

Microwave-assisted extraction of high-molecular-weight hemicelluloses from spruce wood

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1	MICROWAVE ASSISTED EXTRACTION OF HIGH MOLECULAR WEIGHT
2	HEMICELLULOSES FROM SPRUCE WOOD
3	EXTRACTION ASSISTEE PAR MICRO-ONDES DES HEMICELLULOSES DE HAUTS
4	POIDS MOLECULAIRES A PARTIR D'EPICEA DE BOIS
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27 Abbreviations

28	Ac-GGM	Acetyl-Galactoglucomannan
29	ARX	Arabinoglucoronoxylan
30	DW	Dry wood
31	kDa	kilo Dalton
32	FTIR	Fourier Transform InfraRed
33	GGM	Galactoglucomannans
34	HMF	Hydroxymethylfurfural
35	HPLC	High performance liquid chromatography
36	Μ	Mole
37	MAE	Microwave Assisted Extraction
38	Mw	Molecular weight average
39	NREL/TP	National Renewable Energy Laboratory/ Technical Report
40	PD	Polymerization degree
41	W	Watts
42	PDI	Polydispersity index
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48 Abstract

Microwave assisted extraction (MAE) at atmospheric pressure has been demonstrated as an efficient technology for the extraction of polymeric hemicelluloses from spruce sawdust. This technology was shown to be more efficient than conventional extraction. MAE leads to a high solubilization of wood and a selective extraction of hemicelluloses polymers with high molecular weights. To optimize MAE, different treatments power (125-573W) for a period of 60 min of a pre-soaked spruce sawdust in water and 1M sodium hydroxide solution were tested. The yield of hemicellulose extraction increased with the microwave power in both mediums, but with a clear advantage for pre-soaked samples in basic medium. The characterization of extracted hemicelluloses has shown a high extraction selectivity depending on the medium of impregnation of sawdust before MAE: high molecular mass acetylated galactoglucomannans ($M_w \sim 41$ kDa) were isolated after a pre-soaking in water and higher molecular mass arabinoglucoronoxylans ($M_w \sim 66 \text{ kDa}$) in basic medium.

- 60 Key words: Wood, spruce, microwave-assisted extraction, hemicelluloses, polymers

72 Résumé

Le traitement par microondes (MAE) à pression atmosphérique a montré son efficacité pour 73 l'extraction des polymères d'hémicelluloses à partir de la sciure du bois d'épicéa. Cette technologie de 74 traitement s'est avérée plus efficace qu'un traitement thermique conventionnel. Le traitement 75 microondes a conduit à une solubilisation élevée du bois et une extraction sélective des polymères 76 d'hémicelluloses de poids moléculaires élevés. Pour optimiser le traitement MAE, différentes 77 78 puissances de traitements (125-573W pendant 60 minutes) ont été testées sur la sciure de bois imprégnée soit dans de l'eau, soit dans une solution de NaOH à 1 mol/L. Les résultats ont montré que 79 le rendement d'extraction d'hémicelluloses augmentait avec l'augmentation de la puissance des micro-80 81 ondes dans les deux milieux étudiés, avec un avantage pour la sciure imprégnée dans la solution basique. La caractérisation des hémicelluloses extraites a montré une sélectivité d'extraction 82 importante en fonction du milieu d'imprégnation de la sciure de bois avant MAE: des 83 Galactoglucomannanes de masse moléculaire élevée ($M_w \sim 41$ kDa) ont été obtenus après 84 imprégnation dans l'eau et des arabinoglucoronoxylanes de masse moléculaire élevée ($M_w \sim 66$ kDa) 85 en milieu de basique. 86

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88	Mots clés :	Bois, épice	a, extractior	n assistée j	par micro	-ondes,	hémicelluloses,	polymères
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97 **1. Introduction**

Non cellulosic wood polysaccharides are of great interest because of their structural diversity and 98 99 functionality. The potential for application of polymeric hemicelluloses has been demonstrated by 100 several authors [1–5]. As a result, new utilizations of hemicellulosic polysaccharides are emerging in 101 the fields of bioactive polymers, hydrocolloids, papermaking additive, or coating films [6,7]. 102 Softwood hemicelluloses are mainly constituted of galactoglucomannans (GGM), approximately 14-103 20% of the wood [8] and arabinoglucuronoxylans (7-10%) of the wood). Softwood xylans are not acetylated, but the xylan backbone is substituted at carbon 2 and 3 with 4-O-methyl- α -D-glucuronic 104 acid and α-L-arabinofuranosyl residues, respectively. Softwood xylan has a relatively low DP of about 105 106 100 [9]. GGM have been described to have an approximate degree of polymerisation of 100-150, 107 matching to a molar mass of 16–24 kDa [9]. The potential applications of spruce GGM in biomaterials and hydrocolloids, such as barriers in food packaging, bioactive polysaccharides, hydrogels and fibers, 108 and health-promoting agents, have been studied [3,5,10–14]. 109

110 The extraction of polymeric hemicelluloses has been investigated for years. Extraction of softwood 111 hemicelluloses has been studied by hydrothermal treatment at high temperatures (\geq 170 °C) [15,16], by 112 chemical extraction (alkaline) [17–20] and ionic liquids [21], by pressured hot water [22,23], and by 113 microwave heat-fractionation (> 180 °C) ([24–26].

114 The fractionation of wood at high temperatures leads to high solubilization of all macromolecules, 115 including hemicelluloses. However, the extraction at high temperatures for long reaction times led to a 116 low pH level, causing hydrolytic cleavages. As a result, GGMs extracted using these conditions are 117 generally highly deacylated and exhibit low molecular mass. Chemical treatments, especially alkaline 118 extractions are described to produce higher molecular weight hemicelluloses. Nevertheless, alkaline extraction of hemicelluloses has some disadvantages. In addition to causing saponification of 119 120 polysaccharides, extraction with high concentrated alkaline solutions for long times can lead to peeling 121 reactions [27].

122 Nowadays, new process of extraction have been developed in order to reduce the amount of chemical solvents, treatment time and temperature as well as energy consumption, hence improving the 123 124 recovery of valuable compounds with lower costs [28]. Microwave-assisted extraction is one of the 125 advantageous green extraction methods based on electromagnetic radiations with a frequency from 0.3 to 300 GHz. In this process, microwave irradiations are used to extract biomolecules. Unlike 126 127 conventional heat treatment where the energy is transferred to the material by convection, conduction 128 or radiation from the surface of the material, microwave energy is delivered directly to the material 129 through a molecular interaction with the electromagnetic field [29]. This interaction allows the heating 130 of the material and a transfer of energy from the inside to the outside of the cell [30].

131 Several studies have been carried out on the effect of microwaves on the extraction of hemicelluloses from lignocellulosic materials. Microwave heating was shown to be efficient for the extraction of 132 133 softwood hemicelluloses [26,31,32]. In 2014, Panthapulakkal has observed a slightly higher xylans extraction yield from wood fibers using microwave-assisted extraction (58 % of xylans) compared to 134 conventional extraction (54 %) [33]. Similar results have been reported in pretreatment of maize cane 135 136 using water and acid [34], alkaline pretreatment of Panicum virgatum [35]. Free sugars are generally released from the degradation of polysaccharides during the extraction process [36]. Most of the 137 published works are carried out at temperature above 100 °C and at pressure above atmospheric 138 139 pressure, these conditions leading to a degradation and a depolymerization of hemicellulose chains.

In the current work, microwave heating at atmospheric pressure was carried out for the extraction of polymeric hemicelluloses in mild conditions (temperature < 100 °C) in order to limit any possible degradation and depolymerization. The effect of MAE was compared with a conventional treatment at the same temperature. The effect of different conditions (power and/or temperature, pH of extraction) on the yield and the physicochemical properties of extracted hemicelluloses was assessed.

145

2. Material and method

2.1. Raw material

Spruce sawdust was produced from a spruce tree from the Lorraine region of France with a particlesize of (1-5 mm) and stored at room temperature in a sealed polyethylene bags in the dark until use.

The average moisture content of the raw material was 10% w/w. The hemicelluloses content of the
sawdust was approximately 30 % w/w dry biomass. Carbohydrate composition of sawdust includes 72
% glucan, 7.5 % xylan, and 16.3 % of mannan and other sugars.

152 **2.2. Impregnation**

For each experiment, a mass of 20 g of spruce sawdust was soaked with a solution of 1M sodium hydroxide (ratio solid/liquid = 1:7) or water (ratio solid/liquid = 1:7). The sawdust was left for 15 hours before treatment at room temperature.

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2.3.1. Microwave activation

2.3. Experimental methodology of extraction

158 The microwave assisted extraction (MAE) experiments for the extraction of hemicelluloses from spruce sawdust were carried out using a microwave laboratory system (230V-50 Hz, NEOS-GR, 159 160 Milestone, Italy). This mono-mode apparatus operates at 2.45 GHz with maximum delivered power of 900 W regulated in 10 Watt increments with a temperature and power feedback/control. This 161 162 instrument is equipped with time controller. The experiments were performed at atmospheric pressure and time, power were controlled and followed by the software. The MAE experiments were conducted 163 in a glass extraction vessel. The samples were treated for 1h using different selected powers. Indeed, 164 165 the effect of microwave irradiation, nature of solvent and consumed energy were studied. The liquid-166 to-solid ratio was fixed at 7 during microwave treatment.

167 The power values presented in all figures correspond to the effective power received by the sample 168 $(P_{effective})$ which was calculated by the following equations:

169 $P_{effective} = P_{input^{x.}} Y_{MW}$

170 (1)

171
$$Y_{MW} = \frac{m x C_p x \Delta T(373 K - Ti)}{P_{input} x t}$$

172 (2)

with P_{input} is the power immitted by microwave apparatus (Watts), Y_{MW} is the yield of microwave, m is the weight of water (kg), Ti is the initial temperature of water (K), t is the necessary time of treatment to increase temperature from T_i to T_f, C_p is the specific heat of water (4181.3 J/kg/K)

176

2.3.2. Diffusion

After microwave treatment, supplementary diffusion step was done by a heating under reflux at low
temperature to avoid degradation of polymeric chains (70 °C for 3 h). The liquid-to-solid ratio was
fixed at 15 (w/w).

For comparison, conventional extraction, without microwave treatment, from impregnated spruce sawdust was treated in similar extraction vessel heated with a water bath at 100 °C for one hour (to highlight the effect of microwave, the time to reach 100 °C for conventional extraction is not included) and then followed by 3 hours of extraction at 70 °C.

184

2.3.3. Separation of extracted hemicelluloses

185 After each experiment, the extract was filtered on qualitative filter paper with retention particle size of 5–13 µm (VWR International, Fontenay-sous-Bois, France). The volume and end-pH of the extract 186 solutions were measured at room temperature shortly after the extraction. The liquid fraction from 1M 187 188 NaOH impregnated sawdust was neutralized to a pH of 5.5-6 by a solution of 72% H₂SO₄. 10 mL 189 were taken from the filtered extract for analyses. The extract solution was concentrated by vacuum 190 evaporation (BUCHI, France) using a water bath at 50 °C. To precipitate high-molecular weight hemicelluloses, four volumes of 95% ethanol were added to the liquid extracts. The liquid phase was 191 separated from the precipitated hemicelluloses by settling at room temperature and decantation. 192 193 Hemicelluloses were further separated from the remaining liquid by centrifugation at 4000 rpm for 10 min at room temperature. The precipitated hemicelluloses were re-dissolved in distilled water and 194 dialyzed with a dialysis membrane with a cut-off of 3.4 kDa (Spectrum Laboratories, USA). Finally, 195 dialyzed hemicelluloses solutions were freeze-dried. Fig.1. shows the experimental procedure of 196 197 extraction and separation from spruce sawdust.

Fig. 1. Scheme for extraction and separation of hemicelluloses from spruce sawdust using microwave
 assisted extraction and conventional extraction.

200 2.4. Measurement of monosaccharides and uronic acids using HAPE-PAD

The monosaccharide content of the soluble fraction was analyzed according to [37] method. The sugar 201 concentrations were measured by HAPE-PAD (ICS-3000 Dionex, Dionex Corp., Sunnyvale, USA) 202 equipped with a Dionex CarboPac[™] PA-20 (3 × 150 mm) analytical column. Filtered samples (20 µL) 203 were eluted at 35 °C and at 0.4 mL/min with the following composition: pure water 99.2%/250 mM 204 NaOH 0.8%: 0-20 min; pure water 75%/250 mM NaOH 20%/ NaOCOCH₃(1 M)- NaOH (20 mM) 5% 205 206 20-37 min; and pure water 40%/250 mM NaOH 20%/NaOCOCH₃ (1 M)- NaOH (20 mM) 40% 37-41 207 min. Each elution was followed by a wash and subsequent equilibration step. External sugar and uronic acid standards were used for calibration (7 points per curve). The reagents and standards are 208 209 provided by Sigma-Aldrich and Fisher Scientific.

210 **2.5.** Degradation products

Degradation products of hemicelluloses (furfural, HMF, formic acid and levulinic acid) are analyzed according to [38]procedure. All the products are quantified with an Ultimate 3000 HPLC system. The components are separated in 45 min on a Agilent Hi-Plex H column at 55° C with an isocratic flow rate of 0.4 mL·min⁻¹ of sulfuric acid (5. 10⁻³ M). Acids were detected by UV at 210 nm, while HMF and furfural are detected by UV at 280 nm. An external calibration is performed with six calibration points per standard. Reagents and standards are provided by Sigma-Aldrich.

217 **2.6.** Acetyl content

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

221

2.7. Size-exclusion chromatography (SEC)

A Shimadzu Prominence HPLC system is used to determine molecular weight distribution of
 hemicelluloses by gel filtration chromatography. The stationary phase is a Phenomenex Polysep GFC P guard column (7.8 x 35 mm), Phenomenex Polysep GFC-P3000 and GFC-P2000 analytical

columns (7.8 x 300 mm). The mobile phase is 0.25 M aqueous NaNO₃. Samples are filtered with a 0.45 μ m membrane and 20 μ L are injected. Samples are separated at 30 °C with a 0.5 mL min⁻¹ flow and detection is done with a Refractive Index Detector (Shimadzu RID-20A). Each analysis runs for 70 min. aqueous solutions (1 mg/mL) of dextran standards with molecular weights of 1000–80,000 were used to prepare the calibration curve.

230 **2.8. FTIR**

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

236 2.9. 2D 13C–1H HSQC NMR spectroscopy

1 H NMR and 1 H-13C correlation 2D NMR (HSQC) spectra were recorded on a Bruker III 400 MHz
spectrometer equipped with a 5-mm BBFO probe. Experiment was conducted with 20 mg sample
dissolved in 1 ml D₂O. The number of collected complex points was 1024 for the 1H-dimension with a
relaxation of 1.5- and 256-time increments were recorded in 13C-dimension. HSQC spectra were
taken at room temperature.

242

243 **2.10.** Soluble lignin

The acid-soluble lignin (ASL) content was determined from absorbance values at 205 nm according to
the laboratory analytical procedure (LAP) provided by the National Renewable Energy Laboratory
[37].

247 % ASL =
$$\frac{(Abs_{205 nm} * V_t * 100)}{(DMi)}$$
 (3)

248 where V_t is the total volume of sample (L); DM_i is the initial dry matter (g).

249 **2.11.** Wood solubilization

- 250 Wood solubilization (WS) was presented as the ratio of the wood dissolved to the weight of the wood
- used for extraction as expressed in the Eq. (4):

252
$$WS = \frac{Weight of DW before extraction - Weight of DW after extraction}{Weight of DW before extraction}$$
(4)

- where DW stand for the term dry wood.
- 254
- 255

3. Results and discussion

256 **3.1 Wood solubilization**

257 Prior to microwave activation, spruce sawdust was impregnated in a solution of distilled water (neutral 258 medium) or 1 M NaOH (basic medium) for one night. The aim of the impregnation step is to open the wood structure and to enhance the accessibility of hemicelluloses during the microwave extraction 259 [39–41]. After microwave treatment, a diffusion step was carried out during 3h at 70°C. The effect of 260 impregnation and diffusion steps on hemicelluloses yields in neutral medium (+ 19 % and 2.5 %261 respectively) and (+ 16.4 % and +30 % respectively) in basic medium. See (Fig. S1) in SD section. 262 The temperature profiles for conventional extraction and for MAE at different power are also given in 263 264 SD section (Fig. S2).

265 Solubilization of wood was calculated using Eq.(3). As expected, the solubilization of wood is significantly higher in basic medium (Fig.3). The microwave assisted extraction increase notably the 266 effect of sodium hydroxide (+81% for 573W). The advantage of MAE over conventional extraction 267 could be explained by the rapid and the selective heating of wood that lead to destabilization and 268 269 structural damage of wood matrix, thus improving biomolecules extraction, and also the heat and mass 270 transfer which act in the same direction. Furthermore, the effect of MAE is not only related to the fast increase of temperature, but also to the power transmitted to the wood matrix which destabilizes and 271 272 weakens structural linkages between lignin and carbohydrates. In conventional extraction, heat 273 transfer and mass transfer had opposite directions [42].

Wood solubilization steadily increased with the increase of microwave power. The solubilization of sawdust is mainly affected by microwave power which is the main parameter affecting the irradiation strength and the rate of temperature increase (Fig.2). For basic impregnation, alkaline solutions increase the water absorption capacity, swell up cellulosic fibers and decrease their crystallinity, and thus increase the specific surface area [43]. Based on previous reports, the mechanism of action is due to the decrease of intermolecular hydrogen bonds by solvation [44] as well as lignin- hemicelluloses bonds scissions.

281

282

MW, 3h diffusion)

Fig.2. Solubilization of wood after MAE and conventional extractions (1 h under

283 **3.2** Hemicellulose extraction

Polymeric hemicelluloses extracted in the liquid fraction were quantified by subtracting the amount of 284 monomeric sugars (determined by ionic chromatography) from the total sugars content in the liquid 285 fraction obtained after extraction. As expected, Fig.3 (A and B) shows that the extraction of 286 287 hemicelluloses highly depends on the pH of the impregnation step, as it affects the hydrolysis of the hemicelluloses-lignin linkages. In fact, at maximum power (573 Watts) when sodium hydroxide was 288 used as solvent, the effect of microwave on the extraction yield was higher as compared to the 289 conventional extraction (32.3 and 22.5 mg/ g of dry wood respectively). On the other hand, lower 290 291 extraction yields were observed using water as solvent. To explain the effect of MAE on wood, 292 Panthapulakkal et al., (2015) have suggested that the disruption of wood structure and the rupture of 293 wood fibers are due to the selective interaction of the microwaves with the inhomogeneous 294 lignocellulosic materials [35, 45] have found that microwave treatment improves surface disruption 295 and breaking of lignin structures in alkaline treated switch grass, while in conventional heating, the 296 fibers remain intact.

The composition of the liquid fraction in terms of monomeric sugars (after acid hydrolysis) at different conditions of microwave extraction are also given in the **Fig.3** (**A and B**). Polysaccharides extracted in neutral medium are mainly composed of mannose (33%), glucose (18%) and galactose (20%). As a result we can assume that the hemicellulosic extract is mainly composed of galactoglucomannans [9]. On the other hand, in the basic medium the high contents in xylose (57%) and arabinose (10%) suggests the dominant extraction of arabinoxylans. This selectivity of extraction as a function of the 303 pH of the medium is in accordance with previous reports. It has been described that in neutral or mild 304 acid medium (pH \approx 5), hemicelluloses extracted from softwood are rich in mannose, acetyled 305 glucomannans exhibiting a relatively high water solubility [9,32]. In basic medium, the high xylose 306 content can be rationalized by the dominant extraction of arabinoglucuronoxylans. In fact, arabinoglucuronoxylans are substituted with methyl-glucuronic acid and arabinose moiety which 307 affect their solubility [46]. Thornton et al., (1994) have found from the Norway spruce wood that at 308 309 pH values greater than 10, the concentration of extracted arabinose and xylose units increase, 310 suggesting the release of arabino-(4-*O*-methylglucurono)xylans [47].

Fig.3. Yield and sugar composition of extracted hemicelluloses polymers in neutral medium (A) and basic medium (B)

The oligo- and polymeric fraction of the extracted hemicelluloses were calculated by subtracting the amount of monomeric sugars from the total sugars content in the extracts. The monomer proportion is very low (< 2%) whatever the extraction conditions, the mild conditions used in this study (T<100°C, pH>5) limiting the hydrolytic cleavage of hemicelluloses. Based on literature data, the hydrolysis of hemicellulose into monomeric sugars is promoted by extraction at higher temperatures for longer times and lower pH level [48].

319 Solubilization of lignin during the extraction of hemicelluloses is expected especially in basic medium. As shown in (Fig.4.B) the amount of soluble lignin increased after MW sawdust irradiation. A 320 321 significant difference was observed for MAE at high power input (4.8% and 0.49% at 573 W for 1h compared to 3.3 % and 0.3 % at 100 °C for 1h at basic and neutral medium respectively). Acetic, 322 uronic, formic and levulinic acids have also been detected in the liquid effluents. The carboxylic acids 323 324 contents were relatively important in basic medium (Fig.4. A). Acetic and formic acids are the major carboxylic acids formed (6 and 1.7 g/l respectively). Levulinic acid present the lowest concentration 325 326 accounting for 0.3 g/l. The peeling reactions occurring during alkaline treatments lead to endwise degradation of polysaccharides with formation of formic acid and different dihydroxy and 327 dicarboxylic acid [49]. Acetic acid is released from the hemicellulosic backbone by saponification of 328 329 acetate groups.

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Fig.4. Degradation products in the liquid fraction in basic medium (A) and ASL (%) (B)

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3.3 composition of the recovered hemicelluloses powder

332 The sugar composition of polymeric hemicelluloses recovered after precipitation and dialyze is similar 333 to the sugar composition of the liquid fraction (Fig.5). Galactoglucomannans and arabinoxylans are 334 the main polysaccharides extracted in neutral and basic medium respectively. In neutral medium, MAE promotes the extraction of arabinose and galactose compared to conventional extraction. In fact, 335 336 hemicelluloses obtained from neutral medium are accounting for 24% and 19.5 % of galactose, for 337 10.5 % and 5% of arabinose for MW (573W) and conventional extraction respectively. The advantage of MAE seems to be not only due to the temperature, but also owed to the physicochemical 338 interactions induced by microwave heating of the wood matrix. 339

340 Fig.5. Sugar composition of recovered hemicelluloses by MAE and conventional extraction

In native softwood, mannopyranosyl units are acetylated at C-2 or C-3 with a degree of acetylation of about 0.5 [17,9]. In our study, the precipitated hemicelluloses recovered from a neutral medium had a lower degree of acetylation, in the range of 0.10-0.15. In basic medium, the acetyl groups have been totally released from the hemicellulosic backbone by saponification.

These results have been confirmed by a FTIR study. In the **Fig. 6** two weak absorbances at 1735 and 1247 cm⁻¹ due to acetyl groups attached to the hemicelluloses chains were obseverved for the fractions extracted in neutral media but not for the basic extracts. Morover, the absorption at 1515 cm⁻¹ observed in all the hemicellulosic fractions was assigned to lignin's aromatic skeletal vibrations [50]. The higher intensity of this signal for the fractions isolated from basic media confirms the higher lignin contamination for hemicelluloses obtained in basic medium compared to those obtained in neutral medium previously obseved (**Fig. 4.B**).

352

Fig.6. FTIR spectra of extracted hemicelluloses

353 2D ¹H- ¹³C HSQC spectra clearly shows the typical signal spots expected for the hemicellulosic 354 moieties. In basic medium (**Fig 7. B**), β-D-xylopyranoside units are specified by these signals at 355 $\delta C/\delta H$ 60/3.4; 62/4; 72/4.3; 72.5/3.2; 74/3.5; 72.5/3.7. The signals at 72/ 3.5 is assigned to α-D-

356 xylopyranoside of xylans reducing end and at 62/3.3 and 71/3.5 for β -D-xylopyranoside of xylans non 357 reducing end. In neutral medium (Fig 7. A), the signals at 3.4/74; 3.5/74; 3.8/76; 4.2/70 are attributed 358 to β -D-mannopyranoside. The same results were found by [51]. The signal in neutral medium at 5.45/72 was assigned to 6-O-acetyl β-D-mannopyranoside. In fact, as confirmed by the FTIR analysis, 359 the extraction in neutral medium preserved some of the acetyl groups of the backbone units of GGM. 360 These results are in close agreement to those obtained by [47] who found that deacetylation of the O-361 362 acetyl-galactoglucomannans appeared to begin at pH values above 8. The presence of acetyl residues is critical for the water-solubility of GGM. The acetyl moieties are generally cleaved during the 363 extraction at high temperatures leading to the release of acetic acid. The signals at 98/5.25 and 82/3.1 364 are assigned to 4-O-methyl-a-D-glucuronic acids that are spotted only in basic medium which 365 confirms the extraction of arabinoglucuronoxylans in these conditions. 366

367 Fig.7. HSQC spectra of extracted hemicelluloses (A) in neutral medium; (B) in basic medium

Weight-average (M_w) and number-average (M_n) molecular weights, as well as the polydispersity index 368 (PDI) were determined by gel-permeation chromatography. The results in Table 2 show that 369 hemicellulose fractions isolated from microwave irradiation display higher molecular mass as 370 371 compared to the conventionally extracted fractions (for conventional extraction, $M_{\rm w}$ ~38000 and ~56000 g.mol⁻¹ in neutral and basic medium respectively and ~42000 and ~67000 g mol⁻¹ for 372 373 hemicelluloses extracted with microwave activation in neutral and basic medium respectively). It can 374 be also observed that the hemicelluloses extracted in neutral medium had much lower molecular 375 weights than those isolated in basic medium. These results were in close agreement to those reported 376 by [52] who found that increasing the alkali concentration during extraction increased the Mw of 377 extracted hemicelluloses by 46%. They suggested that the extraction of high M_w hemicelluloses was 378 owed to the increase of the co-extraction of lignin-carbohydrates complexes. Considering the sugar 379 composition given in Fig.5, it can be concluded that the neutral medium released relatively low molecular-weight GGM, while basic extraction allowed the recovery of high molecular mass xylans. 380

381	Table 1 . Weight-average (M_w) and number-average (M_n) molecular weights, polydispersity
382	index (PDI) and the polymerization degree (PD) of the hemicellulosic fractions obtained with
383	MAE at 573 W and conventional extraction
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388 380	4. Conclusion
390	In this study the extraction of the hemicelluloses polymers by MAE at atmospheric pressure was
391	investigated. The highest amount of hemicelluloses extracted was 27.5 mg/g of dry wood from pre-
392	soaked wood in 1M NaOH solution using microwave treatment at 573 W for 1 h over. At this
393	condition, the Mw of extracted hemicellulose chains was 66 kDa. At the same conditions, i.e.,
394	temperature and time, the yield and the Mw were lower using a conventional heating than microwave,
395	which highlights the efficacy of MAE for extraction of hemicelluloses chains from spruce sawdust.
396	Pre-soaking in sodium hydroxide improves the solubilization of wood and hence the extraction of high
397	molecular weight hemicelluloses. However, pre-soaking in water preserve acetyl groups in the
398	hemicellulose chains which could be advantageous in various polymer applications. High extraction
399	selectivity was observed as a function of the pre-soaking medium: acetylated galactoglucomannans
400	were isolated after pre-soaking in neutral medium and high molecular mass arabinoglucoronoxylans in
401	basic medium. MAE at atmospheric pressure and mild conditions (temperature < 100°C and pH 5) has
402	shown its effectiveness to extract high molecular weight hemicelluloses chains from spruce wood with
403	less depolymerization and degradation. This technology has a great potential as an alternative method
404	to autohydrolysis and hydrothermal treatments of wood in the aspects of molecular weight of extracted
405	hemicelluloses.
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411	Conflict of interest
412	The authors have no conflict of interest.
413	
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- 606 Figure captions:
- 607 Fig. 1. Scheme for extraction and separation of hemicelluloses from spruce sawdust using microwave
- 608 assisted extraction and conventional extraction.
- **Fig.2.** Solubilization of wood after MAE and conventional extractions (1 h under MW, 3h diffusion)
- 610 Fig.3. Yield and sugar composition of extracted hemicelluloses polymers in neutral medium (A) and
- 611 basic medium (B)
- **Fig.4**. Degradation products in the liquid fraction in basic medium (A) and ASL (%) (B)
- 613 Fig.5. Sugar composition of recovered hemicelluloses by MAE and conventional extraction
- 614 Fig.6. FTIR spectra of extracted hemicelluloses
- 615 Fig.7. HSQC spectra of extracted hemicelluloses (A) in neutral medium; (B) in basic medium

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620 Supplementary data:

- 621 S1. Effect of the impregnation and the diffusion on the yield of extracted hemicelluloses by MAE at
- 622 573 Watts in neutral and basic mediums
- 623 S2. Temperature profile during MAE and conventional extraction

625	Table caption:
626	
627	Table 1. Weight-average (M_w) and number-average (M_n) molecular weights, polydispersity
628	index (PDI) and the polymerization degree (PD) of the hemicellulosic fractions obtained with
629	MAE at 573 W and conventional extraction
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631	





Microwave power (Watts)



Microwave power (Watts)



Microwave power (Watts)







Wave number (cm⁻¹)



Table 1. Weight-average (M_w) and number-average (M_n) molecular weights, polydispersity index (PDI) and the polymerization degree (PD) of the

	Basic medium			Neutral Medium				
	Mn	$M_{ m w}$	PDI	PD	M _n	M _w	PDI	PD
Conventional	27266	56298	2.06	351	20730	37870	1.82	236
MAE 573 W	54227	66564	1.22	416	23875	41589	1.74	259

hemicellulosic fractions obtained with MAE at 573 W and conventional extraction