a 32.6% yield for cellulose, at 170°C for 3 hours. The same authors also concluded that TsOH had better yields than H_2SO_4 (32.6% and 22.0%, respectively), a behavior that was corroborated in the study presented herein. This activity enhancement may be the result of the adsorption of *p*-toluenesulfonic acid onto the biomass surface, which is supported by the repulsion of the hydrophobic tolyl group from the bulk of the water phase, thereby pushing into the lignocellulosic structure, which causes the disruption of the cellulose H-bonding network. This repulsion from water, sustained by the hydrophobic group, is not possible for sulfuric acid, which would explain its relatively weaker activity.[32]

Influence of acid concentration

It is important to know the limit of the acid concentration at which the sugars begin to degrade to HMF and furfural. When a highly concentrated acid is used, the formation of inhibitory products cannot be avoided.[10] The effect of HNO_3 concentration on TRS production by hydrolysis of the different lignocellulosic materials is shown in Figure 2.

An increase in total sugar production when the acid concentration was increased from 5 to 10% was observed in all cases, with an associated increase in the production of degradation products. In the particular case of pine nut shell, a larger amount of acid (15%) was required for the sugar degradation process to start. It is also worth noting that the production of HMF from native cellulose was much higher than from the three lignocellulosic materials under study. This can be explained by the fact that cellulose consists of glucose monomers, which are the precursor of HMF.

The highest TRS production occurred at an acid concentration of 10% in all cases, except for native cellulose. The hydrolysis of cellulose required stronger conditions because the *β*-glycosidic bonds of its glucose moieties are strongly protected by a hermetic structure of cellulose microfibers, hampering the hydrolysis process.[33]

When the acid concentration was increased to 15%, a decrease in TRS concentration values took place. Zhou *et al*.[30] observed this same behavior when studying different HCl concentrations (0, 3, 7 and 10%), obtaining the best results for HCl 7%. Similarly, when studying sugar cane bagasse hydrolysis with different doses of H_2SO_4 (2, 4, 6 and 8%), Dominguez *et al*.[34] found that dilute conditions of the acid (4%) were the optimal choice, since very low acid concentrations were not effective in the hydrolysis and very high acid concentrations resulted in a breakdown of sugars to undesirable by-products. Mateus *et al.*[35] also stated that the degradation to furfural (pentoses) and HMF (hexoses) was enhanced when the acid concentration and temperature were increased.

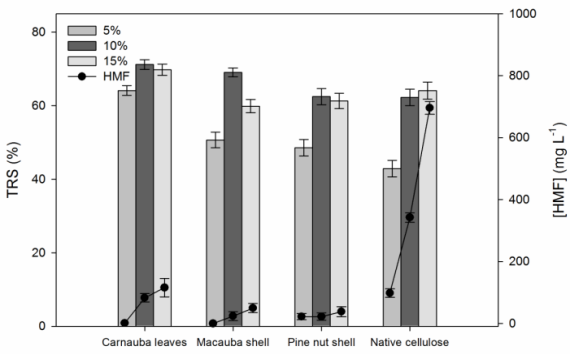

Figure 2: TRS and HMF production from carnauba leaves, macauba shell, pine nut shell and native cellulose, for different doses of acid ($HNO_3$10%, 120°C, 30 min and ChCl/urea reaction medium)

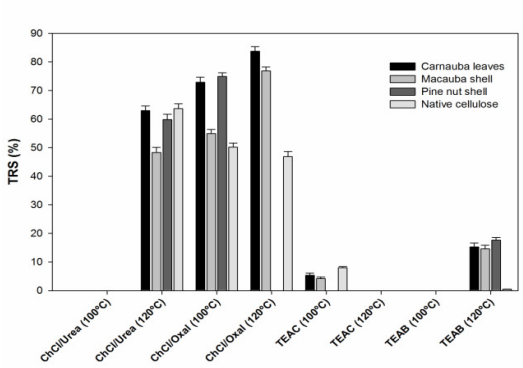

Figure 3: Total reducing sugars production in HNO_3 10% acidic medium, at 100°C/120°C for 30 min

Impact of ionic liquid type

The TRS production yields obtained from lignocellulosic residues and cellulose in four different reaction media at 100°C and 120°C with HNO_3 10% for 30 min are summarized in Figure 3. No sugar production occurred for the ChCl/urea DES at 100°C, but the formation of sugars could be attained by rising the temperature to 120°C. At this temperature, TRS production yields of 63.6%, 62.9%, 59.9% and 48.3% were attained for native cellulose, carnauba leaves, pine nut shell and macauba shell, respectively.

For the ChCl/oxalic acid eutectic mixture, significant TRS production took place at both temperatures, with yields as high as 83.7% for carnauba leaves, followed by macauba shell (76.8%), native cellulose (46.9%) and pine nut shell (0%, i.e., below detection threshold). When conventional ionic liquids (TEAC and TEAB) were used, TRS production was very low: for TEAC, the highest TRS production took place at 100°C, while for TEAB it occurred at 120°C.

From the aforementioned data, it can be inferred that the highest TRS productions are attained when DES are used as reaction media. Further, DES have better characteristics than ILs in terms of cost and toxicity.[36] In comparison with the values reported in the literature, it should be noted that Rinaldi *et al*.[37] found lower yields with the use of 1-butyl-3-methylimidazolium chloride in the hydrolysis of starch, cellulose and palm stem, obtaining reducing sugars with yields of about 19.9% at 130°C for 4 h. It should also be clarified that the ionic liquidswere unable to efficiently hydrolyze the biomasson their own, requiring an acidic medium to optimize the hydrolysis process.

With regard to the HMF/furfural concentrations, they were very low for all reaction media, ranging from 0.069 to 0.10 $g \cdot L^{-1}$ for ChCl/urea, and from 0.10 to 0.40 $g \cdot L^{-1}$for ChCl/ox. Similar values were attained for conventional ionic liquids (TEAB and TEAC), except in the particular case of carnauba leaves with TEAB and HNO_3 10%, in which the by-products concentration reached 0.7 $g \cdot L^{-1}$. This value can be regarded as worryingly high, considering that Keating *et al*.[38] found that values of HMF and furfural higher than 0.08 $g \cdot L^{-1}$ affect the fermentation process.

Effects of temperature and time on reaction yields

The acid-catalyzed hydrolysis was also studied by varying temperatures and reaction times. The effects of these parameters are shown in Figure 3. Keeping the reaction temperature fixed at 120 °C, it could be observed that the peak production of sugars was attained at 30 min, ranging from 43.6 to 68.8% (4.36 to 6.88 $g \cdot L^{-1}$). In the case of carnauba leaves and pine nut shell, it is also worth noticing that 120 °C was not a sufficiently high temperature to hydrolyze the materials in the first 5 minutes, and more time was required to start the hydrolysis process of the materials (Figure 4 a,c). On the other hand, the increase in the reaction temperature entailed an increase in the TRS production yields for all substrates.

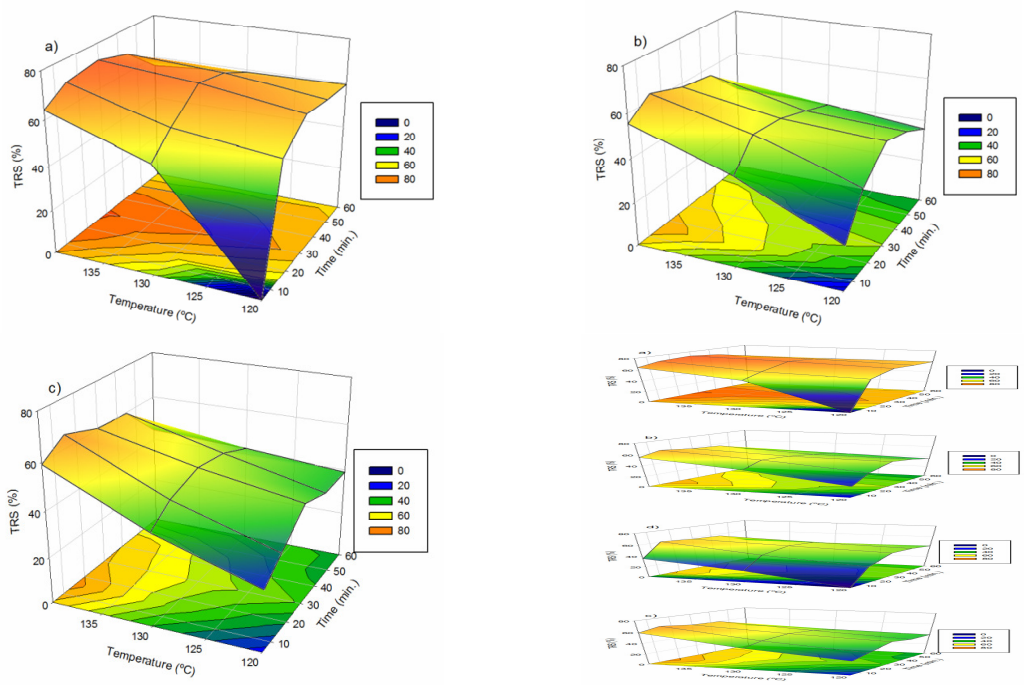

Figure 4: TRS production from carnauba leaves (a), macauba shell (b), pine nut shell (c) and native cellulose (d) at different temperatures (HNO_3 10%, 30 min and ChCl/urea reaction medium)

The highest TRS yield was obtained for carnauba leaves at 140°C and for reaction times in the 15-30 min range (Figure 4a). This result was consistent with the analysis of the composition of each material (Table 1), where a higher proportion of cellulose and hemicellulose and a lower lignin content were observed for this material. Rodriguez-Chong *et al.*[9] obtained similar results using HNO_3 (2%), at 128 °C for 180 min, whereas Tong *et al.*[39] obtained lower yields (cca. 16.9%) at 200 °C for 10 hours, using H_3PO_4(10%). Nonetheless, Wijaya *et al.*[10] reported better results (87.48 to 90.39 $g \cdot L^{-1}$) using more oxidant reaction media by resourcing H_2SO_4 (80%) at 80°C for 30 min.

There was a trade-off between temperature and reaction time, as expected: higher reaction temperature required shorter reaction times, and the other way round. In any case, the production process was followed by the degradation of TRS to HMF-furfural, with an increased reaction rate at higher temperatures, as noted by Mateus *et al.*[35] Therefore, 120°Ccould be regarded as the most convenient choice, since degradation was minimized versus those at 130°C and 140°C.

For all materials, the appearance of two phases in the reaction could be observed when time was increased: one which corresponded to the formation of sugars and the second one associated to the breakdown of sugars to by-products (HMF and furfural). The latter, with continued heating, would lead to levulinic and formic acid production.[20] This same behavior was reported by Wijaya *et al.*[10] in the hydrolysis of oak wood, pine wood and empty fruit bunch.

Kinetic study of TRS production

Lignocellulosic materials can be hydrolyzed into various products, namely glucose, xylose, furfural and 5-HMF.[9,40] Since the acid hydrolysis of lignocellulosic materials is a complex process, simplified first order and pseudo-first order models (which are generally used to determine the reaction kinetics) are not applicable, considering that this type of kinetics assumes that the formation of intermediates (HMF and furfural) is negligible. Consequently, a dilute acid hydrolysis should be described by two consecutive, irreversible, pseudo-homogeneous first-order reactions, as follows:

$$\text{Polymers (P)} \xrightarrow[k_1]{} \text{Monomers (M)} \xrightarrow[k_2]{} \text{Decomposition products} \quad (2)$$

where $k_1(min^{-1})$ is the rate of conversion of glucan to glucose and k_2 (min^{-1}) is the rate of decomposition of glucose. Decomposition products may be furfural, hydroxymethylfurfural,

formic acid, levulinic acid, etc. From this reaction model and solving differential equations, monomer concentration (*M*) as a function of time (*t*) can be represented by:

$$M = M_0 \cdot e^{-k_2 \cdot t} + P_0 \cdot \frac{k_1}{k_2 - k_1}\left(e^{-k_1 \cdot t} - e^{-k_2 \cdot t}\right) \quad (3)$$

where *P* is the polymer concentration ($g \cdot L^{-1}$) and the subscript *0* indicates the initial conditions. In this work, this equation was applied to model the acid hydrolysis of the different substrates using nitric acid.

The degree and rate of hydrolysis depends on many factors: temperature, biomass concentration, type of organic matter and particle size, for example. The same behavior was observed for all materials: when the hydrolysis time increased, the concentration of sugars was reduced, due to decomposition of sugars into furfural, HMF and levulinic acid.

Figure 5 shows the experimental data together with the curves obtained from the kinetic model discussed above. It can be observed that native cellulose had the highest concentration of total reducing sugars (TRS) after 30 min (6.36 $g \cdot L^{-1}$). With regard to the lignocellulosic materials under study, carnauba showed a very similar value to that of native cellulose (6.29 $g \cdot L^{-1}$), followed by pine nut shell (5.99 $g \cdot L^{-1}$) and macauba shell (4.83 $g \cdot L^{-1}$). In all cases, TRS formation peaked at 30 min, after which the increase in reaction time caused a decrease in the TRS content due to degradation. At the point of maximum production of TRS (30 min), the amounts of HMF/furfural varied between 0.02 and 0.10 $g \cdot L^{-1}$. Bellido *et al.*[41] observed that such low amounts of these inhibitors had no effect on the subsequent fermentation process.

Table 3 shows the kinetic coefficients, k_1 and k_2, the initial monomer and polymer concentrations (M_0 and P_0) and the correlation coefficient r^2 for the adjustment of the experimental data with Saeman's model. All r^2 were higher than 0.9, which indicated a good agreement between experimental and predicted data, and it could be inferred that the materials are susceptible to acid hydrolysis under the conditions studied.[9]

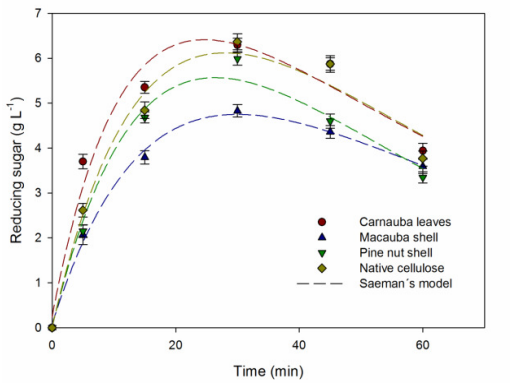

Figure 5: TRS production experimental and predicted values using Saeman's model

Table 3
Kinetic and statistical parameters for total reducing sugars production by microwave-assisted HNO_3 10%catalyzed hydrolysis at 120 °C

Sample	k_1 (min^{-1})	k_2 (min^{-1})	P_0 ($g \cdot L^{-1}$)	M_0 ($g \cdot L^{-1}$)	k_1/k_2	r^2
Carnauba leaves	0.0243	0.0242	11.44	6.39E-09	1.004	0.9508
Macauba shell	0.0497	0.0208	21.11	7.16E-02	2.389	0.9979
Pine nut shell	0.0278	0.0277	9.37	6.94E-09	1.004	0.9135
Native cellulose	0.0308	0.0307	16.42	6.79E-02	1.003	0.9437

P_0: polymer concentration; M_0: monomer concentration

k_1 values varied from0.0243 to 0.0497 min, and k_2 values ranged from0.0242 to 0.0308 min. The highest TRS formation rate corresponded to macauba shell, which had the highest k_1 value (0.0497), while the lowest rate was associated to carnauba leaves, with the lowest k_1 value

(0.0243). Macauba shell showed the lowest TRS decomposition rate, i.e., the lowest k_2 value (0.0208), while the highest rate corresponded to native cellulose, with a k_2 of 0.0307. Chin *et al*.[40] studied the kinetics of sugars production from oil palm empty fruit bunch (EFB) using the same temperature (120°C) and obtained lower k_1 values (0.0007), indicating that our process had a faster rate of TRS production reaction. The ratio of the TRS formation and decomposition rates (k_1/k_2) was close to 1 for all materials, except for macauba shell (2.389). This suggested that, in the particular case of macauba shell, the TRS formation rate was greater than its decomposition rate, so it can be regarded as the lignocellulosic waste that can be most easily decomposed to sugars.

Optimal conditions for each type of biomass

Optimum conditions (in terms of type of acid and its concentration, reaction medium, temperature and reaction time) were defined for each type of biomass, with a trade-off between high TRS yield and low-concentration of fermentation inhibitors (HMF and furfural). For all types of biomass, this goal was attained for the combination of a DES (either ChCl/ox or ChCl/urea) with HNO_3 10%. While temperatures in the 130-140°C range led to a better hydrolysis of lignocellulosic biomass, the increase in HMF and furfural values favored the choice of 120°C as the optimum temperature in terms of efficiency. Regarding the reaction times, which varied for each material and each temperature, 30 minutes was the most suitable for a reaction temperature of 120°C, since it optimized TRS production while minimizing degradation.

CONCLUSION

Microwave-assisted acid-catalyzed hydrolysis was successfully applied to break the structure of cellulose and hemicellulose and extract sugars from carnauba leaves, macauba shell, pine nut shell and native cellulose. In relation to the choice of reaction medium, DES (ChCl/ox and ChCl/urea) systematically led to higher TRS production than conventional ionic liquids (TEAC and TEAB), with the additional advantages of being more environmentally friendly, more economical, less toxic and easily prepared on a large scale. The highest TRS productions were attained for carnauba leaves (83.7% and 62.9% for ChCl/ox and ChCl/urea, respectively), the material with the highest amount of cellulose and lowest crystallinity amongst the chosen lignocellulosic wastes. Although both DES produced large amounts of TRS, ChCl/urea was chosen as a preferred medium, taking into consideration not only the TRS productivity, but placing emphasis on keeping the concentrations of the fermentation inhibitors (HMF/furfural) as low as possible, with a view to subsequent bioethanol production. The optimum operating conditions (ChCl/urea, HNO_3 10%, 120°C, 30 min) were confirmed by a kinetic study.

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