



**HAL**  
open science

## High voltage electric discharges treatment for high molecular weight hemicelluloses extraction from spruce

Morad Chadni, N. Grimi, Isabelle Ziegler-Devin, Nicolas Brosse, Olivier Bals

### ► To cite this version:

Morad Chadni, N. Grimi, Isabelle Ziegler-Devin, Nicolas Brosse, Olivier Bals. High voltage electric discharges treatment for high molecular weight hemicelluloses extraction from spruce. *Carbohydrate Polymers*, 2019, 222, pp.115019. 10.1016/j.carbpol.2019.115019 . hal-02290577

**HAL Id: hal-02290577**

**<https://hal.univ-lorraine.fr/hal-02290577>**

Submitted on 17 Sep 2019

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **High voltage electric discharges treatment for high molecular**  
2 **weight hemicelluloses extraction from spruce**

3 Morad Chadni<sup>1,2</sup>, Nabil Grimi<sup>1</sup>, Isabelle Ziegler<sup>3</sup>, Nicolas Brosse<sup>3</sup>, Olivier Bals<sup>1</sup>

4

5 <sup>1</sup>Sorbonne Universités, Université de Technologie de Compiègne. Laboratoire  
6 Transformations Intégrées de la Matière Renouvelable EA 4297 TIMR Centre de recherches  
7 Royallieu, CS 60319, 60203 Compiègne Cedex, France ;

8 <sup>2</sup>Sofren Groupe, 1 rue Royale 92210 Saint-Cloud, France

9 <sup>3</sup>Université de Lorraine, Faculté des Sciences et Techniques, Laboratoire d'Etude et de  
10 Recherche sur le Matériau Bois (LERMAB), Bld des Aiguillettes, F-54500, Vandoeuvre-les-  
11 Nancy;

12

13 **Corresponding author: Morad Chadni**

14 Sorbonne Université, Université de Technologie de Compiègne, Laboratoire Transformations  
15 Intégrées de la Matière Renouvelable (EA 4297 TIMR), Centre de recherches Royallieu, CS  
16 60 319, 60 203 Compiègne cedex, France

17 E-mail : morad.chadni@utc.fr

18

19

20

21

22

23

24

25

26 **Abbreviations**

27 **HVED** : High voltage electrical discharge

28 **Mw** Molecular weights (g/mol)

29 **GGM** Galactoglucomannans

30 **Mn** Number average molecular weights (g/mol)

31 **PI** Polydispersity index

32 **IR** Infrared

33 **UV** Ultraviolet

34 **AD** Acetylation degree

35 **AcGGM** Acetylated galactoglucomannan

36 **DM** Dry matter

37

38

39

40

41

42

43

44

45

46

## 47        **1. Introduction**

48        Polymeric hemicelluloses are polysaccharides with an important structural diversity and  
49        functionality. Although cellulose is the most widely used polysaccharide, the potential of application  
50        of polymeric hemicelluloses has been demonstrated by several authors (Bai, Hu, and Xu 2012;  
51        Lindqvist et al. 2013; Mikkonen and Tenkanen 2012; Alvira et al. 2010; Hartman, Albertsson, and  
52        Sjöberg 2006). The physicochemical properties of hemicelluloses have made them today an  
53        important untapped resource that can be used in several fields.

54        Spruce hemicelluloses are mainly composed of acetylated galactoglucomannan (AcGGM). AcGGM  
55        has a backbone of  $\beta$ -D-(1 $\rightarrow$ 4) -glucopyranosyl and  $\beta$ -D-(1 $\rightarrow$ 4)-mannopyranosyl units in a linear or  
56        slightly branched chain. Single  $\alpha$ -D-galactopyranose units are attached to the main chain by (1 $\rightarrow$ 6)-  
57        bonds. The mannopyranosyl units are acetylated at C-2 or C-3 with a degree of acetylation of about  
58        0.5 (Capek et al. 2000). An approximate ratio of galactose-to-glucose-to-mannose is around 0.5–  
59        1.1:1:3.5–4.5 for water-soluble AcGGM (Timell 1967). The average weight molar mass  $M_w$  of  
60        native AcGGM has been reported to be between 20 and 50 kDa and the degree of polymerization  
61        varies from 100 to 400 (Timell 1967; Willför et al. 2008).

62        GGMs have the potential to become high added-value products with various applications in food,  
63        health, papermaking, textile and cosmetic industries (Mikkonen et al. 2012, 2010). Different  
64        applications request GGMs in different forms (different molar masses). High molecular masses  
65        GGMs can be used as such or mixed with other polysaccharides to form a raw material for  
66        biodegradable films with promising oxygen barrier properties (Hartman et al. 2006).

67        The extraction of spruce hemicelluloses in general has been investigated for years. The common  
68        studies were focused on the extraction of monomeric form of wood polysaccharides for ethanol  
69        bioconversion. However, due to the discovery of the high potential of application of polymeric  
70        hemicelluloses, many authors have investigated the extraction of the polymeric form of  
71        hemicelluloses from wood. Extraction of softwood hemicelluloses has been studied by hydrothermal  
72        treatment at high temperatures ( $\geq 170$  °C) (Krogell et al. 2013; Song, Pranovich, and Holmbom  
73        2012), by chemical extraction (alkaline) (Capek et al. 2000; Curling, Fowler, and Hill 2007; Alvarez-  
74        Vasco and Zhang 2013), ionic liquids (Anugwom et al. 2012), by pressured hot water (Song et al.

75 2008; Gallina et al. 2017), and by microwave heat-fractionation ( $> 180\text{ }^{\circ}\text{C}$ ) (Gulbrandsen et al.  
76 2015; Palm and Zacchi 2003; Lundqvist et al. 2002).

77 The fractionation of wood at high temperatures leads to high solubilization of all macromolecules,  
78 including hemicelluloses. However, the extraction at higher temperatures led to a lower pH level,  
79 causing deacetylation and partly hydrolytic cleavage of GGMs, and consequently, decreasing the  
80 solubility, the yield and the molar mass of extracted GGMs.

81 Nowadays, electrical pretreatments that provoke electroporation and cell walls destabilization have  
82 also been used for biomolecules extraction (Grimi et al. 2009; Boussetta, 2010; Boussetta et al. 2009;  
83 Loginova et al. 2010). High voltage electric discharges (HVED) have shown very interesting results  
84 for the biomolecules extraction from biomass. HVED showed its effectiveness for polyphenols  
85 extraction from vine shoots (Rajha et al. 2014) and grape pomace (Boussetta et al. 2009; Boussetta et  
86 al. 2012) lignin from rapeseed straw (Brahim et al. 2016), flavonoids from peanut shells based (Yan  
87 et al. 2018), polyphenol and protein extraction from sesame cake (Sarkis et al. 2015), polyphenols  
88 and lignans from flaxseed cake (Boussetta et al. 2013) and sugars from orange peels (El Kantar et al.  
89 2018). Indeed, when applied in a liquid medium, HVED have physical features leading to the  
90 extraction of biomolecules from raw materials located inside the cell walls. The main effect of  
91 HVED is the fragmentation and destruction of raw materials. This destruction occurs through a  
92 certain number of physical phenomena, which will be discussed later. These physical phenomena are  
93 complex and the way they lead to extraction of raw material is insufficiently delighted.

94 There are numerous phenomena that could lead to the fragmentation of biomass during the  
95 application of HVED in liquid medium. Such as electrical breakdown which occurs when the  
96 streamer crosses the two electrodes, the breakdown induces the formation of a powerful shock wave  
97 (hundreds of MPa) in only a few micro- or nanoseconds. Shock waves are known to be responsible  
98 of mechanical cell membrane damages (Boussetta et al. 2009). Directly after shock waves, the  
99 apparition and the collapse of microcavitation bubbles create an intense and very localized shock  
100 waves that can lead to the fragmentation of raw material. The electric arc also emits high-intensity  
101 UV light and induces a large variety of chemical reactions like radical formation, radical molecule  
102 reaction, photochemical reaction, ozone molecules reaction and aqueous electron reaction etc. (Adda

103 et al. 2016). UV light in the range of 200–400 nm is mutagenic to cells (Boussetta and Vorobiev  
104 2014) and hydroxyl radicals lead to oxidative cell damage.

105 To our knowledge, the HVED have not yet been applied to the extraction of hemicelluloses from  
106 sawdust wood. The originality of this study is to suggest an original method to extract high  
107 molecular weight hemicelluloses and reducing the severity of conventional treatments by combining  
108 HVED with chemical and thermal treatment. The main objective of this work is to extract high  
109 molecular weight hemicelluloses at mild conditions (low temperature and suitable pH). Mild  
110 conditions were achieved by combining HVED with chemical extraction and autohydrolysis.

111

112

113

114

115

116

117

118

119

120

121

122

123

124

125

126

127

128

129

130

131           **2. Materials and methods**

132   The objective of this study is to evaluate the effect of HVED pretreatment on the extraction of high  
133   molecular weight hemicelluloses from spruce sawdust. For this purpose, numerous pretreatments at  
134   different impulsions (0-4 ms) were carried out in two different mediums (1M NaOH and water). The  
135   temperature generated during HVED pretreatment was between 40 and 50 °C.

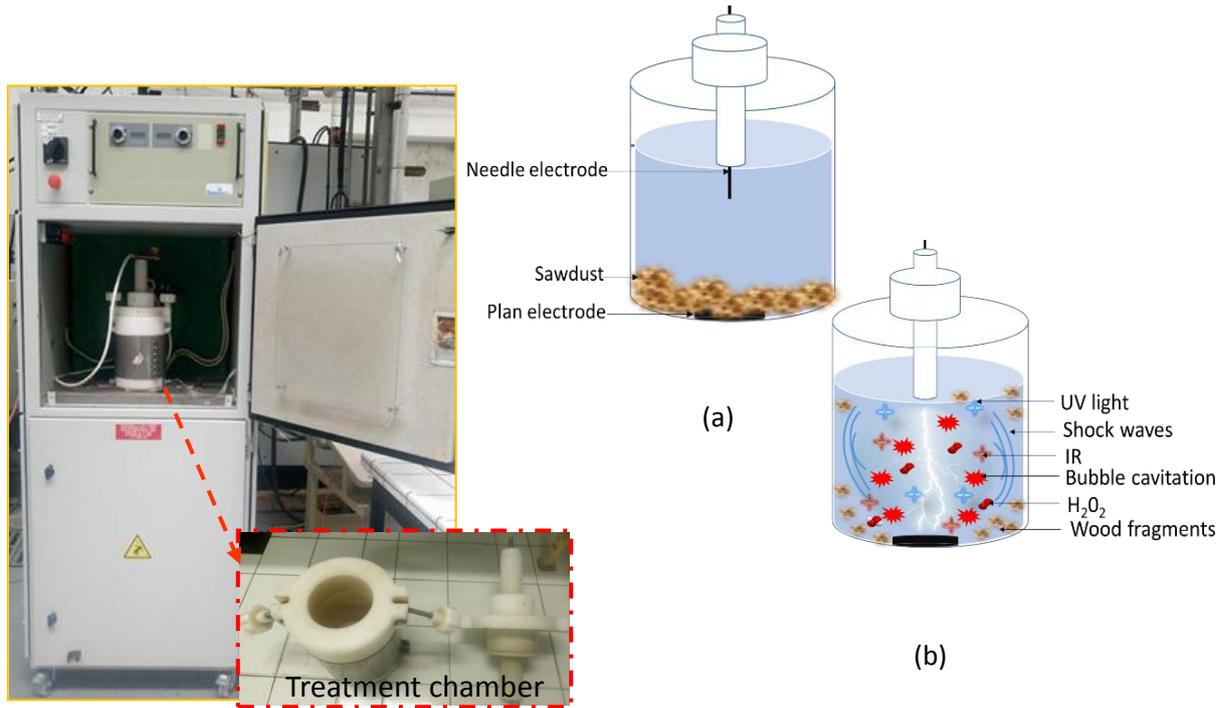
136           **2.1 Raw material**

137   Spruce sawdust with a particle size of (1-5 mm) was produced from a spruce tree from the Lorraine  
138   region in France and stored at room temperature in a sealed polyethylene bags in the dark until  
139   extraction. The average moisture content of the raw material was 10% w/w. The hemicellulose content  
140   of the sawdust (~30 % w/w dry biomass) was calculated by analyzing no-cellulosic monosaccharides  
141   of spruce wood according to NREL/TP-510-42618 method. Carbohydrate composition of sawdust  
142   includes 72 % glucan, 7,5 % xylan, and 16,3 % of mannan and other sugars.

143           **2.2 Extraction procedure**

144           **2.2.1 HVED pretreatment**

145   HVED pretreatment was carried out using a high voltage pulsed power 40 kV-10 kA generator (Basis,  
146   Saint Quentin, France), 1-liter cylindrical batch treatment chamber. The HVED treatment chamber  
147   was equipped with needle-plate geometry electrodes. The diameters of stainless-steel needle and the  
148   grounded disk electrodes were 10 and 35 mm, respectively. The distance between the electrodes was  
149   10 mm. The energy was stored in a set of low-inductance capacitors, which were charged by the high-  
150   voltage power supply. The electrical discharges were generated by electrical breakdown in the  
151   medium of extraction (liquid) at the peak pulse voltage (U) of 40 kV.



152

153 **Fig. 1.** HVED setup and treatment chamber: (a) before HVED pretreatment; (b) during HVED  
 154 pretreatment

155 In all experiments the liquid-to-solid ratio was fixed at 15 (w/w). The HVED generator (40 kV,  
 156 10 kA) provided pulses of an exponential form with a pulse repetition rate of 0.5 Hz. The effective  
 157 time ( $t_{HVED}$ ) of HVED treatment was calculated:

158 
$$t_{HVED} = n \cdot t_p \quad (1)$$

159 Where  $n$  ( $=1-400$ ) is the number of pulses and  $t_p$  ( $\approx 10 \pm 1 \mu s$ ) is the pulse duration.

160 The specific energy input  $W$  (kJ/kg) was obtained from Eq. (2):

161 
$$W = \frac{\sum_{n=1}^n W_{HVED}}{m} \quad (2)$$

162 Where

163  $W_{HVED}$  is the pulse energy (kJ/pulse) and  $m$  is sawdust mass (kg).  $W_{HVED}$  is determined from  
 164 Eq. (3).

165 
$$W_{HVED} = \int_0^n U I dt \quad (3)$$

166 Where U is the voltage (V) and I is the current strength (A)

### 167 **1.2.2 Diffusion**

168 The goal of the diffusion step is to facilitate the transfer of hemicelluloses chains from wood matrix to  
169 the extracellular medium. Temperature during HVED treatment was between 40 and 50 °C. In order to  
170 maintain mild conditions of extraction (low temperature, low decrease of pH), the diffusion was done  
171 under reflux at 50°C during 3 h with agitation.

### 172 **1.2.3 Separation of extracted hemicelluloses**

173 After each experiment, the extract was filtered on qualitative filter paper with retention particle size of  
174 5–13 µm (VWR International, Fontenay-sous-Bois, France). The volume and end-pH of the extract  
175 solutions were measured at room temperature shortly after the extraction. The liquid fraction from 1M  
176 NaOH impregnated sawdust was neutralized to a pH of 5.5-6 by a solution of 72% H<sub>2</sub>SO<sub>4</sub>. 10 mL  
177 were taken from the filtered extract for analyses. The extract solution was concentrated by vacuum  
178 evaporation using a water bath at 50 °C. To precipitate high-molecular weight hemicelluloses, four  
179 volumes of 95% ethanol were added to the liquid extracts. The liquid phase was separated from the  
180 precipitated hemicelluloses by settling at room temperature and decantation. Hemicelluloses were  
181 further separated from the remaining liquid by centrifugation at 4000 rpm for 10 min at room  
182 temperature. The precipitated hemicelluloses were re-dissolved in distilled water and dialyzed with a  
183 dialysis membrane with a cut-off of 3.4 kDa (Spectrum Laboratories, Inc., Rancho Dominguez, CA,  
184 USA). Finally, dialyzed hemicelluloses solutions were freeze-dried.

### 185 **1.3 Measurement of monosaccharides and uronic acids using HAPE-PAD**

186 The monosaccharide content of the soluble fraction was analyzed according to NREL/TP-510-42618  
187 method. The sugar concentrations were measured by HAPE-PAD (ICS-3000 Dionex, Dionex Corp.,  
188 Sunnyvale, USA) equipped with a Dionex CarboPac<sup>TM</sup> PA-20 (3 × 150 mm) analytical column.  
189 Filtered samples (20 µL) were eluted at 35 °C and at 0.4 mL/min with the following composition: pure  
190 water 99.2%/250 mM NaOH 0.8%: 0–20 min; pure water 75%/250 mM NaOH 20%/ NaOCOCH<sub>3</sub> (1  
191 M)- NaOH (20 mM) 5% 20–37 min; and pure water 40%/250 mM NaOH 20%/NaOCOCH<sub>3</sub> (1 M)-

192 NaOH (20 mM) 40% 37–41 min. Each elution was followed by a wash and subsequent equilibration  
193 step. External sugar and uronic acid standards were used for calibration (7 points per curve). The  
194 reagents and standards are provided by Sigma-Aldrich and Fisher Scientific.

#### 195 **1.4 Size-exclusion chromatography (SEC)**

196 A Shimadzu Prominence HPLC system is used to determine molecular weight distribution of  
197 hemicelluloses by gel filtration chromatography. The stationary phase is composed of two columns  
198 linked in series: Phenomenex Polysep GFC-P as a guard column (7.8 x 35 mm), Phenomenex Polysep  
199 GFC-P3000 and GFC-P2000 analytical columns (7.8 x 300 mm). Sodium nitrate (0.25 M) is used as  
200 an eluent with a 0.5 mL.min<sup>-1</sup> flow rate. Samples are filtered with a 0.45 µm membrane and 20 µL are  
201 injected. Samples are separated at 30 °C and detection is done with a Refractive Index  
202 Detector (Shimadzu RID-20A). Each analysis runs for 70 min. aqueous solutions (1 mg/mL) of  
203 dextran standards with molecular weights of 1000; 5000; 12000; 24000; 50000 and 80,000 Da were  
204 used to prepare the calibration curve.

#### 205 **1.5 FTIR**

206 FTIR analysis for extracted hemicelluloses were performed by a FTIR spectrophotometer (Thermo  
207 Scientific iD3ZnSe ATR—Nicolet iS 5 FTIR Spectrometer unit). Hemicelluloses powder was placed  
208 on a ZnSe crystal plate. Each IR spectrum was obtained over the wave number range of 400–  
209 4000 cm<sup>-1</sup> with eight scans at resolution of 4 cm<sup>-1</sup>. The spectra were analyzed using ThermoScientific  
210 OMNIC software.

211

#### 212 **1.6 2D <sup>13</sup>C–<sup>1</sup>H HSQC NMR spectroscopy**

213 <sup>2</sup>H NMR and <sup>1</sup>H–<sup>13</sup>C correlation 2D NMR (HSQC) spectra were recorded on a Bruker III 400  
214 MHz spectrometer equipped with a 5-mm BBFO probe. Experiment was conducted with 20 mg  
215 sample dissolved in 1 ml D<sub>2</sub>O. The number of collected complex points was 1024 for the <sup>1</sup>H-  
216 dimension with a relaxation of 1.5- and 256-time increments were recorded in <sup>13</sup>C-dimension.  
217 HSQC spectra were taken at room temperature.

218  
219  
220  
221  
222  
223  
224  
225  
226  
227  
228  
229  
230  
231  
232  
233  
234  
235  
236  
237  
238  
239  
240  
241  
242  
243

## **2.2 Thermal gravimetric analysis (TGA)**

The thermal behavior of extracted hemicelluloses was investigated by TGA (Thermal Gravimetric Analysis) (Setaram Setsys). The samples were dried before thermal analysis. The heating rate in the experiments was 10 C.min<sup>-1</sup> up to 600 C. A platinum cup was used in these tests. The weight of the sample was recorded during the temperature rise under azote flow (30 ml / min).

## **2.3 Scanning microscopy**

The surface morphology of sawdust fibers was studied by scanning electron microscopy (SEM). An environmental high-resolution electron scanning microscope QUANTA 250 FEG (FEI Company) was used in low-vacuum mode.

244

### 245 **3 Results and discussion**

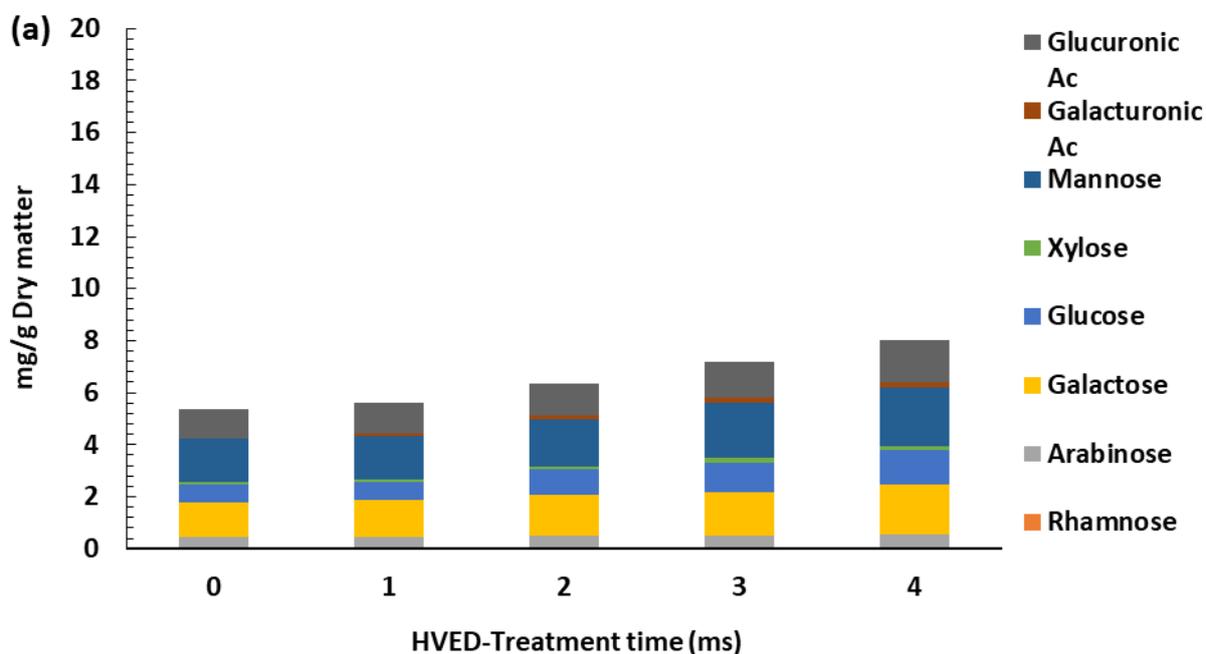
#### 246 **3.1 Yield of hemicellulose extraction**

247 **Fig. 2 (A and B)**, shows the yield of extraction of non-monomeric hemicellulose chains from spruce  
248 sawdust in the liquid fraction in distilled water and in basic media (1M NaOH) respectively. As  
249 expected higher hemicelluloses yields were obtained in basic conditions ( $\approx + 80 \%$ ). This observation  
250 is be explained by the fact that alkaline solutions increase the water absorption capacity, swell up  
251 cellulosic fibers and decrease their crystallinity, and thus increase the specific surface area by  
252 increasing the volume of the solid (Wen et al. 1988). HVED pretreatments increase significantly the  
253 yield of extraction in both media. In water, at 4 ms of HVED pretreatment (376 J/g DM), the yield of  
254 extraction was increased by 38 % compared to the extraction without HVED activation (0 ms) (5.8  
255 mg/g of DM for 0 ms pretreatment and 8 for 4 ms pretreatment). In basic medium, the advantage of  
256 HVED pretreatment step was also apparent. The yield was about 15.8 and 19.7 mg/g of dry wood for 0  
257 and 4 ms of pretreatment respectively.

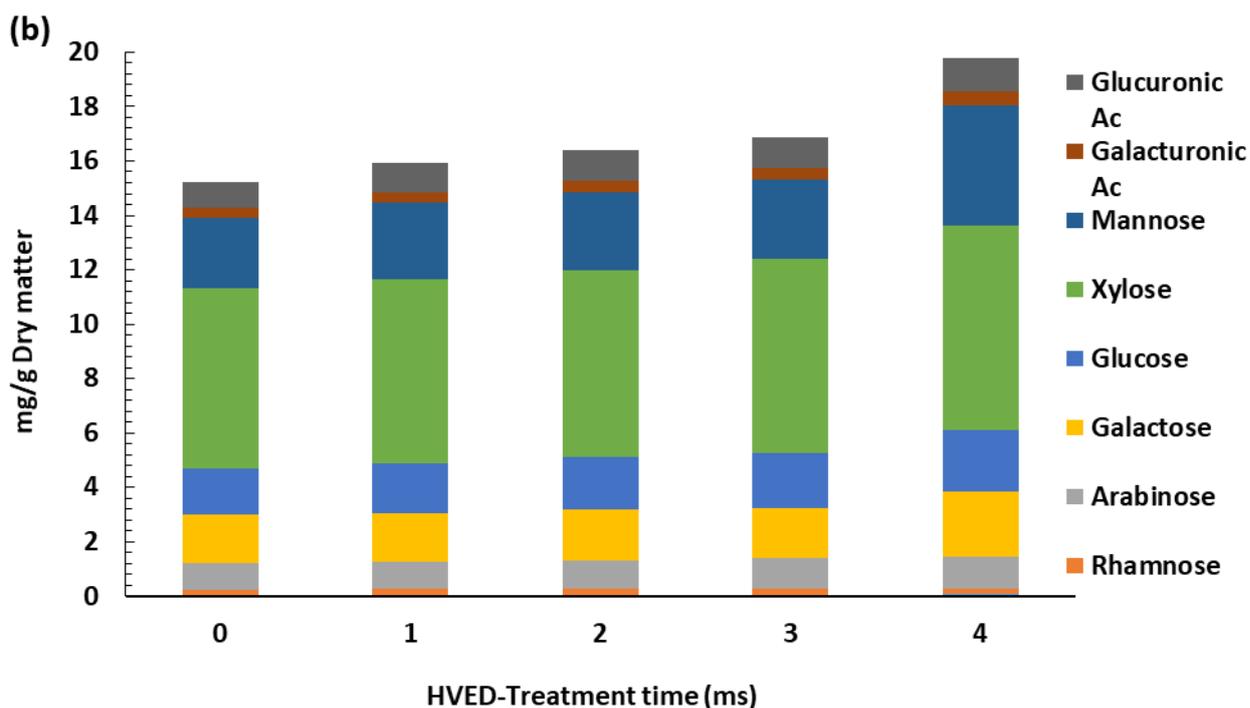
258 The application of HVED-treatment results in generation of shock waves. These shockwaves may play  
259 an important role in the raw material fragmentation, thus facilitating hemicelluloses extraction. The  
260 formation of the shock waves in liquid medium is often followed by the apparition of cavitation  
261 bubbles. The combined effect of shock waves and cavitation bubbles may improve the disruption and  
262 the destabilization of cell walls (Boussetta and Vorobiev 2014; Sarkis et al. 2015; Boussetta 2010).  
263 The pH of extraction is also an important parameter for the extraction of hemicelluloses chains. After  
264 extraction, the final pH of the suspension treated in water had dropped from 7 to 6.1 for untreated  
265 sawdust (0 ms) and from 7 to 5.2-5.3 for HVED pretreated sawdust. The drop of pH in the suspension  
266 treated in water is due to the release of carboxylic acids, mainly acetic acid from GGMs (Casebier,  
267 Hamilton, and Hergert 1969). Acetic acid released in the suspension treated in 1M NaOH solution are  
268 neutralized by hydroxide anions and pH remains stable at 13.5 for both untreated and HVED-treated  
269 samples.

270 **Fig.2 (A and B)** show also the composition of the extracted hemicelluloses in water and 1M NaOH  
 271 solution respectively. Polysaccharides extracted in water are composed of mannose (28%), glucose  
 272 (16%), galactose (23%) xylose (2.1 %), arabinose (6.5%), galacturonic acid (2.25 %) and glucuronic  
 273 acid (20%). The relative sugar composition of these polysaccharides is the same as for a typical GGM  
 274 (Timell 1967). Indeed, in water 64 % of extracted sugars are GGMs. In 1M NaOH, the sugar  
 275 composition of liquid extract is as follows: mannose (22%), glucose (11.3%), galactose (12%), xylose  
 276 (38%), arabinose (10%) galacturonic acid (2.25 %) and glucuronic acid (20%). The increase in  
 277 concentration of arabinose and xylose units at pH 13 suggests the release of arabino-(4-O-  
 278 methylglucurono)xylans (ARX). Indeed, in 1M NaOH solution, approximately 50 % of extracted  
 279 hemicelluloses are ARX and 35% of galactose-poor GGM. These results were in close agreement with  
 280 those found by Thornton et al. 1994 who have found that at pH values above 10, arabino-(4-O-  
 281 methylglucurono) xylans began to dissolve from the Norway spruce.

282



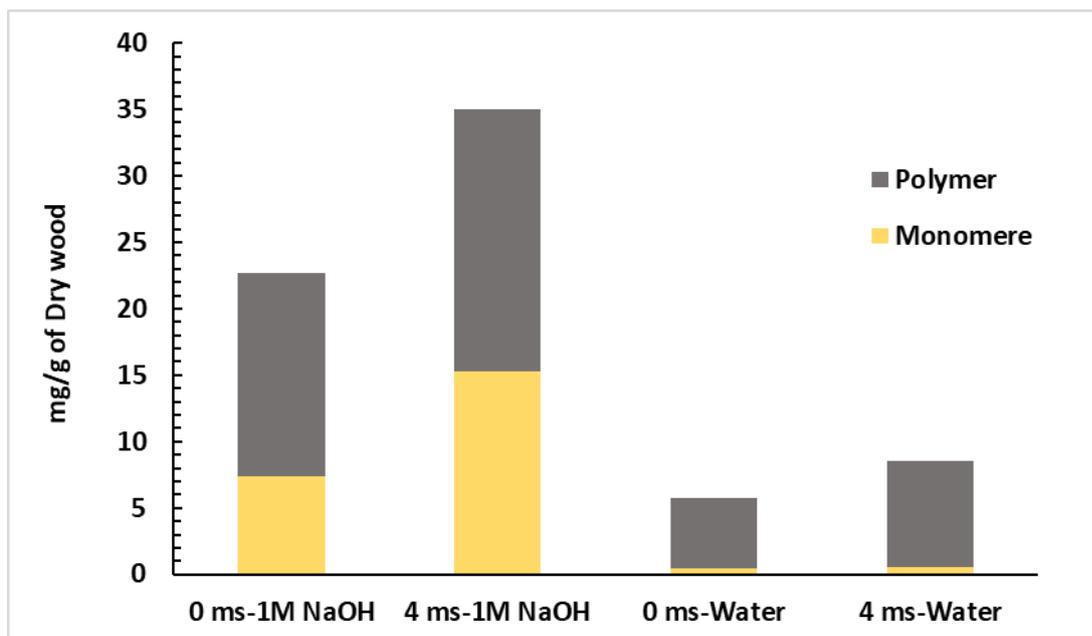
283



284 Fig. 2. Yield of extraction and sugar composition of extracted hemicelluloses versus HVED treatment  
 285 time in (a) water and (b) 1M NaOH  
 286

### 287 3.2 Extracted polymers

288 Fig.4 show the amount of polymers and monomers extracted in water and 1M NaOH solutions. About  
 289 95% of extracted hemicelluloses in water are oligomers and polymers. It can be observed that the  
 290 amount of monomers increased slightly when the HVED treatment duration increased. Extraction in  
 291 1M NaOH shows an increase of the amount of monomers compared to the extraction in water. Indeed,  
 292 about 70 % of the extracted hemicelluloses are polymers. The increase of the amount of monomers is  
 293 assumed to be due to the depolymerization of hemicelluloses chains under alkaline conditions,  
 294 especially by peeling reactions and saponification. The mild extraction conditions induced by HVED  
 295 (temperature around 50 °C) limited the depolymerization of hemicelluloses chains.

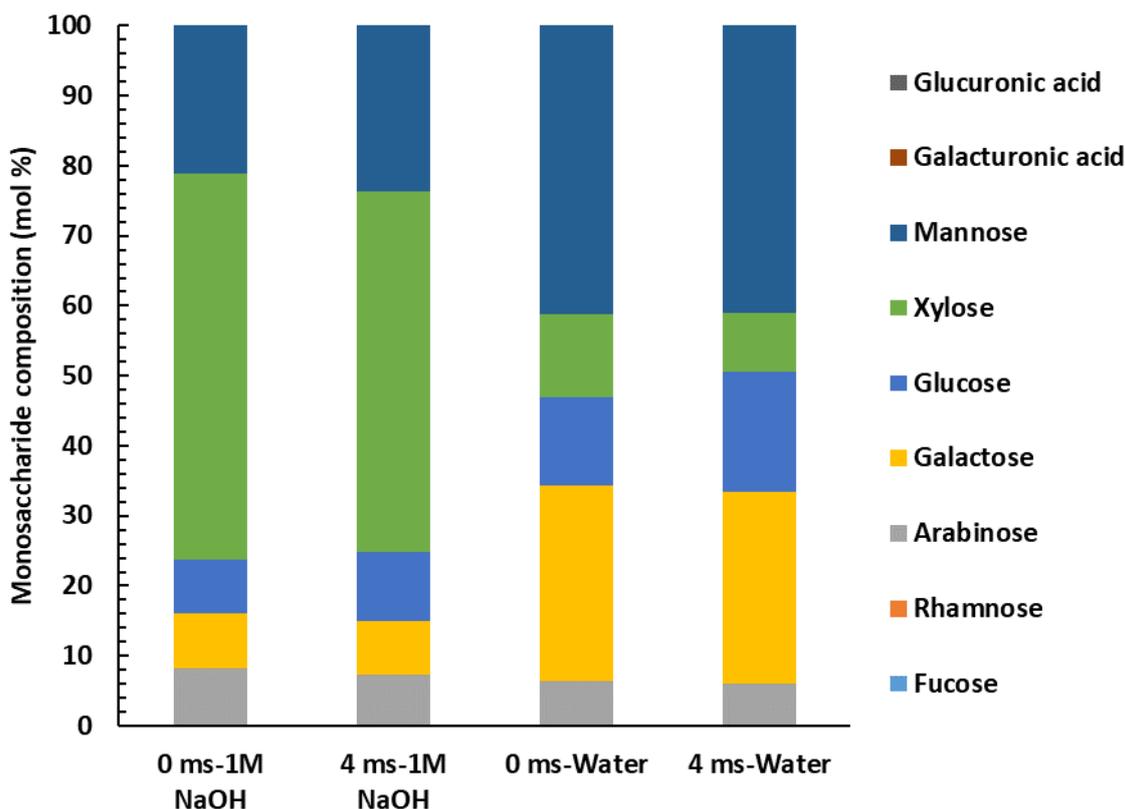


296

297 **Fig.3.** Total monomers and polymers extracted versus HVED-treatment time in (a) water and (b) 1M  
 298 NaOH solutions

299 **3.3 Characterization of the recovered hemicelluloses**

300 **Fig. 4** shows the sugar composition of hemicellulose powder obtained after precipitation of  
 301 hemicellulose chains in water and 1M NaOH solution. As expected from the analyze of the liquid  
 302 fraction, hemicelluloses extracted in 1M NaOH are rich with xylose ( $\approx 60\%$ ), mannose ( $\approx 20\%$ ) and  
 303 arabinose ( $\approx 8\%$ ). The fraction extracted in water is reach in mannose, galactose and glucose (40 %, 27 % and 13 % respectively). The relative sugar composition of these polysaccharides is the same as  
 304 for a spruce galactoglucomannans (Timell 1967). On the other hand, hemicelluloses extracted in 1M  
 305 NaOH solution seem to be mainly composed of arabinoxylans. From these results, it is remarkable to  
 306 note that the medium of extraction (pH) strongly affects the selectivity of the extraction (solubilization  
 307 of hemicelluloses).  
 308



309

310 **Fig. 4.** Sugar composition of hemicellulose chains recovered in water and 1 M NaOH.

311 The average molecular weight (Mw) and molecular weight distribution of extracted hemicelluloses  
 312 were determined by gel permeation chromatography (Table 1). With increasing the time of  
 313 pretreatment by HVED, the average molecular weight of high molecular weight hemicelluloses  
 314 increased. Pretreatment for 4 ms by HVED gave the highest Mw (55 kDa and 47 kDa in 1M NaOH  
 315 solution and water respectively). As expected, the Mw of the extracted carbohydrates clearly increased  
 316 with 1M NaOH solution compared to extraction in water.

317 The polydispersity index (PI) of hemicelluloses is also given in table 1. As shown in table 1, all  
 318 hemicelluloses fractions show a moderate low polydispersity index (1.5-2.8), suggesting a chemical  
 319 and structural homogeneity.

320

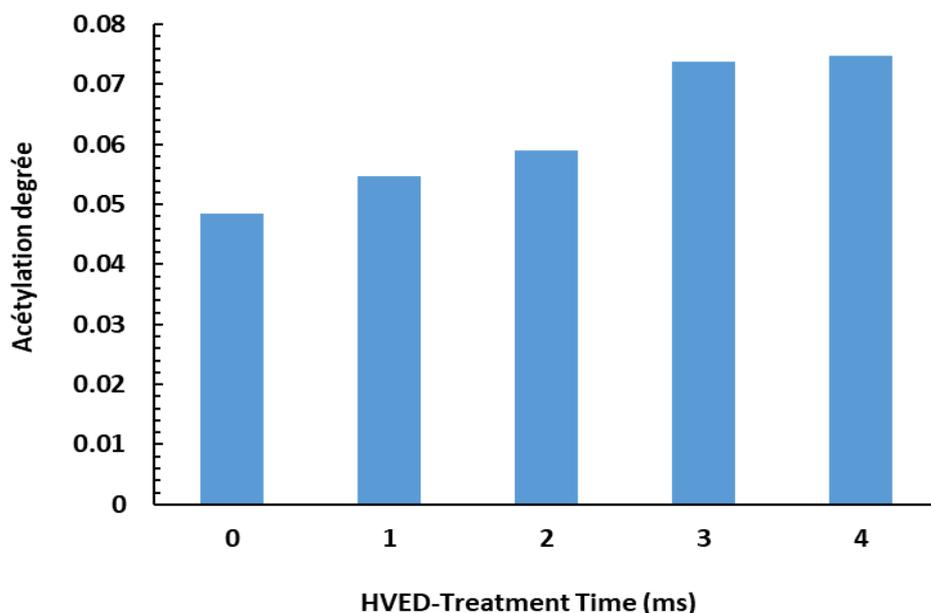
321

322 **Table.1** molecular weight distribution of recovered hemicelluloses

Time of HVED pretreatment	1M NaOH			Water		
	Mn (kDa)	Mw (kDa)	PI	Mn (kDa)	Mw (kDa)	PI
0 ms	28219	44775	1.58	16164	32959	2.03
1 ms	29289	50808	1.73	20700	36034	1.74
2 ms	27343	51808	1.89	19096	36950	1.93
3 ms	18411	51852	2.81	21041	42807	2.03
4 ms	21429	55464	2.58	24788	47662	1.92

323

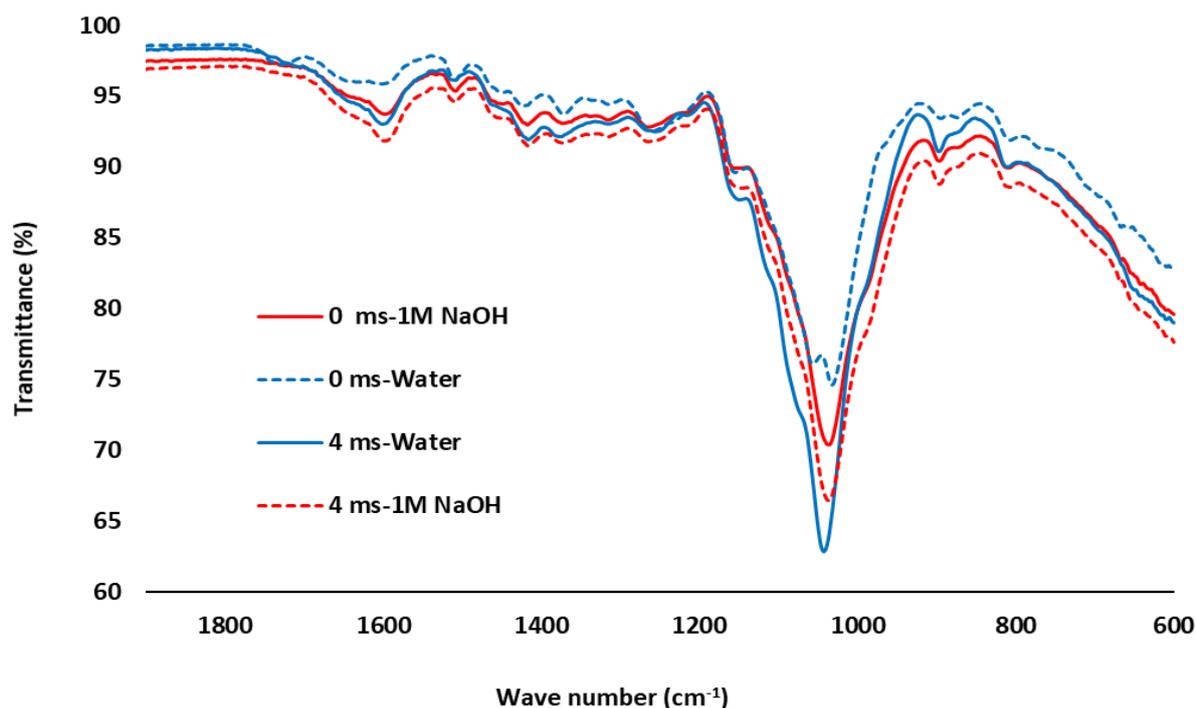
324 Deacetylation of hemicelluloses occurs principally during hot-water extraction (Song, Pranovich, and  
325 Holmbom 2011) and by saponification during the extraction in basic solutions. At higher pH levels  
326 Song et al., 2011 have found that deacetylation was very fast, thus decreasing the extraction of GGM.  
327 The degree of acetylation is calculated as the molar Ac/Man ratio. As shown in **Fig. 6**, all extracted  
328 hemicelluloses in water exhibited an acetylation degree (AD) in the range of 0.05-0.08, lower  
329 compared to that of native GGM in spruce wood (0.45 -0.50) (Timell 1967). It can be observed from  
330 Fig x that the AD increased with the increase of HVED pretreatment time. This observation can be  
331 rationalized by a fragmentation and destruction of sawdust fibers induced by HVED pretreatment  
332 promoting the extraction of acetyl-GGM. As expected, the AD is very low in hemicelluloses extracted  
333 in 1M NaOH due to deacetylation by saponification reactions.



334

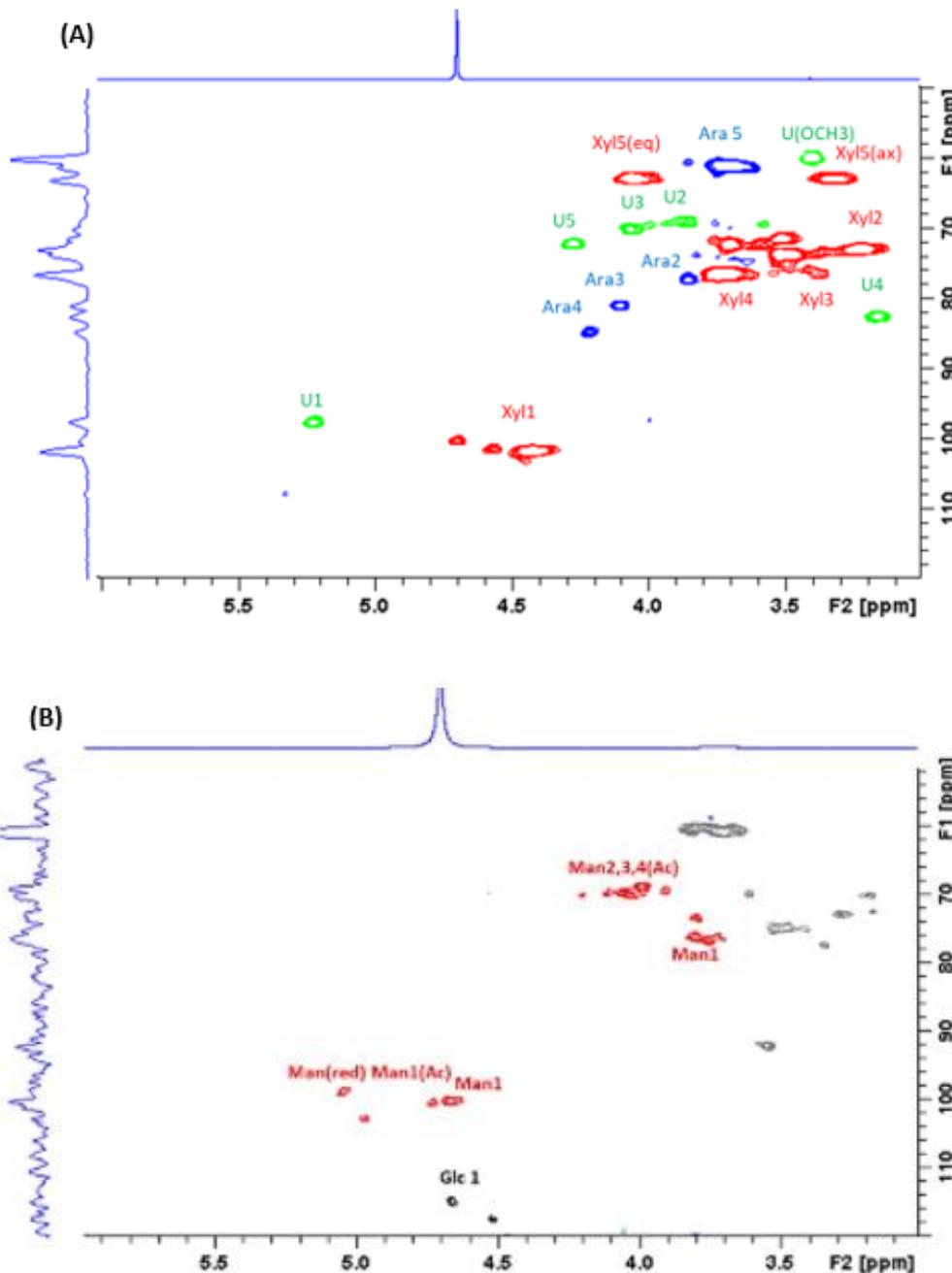
335 **Fig. 5.** Acetylation degree of extracted hemicelluloses in water after HVED pretreatment

336 The FTIR spectra of the extracted hemicelluloses shown in the **Fig. 6.** are in accordance to previous  
 337 papers (Peng et al. 2009). The absorption at  $1025\text{ cm}^{-1}$ , which is assigned to the C-O and C C  
 338 stretching vibration and the glycosidic linkage (C-O-C) contributions in hemicelluloses (Peng et al.  
 339 2009). The weak absorbances at  $1735$  and  $1247\text{ cm}^{-1}$  are due to some acetyl groups attached to the  
 340 hemicelluloses, particularly for 0 ms-Water and 4 ms-Water. These pics are absent in the fractions  
 341 obtained in 1M NaOH solution (0 ms-1M NaOH and 4 ms-1M NaOH) confirming the deacetylation of  
 342 hemicelluloses in basic media previously observed. Furthermore, the presense of the signal at  $1506$   
 343  $\text{cm}^{-1}$  in hemicellulosic fractions obtained in 1M NaOH is originated from the phenolic ring absorbance  
 344 of lignin.



345  
346 **Fig. 6.** FTIR spectra of extracted hemicelluloses in different conditions

347 <sup>1</sup>H-<sup>13</sup>C HSQC spectra clearly shows the typical signal pattern expected for the hemicellulosic moiety.  
 348 The analysis confirms the extraction of arabinoxylans and acetyl-galactoglucomannans (**Figure 7 (A)**  
 349 **and (B)** respectively). **In figure 7, (A)** The xylopyranosyl units of the hemicellulosic extract  
 350 preparation (H3) produce signals at  $\delta C/\delta H$  102.2/4.33 (C1H), 63.6/3.94 and 3.23 (C5H2), 75.9/3.57  
 351 (C4H), 74.4/3.31(C3H) and 73.1/3.10 (C2H) in the HSQC NMR spectrum, in which the two chemical  
 352 shifts of 4 and 3.3 ppm are assigned to the equatorial and axial protons linked at C5, respectively.  
 353 Xylan and arabinan signals, reveals that arabinoxylan was the major hemicellulose extracted in 1M  
 354 NaOH solution. **In figure 7 (B)**, H1/C1 cross peaks assigned to galactose, glucose and mannose units  
 355 are detected at 4.96/98.9 ppm, 4.58/104.4 ppm and 4.68/100.2 ppm respectively. Signals assigned to  
 356 2-O-acetylated- $\beta$ - Manp units are clearly observed at 4.87/99.0 ppm (H1/C1), 5.44/71.6 ppm (H2/C2),  
 357 and the presence of 3-O-acetylated- $\beta$ - Manp units is confirmed by correlation at 5.03/73.2 ppm  
 358 (H3/C3), 4.75/99.9 ppm (H1/C1). These results confirm the previous conclusions drawn from the  
 359 sugar composition of the recovered hemicelluloses powder and the liquid fraction (**Fig 2 and 4**  
 360 **respectively**) about the high selectivity of extraction of hemicelluloses as a function of the pH of  
 361 extraction.



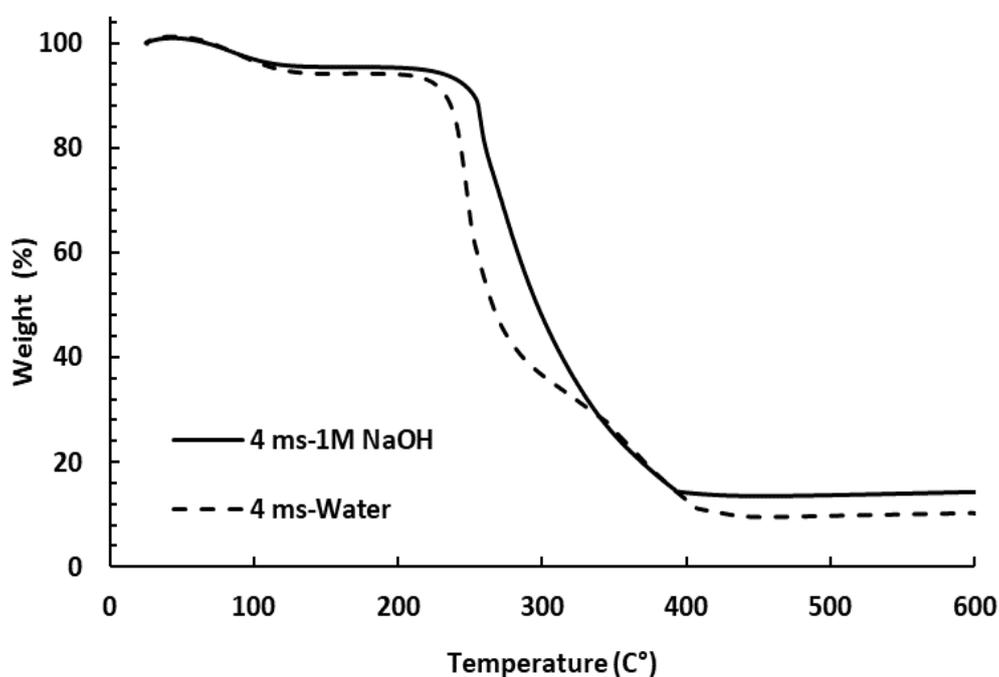
362

363

364 **Fig. 7.** 2D HSQC NMR spectral analysis of extracted hemicelluloses in 1M NaOH (A) and Water (B)

365 TGA curves of hemicellulose fractions 4 ms-Water and 4 ms -1M NaOH are shown in Figure 8. As  
 366 can be seen, the thermal decomposition of hemicelluloses can be divided into four phases. At the first  
 367 step, the weight loss observed below 100 °C was attributed to moisture. At the second phase, 4 ms-  
 368 Water began to decompose, and the maximum rate of weight loss was observed from 217 to 330 °C.  
 369 Compared to 4 ms -1M NaOH, the weight loss mainly occurred between 250 and 335 °C. It was  
 370 concluded that 4 ms-1M NaOH was thermally more stable at this phase. This higher thermal stability

371 can be attributed to their higher molecular weights (see table 1). Outstandingly, this thermal  
372 decomposition process can be attributed to the cracking and abscission of C-C and C-O bonds  
373 connected with the main branch of hemicelluloses, and the decarboxylation and decarbonylation may  
374 also occur (Sun et al. 2013). The third phase involved a gradual loss in weight at temperature above  
375 350 °C, which may be attributed to decomposition of cellulose and continued up to 400 °C. Since the  
376 thermal and chemical condition during extraction of hemicelluloses are so mild??? (low temperature,  
377 low decrease of pH). The presence of a very small quantities of cellulose in the extracted  
378 hemicelluloses owing to the physical and electrical effect of HVED on the wood matrix. Which could  
379 be due to the propagation of high-energy shock waves which could provoke the cleavage of the  $\beta$ -  
380 glycosidic linkage. This observation is in accordance with Brahim et al. 2017, who found that HVED  
381 induced a fragmentation of cellulose macromolecules from rapeseed straw mostly when HVED is  
382 associated to the soda treatment. The fourth phase exhibited weight loss at a temperature above 400 °C  
383 resulted from the decomposition of lignin. It is observed that a higher lignin content was co-extracted  
384 in basic conditions (4 ms -1M NaOH).

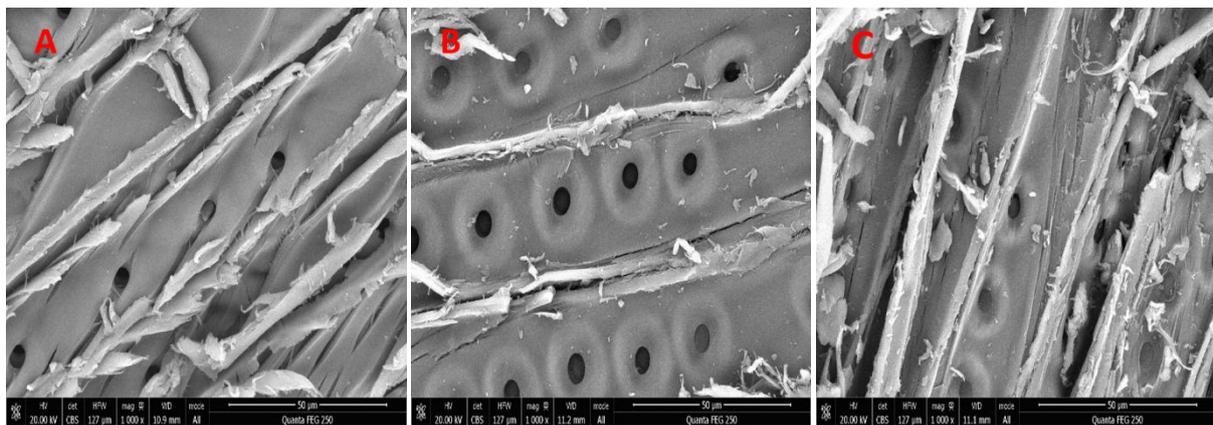


385  
386  
387

**Fig.8.** Thermograms of hemicellulosic fractions

388 **3.4 Scanning electron microscopy**

389 Fig. 9 shows the scanning electron micrographs of spruce sawdust pretreated by HVED which were  
390 associated to the highest extraction yields. Fig. 9 (A and C) shows that the spruce fibers became  
391 ragged and full of cracks due to the structural damage induced HVED. The surface images also  
392 revealed a notable effect of sodium hydroxide on sawdust surface (Fig. 9. (C)), mainly due to the  
393 removal of lignin and its precipitation. Therefore, the hemicellulose extraction increased with the  
394 increase of HVED pretreatment time due to a better breakdown of polysaccharides-lignin matrix and  
395 better accessibility to solvents. Similar observations have been reported in previous works by (Brahim  
396 et al. 2016) who have associated enhancement in material accessibility to higher porosity degree  
397 induced by to the propagation of shock waves and explosion of cavitation bubbles under HVED.



398  
399 **Fig.9.** Scanning electron microscopy images of sawdust submitted to HVED pretreatment: (A) in 1M  
400 NaOH solution, (C) in Water. And (b) untreated sawdust

401  
402  
403  
404  
405  
406

#### 407 4 Conclusion

408 The extraction of high molecular weight hemicelluloses polymers from spruce sawdust induced by  
409 HVED pretreatment was evaluated. The results have shown that HVED could be used as an alternative  
410 technology for the extraction of hemicelluloses polymers. HVED have electrical and mechanical  
411 effects on spruce sawdust caused by shock waves, which can disrupt the cellular walls, reduce the  
412 particle size of wood and increase the overall mass transfer of the intracellular content. The effect of  
413 HVED pretreatment could be enhanced by pH of extraction. The damage caused by HVED on spruce  
414 sawdust fibers increases the accessibility and the penetration of the water into the wood matrix  
415 promoting the hemicelluloses extraction at low temperature. These conditions prevent the decrease of  
416 pH which is responsible for depolymerization of hemicelluloses chains and the degradation of  
417 polysaccharides.

418

419

420

421

422

423

424

425

426

427

428

429

430 **Acknowledgment**

431 This work was financially supported by SOFREN Company and ANRT “Agence National de la  
432 Recherche et de la Technologie”. The authors appreciate the financial support from SOFREN and  
433 ANRT. LERMAB is supported by the French National Research Agency through the Laboratory of  
434 Excellence ARBRE (ANR-12- LABXARBRE-01).

435

436

437

438

439

440

441

442

443

444

445

446

447

448

449

450 **References:**

- 451 Adda, P., N. Boussetta, O. Lesaint, and E. Vorobiev. 2016. "High-Voltage Electrical Discharge  
452 Assisted Extraction of Phenolic Compounds from Grape Seeds." *Handbook of*  
453 *Electroporation*, 1–26.
- 454 Alvarez-Vasco, Carlos, and Xiao Zhang. 2013. "Alkaline Hydrogen Peroxide Pretreatment of  
455 Softwood: Hemicellulose Degradation Pathways." *Bioresource Technology* 150 (December):  
456 321–27. <https://doi.org/10.1016/j.biortech.2013.10.020>.
- 457 Alvira, P., E. Tomás-Pejó, M. Ballesteros, and M. J. Negro. 2010. "Pretreatment Technologies for an  
458 Efficient Bioethanol Production Process Based on Enzymatic Hydrolysis: A Review." *Bioresource Technology*, Special Issue on Lignocellulosic Bioethanol: Current Status and  
459 Perspectives, 101 (13): 4851–61. <https://doi.org/10.1016/j.biortech.2009.11.093>.
- 461 Anugwom, I., P. Mäki-Arvela, P. Virtanen, S. Willför, R. Sjöholm, and J. -P. Mikkola. 2012.  
462 "Selective Extraction of Hemicelluloses from Spruce Using Switchable Ionic Liquids." *Carbohydrate Polymers* 87 (3): 2005–11. <https://doi.org/10.1016/j.carbpol.2011.10.006>.
- 464 Bai, Likun, Hui ren Hu, and Jianfeng Xu. 2012. "Influences of Configuration and Molecular Weight of  
465 Hemicelluloses on Their Paper-Strengthening Effects." *Carbohydrate Polymers* 88 (4): 1258–  
466 63. <https://doi.org/10.1016/j.carbpol.2012.02.002>.
- 467 Boussetta, N., M. Turk, C. De Taeye, Y. Larondelle, J. L. Lanoisellé, and E. Vorobiev. 2013. "Effect  
468 of High Voltage Electrical Discharges, Heating and Ethanol Concentration on the Extraction  
469 of Total Polyphenols and Lignans from Flaxseed Cake." *Industrial Crops and Products* 49  
470 (August): 690–96. <https://doi.org/10.1016/j.indcrop.2013.06.004>.
- 471 Boussetta, N., E. Vorobiev, T. Reess, A. De Ferron, L. Pecastaing, R. Ruscassié, and J. -L. Lanoisellé.  
472 2012. "Scale-up of High Voltage Electrical Discharges for Polyphenols Extraction from Grape  
473 Pomace: Effect of the Dynamic Shock Waves." *Innovative Food Science & Emerging*  
474 *Technologies* 16 (October): 129–36. <https://doi.org/10.1016/j.ifset.2012.05.004>.
- 475 Boussetta, Nadia. 2010. *Intensification de l'extraction Des Polyphénols Par Électrotechnologies Pour*  
476 *La Valorisation Des Marcs de Champagne*. Compiègne.  
477 <http://www.theses.fr/2010COMP1899>.

478 Boussetta, Nadia, Jean-Louis Lanoisellé, Catherine Bedel-Cloutour, and Eugène Vorobiev. 2009.  
479 “Extraction of Soluble Matter from Grape Pomace by High Voltage Electrical Discharges for  
480 Polyphenol Recovery: Effect of Sulphur Dioxide and Thermal Treatments.” *Journal of Food*  
481 *Engineering* 95 (1): 192–98. <https://doi.org/10.1016/j.jfoodeng.2009.04.030>.

482 Boussetta, Nadia, and Eugène Vorobiev. 2014. “Extraction of Valuable Biocompounds Assisted by  
483 High Voltage Electrical Discharges: A Review.” *Comptes Rendus Chimie, Green extraction of*  
484 *natural products (GENP2013)*, 17 (3): 197–203. <https://doi.org/10.1016/j.crci.2013.11.011>.

485 Brahim, M., B. L. Checa Fernandez, O. Regnier, N. Boussetta, N. Grimi, C. Sarazin, E. Husson, E.  
486 Vorobiev, and N. Brosse. 2017. “Impact of Ultrasounds and High Voltage Electrical  
487 Discharges on Physico-Chemical Properties of Rapeseed Straw’s Lignin and Pulps.”  
488 *Bioresource Technology*, 1st International Conference on Bioresource Technology for  
489 *Bioenergy, Bioproducts & Environmental Sustainability (BIORESTEC)*, 237 (August): 11–  
490 19. <https://doi.org/10.1016/j.biortech.2017.04.003>.

491 Brahim, M., S. El Kantar, N. Boussetta, N. Grimi, N. Brosse, and E. Vorobiev. 2016. “Delignification  
492 of Rapeseed Straw Using Innovative Chemo-Physical Pretreatments.” *Biomass and Bioenergy*  
493 95 (December): 92–98. <https://doi.org/10.1016/j.biombioe.2016.09.019>.

494 Capek, P., M. Kubacková, J. Alföldi, L. Bilisics, D. Lisková, and D. Kákoniová. 2000.  
495 “Galactoglucomannan from the Secondary Cell Wall of *Picea Abies* L. Karst.” *Carbohydrate*  
496 *Research* 329 (3): 635–45.

497 Casebier, R. L., J. K. Hamilton, and H. L. Hergert. 1969. “Chemistry and Mechanism of Water  
498 Prehydrolysis on Southern Pine Wood.” *Tappi Tech Ass Pulp Pap Indus.*  
499 <http://agris.fao.org/agris-search/search.do?recordID=US201301228941>.

500 Curling, S. F., P. A. Fowler, and C. A. S. Hill. 2007. “Development of a Method for the Production of  
501 Hemicellulosic Gels from Sitka Spruce.” *Carbohydrate Polymers* 69 (4): 673–77.  
502 <https://doi.org/10.1016/j.carbpol.2007.02.003>.

503 El Kantar, Sally, Nadia Boussetta, Hiba N. Rajha, Richard G. Maroun, Nicolas Louka, and Eugène  
504 Vorobiev. 2018. “High Voltage Electrical Discharges Combined with Enzymatic Hydrolysis

505 for Extraction of Polyphenols and Fermentable Sugars from Orange Peels.” *Food Research*  
506 *International* 107 (May): 755–62. <https://doi.org/10.1016/j.foodres.2018.01.070>.

507 Gabriellii, I, P Gatenholm, W. G Glasser, R. K Jain, and L Kenne. 2000. “Separation, Characterization  
508 and Hydrogel-Formation of Hemicellulose from Aspen Wood.” *Carbohydrate Polymers* 43  
509 (4): 367–74. [https://doi.org/10.1016/S0144-8617\(00\)00181-8](https://doi.org/10.1016/S0144-8617(00)00181-8).

510 Gallina, Gianluca, Álvaro Cabeza, Henrik Grénman, Pierdomenico Biasi, Juan García-Serna, and  
511 Tapio Salmi. 2017. “Hemicellulose Extraction by Hot Pressurized Water Pretreatment at  
512 160oC for 10 Different Woods: Yield and Molecular Weight.” *The Journal of Supercritical*  
513 *Fluids*, October. <https://doi.org/10.1016/j.supflu.2017.10.001>.

514 Grimi, N., N. I. Lebovka, E. Vorobiev, and J. Vaxelaire. 2009. “Effect of a Pulsed Electric Field  
515 Treatment on Expression Behavior and Juice Quality of Chardonnay Grape.” *Food Biophysics*  
516 4 (3): 191–98.

517 Gulbrandsen, Torea A., Ingvild A. Johnsen, Mihaela Tanase Opedal, Kai Toven, K. Øyaas, Andrey  
518 Pranovich, Jyri-Pekka Mikkola, and Bard H. Hoff. 2015. “Extracting Hemicelluloses from  
519 Softwood and Bagasse as Oligosaccharides Using Pure Water and Microwave Heating.” *Cell.*  
520 *Chem. Tech* 49 (2): 117–126.

521 Hartman, Jonas, Ann-Christine Albertsson, Margaretha Söderqvist Lindblad, and John Sjöberg. 2006.  
522 “Oxygen Barrier Materials from Renewable Sources: Material Properties of Softwood  
523 Hemicellulose-Based Films.” *Journal of Applied Polymer Science* 100 (4): 2985–91.  
524 <https://doi.org/10.1002/app.22958>.

525 Hartman, Jonas, Ann-Christine Albertsson, and John Sjöberg. 2006. “Surface- and Bulk-Modified  
526 Galactoglucomannan Hemicellulose Films and Film Laminates for Versatile Oxygen  
527 Barriers.” *Biomacromolecules* 7 (6): 1983–89. <https://doi.org/10.1021/bm060129m>.

528 Krogell, Jens, Ekaterina Korotkova, Kari Eränen, Andrey Pranovich, Tapio Salmi, Dmitry Murzin,  
529 and Stefan Willför. 2013. “Intensification of Hemicellulose Hot-Water Extraction from Spruce  
530 Wood in a Batch Extractor – Effects of Wood Particle Size.” *Bioresource Technology* 143  
531 (Supplement C): 212–20. <https://doi.org/10.1016/j.biortech.2013.05.110>.

532 Lindqvist, Hanna, Jessica Holmback, Ari Rosling, Kristian Salminen, Bjarne Holmbom, Markku Auer,  
533 and Anna Sundberg. 2013. "Galactoglucomannan Derivatives and Their Application in  
534 Papermaking." *BioResources* 8 (1): 994–1010. <https://doi.org/10.15376/biores.8.1.994-1010>.

535 Loginova, K. V., M. V. Shynkaryk, N. I. Lebovka, and E. Vorobiev. 2010. "Acceleration of Soluble  
536 Matter Extraction from Chicory with Pulsed Electric Fields." *Journal of Food Engineering* 96  
537 (3): 374–79. <https://doi.org/10.1016/j.jfoodeng.2009.08.009>.

538 Lundqvist, Jon, Anita Teleman, Linda Junel, Guido Zacchi, Olof Dahlman, Folke Tjerneld, and Henrik  
539 Stålbrand. 2002. "Isolation and Characterization of Galactoglucomannan from Spruce (*Picea*  
540 *Abies*)." *Carbohydrate Polymers* 48 (1): 29–39. [https://doi.org/10.1016/S0144-  
541 8617\(01\)00210-7](https://doi.org/10.1016/S0144-8617(01)00210-7).

542 Mikkonen, Kirsi S., Heikkilä, Mari I, Willf, Stefan M. R, Maija Tenkanen, Kirsi S.  
543 Mikkonen, et al. 2012. "Films from Glyoxal-Crosslinked Spruce Galactoglucomannans  
544 Plasticized with Sorbitol, Films from Glyoxal-Crosslinked Spruce Galactoglucomannans  
545 Plasticized with Sorbitol." *International Journal of Polymer Science, International Journal of*  
546 *Polymer Science* 2012, 2012 (January): e482810. <https://doi.org/10.1155/2012/482810>,  
547 10.1155/2012/482810.

548 Mikkonen, Kirsi S., Mari I. Heikkilä, Harry Helén, Lea Hyvönen, and Maija Tenkanen. 2010. "Spruce  
549 Galactoglucomannan Films Show Promising Barrier Properties." *Carbohydrate Polymers* 79  
550 (4): 1107–12. <https://doi.org/10.1016/j.carbpol.2009.10.049>.

551 Mikkonen, Kirsi S., and Maija Tenkanen. 2012. "Sustainable Food-Packaging Materials Based on  
552 Future Biorefinery Products: Xylans and Mannans." *Trends in Food Science & Technology*,  
553 Special Section: Food Integrity and Traceability, 28 (2): 90–102.  
554 <https://doi.org/10.1016/j.tifs.2012.06.012>.

555 NREL/TP-510-42618, B. Hames, R. Ruiz-Peinado, C. Scarlata, W. J. Sluiter, D. Templaton, A. D.  
556 Crocker, and M. D. Sluiter. 2010. "Determination of Structural Carbohydrates and Lignin in  
557 Biomass, Technical Report NREL/TP-510-42618, National Renewable Energy Laboratory,  
558 Golden, CO," January. [https://www.scienceopen.com/document?vid=a3e04a5d-b049-4506-  
559 9739-b0c71a39b992](https://www.scienceopen.com/document?vid=a3e04a5d-b049-4506-9739-b0c71a39b992).

560 Palm, Magnus, and Guido Zacchi. 2003. "Extraction of Hemicellulosic Oligosaccharides from Spruce  
561 Using Microwave Oven or Steam Treatment." *Biomacromolecules* 4 (3): 617–23.  
562 <https://doi.org/10.1021/bm020112d>.

563 Peng, Feng, Jun-Li Ren, Feng Xu, Jing Bian, Pai Peng, and Run-Cang Sun. 2009. "Comparative Study  
564 of Hemicelluloses Obtained by Graded Ethanol Precipitation from Sugarcane Bagasse."  
565 *Journal of Agricultural and Food Chemistry* 57 (14): 6305–17.  
566 <https://doi.org/10.1021/jf900986b>.

567 Rajha, Hiba N., Nadia Boussetta, Nicolas Louka, Richard G. Maroun, and Eugene Vorobiev. 2014. "A  
568 Comparative Study of Physical Pretreatments for the Extraction of Polyphenols and Proteins  
569 from Vine Shoots." *Food Research International, Recovery and Utilization of Valuable  
570 Compounds from Food Processing By-products*, 65 (November): 462–68.  
571 <https://doi.org/10.1016/j.foodres.2014.04.024>.

572 Sarkis, Júlia Ribeiro, Nadia Boussetta, Christelle Blouet, Isabel Cristina Tessaro, Ligia Damasceno  
573 Ferreira Marczak, and Eugène Vorobiev. 2015. "Effect of Pulsed Electric Fields and High  
574 Voltage Electrical Discharges on Polyphenol and Protein Extraction from Sesame Cake."  
575 *Innovative Food Science & Emerging Technologies, APPLICATIONS OF PEF FOR FOOD  
576 PROCESSING*, 29 (May): 170–77. <https://doi.org/10.1016/j.ifset.2015.02.011>.

577 Song, Tao, Andrey Pranovich, and Bjarne Holmbom. 2011. "Effects of PH Control with Phthalate  
578 Buffers on Hot-Water Extraction of Hemicelluloses from Spruce Wood." *Bioresource  
579 Technology* 102 (22): 10518–23. <https://doi.org/10.1016/j.biortech.2011.08.093>.

580 ———. 2012. "HOT-WATER EXTRACTION OF GROUND SPRUCE WOOD OF DIFFERENT  
581 PARTICLE SIZE." *BioResources* 7 (3): 4214–25. [https://doi.org/10.15376/biores.7.3.4214-  
582 4225](https://doi.org/10.15376/biores.7.3.4214-4225).

583 Song, Tao, Andrey Pranovich, Ivan Sumerskiy, and Bjarne Holmbom. 2008. "Extraction of  
584 Galactoglucomannan from Spruce Wood with Pressurised Hot Water." *Holzforschung* 62 (6):  
585 659–666. <https://doi.org/10.1515/HF.2008.131>.

586 Sun, Shao-Long, Jia-Long Wen, Ming-Guo Ma, and Run-Cang Sun. 2013. "Successive Alkali  
587 Extraction and Structural Characterization of Hemicelluloses from Sweet Sorghum Stem."  
588 Carbohydrate Polymers 92 (2): 2224–31. <https://doi.org/10.1016/j.carbpol.2012.11.098>.

589 Timell, T. E. 1967. "Recent Progress in the Chemistry of Wood Hemicelluloses." Wood Science and  
590 Technology 1 (1): 45–70. <https://doi.org/10.1007/BF00592255>.

591 Wen, L.f., K.c. Chang, G. Brown, and D.d. Gallaher. 1988. "Isolation and Characterization of  
592 Hemicellulose and Cellulose from Sugar Beet Pulp." Journal of Food Science 53 (3): 826–29.  
593 <https://doi.org/10.1111/j.1365-2621.1988.tb08963.x>.

594 Willför, Stefan, Kenneth Sundberg, Maija Tenkanen, and Bjarne Holmbom. 2008. "Spruce-Derived  
595 Mannans – A Potential Raw Material for Hydrocolloids and Novel Advanced Natural  
596 Materials." Carbohydrate Polymers 72 (2): 197–210.  
597 <https://doi.org/10.1016/j.carbpol.2007.08.006>.

598 Yan, Liang-Gong, Yong Deng, Ting Ju, Kejiang Wu, and Jun Xi. 2018. "Continuous High Voltage  
599 Electrical Discharge Extraction of Flavonoids from Peanut Shells Based on 'Annular Gap  
600 Type' Treatment Chamber." Food Chemistry 256 (August): 350–57.  
601 <https://doi.org/10.1016/j.foodchem.2018.02.129>.

602