Hydrothermal fractionation of woody biomass: lignin effect over sugars recovery

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11 Abstract

12 Subcritical water was employed to fractionate woody biomass into carbohydrates and
13 lignin. Nine urban trees species (hardwood and softwood) from Spain were studied. The
14 experiments were carried out in a semi-continuous reactor at 250ºC for 64 min. The
15 hemicellulose and cellulose recovery yields were between 30% wt. and 80% wt. while the
16 lignin content in the solid product ranged between 32% wt. and 92% wt. It was observed
17 that an increment of solubilized lignin disfavored the hydrolysis of hemicelluloses. It was
18 determined that the maximum extraction of hemicellulose was achieved at 20 min of
19 solid reaction time while the extraction of celluloses not exhibited a maximum value. The
20 hydrolysis of hemicellulose and cellulose would be governed by the hydrolysis kinetic and
21 the polymers accessibility. In addition, the extraction of hemicellulose was negatively
22 affected by the lignin content in the raw material while cellulose hydrolysis was not
23 affected by this parameter.

24 Keywords: Biorefinery, Glucose, Process flexibility, Xylose

1. Introduction

Lignocellulosic biomass has emerged as a potential renewable resource for the production of fuels (Thangavelu et al., 2014), energy and added value chemical products (Wijaya et al., 2014). To achieve this, different treatments should be applied to the raw material in a concept industry called: Biorefinery (Bozell, 2008). Lignocellulosic biomass is a complex material composed mainly of three biopolymers: hemicellulose, cellulose and lignin. The nature of these polymers is quite different, hemicellulose is composed of C-5 molecules like xylose, arabinose, mannose and galactose while cellulose is only composed of glucose. On the other hand lignin is the most complex polymer of biomass composed of phenolic units linked in a three dimensional network (Cantero et al., 2015). These polymers interact between them by covalent, hydrogen and Van deer Walls bonds. So, prior the production of chemicals and fuels in a selective way, it is needed to break the interactions between the three main polymers of biomass, which will allow the separation of them.

Sub and supercritical water (SCW) have gained attention as a promising solvent for performing the reactions of fractionation and hydrolysis of biomass. One of the main advantages of SCW is that the used solvent is only water. Water is environmentally friendly and represents an alternative to corrosive and toxic solvents being an attractive reaction media for a large number of applications. The variations in the properties of water near to its critical point (374ºC and 22.1 MPa) only by changing pressure and temperature make it a promising reaction medium to set different reaction conditions depending on the desired product. The main properties of water that can be modified by pressure and temperature and will define the identity of the medium are: dielectric

48 constant, ionic product, density, miscibility and transport properties (Pavlovič et al., 2013;
49 Toor et al., 2011). Water can adopt different roles in the reaction medium: as solvent,
50 reactant or catalyst (Knez et al., 2015). The ionic reactions are favored at high densities
51 and high ionic products while the radical reactions are favored at supercritical water
52 conditions (Peterson et al., 2008). The challenge is to find the experimental conditions
53 which can be adaptable to all kind of biomass contributing to the decentralization and
54 versatility of the process to industrial scale (Arai et al., 2009).

55 The most abundant organic source on the earth is woody biomass with the annual
56 production of approximately $5.64 \cdot 10^{10}$ Mg-C (Liu et al., 2012). The forest activity produces
57 a massive amount of resources and residues which can be used to produce added value
58 products or energy through biorefinery operations, improving the health status of
59 forests. This activity is greatly available in Spain. An increasing trend since 1990 has been
60 observed. According to Eurostat data, since 1990, Spain has increased its forest area to
61 annual rate of 2.19%, being the second country (Sweden leads the first place) with major
62 total forest area in Europe. According to the data from Ministry of Agriculture, Food and
63 Environment (2011), Spain has 8.6 (46.4% of forest area) and 6.4 (34.5% of forest area)
64 millions of hectares of hardwoods and softwoods species and forests and forest land
65 occupy 54% of the national area. Since 1975, has been doubled the woody volume from
66 456.7 to 927.8 millions of m^3 (Forestales, 2013). The use of woody biomass is imperative
67 to world economy and Spain has the resources for a sustainable society.

68 Several studies consider woody biomass as potential feedstock for the production
69 of chemicals and fuels. Numerous pretreatments have been studied to determine the
70 optimum conditions to obtain high yield of carbohydrates from plant biomass (Wijaya et

al., 2014). The pretreatment is necessary to alter the structure of biomass and to increase the hydrolysis of hemicelluloses and celluloses to the enzymes for monosaccharides production (Gong et al., 1999) and increase the porosity of the materials. The fractionation of vegetal biomass using liquid hot water has been investigated obtaining high recovery of the biomass components in relative low treatment times. The hydrolysis enables the depolymerization of hemicelluloses (Grenman et al., 2011), the hydrolysis of lignin (Hu et al., 2014) and the hydrolysis of celluloses. These hydrolysis reactions are usually followed by the formation of byproducts such as furfural, 5-HMF, acetic acid and lactic acid (Du et al., 2010; Li et al., 2014). The hydrothermal fractionation of biomass is usually carried out between 150ºC and 250ºC, at pressures lower than 10 MPa (Mok & Antal Jr, 1992; Sun et al., 2014b; Wei et al., 2011). The recovery yields of hemicellulose usually range between 60% wt. and 100% wt. while cellulose recovery is lower than 60% wt. However, the fractionation yields of plant biomass showed to be highly affected by the nature of the biomass: structural and chemical properties. So, the selection of the experimental conditions play an important role in the obtaining of high yields of the hydrolyzed biopolymer. For instance, hardwood hemicelluloses can be removed/hydrolyzed at lower temperatures than the softwood hemicelluloses (Wei et al., 2011). The main compound in hemicellulose is xylose for hardwood and mannose for softwood. The content of lignin for hardwood is generally lower than softwood (Lim & Lee, 2013). The hardwood has higher content of acetyl groups than softwood, which increase the concentration of acetic acid during the hydrolysis of hemicelluloses being this specie a catalyst in the hydrolysis of carbohydrates (Garrote et al., 1999).

In this work the influence of the composition and nature of the biomass on the hydrothermal treatment of hardwood and softwood material was studied. The

experimentation was focused in the obtaining of high hemicelluloses and cellulose yield with low byproduct generation and a solid rich in lignin. The main objective was studied the system flexibility using different raw materials trying to find a general trend relating these yields with the initial lignin content in biomass. This manuscript will contribute to understanding the effect of the biomass composition in the processes of plant biomass fractionation into sugars and lignin.

2. Materials and methods

2.1. Materials

The raw materials used in this work to conduct the extraction/hydrolysis process were 9 species of urban trees from Valladolid region, in Spain. These species were: Linden (*Large-leaved linden*), Plane (*Platanus x acerifolia*), Eucalyptus (*Eucalyptus globulus*), Catalpa (*Catalpa bignonioides*), Holm oak (*Quercus ilex*), Maple (*Acer saccharum*), Almond (*Prunus dulcis*), Pine (*Pinus pinea*) and Cedar (*Juniperus oxycedrus*).

The standards used in High Performance Liquid Chromatography (HPLC) analysis were: cellobiose (>98%), glucose (>99%), fructose (>99%), glyceraldehyde (95%), pyruvaldehyde (40%), arabinose (>99%), 5-hydroxymethylfurfural (99%), lactic acid (85%), formic acid (98%), acrylic acid (99%), mannose (>99%), xylose (>99%) and galactose (>99%) purchased from Sigma and used without further modification.

For the determination of extractives, n-hexane (96%) supplied by Sigma was used. For the determination of carbohydrates, lignin and ash, sulfuric acid (96%) and calcium carbonate (≥ 99.0%) were purchased from Panreac and used as reagents without further modification. Distilled water was used as reaction medium in the experiment and Milli-Q water was used as mobile phase in the HPLC analysis.

2.2. Methods

Two products were obtained after the hydrothermal treatment (explained in section 2.3) of the raw material: solid and liquid. The solid sample was the remaining amount of biomass in the fixed bed reactor after the treatment. On the other hand, the liquid sample was produced due to the extraction/hydrolysis of the hemicellulose and cellulose fractions of the raw material during the treatment. The raw materials and samples (liquid and solid) taken during hydrothermal fractionation were analyzed to determine the carbohydrates and lignin content. The content of hemicelluloses, celluloses, lignin and ash were determined according to the National Renewable Energy Laboratory (NREL) – Determination of Structural Carbohydrates and Lignin in Biomass (Laboratory, 2011; Sluiter et al., 2010; Sun et al., 2014a). The content of extractives was determined according to the Determination of Extractives in Biomass (A. Sluiter, 2008; Sluiter et al., 2010). The solid samples were dried at 105ºC to constant weight to obtain chemical composition in dry weight basis.

2.2.1. Liquid chemical composition

The liquid samples were subjected to different analysis. First, the pH of the sample was measured just after the sample was taken. The total organic carbon was also determined directly to the taken sample. The composition of sugars from cellulose and hemicellulose was determined by hydrolyzing the extracted oligo- and polysaccharides to their simple monomer: glucose, xylose, arabinose, mannose and galactose. To do so, 10 ml of the liquid sample was set in a hydrolysis bottle (100 mL) together with 4 ml of sulfuric acid. The bottle was incubated at 30ºC for 30 min and then, 84 ml of distilled water was added. After this, the hydrolysis bottle was incubated at 121ºC for 1 h. After

that, the bottle was cooled to room temperature and calcium carbonate was added to neutralize the medium obtaining a final pH between 6 and 7. Then, the sample was filtrated with nylon filters membranes (0.20 µm) and analyzed by HPLC. The HPLC chromatograms were analyzed using Fast Fourier Transform and band-adjustment by Gaussian functions. The yield of hemicellulose products was determined in terms of initial mass and initial hemicellulose as shown in equation 1 and 2 respectively. In the same way, cellulose yield was determined in function of initial mass and initial cellulose as shown in equations 3 and 4 respectively. The hydrolysis product yield was determined by equation 5.

$$Hemicelluloses(g/g_{biomass}) = [xylose(g)+arabinose(g)+galactose(g)+mannose(g)]*m_{biomass}(g)^{-1} \quad (1)$$

$$Hemicelluloses(g/g_{hemibiomass}) = [xylose(g)+arabinose(g)+galactose(g)+mannose(g)]*[m_{biomass}(g)*X_{hemibiomass}]^{-1} \quad (2)$$

$$Celluloses(g/g_{biomass}) = [glucose(g)+fructose\ (g)+cellobiose(g)]*m_{biomass}(g)^{-1} \quad (3)$$

$$Celluloses(g/g_{cellulosebiomass}) = [glucose(g)+fructose\ (g)+cellobiose(g)]*[m_{biomass}(g)*X_{cellulosebiomass}]^{-1} \quad (4)$$

$$Derived\text{-}products(g/g_{biomass}) = [glyceraldehyde(g)+pyruvaldehyde(g)+lactic\text{-}acid(g)+formic\text{-}acid(g) +acrylic\text{-}acid(g)+5\text{-}hydroxymethylfurfural(g)]*m_{biomass}(g)^{-1} \quad (5)$$

The amount of extraction for each component was calculated as it is shown in equation 6.

$$dX_y = C_y.f.dt \quad (6)$$

Where $m_{biomass}$ is the initial content of biomass in the reactor (gr); $X_{hemibiomass}$ is the initial content of hemicelluloses in the biomass (% wt.); $X_{cellulosebiomass}$ is the initial content of celluloses in the biomass (% wt.); X_y is concentration of component y in gr; C is concentration of component y in ppm; f is the flow in ml/min and t is the time in min.

2.2.2. Solid chemical composition

The raw material and the solid samples taken from the reactor after the hydrothermal treatment were analyzed in the same way for carbohydrate and lignin determination. However, the raw materials were subjected to an extraction process using n-hexane as solvent prior the acid hydrolysis. For carbohydrates and lignin content determination, 300 mg (W_1) of the solid samples were set in a hydrolysis bottle together with 3 mL (72%) of sulfuric acid and it was incubated at 30ºC for 30 min. Then, 84 mL of distilled water was added to the bottle and it was incubated at 121ºC for 1 h. Finally, the bottle was cooled to room temperature and the hydrolysates were filtrated under vacuum obtaining two products: a solid composed of insoluble lignin and ash, and a liquid composed of carbohydrates and soluble lignin. The solid was dried at 105ºC for 24 h (W_2) and then it was heated at 550ºC for 24 h in a muffle and weighted (W_3). The content of acid insoluble lignin (AIL) and ash (A) were calculate using the equations (7) and (8) respectively.

$$AIL(g) = \frac{W_2(g) - W_3(g)}{W_1(g)} 100 \quad (7)$$

$$A(g) = \frac{W_3(g)}{W_1(g)} 100 \quad (8)$$

The obtained liquid after acid hydrolysis was divided into two aliquots of 50 ml. Calcium carbonate was added to one of these sample to neutralize the medium obtaining a pH between 6 and 7 and then was analyzed by HPLC. The other sample was analyzed using a UV-visible spectrophotometer to determine the soluble lignin content. The wavelength was set at 320 nm and the absorptivity value was 30 $L \cdot g^{-1} \cdot cm^{-1}$. The soluble lignin content was determined by equation 9 (Laboratory, 2011).

185 $$SL(g) = \frac{UV_{abs} * volume_{filtrate} * Dilution}{\varepsilon * ODW * Pathlength} \quad (9)$$

186 Where SL is the insoluble lignin (g/gbiomass); UV_{abs} is the average UV-Visible
187 absorbance; volume filtrate is the volume hydrolysis liquor (ml); Dilution is the dilution
188 factor (adim); ε is Absorptivity of biomass at specific wavelength; ODW is the weight of
189 sample (milligrams) and Pathlength is pathlength of UV-Vis cell (cm).

190 ### 2.2.3. Analysis

191 The content of total organic carbon (TOC) and pH were analyzed in the liquid
192 samples. TOC was measured using a Shimadzu TOC-VCSH analyzer. The pH was measured
193 by Nahita model 903 pH-meter using an electrode Glass- Body ElectroJelly PH5101-3B.

194 The sugars content was determined by HPLC. A SH-1011 column from Shodex was used
195 to perform the separation of the compounds with 0.01 N of sulfuric acid as mobile phase
196 with a flow of 0.8 ml/min and at 50ºC. The HPLC apparatus is equipped with two
197 detectors: Waters IR detector 2414 (210 nm) to identify the carbohydrates and their
198 derived products and Waters dual λ absorbance detector 2487 at 254 nm to identify the
199 5-hydroxymethylfurfural.

200 The raw materials and the solid obtained after the fractionation-hydrolysis were
201 analyzed by Fourier transform infrared spectroscopy (FTIR) and scanning electron
202 microscopy (SEM). The FTIR analysis were carried out in a Bruker Tensor 27 spectrometer.
203 The analyzed region was between 4000-400 cm^{-1} with a resolution of 4 cm^{-1} and 32 scans
204 was recorded. The surface morphology of these samples was determined by SEM JSM-
205 820 (Joel). A gold evaporator Balzers SCD003 with a gold thickness 25-30 nm was used.

206 The accelerating voltage was 20 kV. The samples were placed under high vacuum
207 conditions.

208 ### 2.2.4. Determination of reaction time

209 Two reaction times were calculated in the semicontinuous system: a solid and a
210 liquid reaction time. The solid reaction time was always defined as 64 min. This is the time
211 that the insolubilized fraction of the raw material remains inside the reactor. The liquid
212 reaction time depends on the flowrate and the mass inside of the reactor (variable value).
213 The liquid reaction time was calculated as the average between the initial and final
214 reaction time determined by equation 10 and 11 respectively.

215 $$LRT_i = [VR - (m_i / density)] / f \quad (10)$$

216 $$LRT_f = [VR - (m_f / density)] / f \quad (11)$$

217 Where LRT_i is initial liquid residence time in min; VR is the volume of the reactor in m^3; m_i
218 is the initial solid inside of the reactor in Kg; density is the density of the material inside
219 of the reactor in $Kg \cdot m^3$; f is the flow in $ml \cdot min^{-1}$; LRT_f is final liquid residence time in min;
220 m_f is the solid inside of the reactor after the solid residence time in Kg.

221 ### 2.3. Heat integration

222 A simplified energy balance to know the recovery energy and the value of overall
223 heat transfer coefficient (U) were calculated. The heat integration was achieved by
224 installing a heat exchanger between water inlet and product outlet.

225 The overall heat transfer coefficient is calculated as shown in equation 12:

226 $$U = \frac{Q}{A * \Delta T_{lm}} \quad (12)$$

Where U is overall heat transfer coefficient (W·ºC·m2), Q is heat load (W), A is internal exchanger area (m2), ΔTlm is logarithmic mean temperature difference (ºC).

The efficiency of a counter flow heat exchanger is calculated as shown in equation 13:

$$\eta = \frac{Q}{Q_{max}} \quad (13)$$

Where η is the efficiency of the system and Qmax (W) is the maximum heat load obtained.

2.4. Experimental setup and operation

The process configuration is shown in the Figure 1. The hydrothermal process was carried out in a semi-continuous reactor. The system essentially consists of a pump feeding system (PU-2080 model), a preheater (E-01, 200 cm of 1/8" AISI 316 piping), a reactor (R-01, 38 cm length, 1/2" O.D. SS316 piping), an oven (HP5680 model) and two heat exchangers (E-02, 15 cm of concentric tube heat exchanger 1/4"-3/8" countercurrent operation and E-03, 70 cm of concentric tube heat exchanger 1/4"-3/8" countercurrent operation). The pressure of the system was controlled using a go-back pressure valve (BPV-01).

The reactor was charged approximately with 5 gr of dried trees sawdust. The particle size was between 3 mm and 6 mm. Two filters were placed at the top and the bottom of the reactor to avoid the loss of raw material during the assays. The heat exchanger E-01 was used to pre-heat the flow and the heater E-02 was used to get the water stream at the desired temperature. The working temperature was 250ºC, the process time was 64 min, the flow was 10 ml/min and the pressure was fixed to 10 MPa.

248 Liquid samples were taken every 10 min. After the process time, the pump was set to
249 zero flow and the system was cooled down back to room temperature and depressurized.
250 The solid inside the reactor was analyzed as well as the liquid samples.

251 3. Results and Discussion

252 Different species of urban trees were hydrothermally treated in a fixed bed
253 reactor using water as solvent. The goal of these experiments was the extraction and
254 hydrolysis of the cellulosic and hemicellulosic sugars of biomass. The used solvent, hot
255 pressurized water, was able to extract and hydrolyze hemicellulose and cellulose while
256 most of the lignin fraction remained insolubilized. So, after hydrothermal treatment of
257 plant biomass two main products were obtained: a liquid and a solid product. These two
258 products can be obtained from hydrothermal treatment at temperatures between 150ºC
259 and 400ºC and pressures between 10 and 25 MPa independently of the operation way
260 (batch, semi-batch or continue) (Cantero et al., 2015). The reaction time is closely related
261 with the treatment temperature. Generally, the reaction times for hydrothermal
262 treatment at mild conditions (250ºC) range between 30 min and 90 min. However, the
263 reaction time is drastically decreased when the hydrolysis temperature is close to the
264 critical point of water. It those situations, the use of continuous reactors is mandatory to
265 set reaction times lower than minutes, which will avoid degradation reactions (Cantero
266 et al., 2015). In this work, the fractionation of different plant biomass was carried out at
267 250ºC in a semi-continuous reactor. This temperature was selected from previous studies
268 because it was the optimum for extracting the maximum amount of carbohydrates (Yedro
269 et al., 2014). The pressure was fixed to 10 MPa for all the experiments in order to ensure
270 a liquid phase extraction and hydrolysis. After 64 min of hydrothermal treatment, it was

observed that most of the cellulose and hemicellulose have been extracted, so, the reaction time of the raw material inside the reactor was set to 64 min. It should be taken into account that 2 different reaction times were defined for a fixed bed reactor as explained in section 2.2.4. The liquid flowing through the reactor has a residence time that is a function of the reactor volume and the flow (reaction time liquid). Although high solid reaction times are desired in order to extract most of the hemicellulose and cellulose fraction, the liquid reaction time should be as low as possible to avoid sugars degradation. Depending on the reaction time of these sugars in the reactor, considerable amount of degradation products can be produced. The addition of small amount of NaOH can be prevented the formation of undesirable degradation products recovering a major amount of hemicelluloses (Li et al., 2014), however in this work any mineral acids or bases were added to avoid the formation of byproducts from mineral acid neutralization and to do the process environmentally friendly using water as solvent . In the experimental system used in this work, the liquid reaction time was between 3 and 3.6 min considering the variations in the porosity of the reactor.

3.1. Raw materials characterization

The content of lignin, cellulose, hemicellulose, ash and extractives for each urban tree were analyzed in duplicate samples following the protocol described in section 2.2.2. The content of lignin was calculated as the sum of acid soluble and insoluble lignin. After the hydrolysis, the content of hemicellulose was calculated as the sum of xylose, arabinose, galactose, mannose and acetic acid in the liquid hydrolysate. In the same way, the concentration of cellulose was determined as the sum of glucose, fructose and cellobiose. In Figure 2 it is shown the composition of the woody raw materials used in this study. The

294 lignin content was between 21.6 and 39.1 %wt. The maximum amount of acid soluble
295 lignin was 2.14% wt. indicating that the lignin content is due mainly to the presence of
296 acid insoluble lignin. It can be seen that Linden contained the lowest amount of lignin
297 while Cedar the highest amount of lignin. The amount of hemicellulose was similar for all
298 urban trees analyzed. These values were between 20.0% wt. (Almond) and 25.5% wt.
299 (Pine). On the other hand, the content of cellulose was variable depending on the raw
300 material. The content of cellulose was between 21.7% wt. (Almond) and 45.2 % wt.
301 (Eucalyptus). A low content of ash and extractives was observed for all the analyzed raw
302 materials. The ash content was lower than 1% wt. for all the studied raw materials and
303 the amount of extractives varied from 0.6 % wt. (Plane) to 5.1% wt. (Linden).

304 ## 3.2. Product distribution

305 In Figure 3 it is shown the removal percentage of hemicellulose, cellulose, derived
306 products and lignin from raw material as well as the solid residue obtained after the
307 hydrothermal treatment. The hemicellulose and cellulose yields were calculated as it was
308 explained in section 2.2.1. The main compound produced from sugars hydrolysis were
309 glyceraldehyde, glycolaldehyde, 5-HMF, formic acid, levulinic acid and acetic acid. Also,
310 lactic acid was observed in the liquid sample from the hydrolysis of Almond wood. The
311 solubilized lignin was calculated as the difference between the content of lignin in raw
312 material and the content of lignin in the solid residue.

313 The mass balance ranged from 0.75 to 1 g/g, however, most of the experiments
314 gave a mass balance higher than 0.8 g/g. These values were acceptable considering the
315 small amount of solid used as raw material. The main loss of material can be attributed
316 to: filling and emptying of the reactor and the difficulty to identify hemicellulose and

cellulose derived products by HPLC. Also, it should be taken into account that 8 samples were taken in each experiment to represent a 64 min experiment. So, an uncertainty should be added to the calculation of the accumulative yield of cellulose and hemicellulose.

The sum of hemicellulose and solubilized lignin yields was similar for all the experiments, representing about 0.3 g/g initial biomass. The studied urban trees exhibited a similar tendency: the presence of high amount of hydrolyzed lignin together with a low amount of hydrolyzed hemicellulose (Mok & Antal, 1992). The cellulosic sugars collected after the hydrothermal treatment ranged from 0.11 to 0.28 g/g initial biomass. The amount of derived products was between 0.10 and 0.27 g/g initial biomass. The content of acetic acid was higher in hardwood than softwood species as it was also observed in literature (Du et al., 2010).

3.3. Liquid Products

The sampling of the liquids products started just before the pumping was started. The samples taken from the reactor outlet were subjected to many analysis as explained in section 2.2.1. One of the easiest ways to follow the fractionation of plant biomass is the measurement of the pH. In Figure 4 it can be seen the pH values of the liquid samples in function of the solid reaction time for the 9 analyzed urban trees. First, the pH values for Eucalyptus globulus were on-line measured every 1 min. From that measurements, it can be observed that the pH shows 3 different behaviors along solid reaction time. First the pH was increased, then it was reduced drastically and finally the pH is increased softly. The values of pH for Eucalyptus fractionation were used to decide the sampling time. The first sample was taken at zero time, then the second sample was taken around the pH

340 peak (4 min). The third sample was taken after the minimum pH is achieved (14 min).
341 Finally, five samples were taken every 10 min in the last part of the experiment. In this
342 way, the analytical and time expenditures were reduced. The pH started at about 5.5
343 corresponding to the distilled water value. The increase in the pH (0 min < t < 4 min) from
344 5.5 (distilled water value) to almost 6 can be attributed to the extraction of ash, which
345 will increase the basicity of the medium. In order to test it, an experiment was run in
346 which the pumping and heating were stopped after 4 min of treatment. The purpose of
347 this experiment was to determine the composition of the solid inside the reactor.
348 Fortunately, it was observed that 40% wt. of the biomass ashes were extracted in the first
349 4 min suggesting that the pH increment in the first minutes of the treatment takes place
350 due to the ash solubility. The decrease in pH from t=4 min to t=10 min is due to the
351 release of acetyl groups linked to hemicellulose during the hydrolysis. The release of
352 acetic acid generate a decrease of the pH (Liu, 2010) and acts as a catalyst in the
353 polysaccharide hydrolysis and degradation of carbohydrates because of the presence of
354 hydronium ions (Kumar et al., 2011). The last behavior of pH was detected from t= 10
355 min to t= 64 min. In that stage of the hydrolysis, the pH value was slightly increase from
356 3.3 to 3.7. This effect can be attributed to a dual effect: (1) an increase because the
357 hydrolysis product concentration decreases along time, so the pH should increase to
358 reach the distilled water value and (2), production of degradation products (Sasaki et al.,
359 1998) such as 5-hydroxymetylfurfural (5-HMF), formic (da Silva et al., 2013), furfural,
360 uronic acid (Li et al., 2014), which will maintain the pH low. This behavior was observed
361 for all the experimented cases. As shown in Figure 3, the minimum pH values for the 9
362 studied species were between 3.2 and 3.6 at around 10 min of hydrothermal treatment.
363 The variability in the minimum pH value and the extraction time which the pH was

364 minimum can be related with the biomass structure and the content of solubilized lignin
365 in the liquid samples (Mok & Antal, 1992). The final pH values were between 3.7 and 3.9.
366 These values were lower compared to the range 4-7 recommended for other researchers
367 to prevent the formation of derived products (Kumar et al., 2011).

368 The liquid products were composed of oligosaccharides and saccharides from
369 hemicellulose and cellulose and also their derived products. The concentration of these
370 components after the hydrothermal process depends strongly on two parameters: the
371 liquid reaction time and temperature. At 180ºC the hemicellulose is hydrolyzed and some
372 lignin is removed at about 200ºC (Bobleter, 1994). At high temperatures, the kinetics of
373 hemicellulose and cellulose hydrolysis are fast, which is desirable to reduce the reactors
374 volume. However, if the reaction time is high a big amount of derived products will be
375 produced (Cantero et al., 2015). The choice of operational conditions is important to
376 avoid the formation of undesirable products.

377 The hemicellulose recovery was plotted along solid reaction time in Figure 5-A. It
378 can be observed that the hemicellulose recovery was quite different depending on the
379 starting biomass. The recovery yield varied from 0.28 to 0.79 g/g hemicellulose in raw
380 material. The main component of hemicellulose was xylose and it can be used as indicator
381 to follow the hydrolysis of this fraction. The maximum extraction of hemicellulose was
382 observed around 20 min of solid reaction time. An increase of the process time favored
383 the formation of undesired products. The cellulose recovery was plotted along solid
384 reaction time in Figure 5-B. As it was observed for hemicellulose, the cellulose recovery
385 was highly dependent of the initial raw material, ranging the yield from 0.36 to 1.00 g/g
386 cellulose in raw material. The process time was not enough to get the maximum

387 extraction value of cellulose indicating that the complete hydrolysis of celluloses was not
388 achieved for 64 min in most of the experiments.

389 The hemicellulose and cellulose have chemical structures and composition very
390 different. Hemicellulose polymerization of its compositional saccharides takes place in a
391 branch way and the hemicellulose has low degree of polymerization compared with
392 cellulose (Liu et al., 2012). For cellulose, the polymerization of glucose take place in a
393 linear way allowing the fibers of cellulose to interact between them forming crystals
394 (Cantero et al., 2015). This difference makes hemicellulose a more accessible polymer for
395 the hydrolysis process. So, it was expected a different behavior under the hydrothermal
396 treatment. This can be one of the main reasons because of cellulose needs more
397 time/temperature than hemicellulose to be completely hydrolyzed. In fact, the
398 hemicellulose recovery yield (Figure 5-A) and the cellulose recovery yield (Figure 5-B)
399 follow different behaviors.

400 The hydrolysis of hemicellulose and cellulose seems to be governed by two
401 parameters: the kinetic of hydrolysis and the accessibility of the polymer. The maximum
402 yield of hemicellulose for each specie was achieved at the same solid reaction time,
403 around 20 min. This suggests that the kinetic of hemicellulose hydrolysis was the same
404 for the different studied species. However, the maximum recovery yield varied a lot
405 depending in the treated biomass. This suggests that the availability of the hemicellulose
406 fraction in the raw material was different for each biomass. As it was analyzed for
407 hemicellulose, the maximum values of cellulose recovery yield would be affected by the
408 accessibility of cellulose in the raw material. Therefore, the hydrolysis of hemicellulose

409 and cellulose will be affect by the composition of the raw material and the distribution of
410 the cellulose and hemicellulose fractions in the biomass.

411 As it can be seen in Figure 5, the hydrolysis of hemicellulose and cellulose depends
412 on the treated raw material. In order to evaluate the interaction of the biomass polymers
413 (hemicellulose, cellulose and lignin), the extraction yield of hemicellulose and cellulose
414 was analyzed in function of the lignin content in biomass. So, the final amount of
415 hemicellulose and cellulose extracted against the lignin content in the raw material was
416 plotted in Figure 6-A and 6-B respectively. The extraction of hemicellulose showed to be
417 improved when the lignin content of the biomass was reduced. In spite of one
418 experimental point (Holm Oak), which did not follow the trend, a general tendency was
419 found for the extraction of hemicellulose (Figure 6-A). A low lignin content may suggests
420 a big hemicellulose accessibility. However, the extraction of cellulose was not influenced
421 by the lignin content of the raw material. In Figure 6-B, it can be seen that the recovery
422 of cellulose yielded 60 ±10 % wt independently of the used biomass. Following the
423 analysis of these results, it can be concluded that the low content of lignin makes more
424 accessible the hemicellulose fraction. Even, it can be though that hemicellulose is mainly
425 placed around/between three dimensional lignin structures. On the other hand, the
426 cellulose fractions seems to be embedded inside the lignin matrix making cellulose
427 hydrolysis independent of lignin content.

428 The carbohydrates produced from cellulose and hemicellulose hydrolysis can
429 follow mainly two different reaction pathways: dehydration or retro-aldol condensation.
430 The formation of 5-HMF is due to a dehydration reaction while that the formation of
431 glycolaldehyde is due to a retro-aldol condensation reaction (Cantero et al., 2015). The

5-HMF is a degradation product from hexose sugars that could act as inhibitor during the fermentation of sugars to produce ethanol (Kumar et al., 2011). In those cases, it is necessary a previous detoxification step, which increase the fermentation cost at industrial scale to obtain this alcohol (Li et al., 2014). After the hydrothermal treatment of the biomass, 5-HMF and glycolaldehyde were observed in the liquid samples (see Figure S.1 in Supplementary Information). The yield of 5-HMF was lower than 0.05 gr/gr biomass and it was below the threshold limit of fermentation inhibitory level (Gong et al., 1999). The content of glycolaldehyde at 64 min was lower than 0.06 gr/gr biomass.

3.4. Solid Products

The content of solid residue after hydrolysis process varied from 0.10 to 0.32 gr/gr initial biomass (see Table 1) and the color of these solids was completely black. These values were lower than the initial content of lignin in the raw material indicating that some lignin was hydrolyzed. The structure of lignin could be composed of an amorphous and crystalline regions; the amorphous region would be easily hydrolysable while that the crystalline region would be more resistant to attack by water molecules (Kumar & Gupta, 2009). The solid residue was analyzed for carbohydrates, lignin and ash content. The ash content was not detected. The carbohydrates were the most common contaminant in the solid (Fengel & Wegener, 1983). The lignin content was between 0.41 and 0.92 gr/gr biomass being the main component in the solid product. These values corresponded to Linden and Pine respectively. The content of lignin in raw material was higher in Pine than in Linden. The difference in the quality of solid (measured as lignin content) can be due to the lignin component is more resistant to thermal degradation.

In the Figure S.2 (in Supplementary Information) it can be seen the structural characterization by FTIR of Cedar and Linden before and after hydrothermal process. The aromatic skeleton vibration can be seen at 1610 and 1460 cm^{-1} while the vibrations at 1135 cm^{-1} represents the aromatic C-H for syringyl type. These bands were observed in the raw material and the solid product suggesting that the aromaticity properties remained after the hydrolysis process (Zhang et al., 2010). At 1735 cm^{-1} it can be observed the linkage between hemicellulose and lignin (Thangavelu et al., 2014). This band was observed in the two raw materials as well as in the Linden solid product but not in the Cedar solid product suggesting that the linkage was broken. This agree with the results presented before, where the presence of low amount of hydrolyzed lignin increases the hydrolysis of hemicelluloses. The results proved that the solid residue was composed of the aromatics groups as it was discussed earlier (see Table 1). The peak at 765 cm^{-1} corresponds to presence of polysaccharide (Thangavelu et al., 2014) and it was observed in all samples indicating the presence of carbohydrates in the raw material as well as in the solid residue. This agree with the results showed in the Table 1.

3.5. Heat integration and recovery energy

The use of high temperatures and pressures in this process leads to the realistic solutions to make the process more economically and energetically efficient. For this reason, the use of a heat exchanger recovering energy was installed (E-01, Figure 1). The reaction temperature was, in all cases, at 250ºC and the flow was 10 mL/min. The overall heat transfer coefficient (U) was 1674 $W \cdot m^{-2} \cdot ºC^{-1}$ and the heat recovery was 95.6%. The high heat recovery is an important point to industrial scale, due practically any demand of heat the system is required. The second heat exchanger (E-03, Figure 1) was not used because the temperature of outlet stream was between 35 and 40ºC.

Renmatix has shown that using the supercritical hydrolysis technology is possible to produce carbohydrates from biomass making this process economically viable (Colakyan, 2012). In the same way, an economic analysis (nine options) was carried out in the ethanol production from surplus lignocellulosic biomass as raw material (Dias et al., 2011). The authors concluded that the integration of two process: first and second production decreased the ethanol production costs.

4. Conclusions

The hydrolysis of carbohydrates using a hydrothermal medium at 250ºC, at 10 mL/min for 64 min was studied. The hemicelluloses and cellulose yield varied from 0.28 to 0.79 gr/grhemicellulose and 0.36 to 1 g/grcellulose, respectively. The hydrolysis kinetic of hemicelluloses was independent of biomass used while that the accessibility to hydrolyze carbohydrates depended on biomass suggesting that the distribution of carbohydrates inside of matrix-biomass depended on the raw material used. Finally, the hydrolysis of hemicelluloses can be improved if the lignin content in the raw material is reduced suggesting that the accessibility of hemicelluloses depended on the low lignin content. Contrary, the hydrolysis of celluloses was not affected by this parameter.

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Table and Figure Captions

Table 1. Amount and characterization of solid obtained after the hydrothermal process.

Figure 1. Schema of the hydrolysis process. Equipment: D-01 Feeder, P-01 Pump, E-01 Heat exchanger, E-02 Preheater, R-01 Reactor, H-01 oven, E-03 Heat exchanger, BPV-01 Go-backpressure valve, D-02 Liquid sampling vessel.

Figure 2. Chemical composition of the raw materials.

Figure 3. Material balance for hydrothermally urban trees.

Figure 4. Behavior of pH during wood autohydrolysis process.

Figure 5. Yield of hemicelluloses (A) and celluloses (B) recovered after hydrolysis process at 250ºC and 64 min.

Figure 6. Yield of hemicelluloses (A) and celluloses extracted (B) after the hydrolysis process using nine species of urban trees at 250ºC.

616 Table 1.

Biomass	Solid after hydrolysis (gr/gr biomass)	Lignin content (gr/gr biomass)
Linden	0,25	0,41
Plane	0,14	0,63
Eucalyptus	0,11	0,56
Catalpa	0,10	0,69
Holm Oak	0,32	0,32
Maple	0,15	0,56
Almond	0,19	0,63
Pine	0,17	0,92
Cedar	0,11	0,78

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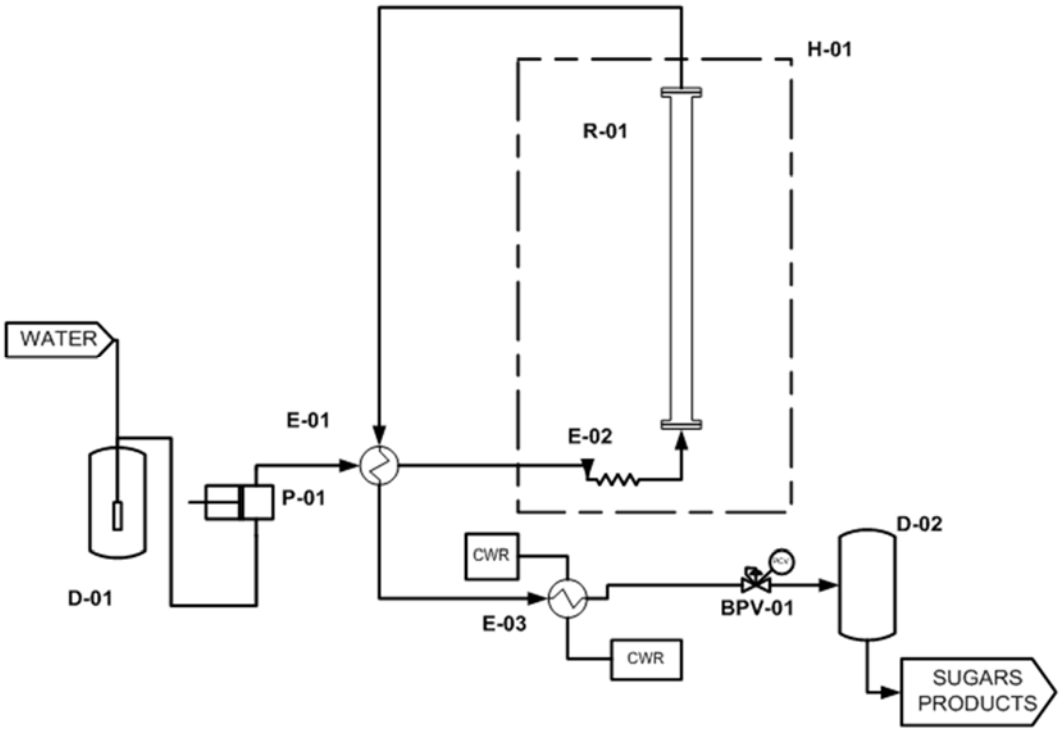
618

619 Figure 1.



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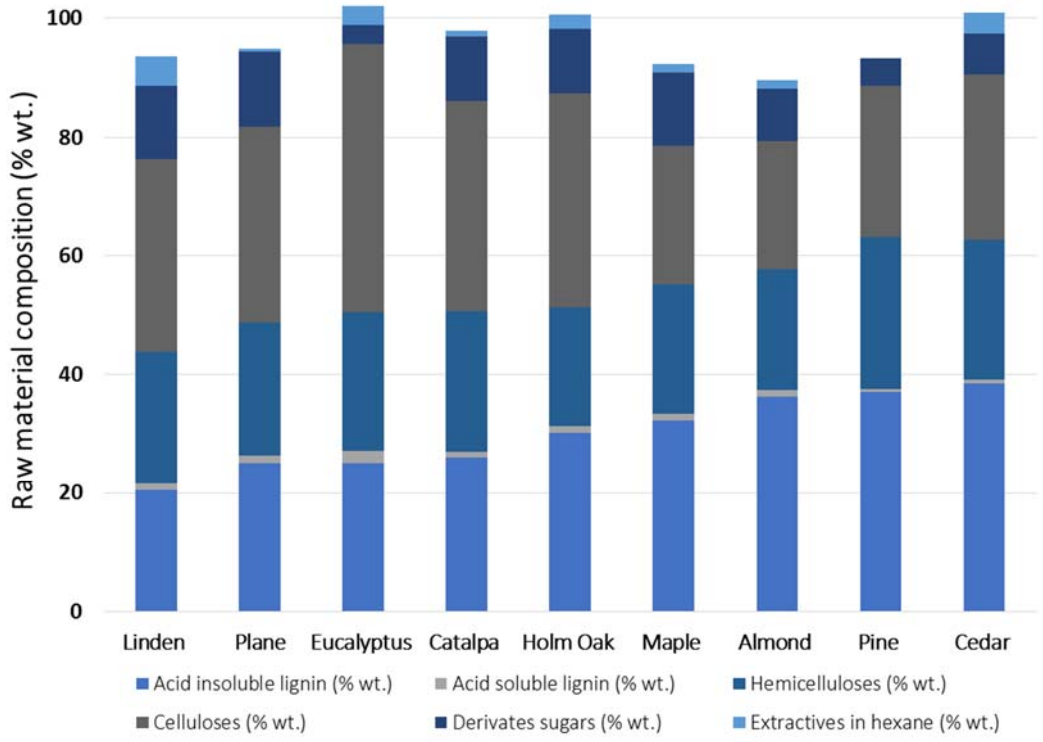

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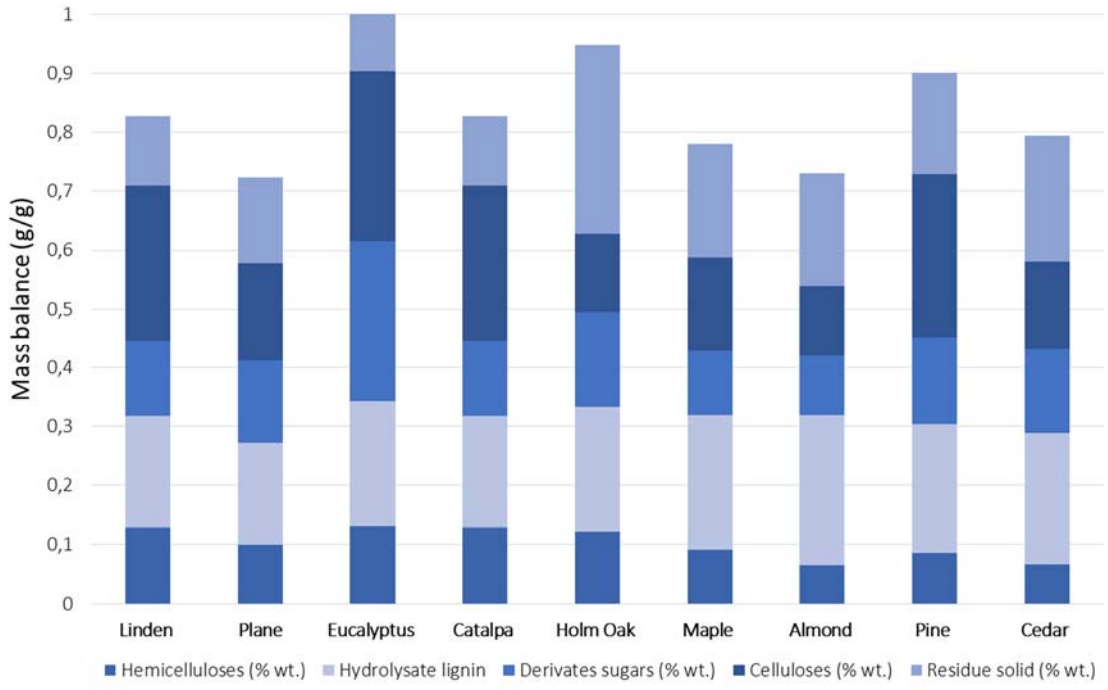

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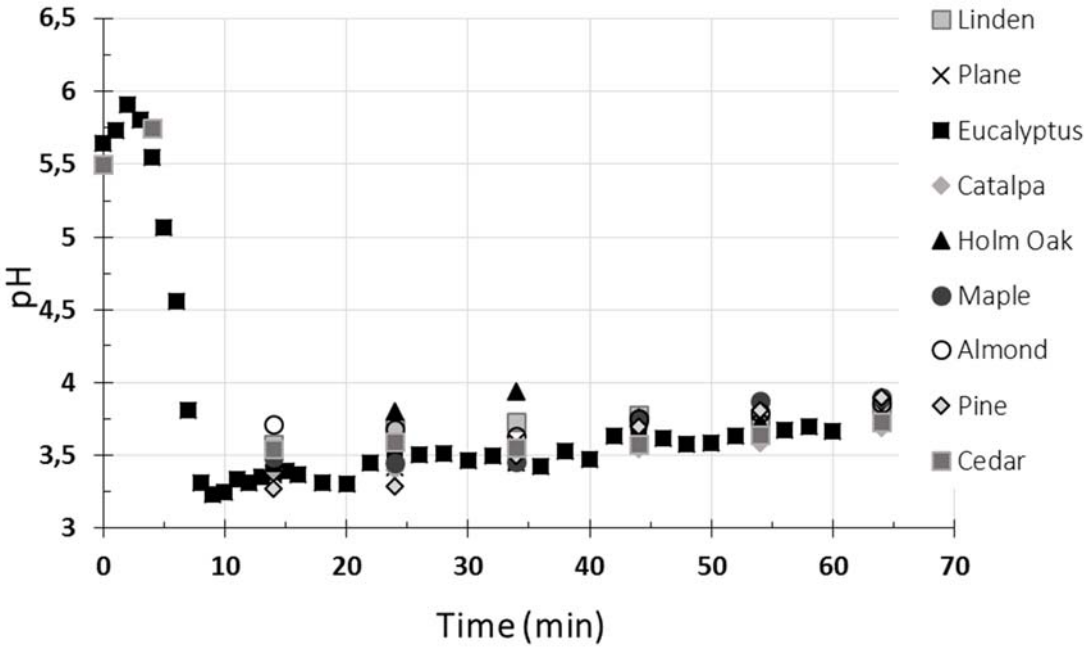

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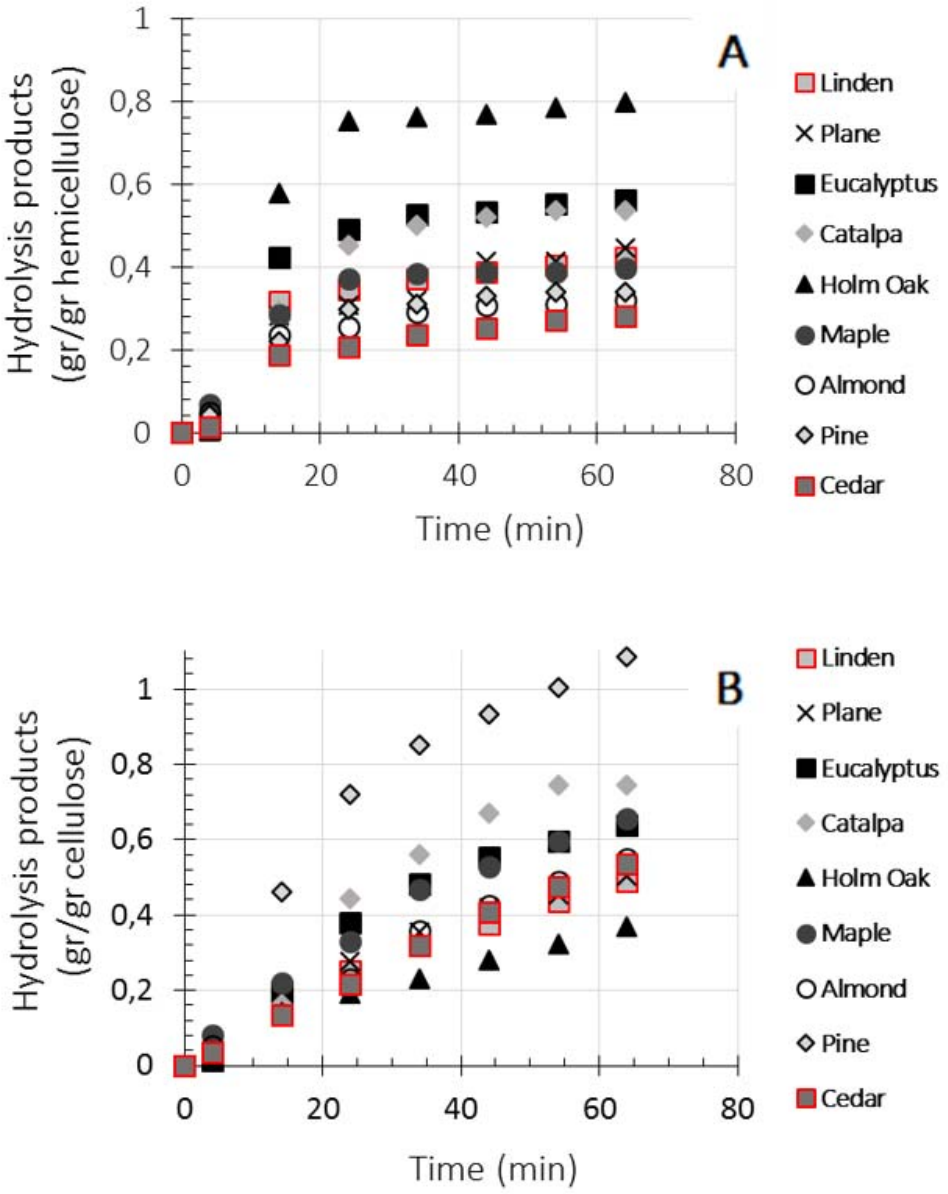

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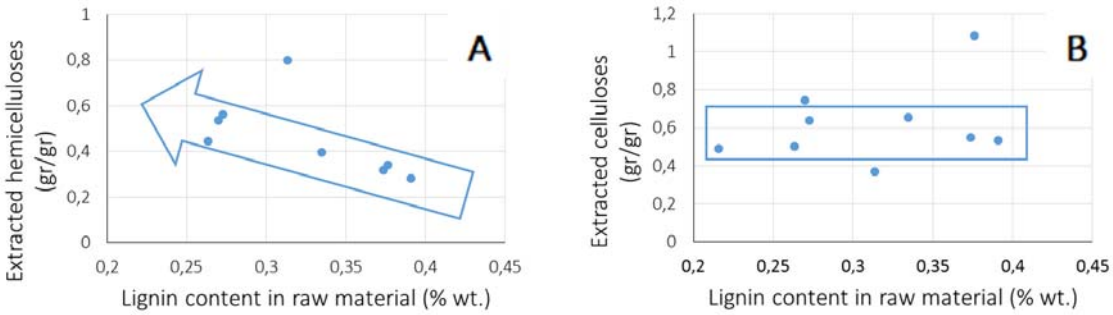

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