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1                   **STEAM EXPLOSION PROCESS FOR THE SELECTIVE EXTRACTION OF**  
2                   **HEMICELLULOSES POLYMERS FROM SPRUCE SAWDUST**

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24 **Abbreviations**

25	AD	Acetylation degree
26	Ac-GGM	Acetyl-Galactoglucomannan
27	AGX	Arabinoglucoronoxylan
28	DW	Dry wood
29	kDa	kilo Dalton
30	FTIR	Fourier Transform InfraRed
31	GGM	Galactoglucomannans
32	HMF	Hydroxymethylfurfural
33	HPLC	High performance liquid chromatography
34	M	Mole
35	Mw	Molecular weight average
36	NREL/TP	National Renewable Energy Laboratory/ Technical Report
37	PD	Polymerization Degree
38	SD	Standard Deviation
39	SE	Steam Explosion
40	SF	Severity Factor
41	SPS	Sodium hydroxide Pre-soaked Sawdust,
42	WPS	Water Pre-soaked Sawdust
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## 46        **1. Introduction**

47        The current industrial utilizations of non-cellulosic and non-starch polysaccharides focus on high molar  
48        mass guar gum, arabic gum, carrageenan, and xanthan gum, the molecular mass being one of the key  
49        features governing their beneficial properties (Rana et al., 2011; Willför et al., 2008). In this context, the  
50        substitution of these polysaccharides by hemicelluloses extracted from wood could be of great interest  
51        because of their abundance and low cost. In recent years, the extraction of polymeric hemicelluloses  
52        with high molecular weight and minimal degradation of their native structure has gained growing  
53        attention and could stimulate the development of novel industrial applications such as hydrogels, food,  
54        health, papermaking, textile and cosmetic industries (Ebringerová et al. 1994; Mikkonen et al. 2012;  
55        Mikkonen and Tenkanen 2012) .

56        Spruce sawdust is a low cost industrial waste material available in large quantities (El-Saied et al., 2017;  
57        Kovacova et al., 2019). In native softwood, the dominant hemicelluloses are acetyl-galactoglucomannan  
58        (Ac-GGM) amounting to 14 – 20% of wood (Willför et al., 2008; Xu et al., 2010) and  
59        arabinoglucuronoxylans (AGX) for approximatively 10%. Ac-GGM has a backbone of  $\beta$ -D-(1 $\rightarrow$ 4) -  
60        glucopyranosyl and  $\beta$ -D-(1 $\rightarrow$ 4)-mannopyranosyl units in a linear or slightly branched chain. Single  $\alpha$ -  
61        D-galactopyranose units are attached to the main chain by (1 $\rightarrow$ 6)-bonds. The mannopyranosyl units are  
62        acetylated at C-2 or C-3 with a degree of acetylation of about 0.5 (Capek et al., 2000). An approximate  
63        ratio of galactose-to-glucose-to-mannose is around 0.5–1.1:1:3.5–4.5 for water-soluble Ac-GGM  
64        (Timell, 1967). The average weight molar mass  $M_w$  of native Ac-GGM has been reported to be between  
65        20 and 50 kDa and the degree of polymerization varies from 100 to 400 (Timell, 1967; Willför et al.,  
66        2008).

67        The potential availability of softwood hemicelluloses is huge. These polysaccharides can be recovered  
68        from thermomechanical pulping (Persson and Jönsson, 2010) or with methods such as hydrolysis,  
69        alkaline extraction, microwaves treatments. (Alvarez-Vasco and Zhang, 2013; Gulbrandsen et al., 2015;  
70        Palm and Zacchi, 2003). However, the extraction of acetylated and high molecular weight  
71        hemicelluloses from the wood tissue with a minimum of degradation and/or depolymerization in an  
72        economically and environmentally feasible way is still challenging. The extraction methods described in

73 the literature generally rely on recovering partially-degraded and generally de-acetylated GGM in  
74 mixture with other hemicelluloses (e.g. xylans) without any selectivity and with a low molecular weight.  
75 However, the presence of acetyl groups in mannose units of high molar mass GGM is described to be an  
76 important structural feature for specific applications, the degree of acetylation being for critical the  
77 solubility in water (Timell, 1967).

78 The quality and composition of extracted hemicelluloses is also important for further applications. The  
79 sugar composition is extremely dependent on wood species and on the extraction conditions. Mannans-  
80 rich hemicelluloses are mainly extracted from softwood (Deloule et al., 2017; Song et al., 2013) while  
81 xylan-rich hemicelluloses are usually extracted from hardwood (Tunc, 2008) or agricultural residues.  
82 However, a few publications reported that the sugar composition of hemicellulose fraction could be  
83 adjusted by the solvent of extraction. Adjusting the pH at values above 8 of suspensions of Norway  
84 spruce thermomechanical pulp (TMP), dissolved deacetylated GGM and at pH values above 10 AGX  
85 began to dissolve from the TMP (Thornton et al., 1994). However, studies dealing with the effect of pH  
86 of impregnation and/or extraction on the composition of extracted hemicelluloses from wood are scarce  
87 (Perez et al., 2011).

88 Steam explosion (SE) is one of the most common pretreatments used in the fractionation of  
89 lignocellulosic biomass (Alvira et al., 2010). This hydrothermal pretreatment involves two main steps:

90 (1) A steam cracking which consists in causing the steam to penetrate under high pressure within the  
91 structure of the wood. Thus, the vapor condenses and the material becomes moistened. The condensed  
92 water coupled with high temperature induce the hydrolysis of the acetyl and methylglucuronic groups  
93 contained in xylans and galactomannan fractions. The acetic and uronic acid released decrease the pH of  
94 the medium and catalyze the depolymerization of hemicellulose fraction (Blecker et al., 2010). More  
95 drastic conditions (high temperature, long time of treatment), lead to the formation of monosaccharides  
96 and to the hydrolysis of a part of the cellulose.

97 (2) An explosive decompression which is caused by a sudden drop in pressure followed by a  
98 revaporization of a part of the condensation water present in the material provoke a mechanical break-  
99 up of the structure of the material (Blecker et al., 2010).

100 SE is traditionally used as pre-treatment for chemical and biochemical conversion of biomass such as  
101 pulping, fermentation and bio-refinery processes (Carvalho et al., 2008; Galbe and Zacchi, 2002).  
102 Several publications described the extraction of hemicelluloses in oligomeric and/or polymeric forms  
103 from agricultural residues (Krawczyk et al., 2008; Schultz et al., 1984; Sun et al., 2014) or wood (Palm  
104 and Zacchi, 2003; Simangunsong et al., 2018; Wojtasz-Mucha et al., 2017) in the liquid effluents of a  
105 steam explosion pretreatment. In fact, this process has been shown to be effective in opening up the  
106 lignocellulose structure, increasing the accessibility of polysaccharides to hydrolysis. Most of the  
107 published studies report a severe hydrolytic depolymerization of hemicelluloses during the steam  
108 process with a depolymerization of the polysaccharide chains into oligomers and monomers and their  
109 subsequent degradation (hydroxymethylfurfural and furfural) (Li et al., 2013; Wojtasz-Mucha et al.,  
110 2017). However, few papers described the recovery of high molecular mass hemicelluloses from  
111 agricultural residues or wood by a steam explosion pretreatment performed after a basic impregnation  
112 (Sabiha-Hanim et al., 2015; Sun et al., 2014; Xing et al., 2014). Taking into account the current  
113 industrial development of the steam explosion process for cellulosic bioethanol production, a full  
114 recovery of the feedstock through optimum utilization of all lignocellulosic components, including  
115 hemicellulosic components as marketable products is a major goal of optimizing a biomass-to-biofuel  
116 process. Therefore, there is a need to better understand the impact of SE conditions for the extraction of  
117 high molecular mass hemicellulose fractions.

118 The aim of this work is to study the effect of different parameters of extraction in the liquid stream of  
119 spruce hemicelluloses by steam explosion (severity factor and soda or water pre-impregnation) on the  
120 molar mass and chemical properties of extracted hemicelluloses from spruce sawdust. The impact of the  
121 experimental parameters of the steam explosion process on the hemicelluloses yields and composition,  
122 molecular mass and on the chemical selectivity has been examined.

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## 129 **2. Materiel and method**

### 130 **2.1 Raw material**

131 Spruce sawdust with a particle size of 1 to 5 mm was produced from spruce wood (*Picea abies*) from  
132 Lorraine forest in France and stored at room temperature in sealed polyethylene bags in the dark until  
133 extraction. The average moisture content of the raw material was 10.01 % w/w. The hemicellulose  
134 content of the sawdust (~30 % w/w dry biomass) was calculated by analyzing no-cellulosic  
135 monosaccharides of spruce wood according to NREL/TP-510-42618 method. Carbohydrate  
136 composition of sawdust includes 72 % glucan, 7.5 % xylan, and 16.3 % of mannan and other sugars.

### 137 **2.2 Soaking**

138 50 g of spruce sawdust was soaked with a solution of 1 M sodium hydroxide (ratio solid/liquid = 1:7)  
139 or water (ratio solid/liquid = 1:7). The sawdust was left for 15 hours before treatment at room  
140 temperature. The impregnated sawdust was treated directly without any filtration or pressing.

### 141 **2.3 Steam explosion**

142 Soaked sawdust was placed into a 2-liter reactor with heat jacket and automatic control for steam  
143 pressure and sampler retention time. The biomass was treated at different temperatures and retention  
144 times by injecting a saturated steam into a pressure resistance chamber. After a given reaction time,  
145 the steam saturated biomass is released into a big discharge tank “explosion chamber” as well the  
146 biomass.

147 The selected experimental conditions are based on literature data for hardwood (Schultz et al., 1984)  
148 and other lignocellulosic biomasses (Vivekanand et al., 2013; Zhuang and Vidal, 1997).

149 The treatment severity for each extraction is determined by a correlation between temperature process  
150 and retention time as described by the following equation (Chornet E and Overend R.P, 1988):

$$S = \text{Log}_{10} \left[ t \cdot \exp \left( \frac{T - 100}{14.75} \right) \right] \quad (\text{Eq. 1})$$

151 where: S = Severity Factor, T(t) = Process temperature (°C), t = Retention time (min), 14.75 =  
152 Activation energy value in the conditions where process kinetics are of first order and obey to  
153 Arrhenius law.

154           **2.4    Hemicellulose recovery**

155    After steam explosion, the condensed vapor and the biomass were recovered and filtered on qualitative  
156    filter paper with retention particle size of 5–13  $\mu\text{m}$  (VWR International, Fontenay-sous-Bois, France).  
157    The volume and end-pH of the extract solutions were measured at room temperature shortly after the  
158    extraction. The liquid fraction from 1 M NaOH impregnated sawdust was neutralized to a pH of 5.5-6  
159    by a solution of 72 %  $\text{H}_2\text{SO}_4$ . 10 mL were taken from the filtered extract for analyses. The extract  
160    solution was concentrated by vacuum evaporation using a water bath at 50  $^\circ\text{C}$ . To precipitate high-  
161    molecular weight hemicelluloses, four volumes of 95 % ethanol were added to the liquid extracts. The  
162    liquid phase was separated from the precipitated hemicelluloses by settling at room temperature and  
163    decantation. Hemicelluloses were further separated from the remaining liquid by centrifugation at  
164    4000 rpm for 10 min at room temperature. The precipitated hemicelluloses were re-dissolved in  
165    distilled water and dialyzed with a dialysis membrane with a cut-off of 3.4 kDa (Spectrum  
166    Laboratories, Inc., Rancho Dominguez, CA, USA) in order to remove salts and low molecular mass  
167    organic molecules. Finally, dialyzed hemicelluloses solutions were freeze-dried.

168           **2.5    Measurement of monosaccharides and uronic acids using HAPE-PAD**

169    The monosaccharide content of the soluble fraction was analyzed according to NREL/TP-510-42618  
170    procedure. The sugar concentrations were measured by HAPE-PAD (ICS-3000 Dionex, Dionex Corp.,  
171    Sunnyvale, USA) equipped with a Dionex CarboPac<sup>TM</sup> PA-20 (3  $\times$  150 mm) analytical column. Filtered  
172    samples (20  $\mu\text{L}$ ) were eluted at 35 $^\circ\text{C}$  and at 0.4 mL/min with the following composition: pure water  
173    99.2 %/250 mM NaOH 0.8%: 0–20 min; pure water 75 %/250 mM NaOH 20%/  $\text{NaOCOCH}_3$  (1 M)-  
174    NaOH (20 mM) 5% 20–37 min; and pure water 40%/250 mM NaOH 20%/ $\text{NaOCOCH}_3$  (1 M)- NaOH  
175    (20 mM) 40 % 37–41 min. Each elution was followed by a wash and subsequent equilibration step.  
176    External sugar and uronic acid standards were used for calibration (7 points per curve). The reagents  
177    and standards are provided by Sigma-Aldrich and Fisher Scientific.

178           **2.6    Degradation products**

179    Degradation products of hemicelluloses (furfural, HMF, formic acid, acetic acid and levulinic acid) are  
180    analyzed according to NREL/TP-510-42623 method. The products are measured with an Ultimate 3000



181 HPLC system. The components are separated in 45 min on an Agilent Hi-Plex H column at 55° C with  
182 an isocratic flow rate of 0.4 mL · min<sup>-1</sup> of sulfuric acid (5 · 10<sup>-3</sup> M). Acids were detected by UV at 210  
183 nm, while HMF and furfural are detected by UV at 280 nm. An external calibration is performed with  
184 six calibration points per standard. Reagents and standards are provided by Sigma-Aldrich.

## 185           **2.7     Acetyl content**

186 Acetyl content of extracted hemicelluloses was calculated by measuring the acetic acid concentration of  
187 hemicelluloses after a saponification reaction. Acid acetic was measured following the same procedure  
188 as degradation products with an UV detection at 210 nm instead of 280 nm.

## 189           **2.8     Size-exclusion chromatography**

190 A Shimadzu Prominence HPLC system is used to determine molecular weight distribution of  
191 hemicelluloses by gel filtration chromatography (GFC). The stationary phase is composed of two  
192 columns linked in series: Phenomenex Polysep GFC-P as a guard column (7.8 x 35 mm), Phenomenex  
193 Polysep GFC-P3000 and GFC-P2000 analytical columns (7.8 x 300 mm). Sodium nitrate (0.25 M) is  
194 used as an eluent with a 0.5 mL·min<sup>-1</sup> flow rate. Samples are filtered with a 0.45 µm membrane and  
195 20 µL are injected. Samples are separated at 30 °C and detection is done with a Refractive Index  
196 Detector (Shimadzu RID-20A). Each analysis runs for 70 min. aqueous solutions (1 mg/mL) of dextran  
197 standards with molecular weights of 1000; 5000; 12000; 24000; 50000 and 80,000 Da were used to  
198 prepare the calibration curve.

## 199           **2.9     Two-dimensional (2D) <sup>1</sup>H–<sup>13</sup>C heteronuclear single-quantum coherence (HSQC)** 200                           **analysis**

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

## 206 **2.10 Fourier Transform Infrared Spectroscopy**

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

## 212 **2.11 Statistical analysis**

213 All experiments were conducted at least in duplicates. The means and error bars were calculated and  
214 were represented in all figures.

## 215 **3. Results and discussions**

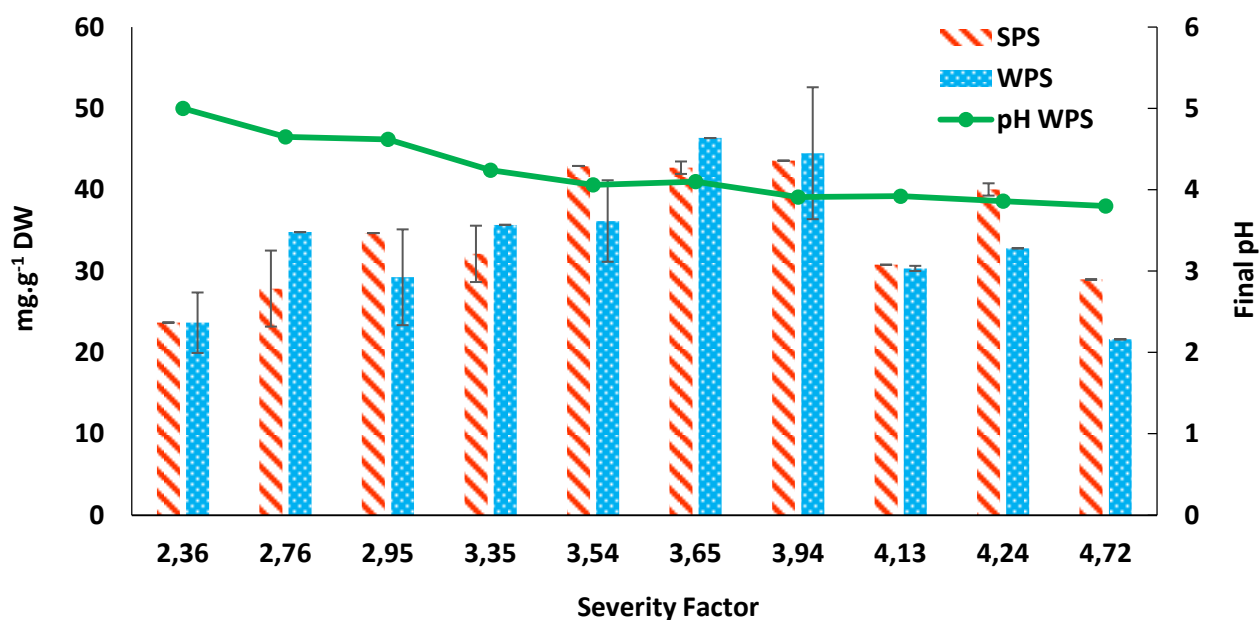
216 The hemicelluloses were recovered after SE of spruce sawdust from the liquid stream by precipitation in  
217 ethanol and dialysis. The influence the severity of treatment and pH of the pre-impregnation step (Water  
218 Pre-soaked Sawdust, WPS, Sodium hydroxide Pre-soaked Sawdust, SPS) on the extraction of high  
219 molar mass hemicelluloses was examined. A visual comparison of the sawdust samples after SE is  
220 given in supplementary data section (S2) as well as Scanning Electron Microscopy (SEM) of untreated  
221 sawdust, sawdust after steam explosion (S3).

### 222 **3.1 Yield of extracted hemicelluloses**

223 Fig. 1 gives the variations of the hemicelluloses yields (recovered after precipitation and dialysis)  
224 related to the duration of SE for the different temperatures. For WPS, during the cooking step of the SE  
225 process acetyl groups present in the raw material are cleaved to produce acetic acid. Fig.1 gives the pH  
226 profiles of the SE liquid streams. As a result, a decrease of the pH is observed with the increase of the  
227 severity of the SE. For SPS, the pH of the liquid after SE remind unchanged at 13 due to the  
228 neutralization of acetic acids and hydronium ions by  $\text{OH}^-$  ions. Hemicellulosic fractions isolated from  
229 the WPS were obtained in high yields compared to those obtained from SPS. In fact, the maximum yield  
230 achieved was  $57.78 \text{ mg} \cdot \text{g}^{-1} \text{ DW}$  at severity factor (SF) of 3.65 for WPS and  $43.52 \text{ mg} \cdot$   
231  $\text{g}^{-1} \text{ DW}$  at SF of 3.94 for SPS. For both presoaking conditions (SPS and WPS, Fig.1), the extraction

232 yields increased with the increase of SF until a SF of 3.94 which is in line with yields obtained after  
 233 treatment of wood chips of Norway spruce (17, 19 and 43 mg. g<sup>-1</sup> at SF of 2.7, 3 and 3.4 respectively)  
 234 (Wojtasz-Mucha et al., 2017). A further increase of the SF of treatment led to a strong decrease in the  
 235 hemicellulose's recovery.

236 This decrease in yield can be explained by degradation reactions and depolymerization of  
 237 hemicelluloses chains (alkali-catalyzed for SPS or acid catalyzed for WPS) under harsher conditions. In  
 238 fact, the increase of the SF led to the formation of furans and carboxylic acids in WPS and SPS media  
 239 respectively. ~~The high concentrations of these degradation products were obtained at SF of 4.13 (Figs.~~  
 240 ~~3A and 3B).~~ Furthermore, the amount of monomers (not precipitated) extracted after SE treatments of  
 241 WPS increased with increasing SF and reached a maximum at values of SF = 4.72 with a yield of 5.3  
 242 mg. g<sup>-1</sup> DW (See Table S1 in supplementary data section). The increase of the amount of monomers at  
 243 higher SF values could be due to the depolymerization of hemicelluloses chains under high  
 244 temperatures and low pH. The amount of monomers after treatment of SPS was very low (less than 0.02  
 245 mg. g<sup>-1</sup> DW).



246

247 Fig. 1. Yield of extraction of hemicelluloses versus severity factor

248 **3.2 Molecular weight**

249 The average molecular masses of the extracted hemicelluloses were assessed by water-based sized  
 250 exclusion chromatography. Table 1 shows the molecular weight as a function of SE parameters for WPS  
 251 and SPS fractions. As expected, the data showed that the molecular weight of the SPS hemicelluloses  
 252 fractions are significantly higher than the WPS hemicelluloses. It has been described that high pH and  
 253 low temperature are known to reduce the kinetic of degradation of polysaccharides (Rissanen et al.,  
 254 2014). On the other hand, low pH, high temperature and long treatment duration increase the rate of  
 255 acid-catalyzed hydrolysis of glycosidic linkages in hemicellulose chains. As a result, the decrease of pH  
 256 previously mentioned for WPS for the highest severities (Fig. 1) justifies the lower molar mass  
 257 obtained. Hemicelluloses extracted from SPS had relatively higher molecular weight than those  
 258 extracted from WPS.

259 Whatever the presoaking conditions, a significant molecular weight decrease was observed as the  
 260 severity of the treatment increase's owing to hydrolysis and depolymerization during the cooking step  
 261 of the process. The higher molecular weight hemicelluloses have been recovered SF of 2.36 and 2.76 for  
 262 WPS (>25 kDa) and at SF of 2.76, 2.95 and 3.35 for SPS (> 68 kDa). Previous works dealing with the  
 263 extraction of neutral polysaccharides from Norway spruce reported higher GGM molecular mass (20-  
 264 60 kDa) (Pranovich, 1995; Sjöström, 1990). The present results show that during the SE process  
 265 performed after a water presoaking, a depolymerization of GGM occurs to some extent.

266 Table 1: Molecular weight ( $M_w$ ) and Polydispersity index (PI) of extracted hemicelluloses versus  
 267 severity factor

Severity factor	WPS		SPS	
	$M_w$	PI	$M_w$	PI
2.36	27.68	2.39	67.6	1.42
2.76	25.12	2.38	69.9	1.29
2.95	21.34	2.55	69.4	1.33
3.35	14.14	2.66	69.4	1.39
3.54	16.47	2.52	67.8	2.04
3.65	14.05	2.50	64.7	2.10
3.94	14.31	2.48	63.5	1.67
4.13	9.98	2.54	35.2	1.30

4.24	13.46	2.24	59.5	1.31
4.72	12.18	2.34	40.2	1.33

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### 272 3.3 Sugar composition of hemicelluloses

273 The composition of the hemicellulosic fractions isolated in terms of monomeric sugars has been  
 274 established, after hydrolysis, by ionic chromatography. The average compositions corresponding to the  
 275 12 extraction conditions from WPS and SPS are given in Table 2. All the data are given in the SM  
 276 section. Whatever the experimental parameters, the data strongly suggest that after a neutral (WPS) and  
 277 a basic (SPS) presoaking, the hemicellulosic fractions are mainly composed of galactoglucomannans  
 278 and arabinoxylans respectively. In fact for WPS, the mannose is the dominant sugar (52–64%), followed  
 279 by glucose (3–16%), galactose (10–13%), the other sugars like xylose (6-13%), glucuronic (0.17%) and  
 280 galacturonic (1.06 %) acids being observed as minor constituents. As a result, we can assume that the  
 281 mannose, glucose and almost all the galactose can be assigned to GGM. On the other hand,  
 282 hemicelluloses extracted from a basic impregnation (SPS) appear to be mainly composed of  
 283 arabinoxylans (AX), xylose and arabinose are the most abundant (68-81 % and 7-13% respectively), and  
 284 mannose is present at low level (0.2-4%). From these results, it is remarkable to note that the pH of the  
 285 pre-soaking medium before SE strongly influences the selectivity of the extraction. In basic medium  
 286 (SPS), ~90% of extracted hemicellulose are arabinoxylans ( $\beta$ -D-Xylp 10;  $\alpha$ -L-Araf 1,5) and ~5 % are  
 287 alkali-soluble galactoglucomannans. In acidic medium (WPS), an opposite trend is observed: ~85% of  
 288 water-soluble galactoglucomannans ( $\beta$ -D-Manp 5;  $\beta$ -D-Glup 1;  $\alpha$ -D-Galp 1) and ~15%  
 289 arabinoglucuronoxylans are recovered.

290 The obtained results were in agreement with those reported by (Thornton et al., 1994) who found that  
 291 for pH values above 10, arabino-(4-0-methylglucurono)xylans began to dissolve from Norway spruce  
 292 thermomechanical pulp, and at pH values below 8, the solubilization of galactoglucomannans is  
 293 promoted. In this paper, a considerable decrease of mannose units was observed when the suspension

294 was prepared at pH 9 and an increase in concentration of arabinose and xylose units was observed for  
295 pH > 10 suggesting the release of arabino-(4-O-methylglucurono)xylans.

296 (Perez et al., 2011) have also found that spruce hemicelluloses extracted in acidic conditions are  
297 primarily composed of galactoglucomannans with a high proportion of mannans (at least 45 %, up to  
298 57 %), then glucans (16 to 25%) and galactans (13 to 19%). Some arabinoxylans are also present (up to  
299 15 %, depending on the extractions conditions), while, the fractions obtained in alkaline conditions are a  
300 mixture of arabinoxylans and galactoglucomannans with a heavily contamination with lignin (up to  
301 18 %). According to (Svård et al., 2017), the nature of the polysaccharides extracted from  
302 lignocellulosic biomass is function of the extraction pH because their solubility is affected by the  
303 substituents on the polymer chain (Ebringerová et al., 2005). As a result, neutral and acidic media could  
304 promote the solubilization of water soluble acetylated galactoglucomannans (Lundqvist et al., 2002). On  
305 the other hand, alkaline medium yielded a xylose-rich extract because of the methyl glucuronic acid and  
306 arabinose substituents of AGX (Andrewartha et al., 1979; CROON, 1961; Kabel et al., 2007). These  
307 results could explain the slight increase in yields for WPS seen in Fig. 1. In fact, the amount of GGM in  
308 spruce wood (20% of wood) is very high compared to AGX (10 %) (Timell, 1967). Therefore, Since  
309 GGM are water-soluble at high temperatures and AGX are alkali-soluble, the yield obtained in WPS is  
310 slightly high (at short time, the effect of autohydrolysis is more operative than the chemical effect of  
311 sodium hydroxide).

312 Table 2 : contents of neutral sugars and uronic acids (mol %) of the extracted hemicelluloses from WPS  
313 and SPS fraction (for more details, see Fig. S2 in supplementary data section)

Sugar composition (% mol)	SPS		WPS	
	Average	SD	Average	SD
Fucose	0.007	0.008	0.001	0.001
Rhamnose	0.225	0.315	0.383	0.199
Arabinose	12.594	1.030	1.594	0.594
Galactose	7.258	0.884	12.558	1.161
Glucose	1.255	0.239	11.096	2.634
Xylose	74.944	2.547	10.868	1.748

Mannose	3.018	0.647	62.269	2.227
Galacturonic acid	0.418	0.364	1.061	0.483
Glucuronic acid	0.282	0.075	0.170	0.066

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### 317 **3.4 Acetylation degree of extracted hemicelluloses**

318 It is well known that the hydroxyl groups of the backbone units of softwood GGM are partly substituted  
319 by *O*-acetyl groups (Timell, 1967) and that this acetyl substitution is critical for further utilization of the  
320 hemicelluloses (Hannuksela et al., 2003; Xu et al., 2010). The degree of acetylation of GGM (WPS  
321 extracts) determined by HPLC after saponification, are given in Fig. 2. The molar Ac/Man ratios were  
322 used as a measure for the acetylation degree (AD) of GGM. Regarding SPS extracts, the acetylation  
323 degree is much lower ( $AD < 0.06$ ) since (1) most of the extracted hemicelluloses are  
324 arabinoglucuronoxylans, native softwood xylans being considered to be non-acetylated, (Fengel and  
325 Wegener, 1983; Sjöström, 1993) and (2) because of a further deacetylation by saponification reaction  
326 under basic conditions. Interestingly, for the SE performed at a  $SF > 2.95$ , the AD observed (0.27-0.35)  
327 are very closed to the values reported for native AcGGM, determined by NMR and titration methods  
328 (Hannuksela and Hervé du Penhoat, 2004; Xu et al., 2010). These acetylation values are higher  
329 compared to those reported in previous studies for AcGGM isolated from thermomechanical pulping or  
330 from spruce wood (Kishani et al., 2018; Song et al., 2011). From this result one can deduce that SE  
331 allow to extract representative and slightly modified AcGGM samples from softwood. At low SF  
332 treatments, the extraction of lower substituted GGM was observed. The optimum SF for the extraction  
333 of highly acetylated GGM is 3.65 after a water presoaking of the spruce sawdust.

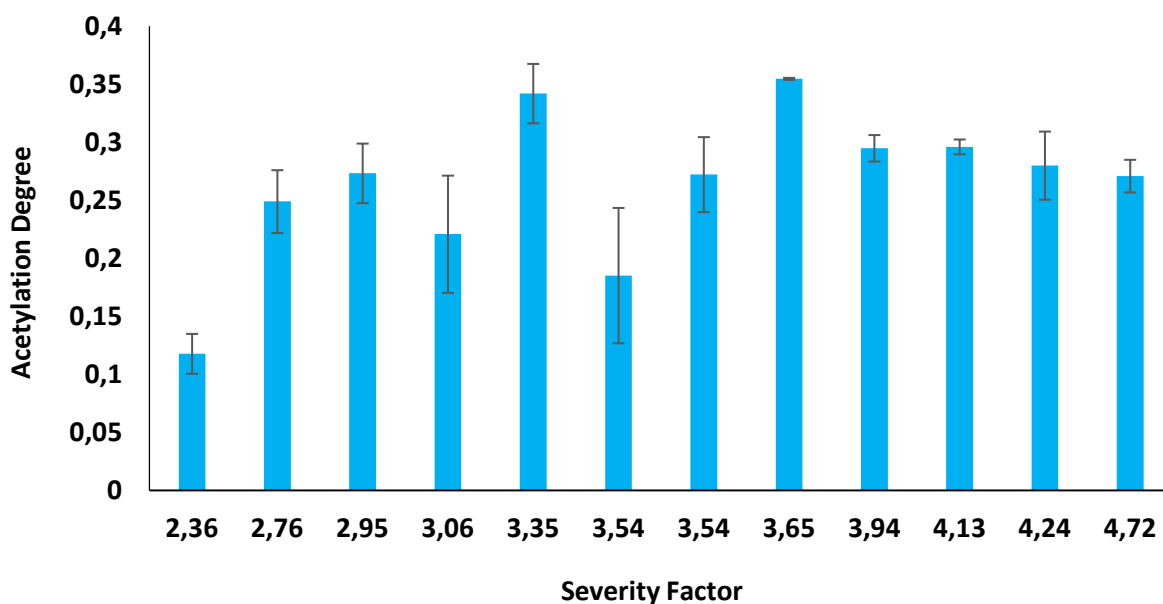


Fig. 2. Acetylation degree of extracted hemicelluloses from WPS

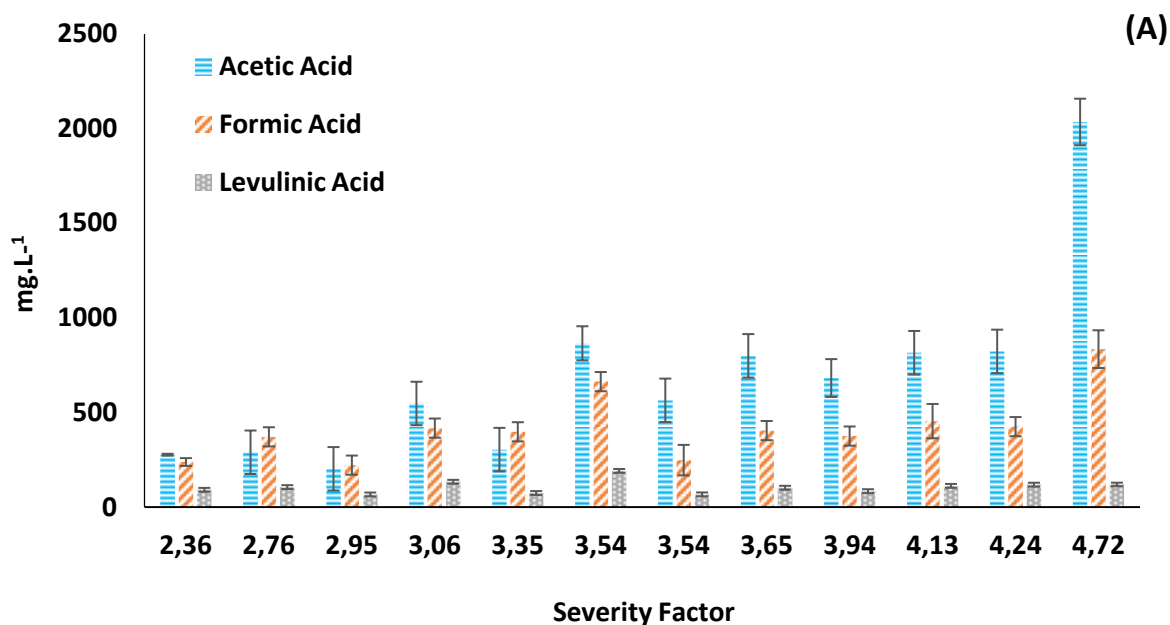
### 3.5 Degradation products

As expected for WPS, the increase of acidity of the media previously observed lead to the formation at high temperature of degradation products, furfural and hydroxymethylfurfural (HMF) formed by dehydration of C5 and C6 sugars respectively (Fengel and Wegener, 1983). The Fig. 3B gives the furans derivatives content as a function of the SE conditions. At SF < 2.95, the furfural and HMF concentrations are very low, under the limit of detection for the short duration treatments. On the other hand, the increase of the severity of SE is followed by a steadily increase in furans content. For SF = 4.13, 15.8 mg/l of HMF and 5.7 mg of furfural were formed in the liquid effluent of SE, while 19,6 mg/l and 6.9 mg/l were formed respectively at SF = 4.72.

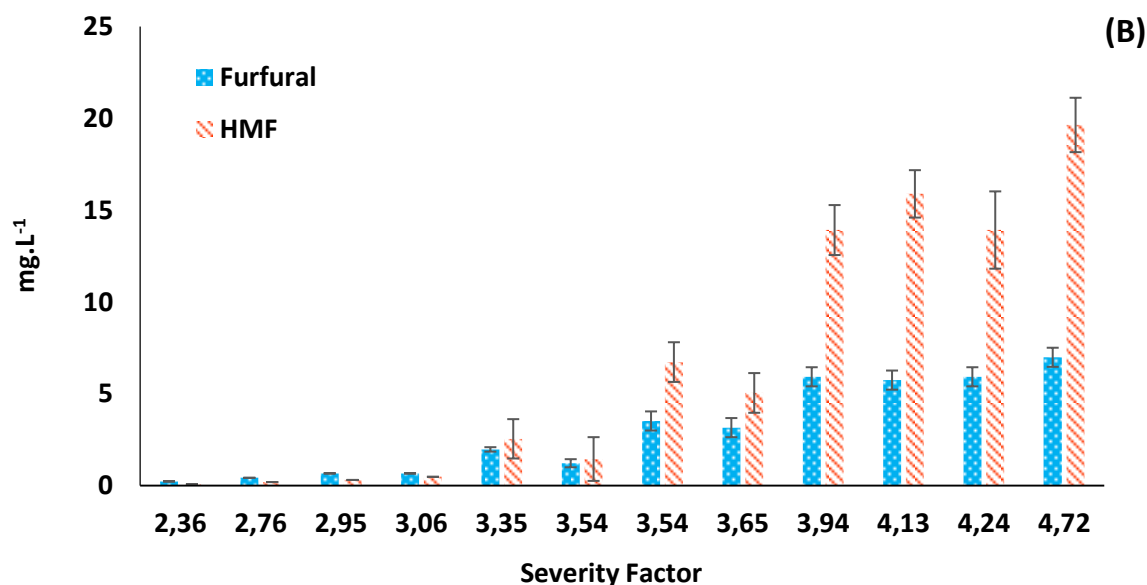
In basic conditions (SPS), the degradation of polysaccharides proceed though distinctly different mechanisms. Primarily by depolymerization reactions, leading to the formation of carboxylic acid (peeling reactions). Acetic acid is also produced primarily by saponification of acetate groups. Fig. 3A gives the concentrations of formic, acetic and levulinic acids according to the SE parameters. It is observed that the concentrations of levulinic acid is low and that formation of formic and acetic acids increases with the treatment severity. At the highest SF (4.72), acetic and formic acid account for 2034



352 mg/l and 834 mg/l respectively probably because of an extensive peeling degradation associated (for  
 353 acetic acid) to the acetate saponification previously mentioned.



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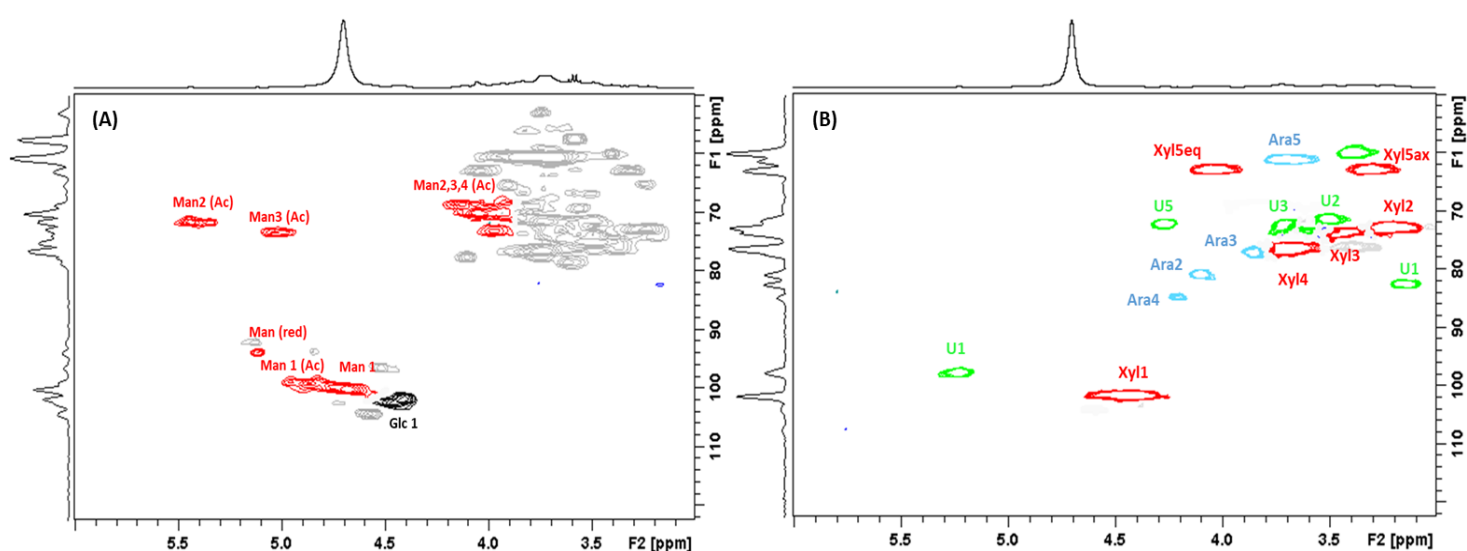
356 Fig. 3. Degradation products in the bulk liquid versus severity factor: (A) SPS fraction; (B) WPS  
 357 fraction

### 358 2.1 Spectroscopic characterization

359 The composition the hemicellulosic Ac-GGM and AGX fractions was confirmed by a 2D NMR study  
 360 <sup>1</sup>H-<sup>13</sup>C HSQC (see Figs. 4A and 4B respectively). The HSQC spectrum of AcGGM is in good

361 accordance with published data of spruce GGM. H1/C1 cross peaks assigned to galactose, glucose and  
362 mannose units are detected at 4.96/98.9 ppm, 4.58/104.4 ppm and 4.68/100.2 ppm respectively. Signals  
363 assigned to 2-*O*-acetylated- $\beta$ - Man<sub>p</sub> units are clearly observed at 4.87/99.0 ppm (H1/C1), 5.44/71.6 ppm  
364 (H2/C2), and the presence of 3-*O*-acetylated- $\beta$ - Man<sub>p</sub> units is confirmed by correlation at 5.03/73.2  
365 ppm (H3/C3), 4.75/99.9 ppm (H1/C1). From the intensity of the cross peak, we can observe that a  
366 slightly larger amount of 2-*O*-acetylated mannose units was detected compared to the 3-*O*-acetylated  
367 counterpart. This is in accordance with a previous study performed on Ac-GGM isolated from spruce  
368 thermomechanical pulp (Hannuksela and Hervé du Penhoat, 2004; Teleman et al., 2003)). The low  
369 intensity signal of reducing end  $\alpha$ -mannopyranosyl unit was also detected through its characteristic  
370 cross-peak at 5.11/93.8 ppm.

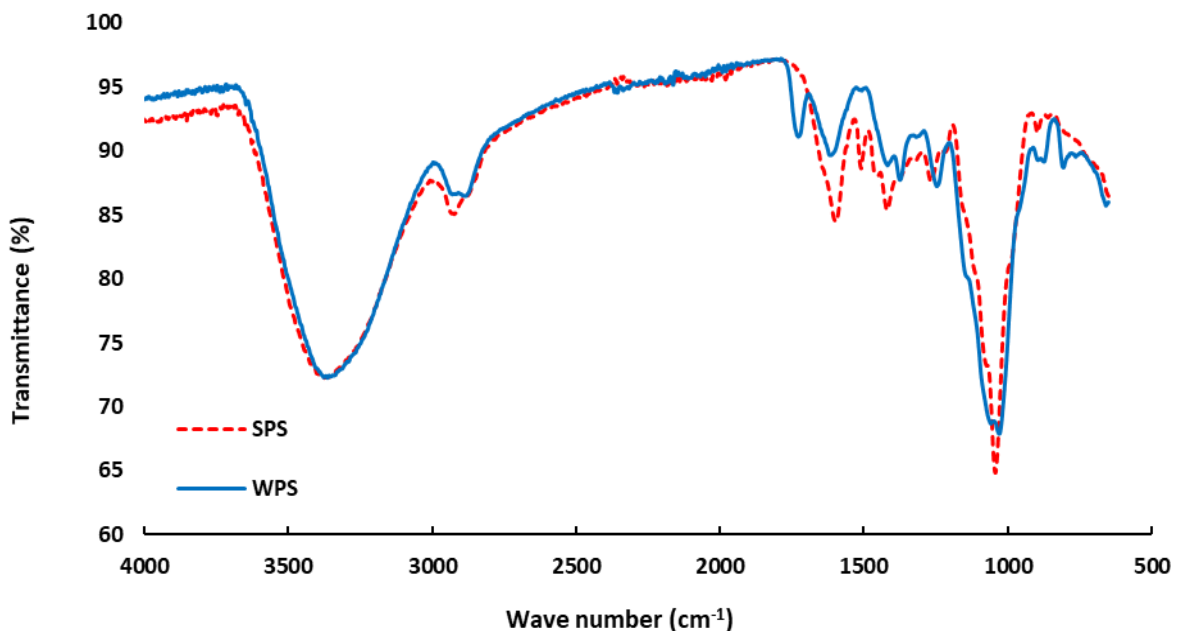
371 Regarding the SPS fraction (Fig. 4B, signals at 5.23/97.6 ppm (H1/C1), 3.70/72.4 ppm (H2/C2),  
372 3.85/77.1 ppm (H3/C3), 3.15/82.4 ppm (H4/C4), 4.28/72 ppm (H5/C5) assigned to 4-*O*-  
373 methylglucuronic acid residues reveal the presence of this residue not detected by ionic  
374 chromatography. The presence of xylan and arabinan signals, reveals that AGX was the major  
375 hemicellulose extracted for a basic impregnation. The chemical shifts of Ara, Xyl and OMeGlcU  
376 residues were in accordance with those described for glucuronoxylans from other material (Moine et al.,  
377 2007).



379 Fig. 4. 2D HSQC NMR spectral analysis of extracted hemicelluloses from WPS (A) and SPS (B)

380 FTIR has been also used for hemicelluloses identification. The results of FTIR spectroscopy are  
381 presented in Fig. 5. The absorptions at 1460, 1424, 1392, 1320, 1247, 1166, 1122, 1119, 1043, 985, and  
382 898  $\text{cm}^{-1}$  are associated with hemicelluloses (Peng et al., 2009). The intense pic at 1043  $\text{cm}^{-1}$  for SPS  
383 spectrum, which is assigned to the C–O and C–C stretching vibration and the glycosidic linkage (C–O–  
384 C) contributions in hemicelluloses, indicates a typical absorbance for xylans in the isolated  
385 hemicelluloses (Peng et al., 2009), which is in agreement with the sugar composition of extracted  
386 hemicelluloses Table 2.

387 The acetyl and uronic ester groups of hemicellulose absorb at 1745  $\text{cm}^{-1}$ . The intensity of this band is  
388 quite intense in the WPS spectrum, confirming that the hemicellulose fraction from WPS is acetylated  
389 while those extracted from SPS are totally deacetylated. The presence of a small band at 1514  $\text{cm}^{-1}$  in  
390 the SPS spectra can be assigned to the presence of small amounts of associated lignin in the  
391 hemicelluloses. In fact, extraction of hemicelluloses in basic medium, promotes the co-extraction of  
392 lignin residues, due to the action of alkali ions on the ester and ether linkage between hemicelluloses  
393 and lignin (Alvarez-Vasco and Zhang, 2013; Perez et al., 2011; Thornton et al., 1994).



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395 Fig. 5. FTIR spectrograms of extracted hemicelluloses from SPS and WPS

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#### **4. Conclusion**

Steam explosion was assessed for the extraction of high molecular weight hemicelluloses from spruce sawdust. It has been shown that the conditions of the pre-impregnation step before SE strongly influence the selectivity of the extraction. After a basic impregnation, ~90% of extracted hemicelluloses are arabinoglucuronoxylans whereas after a water presoaking ~85% of acetylated galactoglucomannans were recovered. In these latter conditions, the GGM isolated are highly acetylated, with an acetylation degree very closed to the values reported for native Ac-GGM. This study show that SE can be an efficient and selective process for a mild extraction in the water stream of close-to-native and highly acetylated GGM or high molecular weight arabinoglucuronoxylans from softwood. This approach paves the way for the recovery of high value-added co-products (e.g. acetylated GGM) from the liquid stream of the steam explosion pretreatment.

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