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

3 **Morad Chadni^{a, b*}, Nabil Grimi^a, Olivier Bals^a, Isabelle Ziegler-Devin^c, Nicolas Brosse^c**

4 ^aSorbonne Université, Université de Technologie de Compiègne, Laboratoire Transformations
5 Intégrées de la Matière Renouvelable (UTC/ESCOM, EA 4297 TIMR), Centre de recherches
6 Royallieu, CS 60 319, 60 203 Compiègne cedex, France

7 ^bSOFREN, 336 Bureaux de la Coline, Bâtiment A - 7ème étage ; 92213 Saint-Cloud Cedex France

8 ^cUniversité de Lorraine – Faculté des Sciences et Technologies, Laboratoire d’Etudes et de
9 Recherche sur le Matériau Bois – EA 4370, Boulevard des Aiguillettes, BP 70239, 54506
10 Vandœuvre-Lès-Nancy Cedex, France

11 **Corresponding author: Morad Chadni**

12 Sorbonne Université, Université de Technologie de Compiègne, Laboratoire Transformations
13 Intégrées de la Matière Renouvelable (UTC/ESCOM, EA 4297 TIMR), Centre de recherches
14 Royallieu, CS 60 319, 60 203 Compiègne cedex, France

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

16 Telephone: +33 (0)3 44 23 44 42

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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 approximately 10%. Ac-GGM has a backbone of β -D-(1 \rightarrow 4) -
60 glucopyranosyl and β -D-(1 \rightarrow 4)-mannopyranosyl units in a linear or slightly branched chain. Single α -
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 μm (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 % H_2SO_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 CarboPacTM PA-20 (3 \times 150 mm) analytical column. Filtered
172 samples (20 μL) 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%/ NaOCOCH_3 (1 M)-
174 NaOH (20 mM) 5% 20–37 min; and pure water 40%/250 mM NaOH 20%/ 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⁻¹ of sulfuric acid (5 · 10⁻³ 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⁻¹ 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) ¹H–¹³C heteronuclear single-quantum coherence (HSQC)** 200 **analysis**

201 ¹ H-¹³C 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 D₂O. The number of collected complex points was 1024 for the ¹H-dimension with a relaxation of 1.5-
204 and 256-time increments were recorded in ¹³C-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 cm^{-1} with eight scans at resolution of 4 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**

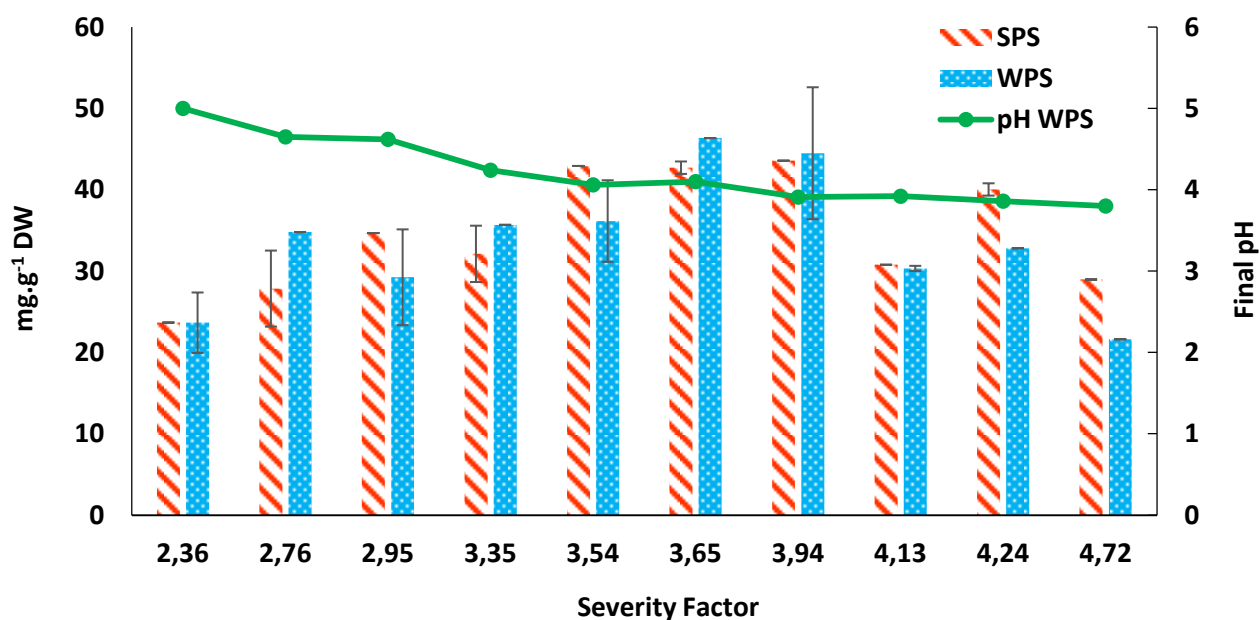
216 The hemicelluloses were recovered after SE of spruce sawdust from the liquid stream by precipitation in
217 ethanol and dialysis. The influence the severity of treatment and pH of the pre-impregnation step (Water
218 Pre-soaked Sawdust, WPS, Sodium hydroxide Pre-soaked Sawdust, SPS) on the extraction 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 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⁻¹ 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⁻¹ 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⁻¹ DW).



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

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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 (β -D-Xylp 10; α -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 (β -D-Manp 5; β -D-Glup 1; α -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.

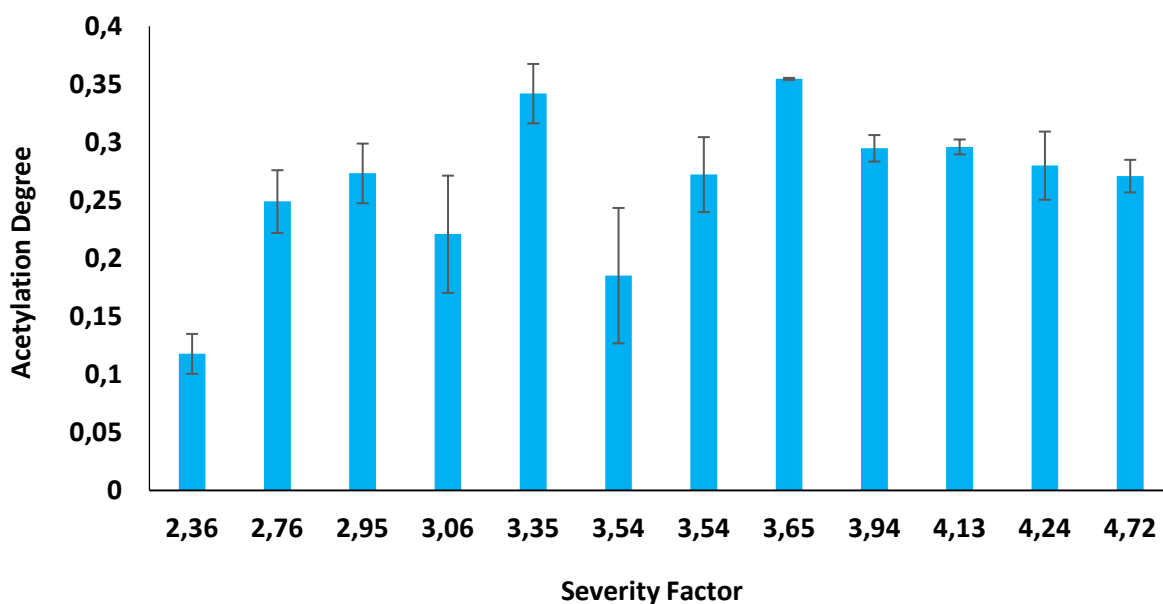


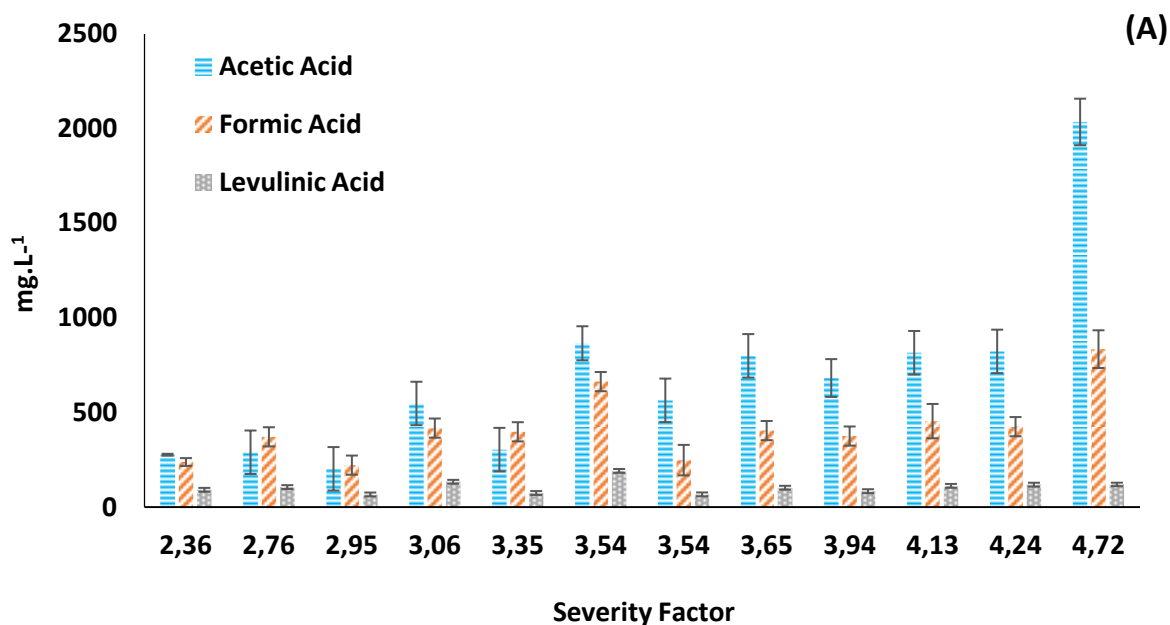
Fig. 2. Acetylation degree of extracted hemicelluloses from WPS

3.5 Degradation products

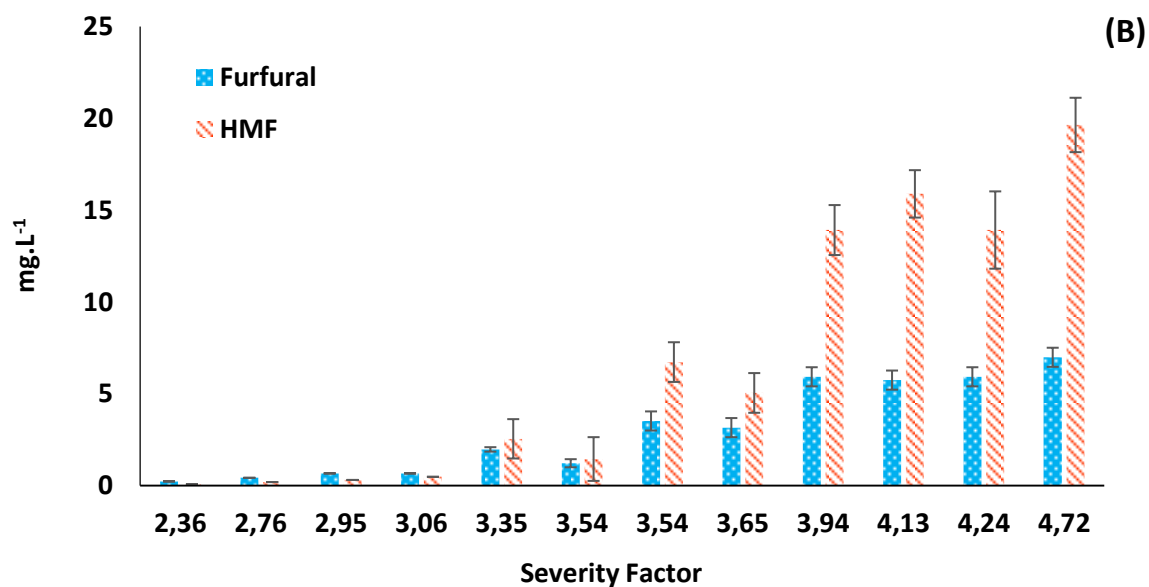
As expected for WPS, the increase of acidity of the media previously observed lead to the formation at high temperature of degradation products, furfural and hydroxymethylfurfural (HMF) formed by dehydration of C5 and C6 sugars respectively (Fengel and Wegener, 1983). The Fig. 3B gives the furans derivatives 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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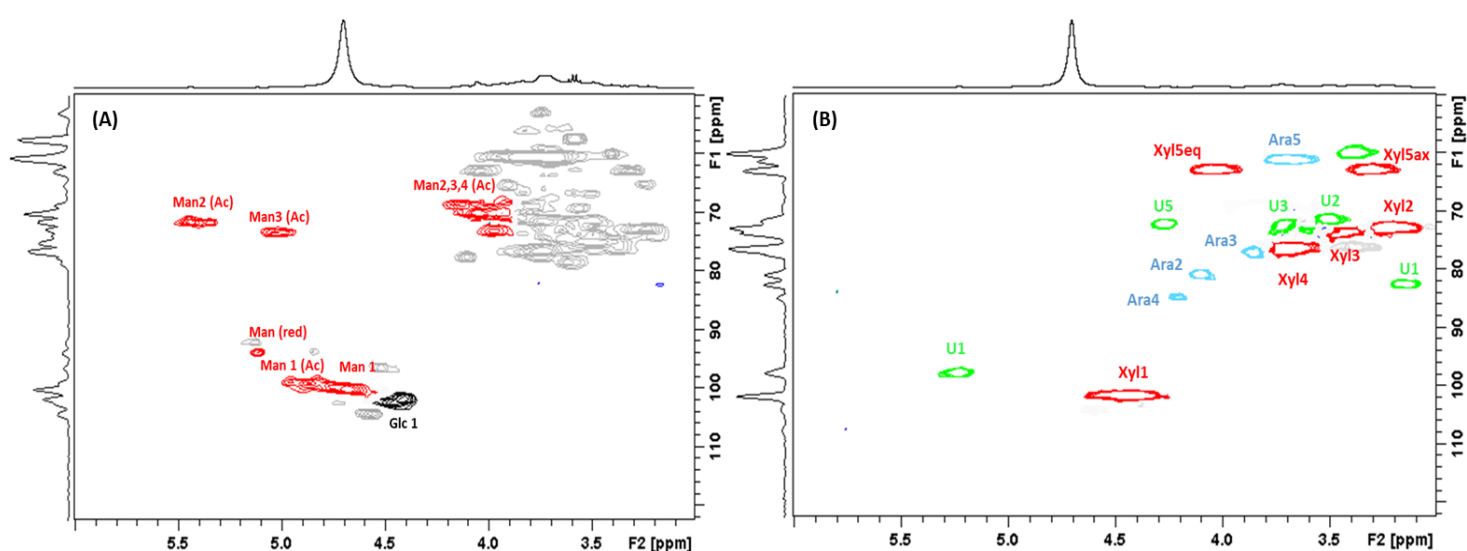
356 Fig. 3. Degradation products in the bulk liquid versus severity factor: (A) SPS fraction; (B) WPS
 357 fraction

358 2.1 Spectroscopic characterization

359 The composition the hemicellulosic Ac-GGM and AGX fractions was confirmed by a 2D NMR study
 360 ¹H-¹³C 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- β - Man_p 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- β - Man_p 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 α -mannopyranosyl unit was also detected through its characteristic
370 cross-peak at 5.11/93.8 ppm.

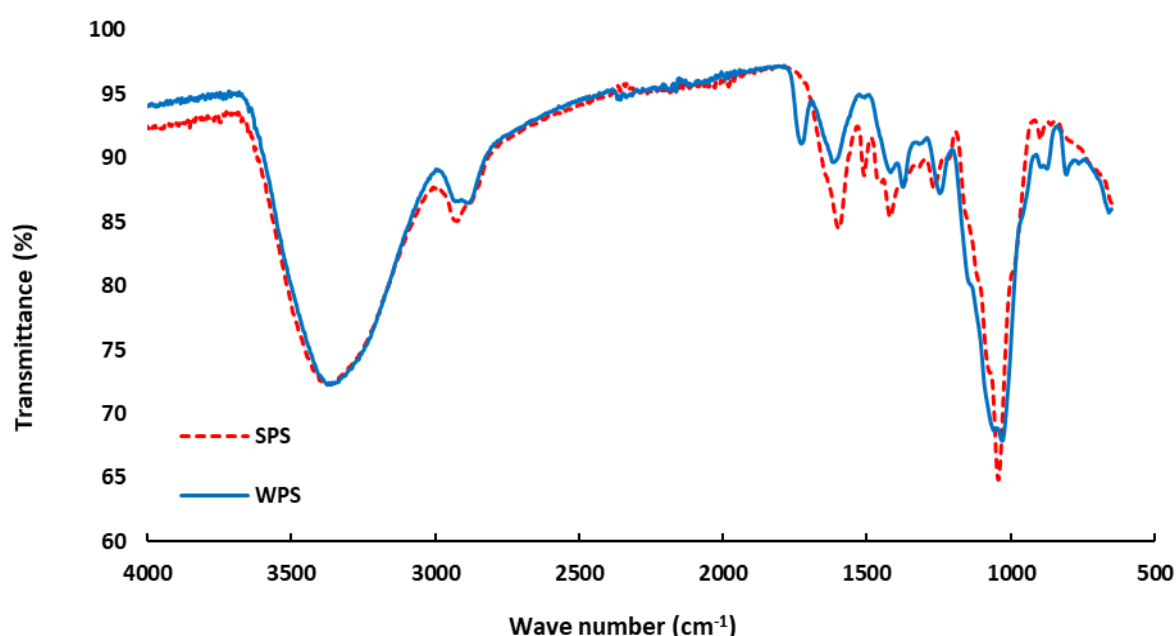
371 Regarding the SPS fraction (Fig. 4B, signals at 5.23/97.6 ppm (H1/C1), 3.70/72.4 ppm (H2/C2),
372 3.85/77.1 ppm (H3/C3), 3.15/82.4 ppm (H4/C4), 4.28/72 ppm (H5/C5) assigned to 4-*O*-
373 methylglucuronic acid residues reveal the presence of this residue not detected by ionic
374 chromatography. The 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 cm^{-1} are associated with hemicelluloses (Peng et al., 2009). The intense pic at 1043 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 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 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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402 **4. Conclusion**

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

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