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## **Steam explosion of *Aucoumea klaineana* sapwood: membrane separation of acetylated hemicelluloses**

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17       **Abstract**

18

19           The fractionation of the aqueous effluent of *Aucoumea klaineana* Pierre (Okoumé)  
20 sapwood steam explosion was examined by a sequential-dilution type membrane  
21 diafiltration. The permeate and retentate fractions were characterized by HPLC-SEC,  
22 HSQC-NMR, FTIR, UV-visible and HPAE-PAD ion chromatography. Diafiltration with 10  
23 kDa regenerated cellulose membrane has been shown to provide efficient fractionation  
24 without fouling. O<sub>2</sub> and/or O<sub>3</sub> acetylated xylans with a lower proportion of O<sub>2</sub> and/or O<sub>3</sub>  
25 acetylated glucomannans were isolated in the retentate ( $\approx 35\%$  w/w and  $1.08\%$   
26 based on initial effluent solid content and on initial dry wood respectively), including  $65\%$   
27 w/w in the range 9-22 kDa). The molecular weights of the polysaccharides were  
28 significantly higher than those obtained by ethanolic precipitation. The permeate  
29 concentrated low molecular mass oligomers ( $90\%$  w/w  $< 2.3$  kDa,  $1.88\%$  w/w based on  
30 initial dry wood) composed of pectic sugars, highly acetylated xylans (DS  $\approx 0.9$ ) and  
31 relatively high proportion of soluble lignin ( $\approx 40\%$  w/w) including Lignin-Carbohydrate  
32 Complexes (LCCs).

33

34

35       **Keywords:** Steam explosion; *Aucoumea klaineana* Pierre; Diafiltration;  
36 Hemicelluloses, *Aucoumea klaineana* Pierre

37

38 **1. Introduction**

39 The search for an optimal use of wood according to the biorefinery concept is leading to a  
40 growing interest in the extraction of polymeric hemicelluloses which constitute the second  
41 component of wood. The potential application of hemicelluloses has been revealed in  
42 several areas, including hydrogels [1] and biodegradable barrier films for food packaging  
43 [2,3]. Also, hemicellulosic chains have demonstrated their importance in the fields of  
44 bioactive polymers, hydrocolloids or as additives for paper making [4].

45 Hardwoods contain two main types of hemicelluloses:

- 46 • *O*-acetyl-4-*O*-methylglucuronoxylan (10 to 35% of the wood mass) composed of a  
47 backbone of  $\beta$ -D-xylopyranosyl (Xylp) base units, attached by  $\beta$ -(1→4) bonds, and  
48 4-*O*-methyl- $\alpha$ -D-glucopyranosyl uronic acid (MeGlc pA) side groups by (1→2)  
49 bonds. Some xylose units are *O*-acetylated at C2 and/or C3.
- 50 • glucomannan (3-5% of wood mass) of low molecular weight with a ratio of one to  
51 two mannose units to one glucose unit forming  $\beta$ -(1,4) bonds. Glucomannans are  
52 usually acetylated on the C2 and/or C3 carbons of mannoses to the extent of one  
53 group every three units [5–8].

54 In wood hemicelluloses are closely interconnected with the other components of the cell  
55 wall. The linkage between hemicelluloses and lignin is provided through covalent bonds  
56 called **Lignin-Carbohydrate Complexes** (LCCs), including phenylglycoside bonds, ester  
57 bonds and benzyl ether bonds. Hemicelluloses and cellulose are connected through a  
58 strong hydrogen bond network [9,10]. Consequently, the use of hemicelluloses for the  
59 elaboration of high value-added products is conditioned by an efficient pretreatment of the  
60 lignocellulosic matrix aiming at destabilizing this complex. In this context, several physical  
61 and/or chemical pretreatment methods have been reported. Alkali extraction using sodium  
62 or potassium hydroxyde is among the simplest and commonly used processes [11]. This  
63 method is broadly used especially for xylans recovery in good yield from biomass. Due to  
64 the basic conditions used, deacetylated and poorly branched hemicelluloses are isolated  
65 [12]. Extraction by organic solvents such as dioxane and DMSO has also been reported by  
66 several authors [13–15]. This approach which promotes the extraction of high molecular-  
67 weight and acetylated hemicelluloses close to the native form is generally used for  
68 characterization purposes. Since few years, the isolation and characterization of Lignin-  
69 Carbohydrate-Complexes have also been attracting increasing interest, LCCs may have  
70 specific properties such as surfactant and/or biological properties [16,17]. The production  
71 of this kind of complex polysaccharides could be of great interest for high value-added

72 applications [18,19]. However, the relatively low yields and the high consumption of  
73 organic solvent limit the large-scale use of this method.

74 Steam explosion (SE) has proven to be a very efficient and promising process for biomass  
75 pretreatment and deconstruction. It is a thermomechanical and chemical process that is  
76 carried out under the combined effect of steam cracking and explosive decompression,  
77 providing an efficient depolymerization and extraction of soluble hemicelluloses, leaving  
78 most of the cellulose and lignin as solid residue [20]. To date, most of the work on SE  
79 focuses on the valorization of the cellulosic fraction for bioethanol production [21,22]. In  
80 2000, Glasser et al. described the extraction from barley husks of non-cellulosic  
81 heteropolysaccharides composed mainly of xylans by SE [23]. More recently and from the  
82 same biomass, Krawczyk et al. [24] studied the SE-extraction and fractionation of  
83 arabinoxylans for the production films for food packaging. In our group, Chadni et al. [25]  
84 described a selective extraction of hemicelluloses from softwood by SE depending on the  
85 pH of the medium.

86 In SE pretreatment, hemicelluloses are mainly recovered as oligo and polysaccharides in  
87 the liquid phase of the treatment in complex mixture with other biomolecules. Therefore,  
88 their recovery is a crucial step in the hemicelluloses upgrading process. Alcoholic  
89 precipitation of hemicelluloses with ethanol and methanol is a method broadly used at the  
90 lab scale. A prior neutralization of the alkaline extracts with an acid to a pH between 5.0  
91 and 5.5 is necessary to limit the depolymerization of hemicelluloses. However, the use of  
92 large volumes of alcohol and the co-precipitation of minerals are inherent limitations of  
93 hemicelluloses precipitation. Alternative methods such as gel permeation chromatography  
94 [26] and CO<sub>2</sub> anti solvent precipitation [14] have also been explored for the separation and  
95 purification of hemicelluloses.

96 Compared to the aforementioned separation techniques, the ultrafiltration membrane  
97 process could have many advantages, such as low cost, energy savings and  
98 environmental friendliness [27]. Ultrafiltration is a well-known and widely used organic  
99 solvent-free membrane process for the separation and purification of complex mixtures of  
100 biomolecules in solution. Because of this, maximum purification of the retained solute is  
101 achieved in diafiltration mode by diluting the retentate with water or a buffer solution for  
102 selective separation of compounds based on their molecular weights. The efficiency of the  
103 separation of hemicelluloses is therefore strongly dependent on the nature of the  
104 membranes (hydrophobic/hydrophylic) used. It has been reported that the feasibility of the  
105 membrane separation process is improved by using hydrophilic membranes made of  
106 polysulfone or surface-modified polyvinylidene fluoride with a cut-off ranging from 1 to 10

107 kDa and possessing a high shear rate inducing lower fouling or concentration polarization  
108 [28]. Indeed, hydrophobic species, such as lignin or extractives (resin) can foul  
109 hydrophobic membranes during ultrafiltration whereas almost no fouling was observed by  
110 using hydrophobic membranes [29,30].

111 Several publications have been published concerning the optimization of membrane  
112 fractionation processes on hemicelluloses hydrolysates. The main problems usually  
113 encountered were membrane fouling and lignin removal [31–34]. Nitzsche et al. recently  
114 described the optimization of ultrafiltration of beechwood hydrolysate using an  
115 experimental design method [35]. By means of response surface methodology, the  
116 multidimensional correlation of the parameters transmembrane pressure, temperature, and  
117 pH value with the performance parameters (permeate flux, hemicellulose sugar retention,  
118 and lignin retention) was studied allowing to determine the optimum parameters of  
119 ultrafiltration. However, the fractionation and fine characterization of highly acetylated  
120 hemicelluloses has never been addressed.

121 *Aucoumea klaineana* Pierre (Okoumé) is a Central African tropical hardwood found  
122 mainly in the Republic of Congo and Gabon. It accounts for about 130,000,000 m<sup>3</sup> of the  
123 total 400,000,000 m<sup>3</sup> of Gabon's forest reserve. Approximately 750,000 m<sup>3</sup>/year of waste  
124 wood (~31% Okoumé) is produced out of approximately 1,500,000 m<sup>3</sup> of logs processed  
125 annually in Gabon [36]. In a recent paper [37], we described the isolation and  
126 characterization of acetylated glucuronoxylans and glucomannans from Okoumé sapwood  
127 by a SE process followed by ethanolic precipitation. The objective of the present work is to  
128 evaluate a fractionation by a sequential-dilution type membrane diafiltration. A  
129 characterization of the molecular weights, the sugars content and the acylation degree has  
130 been carried out with the final goal of anticipating new uses for these insufficiently  
131 valorized wood components.

132

## 133 **2. Materiel and method**

134

### 135 **2.1. Raw material**

136 The samples used in this study were taken from an Okoumé log in the Gabonese natural  
137 forest of Nzamaliqúé by the Société Equatoriale d'Exploitation Forestière (SEEF). The  
138 experiments were performed on the sapwood slices cut with a saw and then ground with a  
139 knife mill and sieved to obtain sawdust of about 2 mm.

140

### 141 **2.2. Pretreatment by steam explosion**

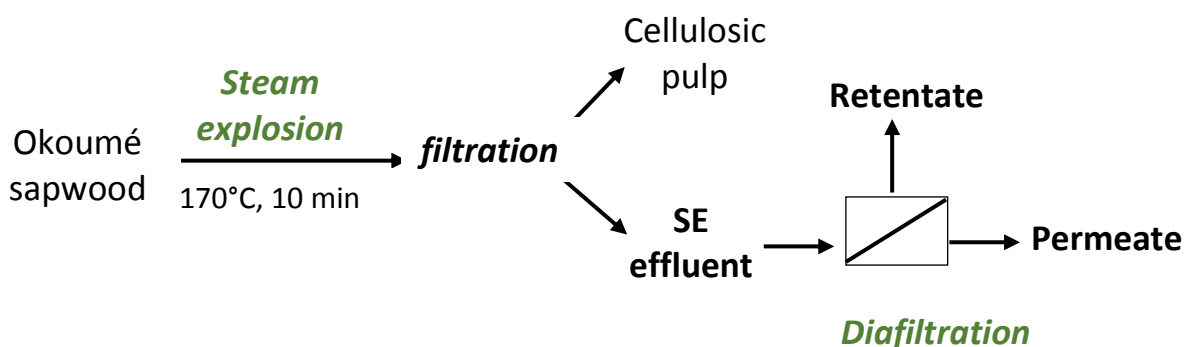
142 50 g of biomass dried at 105°C overnight was impregnated at room temperature for 15 h in  
143 **ultrapure water** (solid/liquid ratio = 1:7). The impregnated biomass was recovered with a  
144 filter paper and introduced directly into a water pressurized reactor. **Saturated steam at**  
145 **high pressure and temperature (8 bars, 170 °C) is injected into this batch reactor by the**  
146 **mean of a steam generator. After a short residence time (10 min) a sudden pressure drop**  
147 **is induced by a ball valve, creating hydrolytic breakdown of the LCC.** Then, after  
148 pretreatment, a solid pulp (rich in both lignine and cellulose) is separated from the  
149 aqueous hydrolysate (rich in hemicelluloses) by vacuum filtration with filter paper with a  
150 retention particle size of 5-13 µm (VWR International, Fontenay-sous-Bois, France).

151

### 152 2.3. Ultrafiltration and diafiltration

153 **A Cogent Process Scale** tangential flow system (Millipore, Molsheim, France) was used to  
154 perform the filtration experiments. Ultrafiltration (UF) was performed on 3, 10, 100 and 500  
155 kDa NMWCO (Nominal Molecular Weight CutOff) regenerated cellulose UF hollow fiber  
156 membranes (Molsheim, Millipore, France) with a surface area of 340 cm<sup>2</sup>. 100 mL of the  
157 explanted hydrolysate was collected and ultrafiltered at room temperature at 2.0 bar  
158 transmembrane pressure (TMP). After 10 minutes of total circulation, permeate and  
159 retentate were sampled for analysis.

160 **The 10 kDa membrane was selected for 100 mL diafiltration (DF) of the hydrolysate.**  
161 **Transmembrane pressure was maintained at 1.5 bar. Diafiltration consisted of keeping the**  
162 **feed volume constant by adding deionized water. The volume of water added at each DF**  
163 **cycle was equal to the volume of the retentate. Sequential-dilution type diafiltration was**  
164 **conducted up to 6 diafiltration volumes (DV). At each diavolume, a flow to the retentate**  
165 **was collected for analyses. At the end of the experiment, the overall permeate**  
166 **concentrated to 100 mL and the final retentate were freeze-dried and then analyzed**  
167 **(Figure 1). The two dry extracts obtained are named **Permeate** and **Retentate****  
168 **respectively.**



169

170 **Figure 1.** Separation of hemicellulose





#### 172 2.4. Determination of sugars, uronic acids and soluble lignin

173 The processing and analysis of the chemical composition of the extracts was adapted from  
174 the National Renewable Energy Laboratory (NREL/TP-510-42623) standard. Briefly, 15  
175 mg of hemicelluloses powder were dissolved in 9.5 mL of ultrapure water. 349  $\mu\text{L}$  of 72 %  
176  $\text{H}_2\text{SO}_4$  was added to reach an acid concentration of 4 %. Samples were then incubated at  
177 121 °C in for 1 h. The sample volume was then completed to 10 mL. For the steam  
178 explosion pretreatment, 2.5 mL of the resulting liquid phase were hydrolyzed by adding 87  
179  $\mu\text{L}$  of 72 %  $\text{H}_2\text{SO}_4$  to reach a final acid concentration of 4 %. Resulting solutions were  
180 diluted then analyzed for monosaccharides and uronic acids content by high-performance  
181 anion exchange chromatography coupled with pulsed amperometric detection (HPAEC-  
182 PAD, ICS-3000 Dionex) with a Dionex CarboPac PA-20 analytical column (3 x 150 mm)  
183 and its guard-column (3 x 30 mm). The separation is done at 35 °C at an isocratic flow rate  
184 of 0.4  $\text{mL}\cdot\text{min}^{-1}$ , with an eluent consisting of soda (250 mM), water and sodium acetate (1  
185 M) in variable proportion (concentration gradient). An external calibration is performed with  
186 7 calibration points per sugar or uronic acid [38]. The acid soluble lignin (ASL) content was  
187 determined from absorbance at 205 nm according to Lin and Dence [39].

#### 189 2.5. Steric exclusion chromatography (SEC)

190 The molar mass distribution of the ultrafiltered and diafiltered fractions was determined  
191 by size exclusion chromatography coupled to a refractive index detector (Shimadzu  
192 Prominence). Two serially coupled columns are used (Phenomenex GFC-P2000, 3000) to  
193 separate hemicelluloses. Samples are separated at 37°C; 0.25 M aqueous sodium nitrate  
194 ( $\text{NaNO}_3$ ) solution is used as eluent with a flow rate of 0.7  $\text{mL}\cdot\text{min}^{-1}$ . A pullulan  
195 polysaccharide calibration kit was used, including 10 pullulan standards, with molecular  
196 weight varying from 180 Da to 700 kDa [ Agilent Technologies, Pullulan Polysaccharide  
197 Calibration Kit, Part number: PL2090-0101]. Mw and Mn values are determined after  
198 slicing the chromatogram and by using Shimadzu LabSolutions software.

#### 200 2.6. Acetyl contents

201 Acetyl content of DF fractions was determined after acetic acid determination by adapting  
202 the protocol of Yuan et al., 2006. 1000  $\mu\text{L}$  of the DF fractions (10 mg of permeate or  
203 retentate in 5 mL of water) are saponified with NaOH (1250  $\mu\text{L}$  at 0.4 M) for 2 h in the  
204 dark, and at room temperature in a 5 mL volumetric flask. The reaction is stopped by  
205 adding 0.4 M  $\text{H}_3\text{PO}_4$  to the mark. The solutions are analyzed by HPLC (Shimadzu  
206 Prominence). The compounds are separated at 30 °C on a C18-Luna 2 column

207 (Phenomenex) with a flow rate of 0.8 mL.min<sup>-1</sup>. The eluent is a 5.10<sup>-3</sup> M sulfuric acid  
208 solution. The detection is performed by UV at 210 nm. An external calibration with 5  
209 calibration points is performed.

210

## 211 **2.7. Fourier transform infrared spectroscopy**

212 Fourier transform infrared spectroscopy (FTIR) analysis was performed on the DF fraction  
213 powders. The infrared spectra were acquired on a Thermo Scientific iD3ZnSe ATRdNicolet  
214 iS 5 FTIR spectrometry unit for a frequency range of 4000 cm<sup>-1</sup> to 650 cm<sup>-1</sup>. The spectra  
215 were analyzed by Thermo Scientific OMNIC software.

216

## 217 **2.8. 2D HSQC NMR**

218 The 2-dimensional heteronuclear singular quantum coherence (HSQC) nuclear magnetic  
219 resonance (NMR) analyses were performed on a 400-MHz Bruker instrument with a  
220 standard Bruker program. The powders (~ 60 mg) were dissolved in 0.5 mL of 99.7 %  
221 deuterium oxide (D<sub>2</sub>O) and filtered on cotton. The analysis was made at room temperature  
222 (25°C). The number of complex points collected was 1024 for the <sup>1</sup>H dimension with a  
223 relaxation of 1.5- and 256 time increments were recorded in the <sup>13</sup>C dimension.  
224 Calculations of DS<sub>Ac</sub> Xyl<sub>p</sub> and DS<sub>Ac</sub> Man<sub>p</sub> are obtained using equations 1 and 2.

225

226

Eq 1.

$$DS(Ac) Xyl_p = \frac{[Ac]^a}{\frac{[Man + Xyl]^b}{1 + \frac{[Man Ac]^c}{[Xyl AC]^c}}}$$

Eq 2.

$$DS(Ac) Man_p = \frac{[Ac]^a}{[Man + Xyl]^b} - DS(Ac) Xly$$

227

228 a) Acetyl content determined by HPLC; b) Sugars content determined by HPEAC-PAD; c)  
229 Integration ratio by HSQC-NMR

230

231 **Table 1.** Sugar composition of okoumé sapwood, SE effluent, Retentate and Permeate.

	wood	SE effluent	Permeate	Retentate
DM % <sup>a</sup>			63.06	35.60
yield % <sup>b</sup>			1.88	1.08
Fuc <sup>c</sup>	0.1 (0.0)	0.7 (0.0)	0.8 (0.0)	0.1 (0.0)
Rha <sup>c</sup>	0.5 (0.0)	4.0 (0.7)	2.5 (0.0)	3.0 (0.1)
Ara <sup>c</sup>	0.8 (0.0)	15.2 (0.1)	15.7 (0.5)	1.5 (0.0)
Gal <sup>c</sup>	1.1 (0.0)	9.9 (0.2)	6.9 (0.2)	6.8 (0.1)
Glc <sup>c</sup>	76.5 (0.2)	15.13 (0.0)	5.6 (0.2)	15.6 (0.8)
Xyl <sup>c</sup>	17.0 (0.0)	31.4 (0.1)	15.0 (1.4)	29.6 (0.4)
Man <sup>c</sup>	3.3 (0.0)	7.3 (0.3)	4.4 (0.4)	5.0 (0.1)
GalUA <sup>c</sup>	0.8 (0.3)	4.8 (0.0)	2.3 (0.1)	4.4 (0.0)
GlcUA <sup>c</sup>	0.2 (0.0)	0.4 (0.0)	0.2 (0.0)	0.3 (0.0)
MeGlcUA <sup>d</sup>	nq <sup>g</sup>	nq	2.8	17.4
Ac <sup>e</sup>	nq	nq	5.4	10.9
ASL <sup>f</sup>	-	11.1 (5.0)	38.5 (0.2)	5.5 (0.3)

232 <sup>a</sup> Dry matter content (%) in Peremate and Retentate based on initial SE effluent DM233 <sup>b</sup> wt% based on initial dry wood.234 <sup>c</sup> Xylose, mannose, glucose, arabinose, galactose, Rhamnose, galacturonic and glucuronic acid %, determined after hydrolysis by HPEAC-PAD.235 <sup>d</sup> Estimation by HSQC-NMR.236 <sup>e</sup> Acetic acid quantified by HPLC after saponification.237 <sup>f</sup> Acid soluble lignin, estimation by UV-visible238 <sup>g</sup> Not quantified

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### 3. Results and discussion

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#### 3.1. Ultrafiltration - Diafiltration

250

In a preliminary study, the fractionation (in full recirculation mode) of SE liquid effluent was

optimized using three ultrafiltration membranes (cutoff 10 kDa, 100 kDa and 500 kDa). The

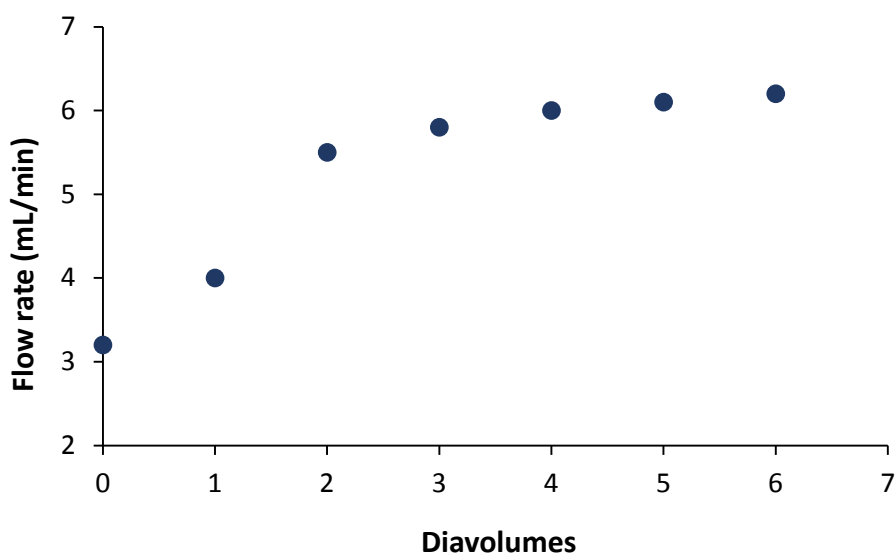
retentate and permeate were recovered and analyzed by steric exclusion chromatography

(Suppl Materials - SM1-3). The 10 kDa membrane, which gave the better results with a

high intensity of the peaks of high molecular weight compounds in the retentate, was

254

255 selected. Ultrafiltration of steam explosion liquor was made on diafiltration mode in order to  
256 get a better separation. Figure 2 shows the permeate flow rate evolution during diafiltration  
257 (up to 6 diavolumes). The flow rate increased until the third diavolume and tends to  
258 stabilize thereafter. After membrane flushing with water, no significant reduction in  
259 permeate flux was observed, indicating that fouling of the membrane was negligible.  
260 Fouling was however expected due to the high polarity of the chosen membrane material  
261 (regenerated cellulose). An increase in flux is observed in Figure 2, which is explained by  
262 a reduction in the viscosity of the permeate probably due to a decrease in the  
263 concentration of low molar weight molecules during DF. This behavior was already  
264 observed during DF of other polysides separation [40].  
265



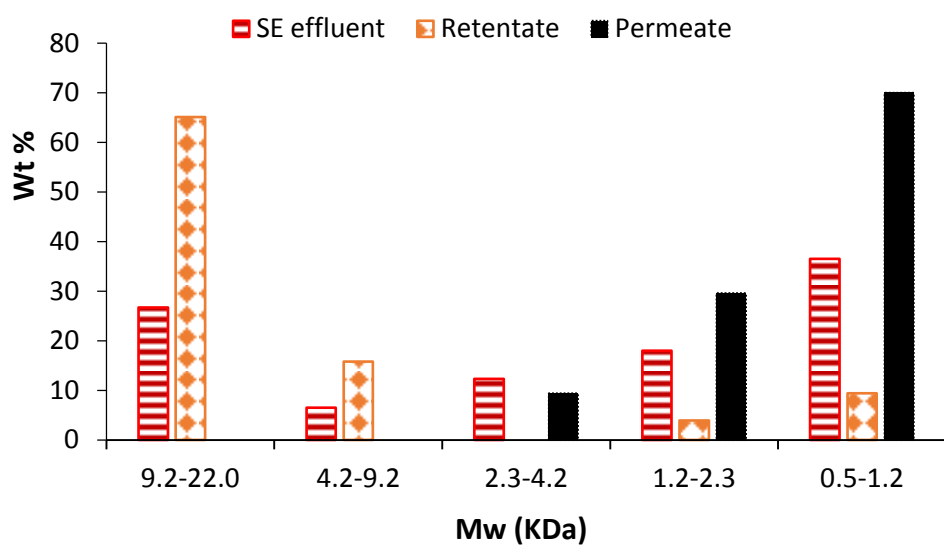
266

267 **Figure 2.** Permeate flow rate evolution during diafiltration

268

269 Dry matter content measurements (Table 1) showed higher proportions of dry matter yield  
270 in the **Permeate** with 63.1 wt% (based on SE effluent dry matter). Based on initial dry  
271 wood, **Permeate** and **Retentate** dry extracts accounted for 1.88 % w/w and 1.08 % w/w  
272 respectively. The efficiency of the fractionation process has been clearly demonstrated by  
273 HPLC-SEC. **SE effluent**, **Permeate** and **Retentate** fractions were analyzed by Size  
274 Exclusion Chromatography. The chromatograms obtained showed five main signals from  
275 0.5 kDa to 22 kDa. The distribution w/w in the 5 compartments is given in Figure 3 for the  
276 **Permeate**, **Retentate** and **SE effluent** (see Supplementary Materials - SM3). The  
277 number-average molecular weight ( $M_n$ ), weight average molecular weight ( $M_w$ ) and  
278 polydispersity ( $IP = M_w/M_n$ ) are also given in the SM 4. It can be seen that the SE effluent  
279 exhibited a broad molecular weight distribution and that the diafiltration process resulted in

280 efficient separation. In the **Retentate**, 65% w/w of the extracts contained hemicelluloses in  
 281 the range 9.2-22 KDa and  $\approx 80\%$  w/w had  $M_w > 4.5$  kDa. Interestingly, the **Retentate**  
 282 fraction concentrated hemicelluloses of significantly higher molecular weight than those  
 283 previously recovered by ethanolic precipitation ( $M_w \approx 9$  kDa) [37]. On the other hand, the  
 284 **Permeate** contained smaller oligomers in the range 0.5-1.2 kDa,  $\approx 90\%$  w/w with  $M_w < 2.3$   
 285 kDa. This observation is in good agreement with the study performed with barley husks  
 286 where the average molecular weight of polysaccharides was 24,000 g/mol after steam  
 287 explosion (liquid A) and 54,000 g/mol after diafiltration [24].  
 288



289  
 290  
 291 **Figure 3.** Mw distribution determined by SEC of Permeate, Retentate and SE effluent  
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293  
 294 **3.2. Composition of hemicelluloses**

295  
 296 The hemicelluloses fractions isolated in this study were analyzed in terms of  
 297 monosaccharides by ion chromatography after controlled acid hydrolysis, acetyl group  
 298 content by saponification and HPLC analysis and soluble lignin by UV quantification. **The**  
 299 **composition of SE effluent, Permeate and Retentate are compared to wood composition**  
 300 **in Table 1. Considering the complexity of the mixture to be analyzed, the results are**  
 301 **reported as relative composition by weight % in order to facilitate the comparison of the**  
 302 **fractions.**

303 In the **SE effluent**, as expected high amounts of xylose (34.7 %) were detected, xylans  
 304 being the predominant non-cellulosic polysaccharides in okoumé sapwood accounting for  
 305 80 % w/w of the hemicellulose [37]. This result is consistent with the hydrolysis of non-

306 cellulosic polysaccharides by autohydrolysis during the SE. The presence of mannose and  
307 glucose (8.1 % and 16.7 % respectively) is justified by the fact that okoumé sapwood  
308 contained  $\approx$  5-6% of glucomannans. The significantly higher proportions of Ara, Gal, Rha  
309 and GalUac were detected in the **SE effluent** compared to sapwood composition (16.8 %,  
310 11.0 %, 4.4 % and 5.3 % compared to 0.5 %, 0.7 %, 0.6 % and 0.1 % respectively). This is  
311 due to the preferential hydrolysis of the primary cell wall and middle lamella during SE  
312 previously reported [41], [42], these tissues being rich in pectic polysaccharides such as  
313 rhamnogalacturonans and arabinans.

314 It can be noticed from Table 1 that diafiltration allowed an efficient separation of arabinose  
315 which is primarily recovered in the **Permeate** fraction. This result is explained by the high  
316 susceptibility of arabinan chains toward hydrolysis producing almost exclusively  
317 monomeric arabinose [43]. An efficient recovery of soluble lignin in the **Permeate** was also  
318 observed. This result is in accordance with Sun and Yomkinson [44] who showed that  
319 soluble lignin produced by uncatalyzed-SE of hardwood was composed of small lignin  
320 fragments ( $M_w \approx 2500$  Da,  $M_w/M_n = 1.5$ ).

321 Xylose is still the dominant sugar in the retentate of diafiltration attesting the recovery of  
322 high molecular weight xylans. Interestingly, the ratio Man/Glc in **Retentate** (Man/Glc  $\approx$   
323 0.40) was lower than that of **Permeate** (Man/Glc  $\approx$  0.77) and did not match that in okoumé  
324 glucomannans previously reported (Man/Glc  $\approx$  1). This relatively high glucan concentration  
325 in the retentate can be explained by the retention of high molecular weight cellulose  
326 fragments produced during the explosive decompression of SE.

327

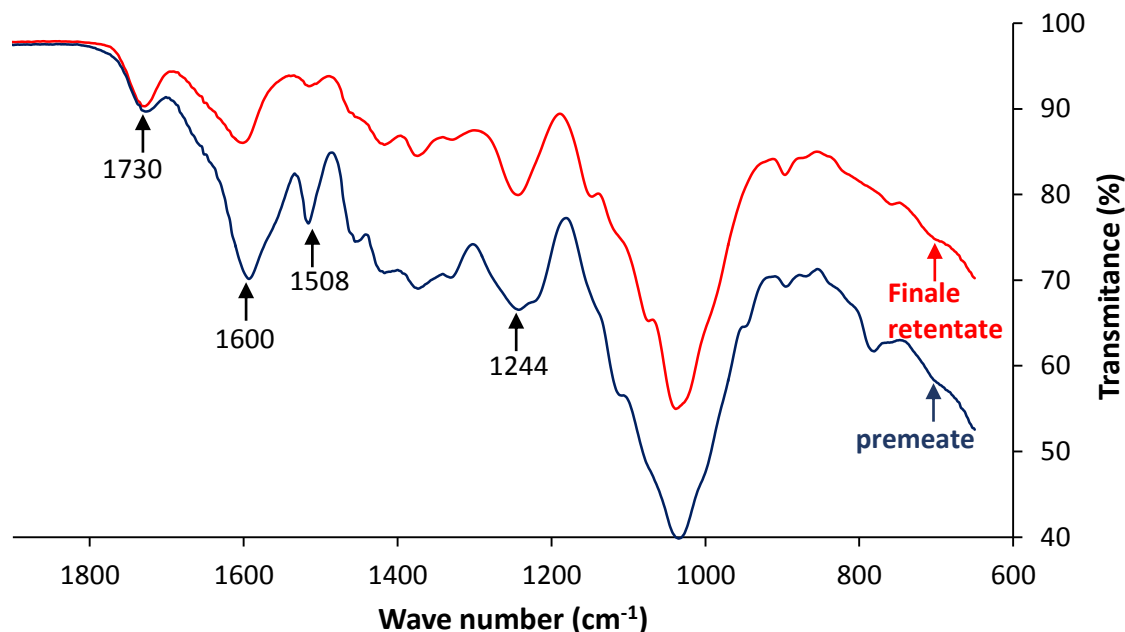
328 The FTIR spectra of the **Permeate** and **Retentate** are shown in **Figure 4** and are in good  
329 agreement with other works ([15], [45]). The acetylation of all the fractions has been  
330 confirmed by the band at  $1730\text{ cm}^{-1}$  characteristic of the stretching of the carbonyl group  
331 (C=O) of the ester group and by the peak at  $1244\text{ cm}^{-1}$  assigned to C-O  $\delta$  (C-O)  
332 deformation of the ester functions. All hemicellulose fractions contain the band at  $1595$ -  
333  $16031\text{ cm}^{-1}$  attributed to adsorbed water and/or  $\nu(\text{COO}^-)$  uronic acids of hemicelluloses  
334 chains [46]. In agreement with the UV quantification (Table 1), the stronger signal at  $1508$   
335  $\text{cm}^{-1}$  for the **Permeate**, attributed to C=C stretching in the aromatic ring of the lignin,  
336 confirmed the higher amounts of residual lignin than for the **Retentate** (Table 1).

337

338 2D HSQC NMR analysis was used to better understand the molecular structure of the  
339 diafiltration **Retentate** and **Permeate** fractions. The NMR spectra are presented in Figure

340 5 and in Supplementary Material section (SM 5). Assignments of the different signals were  
341 made with respect to literature data [47–50].

342



343

344

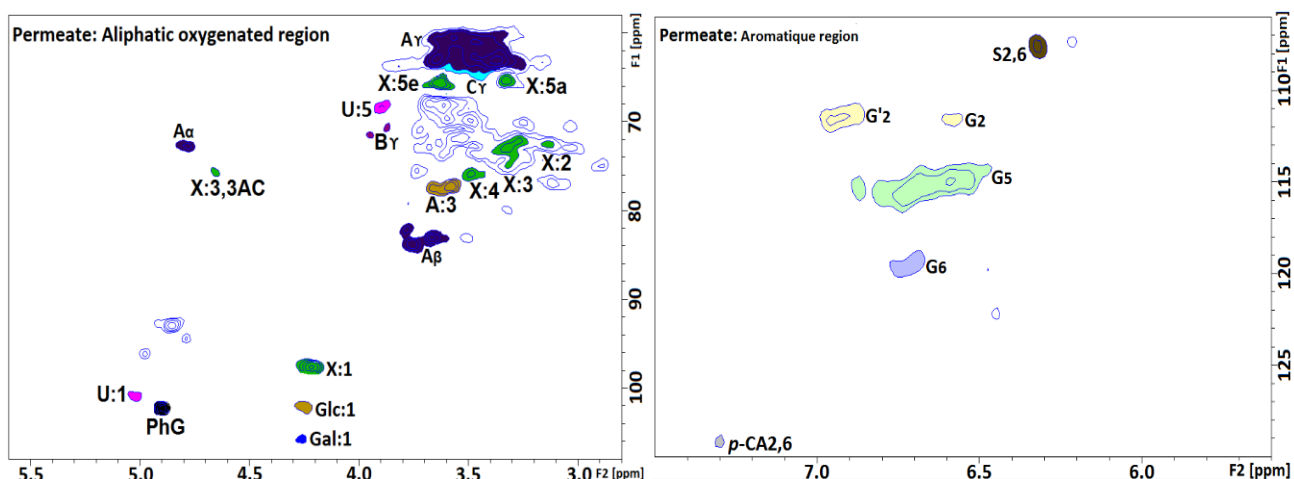
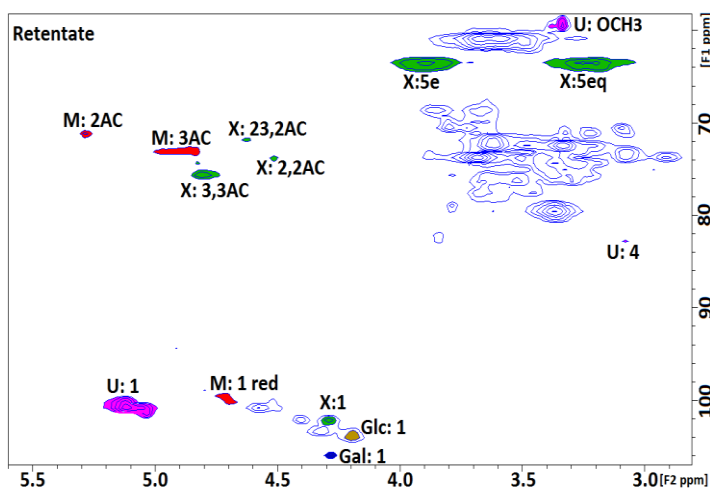
345 **Figure 4.** FTIR spectra of **Retentate** and **Permeate**

346

347 The  $^{13}\text{C}/^1\text{H}$  NMR correlations corresponding to the O-acetyl groups of hemicelluloses  
348 through the signals of of [O-Ac(1→2/3)- $\beta$ -D-Xylp] and [O-Ac(1→2/3)- $\beta$ -D-Manp] were  
349 gathered in Table 2 and determined the distribution pattern of signals. The acetic acid  
350 content presented in Tables 1 and 2 was determined by HPLC after saponification. The  
351 acetylation of **Permeate** and **Retentate** clearly appeared in both HSQC spectra (Figures 5  
352 and SM 5). The acetylation degrees were determined by HPLC and are given in Table 1.  
353 Interestingly, it appears that diafiltration allowed to recover highly acetylated fraction in the  
354 retentate (13.2 % w/w). Based on previous report, a semi quantification of acetyl units in  
355 xylan and mannan backbone was performed using NMR and HPLC data [37] and is  
356 gathered in Table 2. For **Retentate**, it is observed that both xylose and mannose residues  
357 are O2 and/or O3 acetylated with a comparable degree of acetylation ( $\text{DS}_{\text{Ac}} \approx 0.40$ ). The  
358 permeate displayed globally a lower acetylation content (5.4 % w/w). However, according  
359 to the NMR data the acetylation in **Permeate** involved mostly the xylose units which are  
360 highly acetylated ( $\text{DS}_{\text{Ac}} \approx 0.9$ ). A lower proportion of MeGlcUA was also detected in  
361 **Permeate** compared to **Retentate**. Soluble lignin was identified in the aromatic region of  
362 the HSQC NMR spectrum of the **Permeate** (SM 5). An intense cross peak at  $\delta\text{H}/\delta\text{C}$ -  
363 3.60/56.19 ppm attested the presence of lignin methoxy groups. Guaiacyl and syringyl



364 residues in soluble lignin were confirmed by the cross peak at  $\delta\text{H}/\delta\text{C}$ -6.92-111.33 ppm  
 365 ( $\text{C}_2/\text{H}_2$  in G),  $\delta\text{H}/\delta\text{C}$ -6.69/115.28 ppm ( $\text{C}_5/\text{H}_5$  in G),  $\delta\text{H}/\delta\text{C}$ -6.70/119.28 ppm ( $\text{C}_6/\text{H}_6$  in G)  
 366 and  $\delta\text{H}/\delta\text{C}$ -6.57/104.42 ppm ( $\text{C}_{2,6}/\text{H}_{2,6}$  in S). Small amounts of H units were also detected  
 367 at 7.30/129.18 ppm ( $\text{C}_{2,6}/\text{H}_{2,6}$  in H). The presence of aryl-ether bonds seems to be attested  
 368 by the cross signals at  $\delta\text{H}/\delta\text{C}$ -4.79/72.66 ppm and  $\delta\text{H}/\delta\text{C}$ -3.70/82.90 ppm assigned to  
 369  $\text{CH}_\alpha$  and  $\text{CH}_\beta$  in  $\beta$ -O-4 interunit linkages respectively. According to previous papers,  
 370 lignin-carbohydrate linkages in LCC can be detected by HSQC NMR especially the phenyl-  
 371 glycosidic bond correlation signal which was clearly assigned in the lateral chain region.  
 372 The detection of this cross signal at  $\delta\text{H}/\delta\text{C}$ -4.90/102.08 ppm in the **Permeate** suggested  
 373 the presence of LCCs and justified the high-water solubility of these sugar-lignin  
 374 fragments. **This observation is in agreement with previous results showing that LCCs**  
 375 **extracted by TMP or hydrolytic processes exhibited low molecular mass (0.4 - 7 kDa) and**  
 376 **high water solubility [51,52].**



377

378

379 **Figure 5.** 2D HSQC NMR Spectral analysis of hemicellulose: X: (1 → 4)  $\beta$ -D-Xylopyranosyl ; U: 4-  
 380 O-methyl- $\alpha$ -D-glucuronic acid ; M: (1 → 4)  $\beta$ -D-Mannosyl ; G: (1 → 4)  $\beta$ -D- Glucosyl ; A:  $\alpha$ -L-  
 381 Aranosyl ; Ac: acetyl; A: C $\gamma$ /H $\gamma$  in  $\beta$ -O-4 linkage ; A: C $\beta$ /H $\beta$  in  $\beta$ -O-4 linkage from guaiacyl units ; C:  
 382 C $\gamma$ /H $\gamma$  in phenylcoumaran substructure ; S: C<sub>2,6</sub>/H<sub>2,6</sub> in syringyl units ; G: C<sub>2</sub>/H<sub>2</sub> in guaiacyl units;



383 G': C2/H2 in oxidized guaiacyl units; G: C5/H5 in guaiacyl units ; G: C6/H6 in guaiacyl units ; p-  
384 CA,6: C2,6/H2,6 in p-hydroxybenzoate units;  
385

386

## 387 **Conclusion**

388 The steam explosion process of okoumé wood produced not only a pretreated  
389 cellulosic pulp but also a water effluent containing acid soluble lignin and  
390 oligo/polysaccharides displaying a broad of molecular weight distribution. The fractionation  
391 of this aqueous stream by diafiltration with a 10 kDa membrane allowed the separation of  
392 acetylated xylans and glucomannans of relatively high molecular weight from small pectic  
393 oligomers, low molecular mass xylans and soluble lignin including lignin carbohydrate  
394 complexes. The results presented in this paper are part of a research program aiming to  
395 valorize the co-products of the okoumé industry in Gabon according to the concept of  
396 biorefinery by using all the components of the feedstock including non-cellulosic  
397 biopolymers.

398

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