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# 1 Efficiency of acetic acid and formic acid as a catalyst in catalytical 2 and mechanocatalytical pretreatment of barley straw

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8

## 9 **Abstract**

10 In this study, the potential of organic acids (formic acid, acetic acid) in a catalytical and  
11 mechanocatalytic conversion of lignocellulosic barley straw to valuable sugars is explored using  
12 sulfuric acid as a reference. Acid-catalyzed hydrolysis has been carried out with acid-impregnated  
13 samples as well as unmodified barley straw. In the mechanocatalytical approach, pretreatment  
14 consists of impregnation with the acid catalyst and mechanical treatment by ball milling following  
15 chemical hydrolysis. Straw samples and residues were analyzed by Fourier transform infrared  
16 spectrometry (FT-IR) whereas hydrolysate analysis was based on total reducing sugar (TRS)  
17 determination following the DNS method and capillary electrophoresis (CE) analysis. The results  
18 indicated that acetic acid and formic acid are rather mild acids yielding low TRS levels compared to  
19 the reference acid. Mechanocatalytical pretreatment slightly increased TRS yields, but not  
20 significantly. Strikingly, sulfuric acid showed an efficient conversion efficiency yielding almost 45 %  
21 of TRS. Furthermore, this study provided evidence for the acetylation of straw components when  
22 acetic acid was used as catalyst. Alkali hydrolysis induced the de-esterification, but revealed no  
23 significant increase of TRS yields.

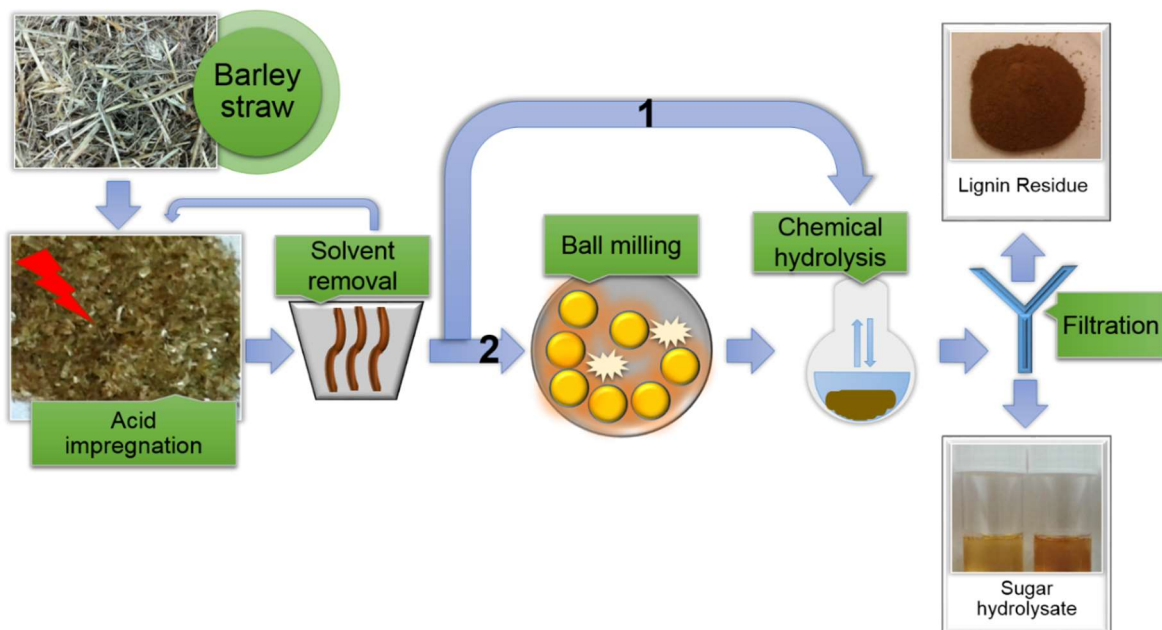
24 *Keywords:* Barley straw, Lignocellulose, Formic acid, Acetic acid, Mechanocatalytical conversion

25

## 26 **1. Introduction**

27 Strengthening the commitment of renewable energy sources is one of the major bioeconomy  
28 challenges in order to safeguard a sufficient supply of energy and to reduce greenhouse gas  
29 emissions. The European Commission states the binding target in the EU's Renewable Energy  
30 Directive (2009/28/EC) that 20 % of final energy consumption should be derived from renewable  
31 sources as well as a minimum of 10 % of transportation fuels, both by 2020 [1]. Moreover the targets  
32 for 2030 include at least a 27 % share of renewable energy consumption, a 30 % improvement in  
33 energy efficiency as well as a 40 % cut in greenhouse gas emissions compared to the levels in 1990  
34 [2]. Biomass is a strong growing stock of natural raw material source which has high potential for  
35 contribution in the sustainable production of energy, biofuels and biochemicals. Generation of

36 bioenergy from renewable resources lowers CO<sub>2</sub> emissions and decreases the dependence on  
 37 energy imports and fossil materials whose reservoirs are about to run out.  
 38 Biomass can be efficiently recycled by catalytic conversion to carbohydrates which can in turn be  
 39 converted to biofuels such as ethanol and butanol, e.g. by gasification (Fischer-Tropsch process) or  
 40 fermentation [3]. These days, bioethanol production derives predominantly from the turnover of food  
 41 supply chain products such as corn, beet and cane sugar. This is clearly not sustainable and conflicts  
 42 with food and feed production security. Biomass does not interfere with food production. In this study,  
 43 barley straw is explored for its potential as a raw material for catalytic and mechanocatalytic  
 44 conversion to valuable sugars. Barley straw, as a second generation biomass, belongs to the  
 45 lignocellulosic resources group whereas starch and sugar-based raw materials represent first  
 46 generation biomass.  
 47 Currently, less commercial technological applications for biofuel production exist which requires the  
 48 establishment of innovative techniques. The present research aims at the determination of the  
 49 efficiency of acetic acid and formic acid as a catalyst in catalytic and mechanocatalytic fractionation  
 50 of lignocellulosic barley straw to valuable carbohydrates. The mechanocatalytic approach,  
 51 schematically illustrated in Figure 1, is carried out under solvent-free conditions by the combination  
 52 of chemical catalysis and mechanical assisted processing. The conversion of lignocellulosic barley  
 53 straw into sugars is a two-step process which includes the impregnation of the material with an acid  
 54 catalyst in the first step and mechanical treatment driven by milling in the second leading to the  
 55 disruption of the lignocellulosic matrix [4]. Resulting oligosaccharides from the conversion reaction  
 56 are hydrolyzed yielding monosaccharides [3]. These mechanocatalytically obtained sugars  
 57 constitute the basic product for the formation of biofuels with high conversion efficiency.



58  
 59 Figure 1. Scheme of the fractionation of lignocellulosic barley straw excluding (1) and including (2) mechanical treatment  
 60 using a ball mill.

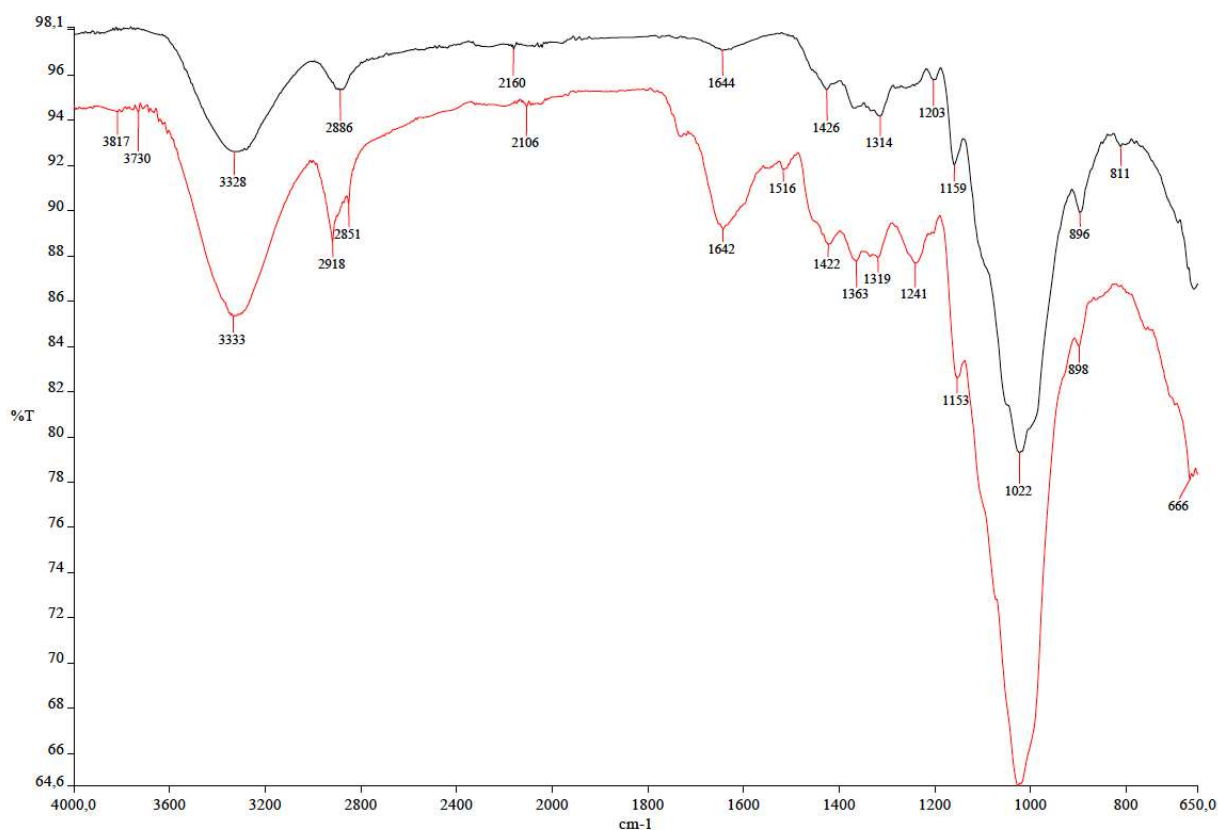
61 **2. Results and Discussion**

62

63 **2.1. Elemental and structural analysis of barley straw**

64 Elemental analysis revealed a total carbon content of 45.4 wt.%, nitrogen content of 2.5 wt.% and  
65 hydrogen content of 5.7 wt.% calculated from dry material which is comparable to literature [5, 6].  
66 Nitrogen content appeared to be increased by about a factor of 4 which might indicate an increased  
67 protein level. However, the protein content is of minor interest in this study and was not separately  
68 determined.

69 Structural analysis of barley straw was carried out by Fourier transform infrared spectrometry (FT-IR)  
70 which provides detailed information on the chemical bonds in the molecules. The recorded spectrum  
71 is illustrated with a reference spectrum of alpha-cellulose in Figure 2. The absorption band at  
72  $3333\text{ cm}^{-1}$  shows a broad peak for intra and intermolecular hydrogen bonded OH-groups in C2, C3  
73 and C6 position of the glucose units of cellulose. The two bands at  $2918\text{ cm}^{-1}$  and  $2851\text{ cm}^{-1}$   
74 represent C-H stretching for aliphatic saturated compounds which is attributed to the  $\text{CH}_2$  stretch  
75 vibration from the  $\text{CH}_2\text{OH}$  group of C6 in cellulose. Characteristic in the fingerprint region is the sharp  
76 and strong band at  $1026\text{ cm}^{-1}$  which also occurs in the alpha-cellulose FT-IR spectrum. It represents  
77 the C-O-C ether vibration in cellulose and hemicellulose and the C-O stretching in cellulose,  
78 hemicellulose and lignin [7]. The band of the barley straw spectrum is more distinct and intense due  
79 to its complex matrix including hemicellulose [8].



80

81 Figure 2. FT-IR spectrum of barley straw (red) (particle size: 0.5 mm) and alpha-cellulose (black).

**2.2. Effect of different acid catalysts on the total reducing sugar (TRS) yield of barley straw hydrolysates**

Prior to the mechanocatalytic treatment with the ball mill, solely acid-catalyzed hydrolysis of impregnated as well as non-impregnated barley straw samples was performed. The results are shown in Figure 3. It can be clearly seen that total reducing sugar (TRS) analysis of non-impregnated straw revealed significant high yield for barley straw when it is hydrolyzed with 2 mmol of sulfuric acid per gram straw sample at 100°C. TRS release decreased 5 to 9 times when the same concentration of formic acid and acetic acid was applied. Similar results could also be observed for acid-impregnated straw samples which have been in contact with the respective acid for 30 min prior to hydrolysis.

To find out whether a higher concentration of organic acid results in higher TRS yields, barley straw was impregnated with a 3 times higher formic acid concentration (6 mmol/ g). However, similar TRS yields were achieved. In general, hydrolysis at room temperature yielded lower TRS release compared to hydrolysis at 100°C. Exclusively acetic acid yielded a higher TRS result at room temperature than at 100°C which might be associated with its boiling point at 118°C. However, straw hydrolysis with acetic acid performed at a lower temperature (80°C) revealed only a slight increase in TRS yields. Surprisingly, hydrolysis merely with distilled water at 100°C without the addition of an acid catalyst yielded higher TRS levels than organic acid hydrolysis.

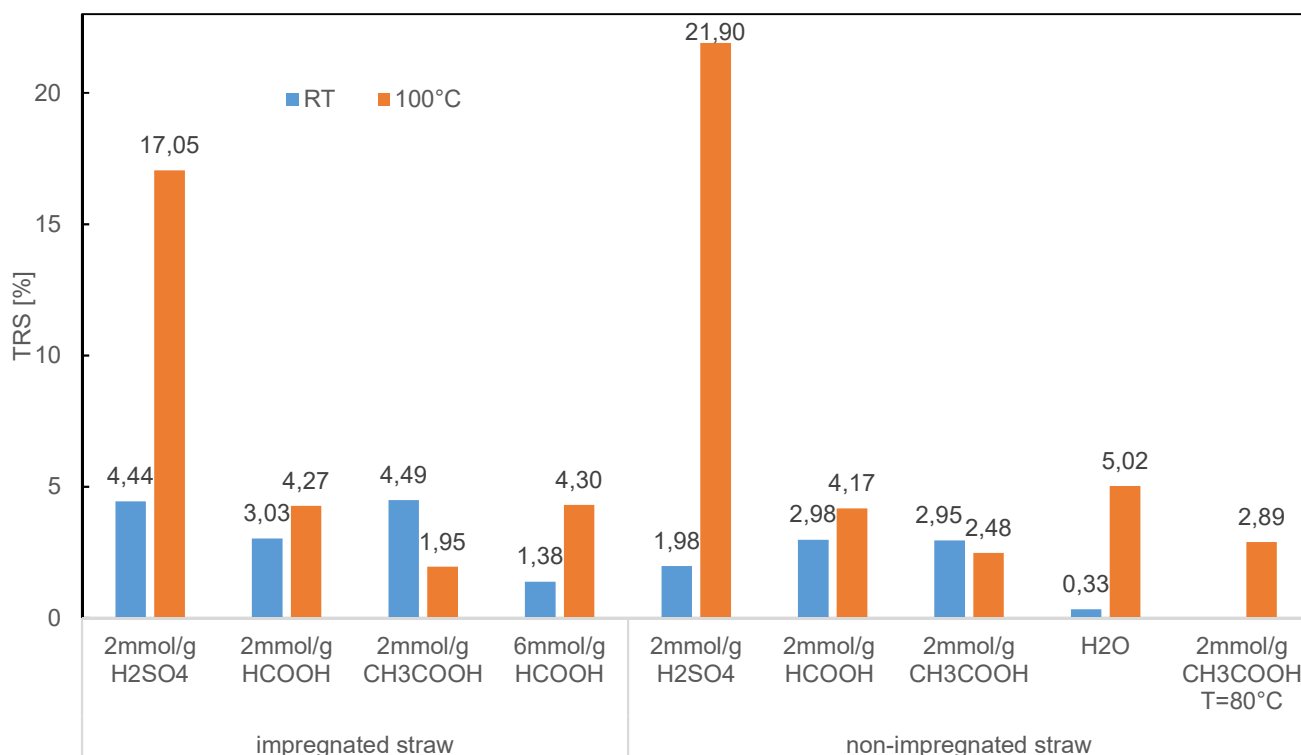
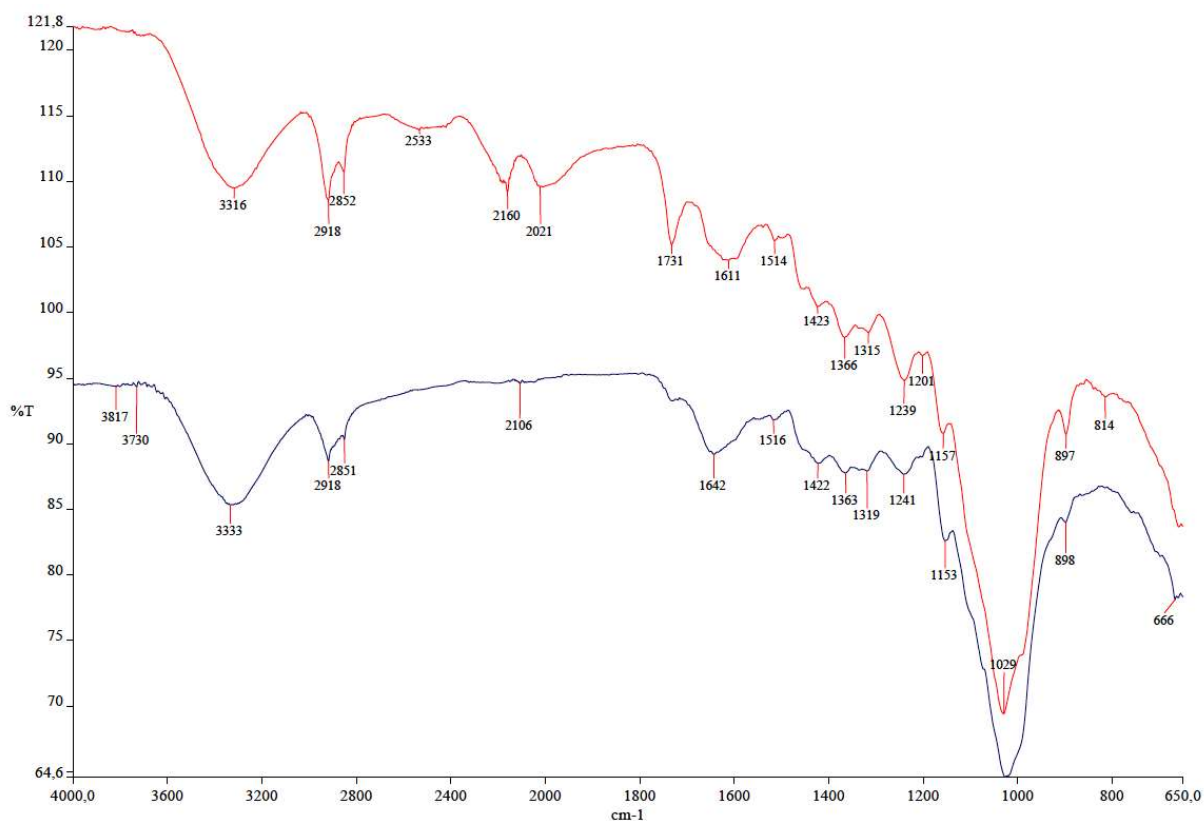


Figure 3. TRS yield of hydrolysates from impregnated and non-impregnated barley straw with different acid catalysts at room temperature (RT) and 100°C.



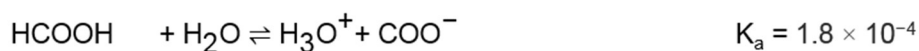
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Figure 4. FT-IR spectrum of unmodified barley straw (black) (particle size: 0.5 mm) and impregnated straw with acetic acid (red).

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108 Since TRS yields from straw hydrolysates catalyzed with organic acids were relatively low compared  
 109 to hydrolysis with the mineral acid, it raised the question whether barley straw was sufficiently loaded  
 110 with the respective organic acids. Figure 4 shows the FT-IR spectrum of unmodified barley straw  
 111 and acetic acid-impregnated barley straw. Major differences between the two spectra occurred in  
 112 the band at  $1731\text{ cm}^{-1}$  which was only present in the impregnate. The band represents the acetyl  
 113 C=O stretching band from acetic acid which confirmed the successful loading of the straw sample.  
 114 However, the band is not as strong as expected. Hence, the amount of loaded acid was identified  
 115 by titration with a sodium hydroxide solution. Strikingly, impregnated straw samples revealed an acid  
 116 loading of 14.8 % acetic acid, 30.9 % formic acid and 70.8 % sulfuric acid. These results clearly  
 117 indicated that the impregnation of barley straw with the tested organic acids is somehow hampered  
 118 whereas sulfuric acid could be loaded relative efficiently. However, the acid loading appears to not  
 119 have a significant effect on TRS yields. It has been shown above that TRS yields of hydrolyzed  
 120 impregnated straw samples were similar to the TRS releases of non-impregnated hydrolysates in  
 121 which the acid catalyst was added shortly before the hydrolysis process in the same concentration.  
 122 Another possible explanation for the low TRS yields could be the inhibition of sugar generation due  
 123 to the formation of by-products. It has been reported that the hydrolysis of lignocellulosic biomass

124 leads to the formation of furfural, hydroxymethyl furfural, complex esters, humins and tars.  
 125 Depending on the hydrolysis process conditions, these by-products can reduce the TRS yields [9].  
 126 Furthermore, formic acid and acetic acid represent mild C1 and C2 organic acids with a pKa of 3.7  
 127 and 4.7, respectively. Sulfuric acid, in contrast, is a strong diprotic acid whose proton is more likely  
 128 donated to the solvent. Formic acid and acetic acid stay as non-ionized acids following the equations  
 129 with acidity constants ( $K_a$ ) below [10].



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132 In addition, it has been pointed out that contacting lignocellulosic biomass with acetic acid may result  
 133 in the acetylation of its compounds such as cellulose and hemicellulose [9]. Solely, hydrolysis of the  
 134 impregnated sample without any additional catalyst may not break the acetylated cellulose and  
 135 hemicellulose and moreover, affect their turnover to sugars. Deacetylation is achieved by chemical  
 136 hydrolysis with an alkali compound. Hence, supplementation of a base catalyst was carried out in  
 137 order to induce de-esterification which might result in increased TRS yields. The results will be  
 138 discussed in the following section.

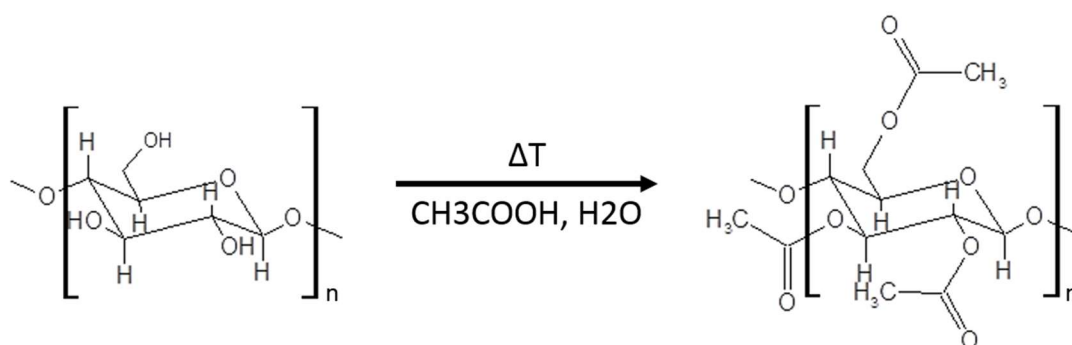
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### 141 2.3. Effect of alkali hydrolysis on the TRS yield from barley straw impregnates

142 Chemical reaction between the barley straw components and acetic acid may occur in form of an  
 143 acetylation [9]. Hemicellulose and cellulose hold reaction sites which enable an acetylation on an  
 144 alcohol function as shown schematically in the following figure:

145



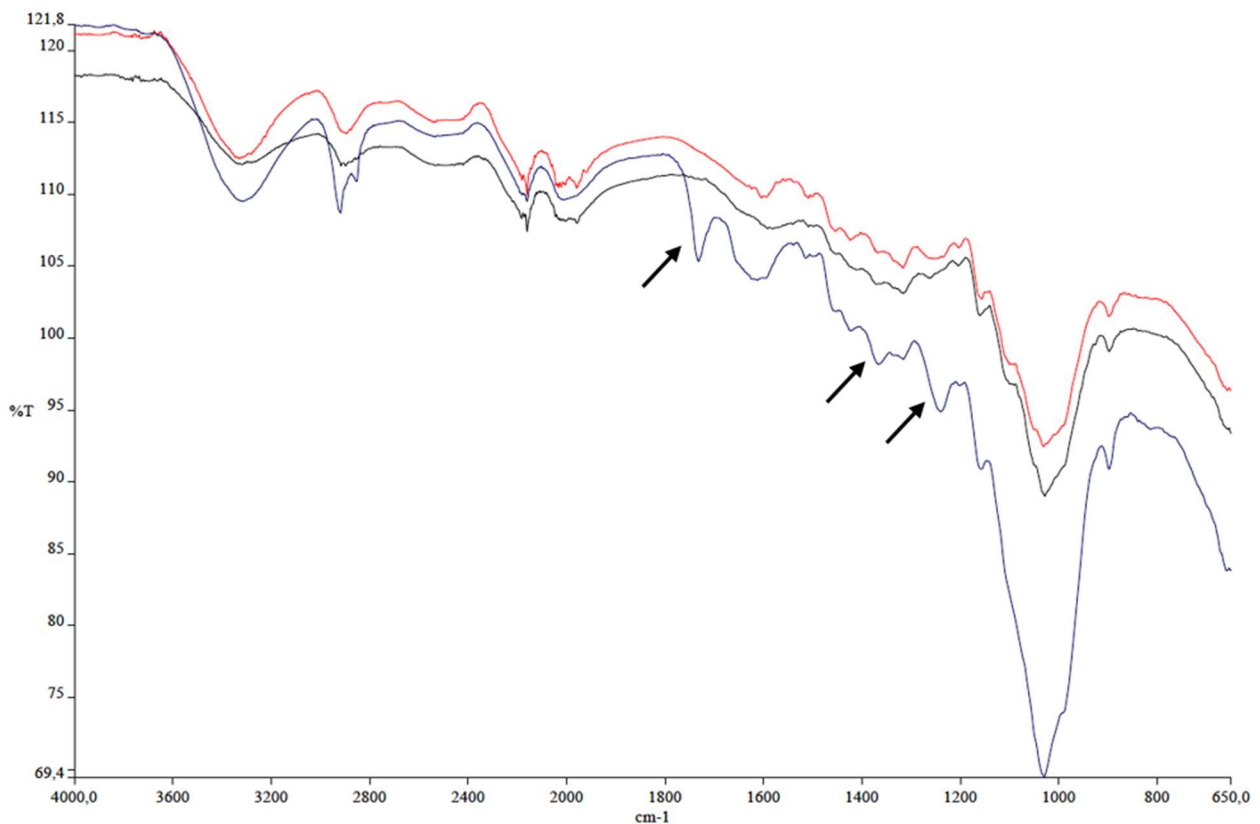
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147 Figure 5. Schematic reaction of the acetylation of cellulose during hydrolysis with acetic acid.

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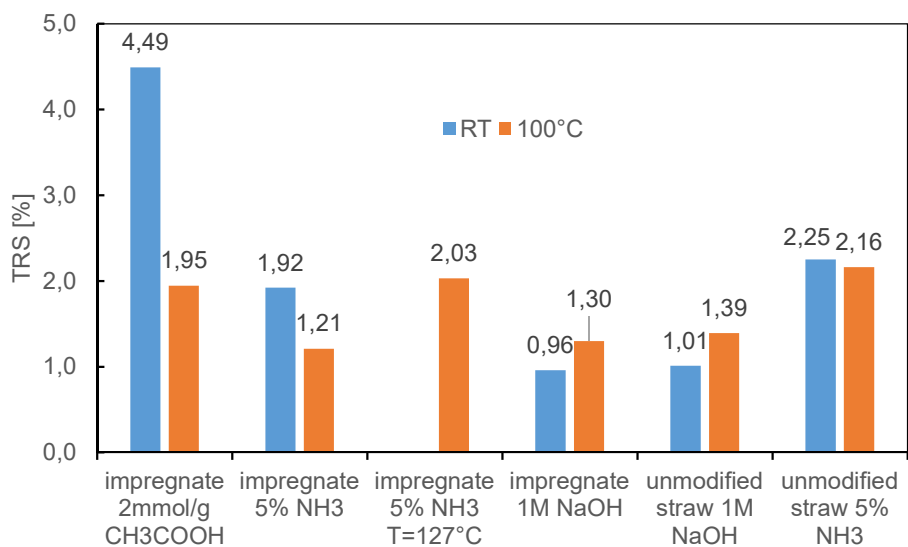
149 De-esterification of the acetate groups is catalyzed by alkali hydrolysis. In the present study, the  
150 acylated compounds are broken by chemical hydrolysis using sodium hydroxide and ammonium  
151 hydroxide as the catalyst. Straw was impregnated with acetic acid assuming that hemicellulose and  
152 cellulose undergo acetylation. The hydrolysis was subsequently carried out with an excess of  
153 catalyst attaining pH 14 and pH 10, respectively. During hydrolysis with NaOH, the formation of a  
154 swollen bulk could be observed which is associated with the mercerization process, a chemical  
155 treatment applied in the textile industry.

156 Strikingly, FT-IR analysis of the hydrolysis residue demonstrated that the acetyl groups were  
157 successfully removed. Figure 6 illustrates the spectra of barley straw impregnated with acetic acid,  
158 NaOH-hydrolyzed impregnate and NH<sub>3</sub>-hydrolyzed impregnate. The spectra show that the ester  
159 bands of the impregnated straw disappear in the alkali hydrolyzed samples. The ester bands  
160 comprise the acetyl C=O stretching band of esters at 1731 cm<sup>-1</sup>, the signal at 1369 cm<sup>-1</sup> attributed to  
161 the C-H in -O(C=O)-CH<sub>3</sub> and the C-O stretching of acetyl group at 1234 cm<sup>-1</sup> [7, 11]. Furthermore,  
162 alkali hydrolysates were free of esterified acetic acid which is demonstrated by the missing band at  
163 1700 cm<sup>-1</sup> for a carboxylic group [9]. These results indicated that barley straw compounds were  
164 partly acetylated when contacted with acetic acid. In addition, hydrolysis of the impregnate with alkali  
165 compound succeeded in de-esterification of the acetylated straw constituents.



166  
167 Figure 6. FT-IR spectrum of barley straw impregnated with acetic acid (blue), NaOH-hydrolyzed impregnate (black) and  
168 NH<sub>3</sub>-hydrolyzed impregnate (red). Arrows indicate the three characteristic ester bands at 1731 cm<sup>-1</sup>, 1369 cm<sup>-1</sup> and  
169 1234 cm<sup>-1</sup>.





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Figure 7. TRS yield from alkali hydrolysis of unmodified straw and samples impregnated with acetic acid at room temperature and 100°C.

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The straw hydrolysates which were obtained by alkali hydrolysis either with sodium hydroxide or ammonium hydroxide, have been analyzed for their TRS yield. The results are depicted in Figure 7 which revealed that hydrolysis of the acetic acid-impregnated samples with ammonium hydroxide is more effective than hydrolysis with sodium hydroxide. Furthermore, an increase in TRS yield by a factor of 1.5 could be achieved by raising the hydrolysis temperature to 127°C. Alkali hydrolysis of unmodified barley straw which was not impregnated with acetic acid, was also analyzed. TRS results were similar or slightly higher compared to acid-impregnated straw samples hydrolyzed with the corresponding base catalysts.

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Comparing the results of alkali hydrolysis with the earlier results obtained for hydrolysis of acetic acid-impregnated straw, it can be pointed out that TRS yields were higher when no base catalyst was supplemented for the hydrolysis reaction. The results indicated that sodium hydroxide and ammonium hydroxide were not efficient enough to convert straw compounds to valuable carbohydrates under the conditions applied in this study. Though an excess of base catalyst induced de-esterification, the efficiency for the conversion of lignocellulosic biomass was not sufficient.

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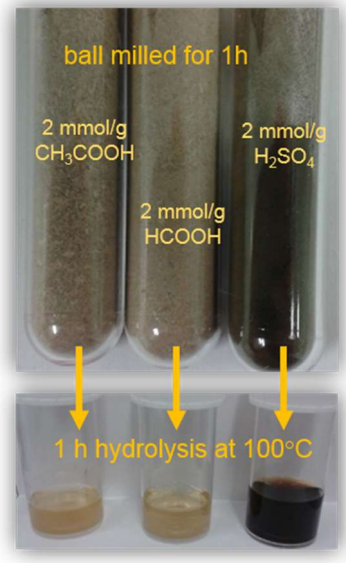
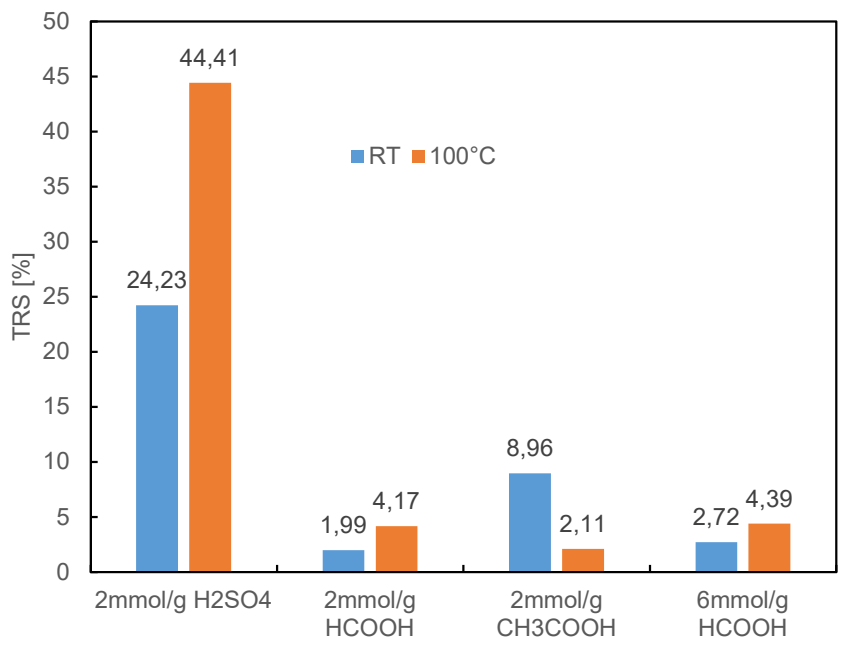
#### 2.4. Effect of mechanically solid state depolymerisation on the TRS yield of barley straw

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Mechanical treatment by milling is a potential method for “cutting” the lignocellulosic biomass and increasing the availability of cellulose for catalytic conversion into sugars. Milling reduces the straw particle size and crystallinity and at the same time increases the specific surface area and supports the depolymerisation of the lignocellulosic material. The structural changes induced by the milling process imply an increase in TRS release.

195 In this section, the effect of ball milling prior to acid-catalyzed hydrolysis will be analyzed and  
196 discussed. According to the above mentioned results acetic acid and formic acid only released small  
197 amounts of total reducing sugar from the cellulosic material by hydrolysis. Sulfuric acid was  
198 emphasized as an effective acid catalyst for the conversion of lignocellulosic material from barley  
199 straw to valuable sugars. Figure 8 depicts the TRS yields of mechanocatalytically pretreated barley  
200 straw samples impregnated with sulfuric acid, formic acid and acetic acid. In principal, TRS release  
201 increased when milling was applied, except for impregnation with 2 mmol of formic acid per gram  
202 straw sample. Sulfuric acid-impregnated straw significantly produced the highest sugar amount.  
203 When hydrolysis was carried out with high temperatures, an 11 to 21 times increase in TRS  
204 compared to organic acid-impregnates was observed. Strikingly, 44.41 % fermentable sugars were  
205 obtained when sulfuric acid was used as a catalyst. Capillary electrophoresis (CE) measurement  
206 revealed a yield of 6.4 g glucose and 7.4 g xylose per 100 g of starting material at elevated  
207 temperature whereas 2.1 g glucose and 3.1 g xylose were formed at room temperature. Formic  
208 acid-based conversion generated little glucose (0.8 % at 100°C, 1.5 % at RT) and arabinose (0.32 %  
209 at 100°C, 0.8 % at RT). Xylose yields were under the limit of quantification. Surprisingly, acetic  
210 acid-catalyzed turnover showed a yield of 7.1 g glucose and only 0.8 g xylose per 100 g of barley  
211 straw when hydrolysis was carried out at room temperature which accounts for a total fermentable  
212 sugar release of 8.96 %. It can be pointed out that a higher glucose fraction in relation to total  
213 reducing sugar yield was released when formic acid was used as a catalyst. However, the total  
214 reducing sugar yield is five times less compared to sulfuric acid-based conversion. Xylose is  
215 additionally generated in adequate yields when sulfuric acid is used as catalyst. Furthermore,  
216 arabinose, galactose and sucrose could be determined, however in insignificant concentrations. The  
217 comparatively high TRS release of almost 45 % may be due to the presence of gluco- and  
218 xylo-oligosaccharides which have not been hydrolyzed completely [12].

219 The corresponding FT-IR spectra in Figure 9 show the changes in the cellulosic material by milling.  
220 The relative band height (distance from baseline to adjacent valley) at 1029 cm<sup>-1</sup> which represents  
221 the C-O-C ether vibration in cellulose and hemicellulose, significantly decreased in the grinded straw  
222 sample, impregnated with sulfuric acid. These changes in cellulosic material arise from the decrease  
223 in the degree of crystallinity and polymerization which indicate the conversion of cellulose [8]. An  
224 increase in organic acid concentration repeatedly did not affect the TRS release. These results  
225 highlighted sulfuric acid as the most efficient conversion catalyst for barley straw among the tested  
226 acids. The enhancing effect of ball milling was substantially visible for sulfuric acid-impregnated  
227 straw samples which yielded almost 45 % TRS release. Absence of a mechanical treatment revealed  
228 a TRS yield of 17 % (see Figure 3) indicating that with the inclusion of mechanical treatment, TRS  
229 release almost triples under the milling conditions applied in this study.



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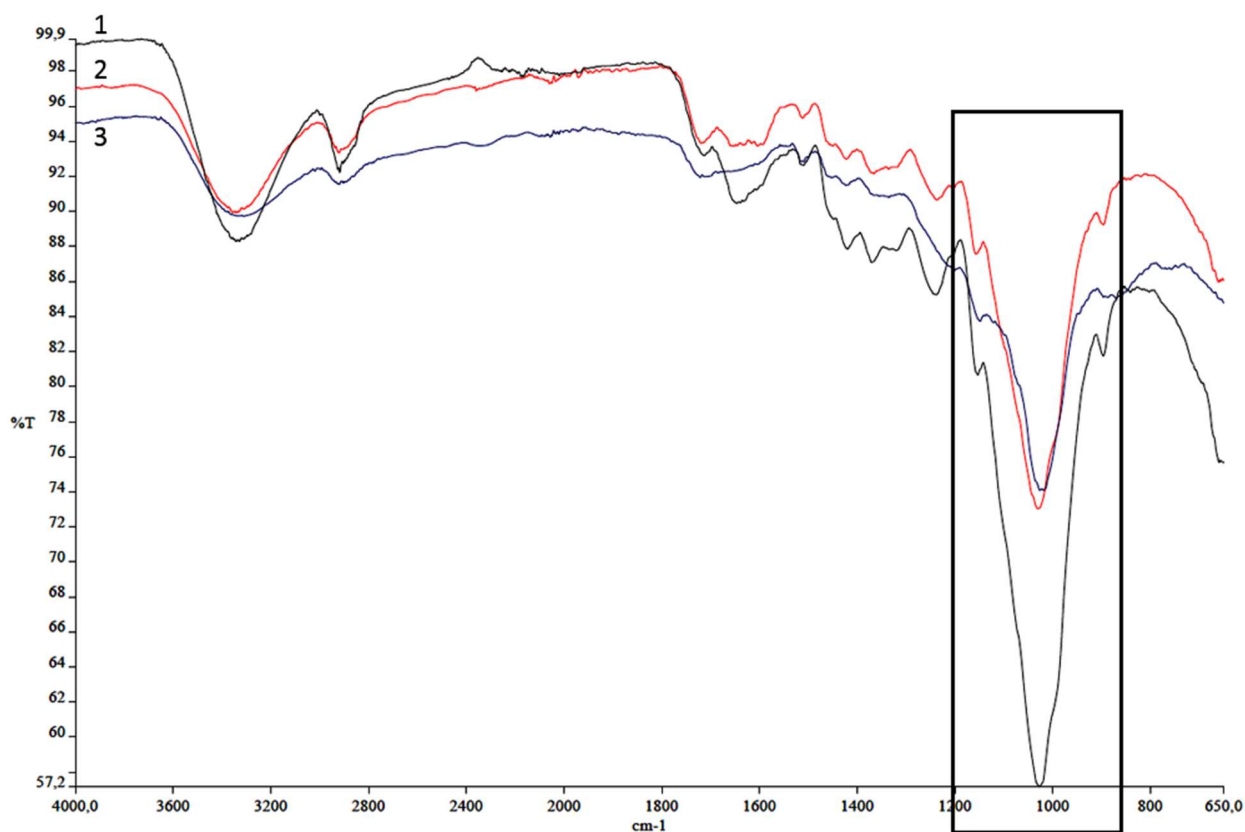
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Figure 8. TRS yield from mechanocatalytically pretreated barley straw with different acid catalysts (left) and illustration of the mechanical treated acid-impregnated straw as well as the hydrolysates (right).

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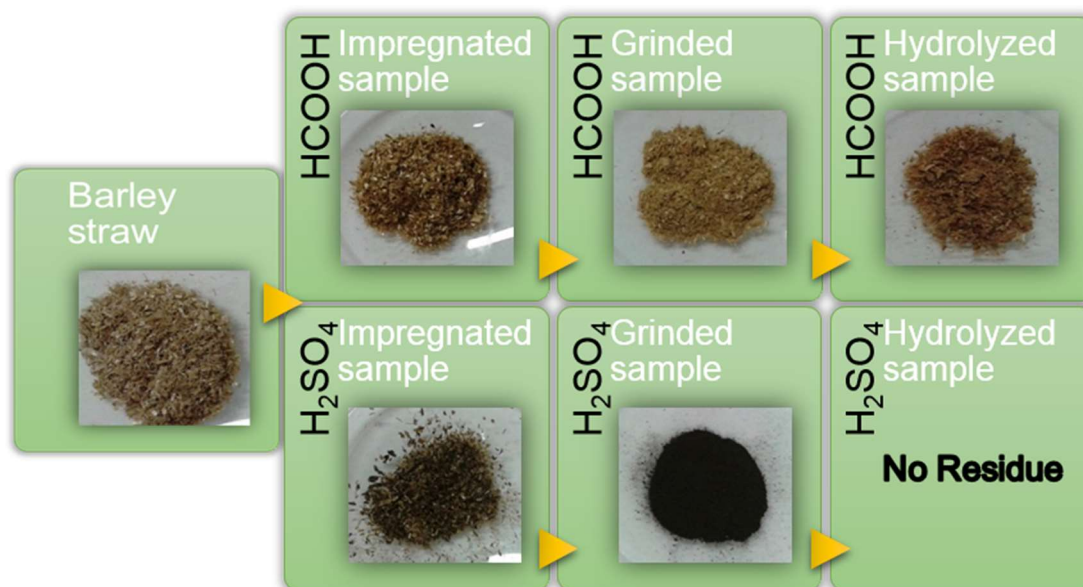
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Figure 9. FT-IR spectrum of ball milled barley straw samples impregnated with acetic acid (1), formic acid (2) and sulfuric acid (3) before hydrolysis revealing the changes in the cellulose backbone with the band at 1029 cm<sup>-1</sup>.

238 Remarkably, the color of the straw sample which was impregnated with sulfuric acid, turned dark  
 239 greenish-brown after mechanical treatment (see Figure 8). Impregnation with organic acid did not  
 240 drastically change the typical straw color. Each sample was treated the same and grinded for a total  
 241 milling time of 1 h including breaks of 10 min after every 5 min grinding in order to avoid overheating  
 242 and burning of the sample. The hydrolysate solutions of organic acid hydrolysis were pale yellow  
 243 whereas the hydrolysate using sulfuric acid as a catalyst turned dark red-brownish. The reason for  
 244 the color change is not yet known and should be subject to further analysis. It can be assumed that  
 245 a colored substance was formed rather than the event of a carbonization process. The corrosive  
 246 character of sulfuric acid combined with mechanical treatment which enhances the reaction contact  
 247 sites between acid and organic material, may contribute to the appearance of the red-brownish color.  
 248 Figure 10 depicts the barley straw sample at different stages of the mechanocatalytical process  
 249 using formic acid and sulfuric acid as catalysts. Here, the color change is clearly visible when sulfuric  
 250 acid was used as a catalyst. Already the unground, but impregnated sample was slightly darker in  
 251 color compared to the pale yellow untreated barley straw. Interestingly, no residue was found after  
 252 hydrolysis of the grinded sample which assumes that the straw compounds have been completely  
 253 converted. Another possibility is the formation of a colloidal solution. Related to formic  
 254 acid-impregnated samples, the color stayed pale yellow during the whole treatment process and  
 255 resulted likewise in a pale yellow-colored hydrolysate as illustrated in Figure 8.  
 256  
 257



258  
 259 Figure 10. Barley straw processing chart for formic acid (up) and sulfuric acid (down) treated samples revealing the change  
 260 in particle size and color.

261  
 262

### 263 3. Conclusions

264 Biomass is a significant resource for biofuel production which is not yet used to its full potential. New  
265 innovative scientific research concerning the construction of efficient technologies, methods and  
266 installations plays a key role in order to enhance the contribution of biomass as a renewable energy  
267 source for biofuel production. In this study, barley straw was investigated as a potential candidate  
268 for its fractionation into valuable carbohydrates using acetic acid, formic acid and sulfuric acid as a  
269 catalyst. It turned out that sulfuric acid significantly generated the highest yield of total reducing sugar  
270 in a mechanocatalytic approach, though a bioeconomic solution including formic acid and acetic acid  
271 was prioritized in this study. The catalytic conversion efficiency on hydrolysis of lignocellulosic straw  
272 material can be displayed as follows: Sulfuric acid >> formic acid > water > acetic acid. Sulfuric  
273 acid-catalyzed hydrolysis combined with mechanical treatment revealed a reasonable yield of 45 %  
274 fermentable sugars. The results verified barley straw as a feasible feedstock in sugar generation for  
275 the production of transportation biofuels and biochemicals yielding the replacement of conventional  
276 biofuels combined with greenhouse gas emission savings.  
277 Continuing research will focus on the optimization of the mechanocatalytic fractionation of barley  
278 straw using sulfuric acid as a catalyst. The present study showed that mechanical treatment in  
279 combination with high temperature hydrolysis in an acidic environment were required for a sufficient  
280 conversion efficiency of cellulose and hemicellulose into carbohydrates.

281

282

### 283 4. Experimental Section

284

#### 285 4.1. Raw materials

286 Barley straw examined in the present study was provided from a local Finnish farmer. The straw was  
287 dried and grinded to a particle size of 0.5 mm using a Retsch SM100 Comfort cutting mill. Other  
288 chemicals included: Formic acid (98-100 %, Merck), acetic acid (99-100 %, J.T.Baker), sulfuric acid  
289 (95 %, VWR), sodium hydroxide pellets (Merck), ammonium hydroxide (25 %, J.T. Baker).

290

#### 291 4.2. Mechanocatalytical pretreatment

292 The acid catalyst (2 mmol per gram straw) was dissolved in 150 mL diethyl ether. 10 g of barley  
293 straw sample was added to the acid solution and kept for 30 min shaking at room temperature (Stuart  
294 orbital incubator S1500, 170 rpm). The solvent was removed using a vacuum rotary evaporator  
295 (Heidolph Laborota 4010 digital, 40 C). Dry acid-impregnated straw (2.4 g) was applied to a stainless  
296 steel container (45 mL, 16 milling balls each 2.93 g and 1 cm in diameter) and grinded in a ball mill  
297 (Fritsch premium line Pulverisette 7) at 800 rpm. The grinding time was set at 1 h including 12 cycles  
298 of 5 min grinding and a break time of 10 min in order to avoid overheating and burning of the sample.  
299 The temperature was controlled manually after every run (TM-903 LT Lutron).

300 **4.3. Acid-catalyzed hydrolysis**

301 Acid hydrolysis was carried out without any further addition of acid catalyst with the pretreated barley  
302 straw. Pretreated straw samples of 5 wt.% in distilled water were hydrolyzed for 1 h either by shaking  
303 at room temperature (RT) or by heating in an oil bath at 100°C. The hydrolysis causes the formation  
304 of a precipitate which is separated from the sugar solution by filtration. Additionally, direct hydrolysis  
305 of the non-impregnated straw has been performed by adding the acid catalyst directly to the 5 wt.%  
306 suspension without prior impregnation and mechanical pretreatment.

307

308 **4.4. Alkali hydrolysis**

309 Alkali hydrolysis was carried out with either 1 M sodium hydroxide (2 mL) or 25 % ammonium  
310 hydroxide in a 5 wt.% sample suspension with a total volume of 10 mL. The samples were hydrolyzed  
311 for 1 h either by shaking at room temperature (RT) or by heating in an oil bath at 100°C. Additionally,  
312 direct hydrolysis of unmodified straw has been performed by adding the base catalyst directly to the  
313 5 wt.% suspension without prior impregnation and mechanical pretreatment.

314

315 **4.5. Analytical methods**

316 **4.5.1. Determination of acid loading by titration**

317 Acid loading of the impregnate was determined by titration. 1 g of straw impregnate was suspended  
318 in 40 mL of distilled water and titrated with 0.01 M and 0.1 M NaOH for organic acid-impregnated  
319 and sulfuric acid-impregnated straw, respectively.

320

321 **4.5.2. Elemental and structural analysis**

322 The total carbon content (TC) of the dry straw sample was determined by elemental analysis with a  
323 Perkin Elmer CHNS analyzer. Structural analysis of the raw materials were carried out by Fourier-  
324 transformed infrared spectrometry (FT-IR) which provided detailed information on the chemical  
325 bonds in the molecules.

326

327 **4.5.3. Determination of Total Reducing Sugar by UV/ VIS spectrometry**

328 The amount of total reducing sugar (TRS) was determined according to the DNS (dinitrosalicylic  
329 acid) method using a Shimadzu UV-1800 spectrometer [13]. The measurement provides fast TRS  
330 analysis which allows an estimation of the sugar concentration. The reaction is based on a  
331 simultaneous oxidation of functional sugar groups, accompanied with the reduction reaction of the  
332 DNS reagent. A color change from light yellow to orange, brownish or reddish-brown occurs  
333 depending on the amount of fermentable sugars. A (diluted) portion of the hydrolyzed biomass filtrate  
334 was mixed with a 1 % 3,5-dinitrosalicylic acid reagent in a volume ratio of 1:1 and subsequently  
335 heated in a boiling water bath for 5 min. The TRS was determined at a wavelength of 540 nm based  
336 on a glucose calibration. The analysis was carried out in triplicates.

337 **4.5.4. Capillary electrophoresis**

338 The hydrolysates were filtered with a 0.45 µm GHP Acrodisc syringe filter and diluted with water. CE  
339 analyses were performed with a P/ACE MDQ CE instrument (Beckman-Coulter, Fullerton, CA, USA)  
340 equipped with a diode array detector (DAD), using a modified method of Rovio et al. (2007) [14].  
341 Uncoated fused-silica capillaries of I.D. 25 µm and length 30/40 cm (effective length/total length)  
342 were used. The samples were injected at a pressure of 0.5 psi for 10 s and the separation voltage  
343 was +16 kV. Calibration curves for the external quantification of HMF, sucrose, lactose, cellobiose,  
344 galactose, glucose, mannose, arabinose and xylose. All samples were measured as duplicates. In  
345 addition, each sample run was performed with spiked standards to confirm the identity of the  
346 analytes.

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