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Title: Sustainable conversion of Pinus pinaster wood into biofuel precursors: a biorefinery approach

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Abstract: Pinus pinaster wood was fractionated by aqueous treatments to yield a solution containing hemicellulose-derived saccharides (mainly of polymeric or oligomeric nature) and a solid phase enriched in cellulose and lignin. The solution containing polymeric or oligomeric hemicellulosic saccharides was acidified with sulfuric acid (up to 4 wt%) and heated (up to 130 °C) to assess the conversion of substrates into sugars. The interpretation of the experimental data was done on the basis of a mechanism involving 10 first-order, kinetic parameters. Almost total saccharification was achieved under a range of operational conditions, and the hydrolyzates presented compositions favorable for their further utilization as fermentation media. The solid phase coming from aqueous processing was mixed with HCl solutions and reacted under microwave irradiation to yield the target products levulinic acid (precursor of valeric biofuels) and formic acid (suitable for fuel cell devices). The effects of the most influential parameters (temperature, reaction time and acid concentration) were assessed by empirical modeling. The highest cellulose conversion into levulinic acid (61.9% of the stoichiometric value as an average) was achieved at 190 °C operating for 15 min in a medium containing 1 wt% HCl.

Dear Editor,

We are submitting our work entitled

**Sustainable conversion of *Pinus pinaster* wood into biofuel precursors:
a biorefinery approach**

for possible publication in Fuel.

In this article we report our original research on the processing of pine wood by consecutive stages of aqueous fractionation (to yield a solution containing hemicellulose-derived saccharides, mainly of polymeric or oligomeric nature) and acidic processing of solid and liquid phases. The liquid phases from aqueous treatments were heated in the presence of sulfuric acid to assess their conversion into sugars. The interpretation of the experimental data was done on the basis of a kinetic model that allowed the identification of a range of operational conditions, under which hydrolyzates with favorable composition to be used as fermentation media (for example, for biofuel production) were obtained.

In order to achieve an integrated utilization of the raw material, the solid phase coming from aqueous processing (mainly made up of cellulose and lignin) was mixed with HCl solutions and reacted under microwave irradiation to yield the target products levulinic acid (precursor of valeric biofuels) and formic acid (suitable for fuel cell devices). The effects of the most influential parameters (temperature, reaction time and acid concentration) were assessed by empirical modeling. The highest cellulose conversion into levulinic acid (61.9% of the stoichiometric value) was achieved at 190 °C operating for 15 min in a medium containing 1 % HCl). This result compares favorably with reported data. The residual solid was mainly made up of lignin, and can be used for multiple purposes (for example, liquefaction).

In summary, this work provides a sound assessment on the production of biofuel precursors from the polysaccharides components of the raw material, leaving lignin as a valuable byproduct.

Sincerely,

J. C. Parajó.

REPLIES TO Reviewer #2:

QUERY 1. The quotations to the literature should be uniformed according to the formatting specifications of the journal.

REPLY. The quotations to the literature have been modified to fit the journal specifications.

QUERY 2. Details about the conditions used for aqueous extraction should be provided.

REPLY. The requested information has been included in the revised version of the article (section 2.2).

QUERY 3. Consider reducing the frequency of utilization of abbreviations (for example, for furfural, levulinic acid, formic acid)

REPLY. Modifications have been introduced in sections 1, 2.6, 3.2, 3.3 to reduce the utilization of abbreviations

QUERY 4. p. 5, l. 116-120. Provide details of the experimental design

REPLY. Additional details on the structure of the design have been introduced in the revised version of the article (section 3.2).

QUERY 5. p. 13, l. 334. Please, check the optimal conditions in the conclusions

REPLY. The text has been rewritten for better understanding

QUERY 6. The figure captions are not complementary materials. They should be shown in the main document.

REPLY. The figure captions have been included in the main document.

QUERY 7. Correct the spelling of Hydroxymethylfurfural and Furfural in Fig. 3.

REPLY. The format of Figure 3 has been modified to make the complete word visible.

Highlights (for review)

- Pine wood was subjected to aqueous fractionation to solubilize hemicelluloses
- Hemicellulosic saccharides were efficiently saccharified by posthydrolysis
- Solids from fractionation were processed in acidic media under microwave heating
- The conversion of cellulose into formic acid and levulinic acid was optimized
- Lignin was recovered as the insoluble residue from acidic processing

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Sustainable conversion of *Pinus pinaster* wood into biofuel precursors: a biorefinery approach

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ABSTRACT

Pinus pinaster wood was fractionated by aqueous treatments to yield a solution containing hemicellulose-derived saccharides (mainly of polymeric or oligomeric nature) and a solid phase enriched in cellulose and lignin. The solution containing polymeric or oligomeric hemicellulosic saccharides was acidified with sulfuric acid (up to 4 wt%) and heated (up to 130 °C) to assess the conversion of substrates into sugars. The interpretation of the experimental data was done on the basis of a mechanism involving 10 first-order, kinetic parameters. Almost total saccharification was achieved under a range of operational conditions, and the hydrolyzates presented compositions favorable for their further utilization as fermentation media. The solid phase coming from aqueous processing was mixed with HCl solutions and reacted under microwave irradiation to yield the target products levulinic acid (precursor of valeric biofuels) and formic acid (suitable for fuel cell devices). The effects of the most influential parameters (temperature, reaction time and acid concentration) were assessed by empirical modeling. The highest cellulose conversion into levulinic acid (61.9% of the stoichiometric value as an average) was achieved at 190 °C operating for 15 min in a medium containing 1 wt% HCl.

Keywords: cellulose, formic acid, hemicellulosic sugars, levulinic acid, *Pinus pinaster*, wood

36 1. Introduction

37

38 The sustainable growth of the chemical and energy industries has become an
39 indispensable component of our society (Cheng & Zhu, 2009). The current development pattern
40 of the industrial sector, defined by a strong dependence on fossil resources, is far from being
41 sustainable (Ghatak, 2011), and must be replaced by an alternative one, based on renewable
42 resources. Lignocellulosic materials are a widespread, renewable resource with an enormous
43 potential as feedstocks for the industry. Their extensive utilization for this purpose is expected
44 to enable the development of a biobased economy (Jenkins, 2008), in which biorefineries could
45 play a key role. Cellulose and hemicelluloses are polysaccharide constituents of biomass, which
46 can be depolymerised to free sugar monomers that can be further transformed into different
47 platform chemicals (Hu, y otros, 2015).

48 *Pinus pinaster* is widely distributed in the South-West of Europe and North Africa, and
49 represents a major resource in Galicia (North-West of Spain). In previous studies, the
50 fractionation of *Pinus pinaster* wood by two-stage aqueous processing (extraction to remove
51 water-soluble extractives followed by autohydrolysis to cause the partial breakdown of
52 hemicelluloses into polymeric and oligomeric saccharides, here denoted POHS) has been
53 assessed [5, 6]. The extraction stage leads to a complex mixture of compounds (among which
54 phenolic compounds concentrate the added value) (Conde, y otros, 2013), whereas the soluble
55 hemicellulose-derived products solubilized upon autohydrolysis can be converted into
56 monosaccharides by acidic postprocessing, yielding a solution of hemicellulosic sugars suitable
57 as a fermentation media (for example, for ethanol or butanol production). On the other hand, the
58 solid leaving the autohydrolysis stage (here denoted extracted, autohydrolyzed solid EAS) is
59 enriched in cellulose and lignin, and can be processed in acidic media to convert its cellulosic
60 fraction into organic acids (levulinic acid, denoted LA, and formic acid, denoted FA). The
61 production of these organic acids from cellulose pulps has been reported in a previous study
62 (Rivas, Galletti, Antonetti, Santos, & Parajó, In press).

63 The insoluble fraction remaining after aqueous extraction and acidic processing is
64 essentially made up of lignin, and could be employed for a number of purposes, including
65 power generation, chemical modification (for example, by liquefaction or gasification), or as an
66 intermediate for manufacturing polymers or chemicals (including antioxidants for foods or
67 cosmetics, gels or emulsifiers). The processing scheme considered in this work is shown in
68 Figure 1.

69

Figure 1

70

71 This study focuses on the production of hemicellulosic sugars from POHS and on the
72 generation of lactic and formic acids from EAS under microwave heating. Both routes are

73 compatible and lead to fuel precursors, since: i) hemicellulosic sugars can be fermented into
74 fuels (for example ethanol, butanol or biogas) [9 - 12]. Even if the fermentability of
75 conventional hemicellulose-derived hydrolyzates can be limited by the presence of inhibitors
76 such as weak acids, furan derivatives and/or phenolic compounds (Palmqvist & Hahn-Hägerdal,
77 2000), it can be noted that the approach followed in this work limits this drawback in a multiple
78 way: POHS are obtained from an extractive free substrate; the acidic post-processing of POHS
79 is carried in absence of solids that could release unwanted compounds; and monosaccharides are
80 obtained under mild conditions (limited temperature and low acid concentration), keeping the
81 concentration of furans far below the threshold resulting in inhibitory effects; ii) LA is an
82 intermediate for the production of liquid fuels, including γ -valerolactone (GVL) and related
83 compounds [14 – 16], in a way that LA has been identified as one of the “12 top value-added
84 chemicals from biomass” (Manzer, 2006); and iii) FA is the power source for “Direct Formic
85 Acid Fuel Cells” suitable for portable devices (Aslam, Masdar, Kamarudin, & Daud, 2012).

86

87 **2. Materials and methods**

88 2.1. Materials

89 *Pinus pinaster* wood chips were kindly provided by a local particleboard industry
90 (Oremer-Finsa, Ourense, Spain). Samples were air-dried, milled to pass an 8 mm screen,
91 homogenized in a single lot to avoid compositional differences among samples and stored until
92 use.

93 2.2. Aqueous extraction and autohydrolysis

94 *Pinus pinaster* wood samples were first subjected to an aqueous extraction under non
95 isothermal-conditions up to reach 130°C in a stirred, pressurized reactor; and then immediately
96 cooled by circulating tap water through an internal coil. This method enables the removal of
97 water-soluble extractives, as discussed in an earlier work (Conde, y otros, 2013). The resulting
98 solids were autohydrolyzed at 175°C for 26 minutes, conditions reported as optimal for POHS
99 production [5, 6]. Both treatments were performed in a 3.75 L stainless steel reactor (Parr
100 Instrument Co., Moline, IL, USA), operating with 8 kg water/kg oven-dry solid. The solid
101 obtained after extraction and autohydrolysis (EAS) was employed as a substrate for producing
102 LA and FA under microwave irradiation (see below).

103

104 2.3. Wood and solid analysis

105 Wood was assayed for ethanol extractives (TAPPI 264 method). Wood and EAS were
106 assayed for moisture (oven-drying at 105 °C until constant weight) and subjected to two-step
107 quantitative acid hydrolysis (with 72% and 4% sulfuric acid, respectively) following the TAPPI

108 249 method. The solid residue from the quantitative acid hydrolysis was considered as Klason
109 lignin, and the liquid phase was assayed by HPLC for sugars, sugar oligomers, acetic acid and
110 uronic acids (see below).

111

112 2.4. Production of furans and levulinic acid in microwave-heated, stirred vessels from
113 hemicellulose free solid

114 EAS samples were mixed with aqueous HCl solutions, and processed in a Microwave
115 Accelerated Reaction System (MARS 6, CEM Corporation, Matthews, NC, USA). Reactions
116 were carried out in 100 mL Teflon vessels fitted with magnetic drive stirrers. Temperature was
117 controlled by fiber optic and IR sensors. One vessel, used as a reference, also contained a
118 pressure sensor. The liquor to solid ratio was fixed in 20 kg solution/kg oven-dry EAS.

119

120 2.5. Production of monosaccharides from hemicellulosic liquors

121 Sulfuric acid was added to the liquid phases coming from autohydrolysis treatments to
122 reach concentrations in the range 0.5-4 wt% H₂SO₄, and the resulting solutions were treated in
123 autoclave at 110, 120, or 130 °C for the desired reaction time. The reaction time indicates the
124 duration of the isothermal reaction stage (time zero min correspond to the moment when the
125 target temperature was reached). The reaction media were analyzed by HPLC (see next section).

126

127 2.6. Analytical methods

128 The compositions of the liquid phases from quantitative acid hydrolysis, the
129 autohydrolysis liquors and the solutions obtained after acidic processing of autohydrolysis
130 liquors were determined by HPLC using an Agilent 1100 instrument fitted with a refractive
131 index detector. Samples were filtered through 0.45 µm PTFE membranes before analysis.

132 Monosaccharides (glucose, xylose, galactose, arabinose and mannose) were analyzed
133 using a 300x7.8 mm CARBOsep CHO 682 column with a guard column (Transgenomic,
134 Glasgow, U.K.) kept at 80°C, employing distilled water as mobile phase (flow rate, 0.4
135 mL/min). Before analysis with the CARBOsep CHO 682 column, liquors from acid hydrolysis
136 were diluted, neutralized with BaCO₃, centrifuged and filtered.

137 Acetic acid, formic acid, furfural, 5-hydroxymethylfurfural (HMF), and LA were
138 determined using a 300x7.8 mm Aminex HPX-87H column (BioRad, Life Science Group
139 Hercules, CA) and a guard column. The mobile phase (0.006 N H₂SO₄) was eluted at 0.6
140 mL/min, and the column was kept at 50 °C.

141 Sugars, acetic acid, FA, furfural, HMF and LA in water-HCl media were determined by
142 HPLC using a Perkin Elmer Flexar instrument fitted with a RI detector and a PolyporeCA

143 column (4.6 x220 mm) eluted with 0.005 M H₂SO₄ at 0.2 mL/min. The column was kept at
144 40°C.

145 Concentrations of POHS and bound acetyl groups were measured from the increases in
146 the concentrations of monosaccharides and acetic acid after a quantitative post-hydrolysis
147 (NREL/TP-510-42623 method).

148 Uronic acids were determined spectrophotometrically by the method described by
149 Blumenkrantz and Asboe Hansen (Blumenkrantz & Asboe Hansen, 1973), using galacturonic
150 acid as reference compound for quantification.

151 Nonvolatile compounds (NVCs) in solutions were measured by oven-drying at 105°C
152 until constant mass was achieved.

153 **3. Results and Discussion**

154 **3.1. Autohydrolysis of wood**

155 The *Pinus pinaster* wood samples employed in this study contained (in oven-dry basis)
156 40.4 wt% of anhydroglucose units in polysaccharides (including cellulose and hemicelluloses),
157 9.92 wt% anhydromannose units, 3.35 wt% anhydrogalactose units, 4.7% anhydroxylose units,
158 1.67 anhydroarabinose units, 32.4 wt% of lignin, 1.35 wt% of acetyl groups, 3.30 wt% uronic
159 groups and 2.91 wt% of other compounds of minor importance for the purposes of this study.

160 Upon extraction and hydrothermal processing, 27.1 wt% of the original wood was
161 dissolved. The composition of autohydrolysis liquors (in g/L) was as follows: glucose (G), 0.13;
162 xylose (X), 1.10; galactose (Ga), 0.63; arabinose (Ar), 1.51; mannose (Mn), 0.29; acetic acid,
163 0.35; anhydroglucose units in POHS (denoted GOS), 2.13; anhydroxylose units in POHS
164 (denoted XOS), 3.03; anhydrogalactose units in POHS (denoted GaOS), 3.28; anhydroarabinose
165 units in POHS (denoted ArOS), 0.21; anhydromannose units in POHS (denoted MnOS), 8.11;
166 acetyl groups in POHS (denoted AcOS), 1.10; uronic acid substituents (UA), 0.78. The
167 identified compounds accounted for about 100 wt % of the total nonvolatile compounds (NVC).
168 A part of the anhydroglucose units in wood polysaccharides were converted into GOS (4.36%)
169 or glucose (0.27%), in amounts compatible with the anhydroglucose content of glucomannan,
170 confirming that cellulose was not significantly affected by autohydrolysis. In comparison,
171 anhydrogalactose units were extensively removed from the solid material, appearing either
172 making part of GaOS (81% of the total amount) or as free galactose. Anhydromannose units
173 presented an intermediate behaviour, since 67.6% of them were recovered in MnOS, and 2.4%
174 were converted into mannose. Similarly, the solubilized anhydroxylose units appeared either
175 making part of XOS (53.3%) or as xylose (19.3%).

176 The remaining 72.9 wt% of original wood remained as a solid (EAS), which presented
177 the following composition (in wt%): anhydroglucose units, 48.7%; anhydroxylose units, 2.83%;

178 anhydrogalactose units, 0.71%, anhydromannose units, 3.94%; anhydroarabinose units, 0.2%;
179 Klason lignin, 40.0%; uronic substituents, 2.45%; acetyl groups, 0.60%; and other components,
180 0.55%.

181 3.2. Acidic processing of autohydrolysis liquors

182 POHS-containing autohydrolysis liquors were converted into monosaccharides by
183 acidic posthydrolysis, in order to identify operational conditions leading simultaneously to both
184 high substrate conversion and limited generation of furans (resulting from sugar dehydration).
185 For an easier interpretation of data, the kinetic assessment presented in this work is expressed in
186 terms of volumetric concentrations. The experimental plan was defined to assess the effects of
187 the three major experimental variables (temperature in the range 110-130 °C, reaction time up to
188 60 min and sulfuric acid concentration in the range 0- 4%), which covers the domain of interest.
189 The operational conditions considered in the various assays corresponded to an incomplete,
190 factorial, centered, second-order design of experiments with minimum dead volume.

191 When no sulfuric acid was added, no significant amounts of hexoses were produced
192 from POHS even under the harshest conditions assayed (130 °C, 60 min). Under the same
193 conditions, some xylose production from XOS was observed.

194 When sulfuric acid was added at the lowest concentration assayed (0.5%), its catalytic
195 action was evident even at the lowest temperature (110 °C), leading to near 50% POHS
196 conversion into hexoses after 90 min. In the same experiment, about half of the C₅ units in
197 POHS were converted into xylose or arabinose along the heating-up period, and reached total
198 conversion after 90 min. As a representative example, Figure 2 shows the results achieved in the
199 experiment performed at 130 °C with 0.5% sulfuric acid. The anhydrohexose units in POHS
200 were completely converted into monosaccharides after 60 min (Figure 2A), whereas some
201 decomposition into HMF was observed, and lactic acid was obtained as a HMF rehydration
202 product at very low yields. In comparison, XOS were completely saccharified during the
203 heating-up period (Figure 2B), and xylose-decomposition reactions led to the formation of
204 furfural (Fur).

205

206

Figure 2

207

208 After 45 min, the anhydrohexose units in POHS were converted into the corresponding hexoses
209 at almost quantitative yields, whereas the anhydropentose units in POHS were first converted
210 into pentoses and then into decomposition products (which accounted for up to 9% of the
211 stoichiometric amount).

212 Related variation patterns were observed when the sulfuric acid concentration was fixed at
213 intermediate values (1 - 2 wt%), achieving similar yields after 15 or 10 min, respectively. Under

214 these conditions, HMF and LA reached very low concentrations, whereas the maximum furfural
215 concentration was about 3 mmol/L. Considering the aspects cited before (no external catalyst in
216 the autohydrolysis stage, no presence of lignocellulosic solids in the posthydrolysis stage, very
217 low concentrations of furfural and LA), it can be noted that the sugar solutions produced in the
218 posthydrolysis stage meet the desirable compositional requirements for fermentability.

219 In order to assess the kinetics of the posthydrolysis step, the experimental data were
220 modelled according to the mechanism shown in Figure 3: the various types of oligomers making
221 part of POHS were assumed to be hydrolyzed into the correspondent mososaccharides, which
222 can be dehydrated to the corresponding furan (Fur from pentoses and HMF from hexoses). All
223 reactions were assumed to follow first-order kinetics. Table 1 lists the results determined for the
224 various kinetic coefficients involved in the model, whereas the agreement between experimental
225 data and model predictions (see Figure 2) confirms the suitability of the approach presented in
226 this work for providing a quantitative interpretation of the experimental data.

227

228 Figure 3

229 Table 1

230

231 3.3. LA production by acidic processing of extracted-autohydrolyzed solids EAS

232 LA is a versatile platform chemical with multiple applications (Rackemann & Doherty, 2011),
233 including fuel additives and liquid hydrocarbon fuels (Raspolli Galletti A. , Antonetti, De Luise,
234 & Martinelli, A sustainable process for the production of γ -valerolactone by hydrogenation of
235 biomass derived levulinic acid, 2012). Lactic can be obtained together with formic acid by
236 acidic processing of biomasses [21 – 26], through the dehydration-rehydration of free or
237 polysaccharide-derived sugars. The reaction mechanism is complex, and involves a number of
238 secondary reactions leading to the formation of unwanted byproducts [20, 21] that limit the
239 yields in the target products.

240 Since the heating temperature profile is an important factor affecting the product
241 distribution in the above mentioned set of reactions, LA production has been carried out using
242 microwave heating, which provides high reaction rates at reduced reaction times, and enables
243 significant savings of energy and time (Raspolli Galletti A. M., Antonetti, Ribechini,
244 Colombini, Nasso, & Bonari, 2013).

245 In this work, LA production has been achieved by microwave heating of EAS in HCl-
246 catalyzed aqueous media. Since the reaction media is biphasic and the reaction kinetics is
247 complex, the LA yield depends on a large number of variables, in a way that a detailed
248 assessment would require an extensive experimental plan. In this work, the optimization of the
249 target product yields was performed using an empirical method widely employed in literature

250 (Response Surface Methodology, RSM), which entails much less experimental work and
251 provides solid results.

252 The liquid to solid ratio and type of catalyst were chosen on the basis of literature [8,
253 24] and the results obtained in preliminary assays. The independent variables, their
254 nomenclature and the respective variation ranges were as follows: temperature (T, 180 - 200
255 °C), reaction time (t, 5 - 25 min) and catalyst concentration (CC, 0.6 - 1.4 HCl wt %). The
256 dependent variables selected to measure the effects of the chemical reactions were:

257 -y₁: cellulose conversion into glucose, defined as mol glucose/100 mol of potential
258 glucose,

259 -y₂: hexose conversion into HMF, defined as mol HMF/100 mol of potential HMF,

260 -y₃: hexose conversion into LA, defined as mol LA/100 mol of potential LA,

261 -y₄: hexose conversion into FA, defined as mol FA/100 mol of potential FA

262 where the potential amounts were calculated assuming 100% conversion of the substrates
263 (cellulose or hexoses) into the considered product (glucose, HMF, LA or FA). The potential
264 concentrations variables y₂, y₃ and y₄ were calculated considering the various types of
265 anhydrohexoses present in EAS as suitable substrates.

266 According to the stoichiometry of the hydrolysis-dehydration-rehydration reactions
267 taking place in the media, 1 mol of a given anhydrohexose in EAS may yield (as maximum
268 amount) either 1 mol of the correspondent hexose upon hydrolysis, or 1 mol of potential HMF
269 under further dehydration, or 1 mol of potential LA plus 1 mol of FA under further rehydration.

270 The incomplete, factorial, centered experimental design listed in Table 2 was employed
271 to assess the generation of the target products. The same Table includes the operational
272 conditions expressed in terms of dimensionless, normalized variables (with variation ranges [-1,
273 1]), defined as follows:

274 x_1 (dimensionless temperature) = $2 \cdot [T (\text{°C}) - 190] / (200 - 180)$ (eq. 1)

275 x_2 (dimensionless time) = $2 \cdot [t (\text{min}) - 15] / (25 - 5)$ (eq. 2)

276 x_3 (dimensionless catalyst conc.) = $2 \cdot [CC (\text{wt}\%) - 1] / (1.4 - 0.6)$ (eq. 3)

277

278 Table 2

279

280 Table 3 lists the experimental results. Cellulose conversion into glucose was only
281 important in experiment 1, which was performed at the lowest temperature and shortest time
282 considered, and led to low yields in the target products. The limited generation of LA and FA
283 determined in other experiments performed under mild conditions resulted from incomplete
284 cellulose hydrolysis.

285

Table 3

286

287 It can be noted that variable y_2 (measuring the HMF generation from hexoses) achieved
288 low values in all the experiments assayed, a fact ascribed to the rapid conversion of HMF into
289 LA and FA in aqueous acid media (Rivas, González-Muñoz, Santos, & Parajó, 2014). As HMF
290 is not a target compound in this study, the importance of y_2 lies on the possibility of assessing
291 the performance of the whole set of chemical reactions in combination with the results achieved
292 for the concentrations of glucose and/or organic acids.

293 Owing to the structure of the experimental design, the dependence of y_3 (cellulose
294 conversion into LA) on variables x_1 to x_3 was established using an equation containing first- order,
295 second- order and interaction terms:

296
$$y_3 = b_{03} + \sum_i b_{i3} x_i + \sum_i \sum_k b_{ik3} x_i x_k \quad (\text{eq. 4})$$

297 where x_i or x_k (i or k : 1 to 3, $k \geq i$) stand for the independent variables, and $b_{03} \dots b_{ik3}$ are the
298 regression coefficients, whose values were obtained by data fitting.

299 Table 4 lists the regression coefficients and their statistical significance (based on a t-test).
300 The same Table includes the statistical F and R^2 parameters measuring the significance and the
301 correlation of the model, respectively

302

303

Table 4

304

305 The coefficients of the interaction terms (b_{ik3} , $i \neq k$) were relatively small, whereas first-
306 order terms (b_{i3}) were positive and the second order ones (b_{ik3} , $i = k$) negative. The values of the
307 linear, interaction and second-order terms affecting variable x_3 reached comparatively high
308 absolute values, confirming that the catalyst concentration was the most influential variable within
309 the tested ranges. Figures 4A, 4B and 4C show the response surfaces calculated for y_3 as a
310 function of two independent variables, when the third one was fixed at its intermediate value.

311 The operational conditions leading to a maximum yield of levulinic acid were calculated
312 by optimizing the value of y_3 respect to each operational variable:

313
$$\frac{\partial y_3}{\partial x_1} = \frac{\partial y_3}{\partial x_2} = \frac{\partial y_3}{\partial x_3} = 0 \quad (\text{eq 5})$$

314 The maximum value predicted for y_3 corresponded to experimental conditions defined by
315 $T = 190.8$ °C, $t = 15.4$ min and $CC = 1.06$ wt%, very close to central point of the experimental
316 design (experiments 15, 16 and 17, for which the average values of y_3 and y_4 were 61.9 and
317 71.7, respectively). Additional experiments performed under the conditions predicted as optimal

318 by the model did not improve the results obtained in 15, 16 and 17, a fact ascribed to the
319 similarity of conditions and to the influence of experimental and fitting errors.

320 The results obtained in this work compare well with the data reported for LA production
321 in acid-catalyzed aqueous media operating with other substrates such as sugar cane bagasse
322 (Girisuta, Dussan, Haverty, Leahy, & Hayes, 2013), water hyacinth (Girisuta, Danon,
323 Manurung, Janssen, & Heeres, 2008), poplar wood (Runge & Zhang, 2012), pine wood pulp
324 (Rivas, Galletti, Antonetti, Santos, & Parajó, In press), cotton straw (Yang, y otros, 2013) or
325 cellulose (Girisuta, Janssen, & Heeres, Kinetic study on the acid-catalyzed hydrolysis of
326 cellulose to levulinic acid, 2007)(Shen & Wyman, 2012)(Szabolcs, Molnár, Dibó, & Mika,
327 2013).

328 Figure 4
329

330 **4. Conclusion**

331
332 *Pinus pinaster* wood is an important resource in quantitative terms, and presents an
333 remarkable content in hemicelluloses (including pentosans and hexosans). On the other hand,
334 the extractive fraction contains a number of added-value phenolics. Two sequential aqueous
335 treatments enabled the separation of two liquid streams (containing water-soluble extractives
336 and hemicellulose-derived saccharides, respectively) from a solid phase mainly made up of
337 cellulose and lignin. Hemicellulosic saccharides were hydrolyzed by sulfuric acid in a set of
338 experiments (covering a scope of operational conditions) that allowed the kinetic modeling of
339 the individual hydrolysis-decomposition reactions. Operating in a selected range of severities
340 (defined by temperature, reaction time and sulfuric acid concentration), the various substrates
341 were almost completely converted into sugars (hexoses and pentoses), leading to reaction media
342 with favorable composition for further use as fermentation media. On the other hand, the solids
343 leaving the second aqueous treatment (autohydrolysis stage) were heated by microwave
344 irradiation and reacted in the presence of HCl and water, to yield both levulinic acid and formic
345 acid. An empirical optimization enabled the identification of the operational conditions. The
346 predicted maximum was very close to the central point of the experimental design (190 °C, 15
347 min, 1 wt% HCl), conditions under levulinic acid yields accounting for 61.9 – 62.2% of the
348 stoichiometric value were obtained. The residual solid, which was mainly made up of lignin,
349 could be utilized for a number of purposes, enabling an integral utilization of the *Pinus pinaster*
350 wood.

351

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Figure Captions

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451

452 Figure 1. Processing scheme proposed for *Pinus pinaster* wood utilization

453

454 Figure 2. Concentration profiles determined for substrates and reaction
455 products in posthydrolysis assays performed at 130 °C with 0.5 wt%
456 H₂SO₄. A) Hexoses; B) Pentoses.

457

458 Figure 3. Kinetic mechanism employed for the interpretation of the
459 experimental data

460

461 Figure 4. Surface responses determined for the cellulose conversion into LA as
462 a function of two the independent variables (in all cases, the other
463 independent variable was fixed at its intermediate value)

464

465

Table 1. Values determined for the various kinetic parameters involved in the reaction mechanism (data expressed as $\text{min}^{-1} \cdot 10^3$)

[H ₂ SO ₄]	0.5%			1%			2%			4%		
Temp. (°C)	110	120	130	110	120	130	110	120	130	110	120	130
k _{0 Mn}	6.04	22.6	92.8	17.4	52.7	175.8	60.0	81.8	181	98.2	110	n.d.
k _{1 Mn}	0.00	0.00	0.20	0.00	0.00	0.31	0.00	0.30	1.69	0.80	1.40	2.40
k _{0 G}	5.71	16.9	47.6	16.7	40.3	89.3	27.7	72.3	308	51.4	87.7	n.d.
k _{1 G}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.80	1.00	1.30	1.40
k _{0 Ga}	17.9	36.1	91.2	27.9	54.2	226	37.5	63.4	312	47.9	136	n.d.
k _{1 Ga}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.30	0.40	0.50
k _{0 X}	50.9	99.7	n.d.	110	130	n.d.	175	210	n.d.	n.d.	n.d.	n.d.
k _{1 X}	0.00	0.00	1.08	0.00	0.00	1.35	0.69	0.90	2.60	1.60	2.30	6.06
k _{0 Ar}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
k _{1 Ar}	0.00	0.00	0.59	0.00	0.00	1.36	0.37	1.00	1.60	1.36	1.72	2.76

n.d.: Not determined due to the limited concentrations of the products involved in reactions

Table 2. Operational conditions assayed for manufacturing LA and FA

Experiment	Independent variables			Dimensionless, normalized, independent variables		
	T (°C)	t (min)	CC (wt%)	x_1	x_2	x_3
1	180	5	0.6	-1	-1	-1
2	180	25	0.6	-1	1	-1
3	180	5	1.4	-1	-1	1
4	180	25	1.4	-1	1	1
5	200	5	0.6	1	-1	-1
6	200	25	0.6	1	1	-1
7	200	5	1.4	1	-1	1
8	200	25	1.4	1	1	1
9	180	15	1.0	-1	0	0
10	200	15	1.0	1	0	0
11	190	5	1.0	0	-1	0
12	190	25	1.0	0	1	0
13	190	15	0.6	0	0	-1
14	190	15	1.4	0	0	1
15	190	15	1.0	0	0	0
16	190	15	1.0	0	0	0
17	190	15	1.0	0	0	0

T: Temperature; t: Time; CC: Catalyst concentration

Table 3. Experimental results achieved in the experiments listed in Table 2

Experiment	y_1	y_2	y_3	y_4
1	24.9	2.04	11.5	17.1
2	8.44	0.69	24.8	30.3
3	0.00	0.12	31.3	34.3
4	0.00	0.00	31.8	34.1
5	0.00	0.18	28.7	31.4
6	0.00	0.00	28.8	28.1
7	0.00	0.13	32.8	28.6
8	0.00	0.13	32.6	28.5
9	0.00	0.00	62.6	73.3
10	0.00	0.25	60.6	60.7
11	0.00	0.00	61.0	68.1
12	0.00	0.00	59.9	56.4
13	3.23	0.34	51.5	58.9
14	0.00	0.00	62.8	58.4
15	0.00	0.20	62.2	73.1
16	0.00	0.24	61.5	71.5
17	0.00	0.27	62.1	70.6

y_1 : cellulose conversion into glucose, %

y_2 : hexoses conversion into HMF, %

y_3 : hexoses conversion into LA, %

y_4 : hexoses conversion into FA, %

Table 4. Regression coefficients and statistical parameters measuring the model correlation and significance of model and coefficients

Coefficient	Value
b_{03}	68.1*
b_{13}	2.15
b_{23}	1.26
b_{33}	4.59***
b_{123}	-1.74
b_{133}	-2.38
b_{233}	-1.63
b_{113}	-11.2**
b_{223}	-12.3**
b_{333}	-15.6*

*:Significance >99%; **:Significance >95%; ***:Significance>90%

$F_{st} = 11.53$ (significance > 99%); $R^2 = 0.937$

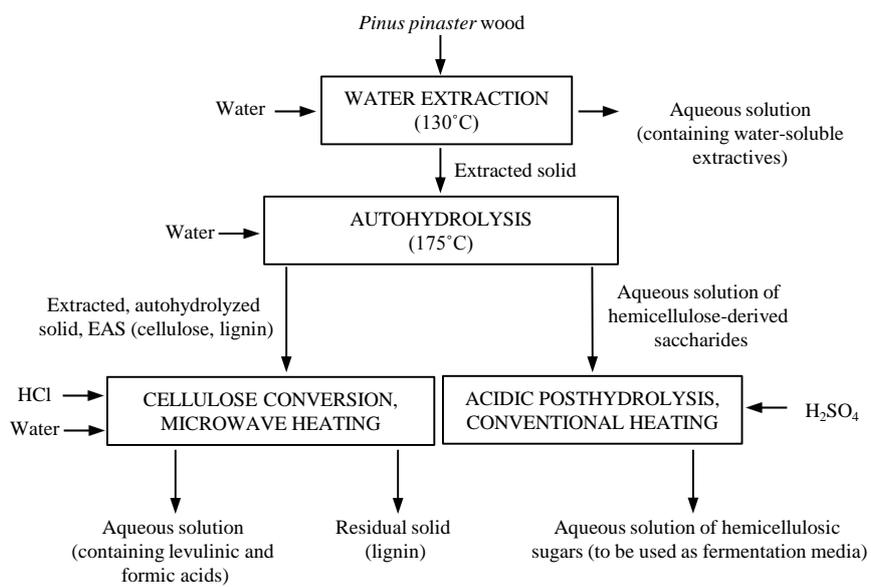


Figure 1

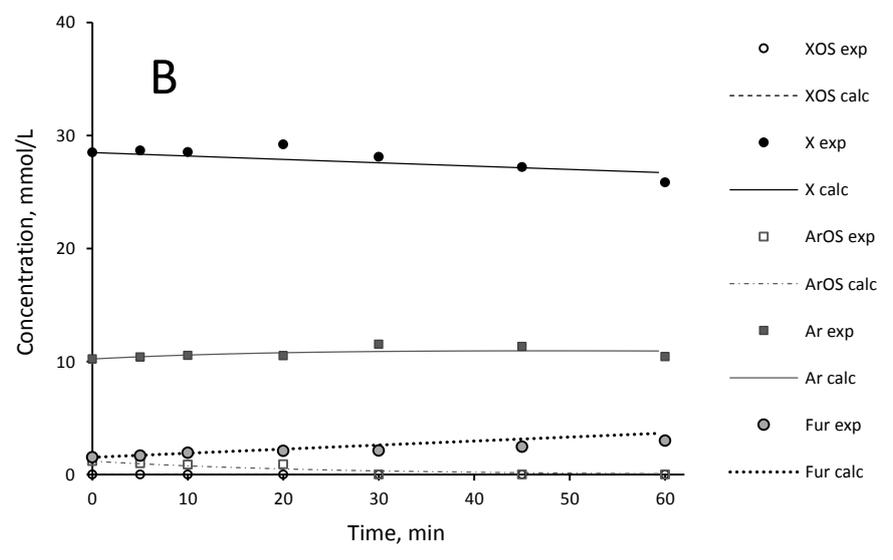
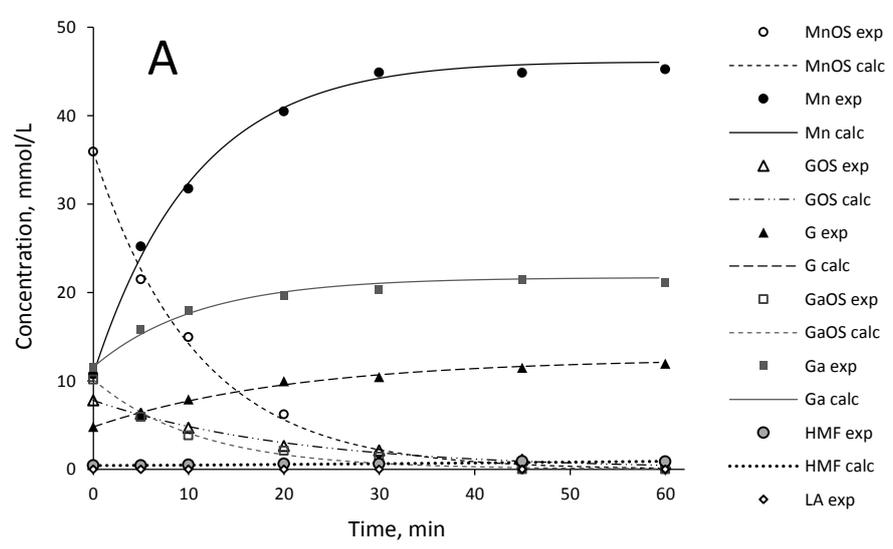


Figure 2

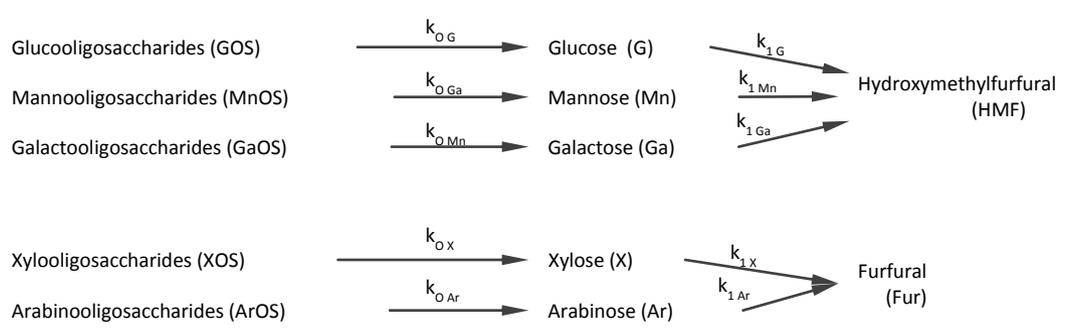


Figure 3

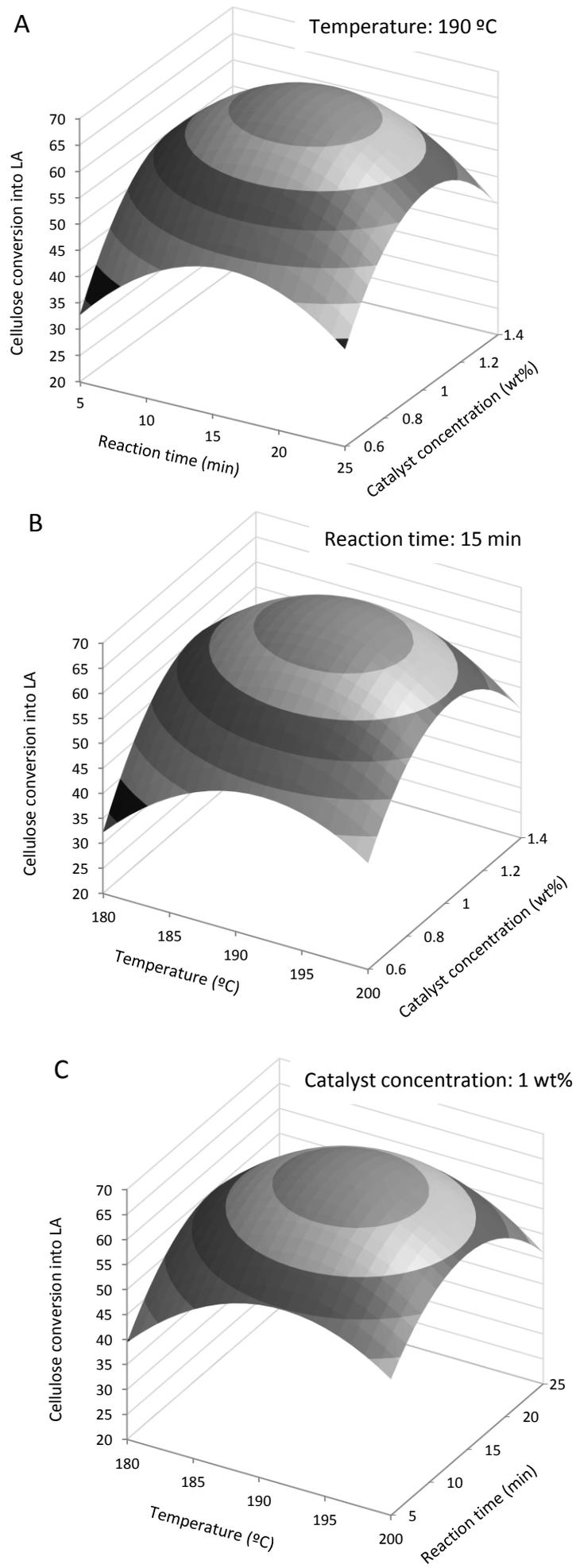


Figure 4