



Steam explosion of *Aucoumea klaineana* sapwood: Membrane separation of acetylated hemicelluloses

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

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Abstract

The fractionation of the aqueous effluent of *Aucoumea klaineana* Pierre (Okoumé) sapwood steam explosion was examined by a sequential-dilution type membrane diafiltration. The permeate and retentate fractions were characterized by HPLC-SEC, HSQC-NMR, FTIR, UV-visible and HPAE-PAD ion chromatography. Diafiltration with 10 kDa regenerated cellulose membrane has been shown to provide efficient fractionation without fouling. O2 and/or O3 acetylated xylans with a lower proportion of O2 and/or O3 acetylated glucomannans were isolated in the retentate ($\approx 35\%$ w/w and 1.08% w/w% based on initial effluent solid content and on initial dry wood respectively, including 65% w/w in the range 9-22 kDa). The molecular weights of the polysaccharides were significantly higher than those obtained by ethanolic precipitation. The permeate concentrated low molecular mass oligomers (90% w/w < 2.3 kDa, 1.88% w/w% based on initial dry wood) composed of pectic sugars, highly acetylated xylans (DS ≈ 0.9) and relatively high proportion of soluble lignin ($\approx 40\%$ w/w) including Lignin-Carbohydrate Complexes (LCCs).

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

1. Introduction

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

Hardwoods contain two main types of hemicelluloses:

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

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

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

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

In SE pretreatment, hemicelluloses are mainly recovered as oligo and polysaccharides in the liquid phase of the treatment in complex mixture with other biomolecules. Therefore, their recovery is a crucial step in the hemicelluloses upgrading process. Alcoholic precipitation of hemicelluloses with ethanol and methanol is a method broadly used at the lab scale. A prior neutralization of the alkaline extracts with an acid to a pH between 5.0 and 5.5 is necessary to limit the depolymerization of hemicelluloses. However, the use of large volumes of alcohol and the co-precipitation of minerals are inherent limitations of hemicelluloses precipitation. Alternative methods such as gel permeation chromatography [26] and CO₂ anti solvent precipitation [14] have also been explored for the separation and purification of hemicelluloses.

Compared to the aforementioned separation techniques, the ultrafiltration membrane process could have many advantages, such as low cost, energy savings and environmental friendliness [27]. Ultrafiltration is a well-known and widely used organic solvent-free membrane process for the separation and purification of complex mixtures of biomolecules in solution. Because of this, maximum purification of the retained solute is achieved in diafiltration mode by diluting the retentate with water or a buffer solution for selective separation of compounds based on their molecular weights. The efficiency of the separation of hemicelluloses is therefore strongly dependent on the nature of the membranes (hydrophobic/hydrophilic) used. It has been reported that the feasibility of the membrane separation process is improved by using hydrophilic membranes made of 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 m³ of the
123 total 400,000,000 m³ of Gabon's forest reserve. Approximately 750,000 m³/year of waste
124 wood (~31% Okoumé) is produced out of approximately 1,500,000 m³ 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 Nzamaligué 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

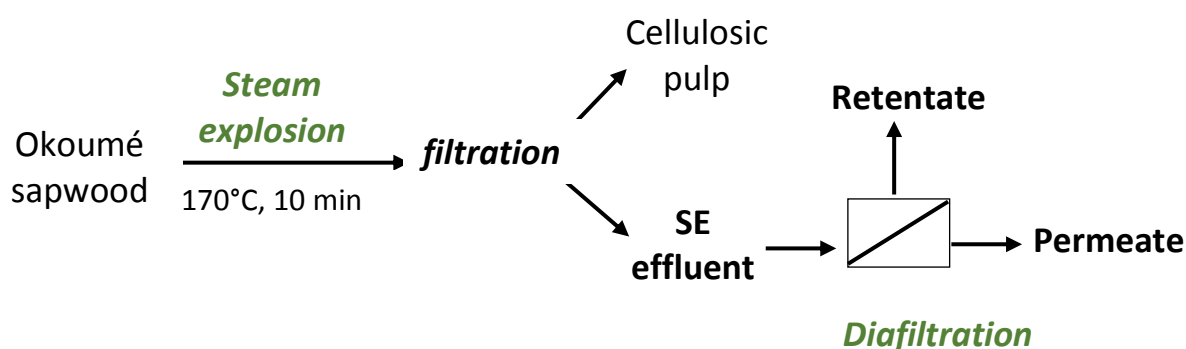
142 50 g of biomass dried at 105°C overnight was impregnated at room temperature for 15 h in
 143 **ultrapure water** (solid/liquid ratio = 1:7). The impregnated biomass was recovered with a
 144 filter paper and introduced directly into a water pressurized reactor. **Saturated steam at**
 145 **high 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 cm². 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

2.4. Determination of sugars, uronic acids and soluble lignin

The processing and analysis of the chemical composition of the extracts was adapted from the National Renewable Energy Laboratory (NREL/TP-510-42623) standard. Briefly, 15 mg of hemicelluloses powder were dissolved in 9.5 mL of ultrapure water. 349 μL of 72 % H_2SO_4 was added to reach an acid concentration of 4 %. Samples were then incubated at 121 $^{\circ}\text{C}$ in for 1 h. The sample volume was then completed to 10 mL. For the steam explosion pretreatment, 2.5 mL of the resulting liquid phase were hydrolyzed by adding 87 μL of 72 % H_2SO_4 to reach a final acid concentration of 4 %. Resulting solutions were diluted then analyzed for monosaccharides and uronic acids content by high-performance anion exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD, ICS-3000 Dionex) with a Dionex CarboPac PA-20 analytical column (3 $\times$ 150 mm) and its guard-column (3 $\times$ 30 mm). The separation is done at 35 $^{\circ}\text{C}$ at an isocratic flow rate of 0.4 $\text{mL}\cdot\text{min}^{-1}$, with an eluent consisting of soda (250 mM), water and sodium acetate (1 M) in variable proportion (concentration gradient). An external calibration is performed with 7 calibration points per sugar or uronic acid [38]. The acid soluble lignin (ASL) content was determined from absorbance at 205 nm according to Lin and Dence [39].

2.5. Steric exclusion chromatography (SEC)

The molar mass distribution of the ultrafiltered and diafiltered fractions was determined by size exclusion chromatography coupled to a refractive index detector (Shimadzu Prominence). Two serially coupled columns are used (Phenomenex GFC-P2000, 3000) to separate hemicelluloses. Samples are separated at 37 $^{\circ}\text{C}$; 0.25 M aqueous sodium nitrate (NaNO_3) solution is used as eluent with a flow rate of 0.7 $\text{mL}\cdot\text{min}^{-1}$. A pullulan polysaccharide calibration kit was used, including 10 pullulan standards, with molecular weight varying from 180 Da to 700 kDa [Agilent Technologies, Pullulan Polysaccharide Calibration Kit, Part number: PL2090-0101]. Mw and Mn values are determined after slicing the chromatogram and by using Shimadzu LabSolutions software.

2.6. Acetyl contents

Acetyl content of DF fractions was determined after acetic acid determination by adapting the protocol of Yuan et al., 2006. 1000 μL of the DF fractions (10 mg of permeate or retentate in 5 mL of water) are saponified with NaOH (1250 μL at 0.4 M) for 2 h in the dark, and at room temperature in a 5 mL volumetric flask. The reaction is stopped by adding 0.4 M H_3PO_4 to the mark. The solutions are analyzed by HPLC (Shimadzu Prominence). The compounds are separated at 30 $^{\circ}\text{C}$ on a C18-Luna 2 column

207 (Phenomenex) with a flow rate of 0.8 mL.min⁻¹. The eluent is a 5.10⁻³ 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⁻¹ to 650 cm⁻¹. 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 (D₂O) and filtered on cotton. The analysis was made at room temperature
222 (25°C). The number of complex points collected was 1024 for the ¹H dimension with a
223 relaxation of 1.5- and 256 time increments were recorded in the ¹³C dimension.
224 Calculations of DSAc Xylp and DSAc Manp are obtained using equations 1 and 2.

225

Eq 1.

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

226

Eq 2.

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

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

232 ^a Dry matter content (%) in Peremate and Retentate based on initial SE effluent DM

233 ^b wt% based on initial dry wood.

234 ^c Xylose, mannose, glucose, arabinose, galactose, Rhamnose, galacturonic and glucuronic acid %, determined after
235 hydrolysis by HPEAC-PAD.

236 ^d Estimation by HSQC-NMR.

237 ^e Acetic acid quantified by HPLC after saponification.

238 ^f Acid soluble lignin, estimation by UV-visible

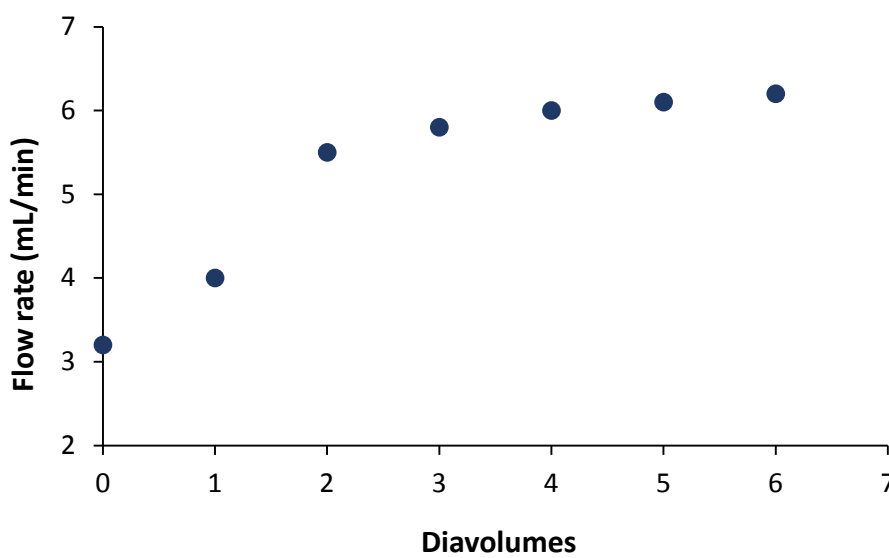
239 ^g Not quantified

247 **3. Results and discussion**

249 **3.1. Ultrafiltration - Diafiltration**

250 In a preliminary study, the fractionation (in full recirculation mode) of SE liquid effluent was
251 optimized using three ultrafiltration membranes (cutoff 10 kDa, 100 kDa and 500 kDa). The
252 retentate and permeate were recovered and analyzed by steric exclusion chromatography
253 (Suppl Materials - SM1-3). The 10 kDa membrane, which gave the better results with a
254 high intensity of the peaks of high molecular weight compounds in the retentate, was

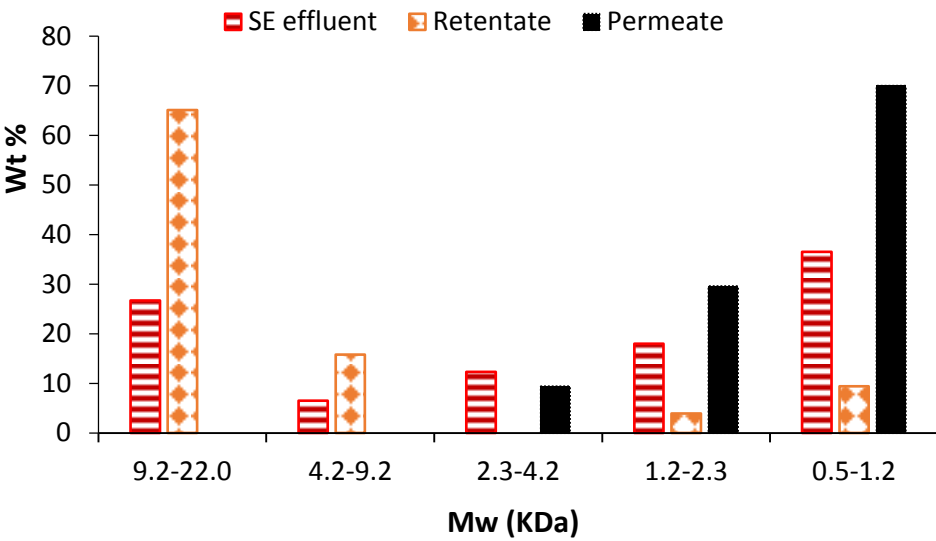
255 selected. Ultrafiltration of steam explosion liquor was made on diafiltration mode in order to
256 get a better separation. Figure 2 shows the permeate flow rate evolution during diafiltration
257 (up to 6 diavolumes). The flow rate increased until the third diavolume and tends to
258 stabilize thereafter. 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
 292

293
 294
 295

3.2. Composition of hemicelluloses

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-

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

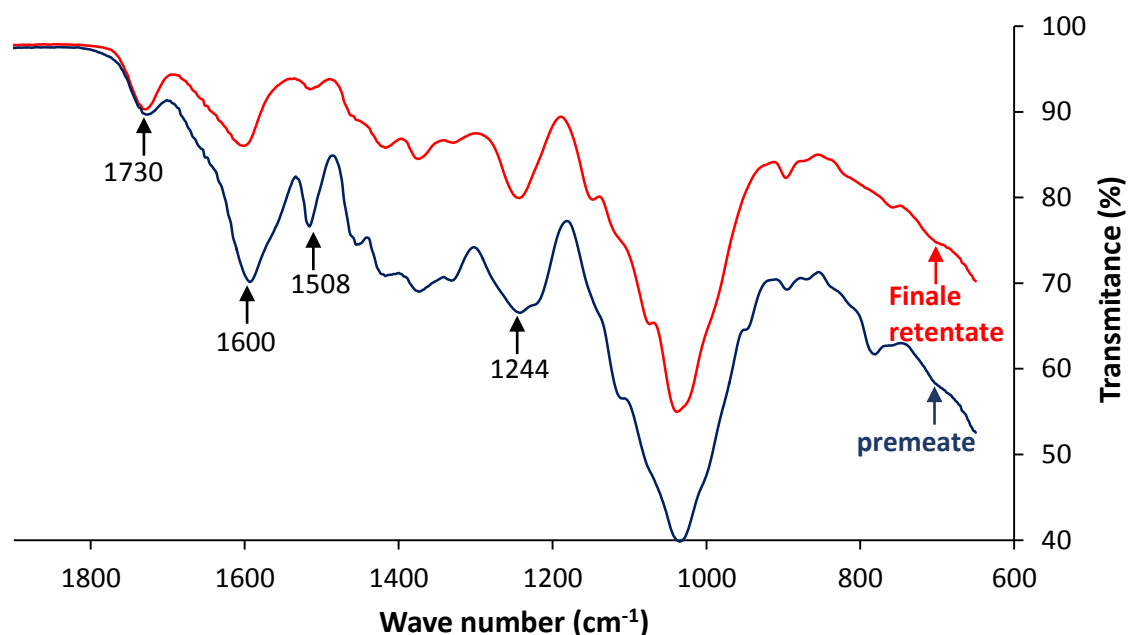
It can be noticed from Table 1 that diafiltration allowed an efficient separation of arabinose which is primarily recovered in the **Permeate** fraction. This result is explained by the high susceptibility of arabinan chains toward hydrolysis producing almost exclusively monomeric arabinose [43]. An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

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

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

2D HSQC NMR analysis was used to better understand the molecular structure of the 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



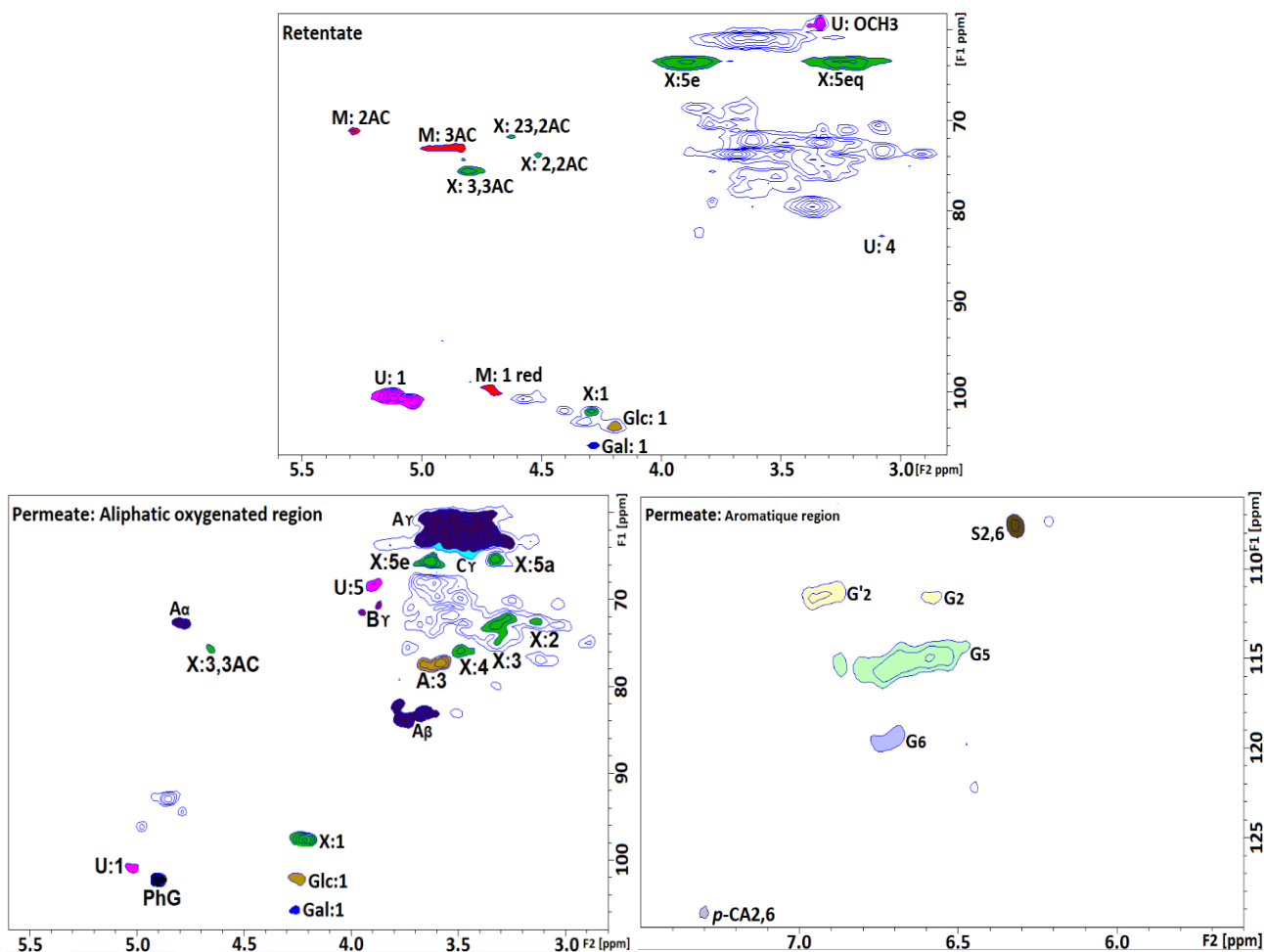
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345 **Figure 4.** FTIR spectra of **Retentate** and **Permeate**
346

347 The $^{13}\text{C}/^1\text{H}$ NMR correlations corresponding to the *O*-acetyl groups of hemicelluloses
348 through the signals of $[\text{O-Ac}(1\rightarrow2/3)\text{-}\beta\text{-D-Xylp}]$ and $[\text{O-Ac}(1\rightarrow2/3)\text{-}\beta\text{-D-Manp}]$ 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 *O*2 and/or *O*3 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 (C_2/H_2 in G), $\delta\text{H}/\delta\text{C}$ -6.69/115.28 ppm (C_5/H_5 in G), $\delta\text{H}/\delta\text{C}$ -6.70/119.28 ppm (C_6/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 β -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) β -D-Xylopyranosyl ; U: 4-
 380 O-methyl- α -D-glucuronic acid ; M: (1→4) β -D-Mannosyl ; G: (1→4) β -D- Glucosyl ; A: α -L-
 381 Arabinosyl ; Ac: acetyl; A: C γ /H γ in β -O-4 linkage ; A: C β /H β in β -O-4 linkage from guaiacyl units ; C:
 382 C γ /H γ in phenylcoumaran substructure ; S: C $_{2,6}$ /H $_{2,6}$ in syringyl units ; G: C $_2$ /H $_2$ in guaiacyl units;

G': C2/H2 in oxidized guaiacyl units; G: C5/H5 in guaiacyl units ; G: C6/H6 in guaiacyl units ; p-CA2,6: C2,6/H2,6 in p-hydroxybenzoate units;

Conclusion

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

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Bibliography

- [1] M. S. Lindblad, E. Ranucci, and A.-C. Albertsson, "Biodegradable Polymers from Renewable Sources. New Hemicellulose-Based Hydrogels," p. 6.
- [2] J. Hartman, A.-C. Albertsson, M. S. Lindblad, and J. Sjöberg, "Oxygen barrier materials from renewable sources: Material properties of softwood hemicellulose-based films," *J. Appl. Polym. Sci.*, vol. 100, no. 4, pp. 2985–2991, May 2006, doi: 10.1002/app.22958.
- [3] M. Gröndahl, L. Eriksson, and P. Gatenholm, "Material Properties of Plasticized Hardwood Xylans for Potential Application as Oxygen Barrier Films," *Biomacromolecules*, vol. 5, no. 4, pp. 1528–1535, Jul. 2004, doi: 10.1021/bm049925n.
- [4] S. Willför, K. Sundberg, M. Tenkanen, and B. Holmbom, "Spruce-derived mannans – A potential raw material for hydrocolloids and novel advanced natural materials," *Carbohydrate Polymers*, vol. 72, no. 2, pp. 197–210, May 2008, doi: 10.1016/j.carbpol.2007.08.006.
- [5] S. Wang, B. Ru, H. Lin, and W. Sun, "Pyrolysis behaviors of four O-acetyl-preserved hemicelluloses isolated from hardwoods and softwoods," *Fuel*, vol. 150, pp. 243–251, Jun. 2015, doi: 10.1016/j.fuel.2015.02.045.

- 423 [6] T. E. Timell, "Recent progress in the chemistry of wood hemicelluloses," *Wood*
424 *Sci. Technol.*, vol. 1, no. 1, pp. 45–70, 1967, doi: 10.1007/BF00592255.
- 425 [7] E. Sjöström and U. Westermark, "Chemical Composition of Wood and Pulps: Basic
426 Constituents and Their Distribution," in *Analytical Methods in Wood Chemistry, Pulping, and*
427 *Papermaking*, E. Sjöström and R. Alén, Eds. Berlin, Heidelberg: Springer Berlin Heidelberg, 1999,
428 pp. 1–19. doi: 10.1007/978-3-662-03898-7_1.
- 429 [8] E. Sjöström and U. Westermark, "Chemical Composition of Wood and Pulps: Basic
430 Constituents and Their Distribution," in *Analytical Methods in Wood Chemistry, Pulping, and*
431 *Papermaking*, E. Sjöström and R. Alén, Eds. Berlin, Heidelberg: Springer Berlin Heidelberg, 1999,
432 pp. 1–19. doi: 10.1007/978-3-662-03898-7_1.
- 433 [9] K. Iiyama, T. B. Lam, and B. A. Stone, "Covalent Cross-Links in the Cell Wall.," *Plant*
434 *Physiol.*, vol. 104, no. 2, pp. 315–320, Feb. 1994, Accessed: Mar. 25, 2022. [Online]. Available:
435 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC159201/>
- 436 [10] M. Tenkanen, T. Tamminen, and B. Hortling, "Investigation of lignin-carbohydrate
437 complexes in kraft pulps by selective enzymatic treatments," *Applied Microbiology and*
438 *Biotechnology*, vol. 51, no. 2, pp. 241–248, Feb. 1999, doi: 10.1007/s002530051388.
- 439 [11] A. Ebringerová, Z. Hromádková, and T. Heinze, "Hemicellulose," in *Polysaccharides I:*
440 *Structure, Characterization and Use*, T. Heinze, Ed. Berlin, Heidelberg: Springer, 2005, pp. 1–67.
441 doi: 10.1007/b136816.
- 442 [12] X.-F. Sun, P. Fowler, and M. S. Baird, "Extraction and Characterization of Original
443 Lignin and Hemicelluloses from Wheat Straw," *J. Agric. Food Chem.*, vol. 53, no. 4, pp. 860–870,
444 Feb. 2005, doi: 10.1021/jf040456q.
- 445 [13] E. Hägglund, B. Lindberg, J. Mcpherson, L. G. Sillén, and B. Thorell, "Dimethylsulphoxide,
446 a Solvent for Hemicelluloses.," 1956, doi: 10.3891/ACTA.CHEM.SCAND.10-1160.
- 447 [14] E. Haimer, M. Wendland, A. Potthast, U. Henniges, T. Rosenau, and F. Liebner,
448 "Controlled precipitation and purification of hemicellulose from DMSO and DMSO/water mixtures
449 by carbon dioxide as anti-solvent," *The Journal of Supercritical Fluids*, vol. 53, no. 1–3, pp. 121–
450 130, Jun. 2010, doi: 10.1016/j.supflu.2010.02.009.
- 451 [15] J. Rowley, S. R. Decker, W. Michener, and S. Black, "Efficient extraction of xylan from
452 delignified corn stover using dimethyl sulfoxide," *3 Biotech*, vol. 3, no. 5, pp. 433–438, Oct. 2013,
453 doi: 10.1007/s13205-013-0159-8.
- 454 [16] Y. Uraki, Y. Usukura, T. Kishimoto, and M. Ubukata, "Amphiphilicity of a lignin-
455 carbohydrate complex," vol. 60, no. 6, pp. 659–664, Nov. 2006, doi: 10.1515/HF.2006.111.
- 456 [17] X. Zhao *et al.*, "Characterization of hemicelluloses from *Neolamarckia cadamba*
457 (Rubiaceae) during xylogenesis," *Carbohydr Polym*, vol. 156, pp. 333–339, Jan. 2017, doi:
458 10.1016/j.carbpol.2016.09.041.
- 459 [18] A. Ebringerová, Z. Hromádková, M. Kačuráková, and M. Antal, "Quaternized xylans:
460 synthesis and structural characterization," *Carbohydrate Polymers*, vol. 24, no. 4, pp. 301–308, Jan.
461 1994, doi: 10.1016/0144-8617(94)90075-2.
- 462 [19] K. S. Mikkonen and M. Tenkanen, "Sustainable food-packaging materials based on future
463 biorefinery products: Xylans and mannans," *Trends in Food Science & Technology*, vol. 28, no. 2,
464 pp. 90–102, Dec. 2012, doi: 10.1016/j.tifs.2012.06.012.
- 465 [20] I. Ziegler-Devin, L. Chrusciel, and N. Brosse, "Steam Explosion Pretreatment of
466 Lignocellulosic Biomass: A Mini-Review of Theoretical and Experimental Approaches," *Front.*
467 *Chem.*, vol. 9, p. 705358, Nov. 2021, doi: 10.3389/fchem.2021.705358.
- 468 [21] M. Tutt, T. Kikas, H. Kahr, M. Pointner, P. Kuttner, and J. Olt, "Using steam explosion
469 pretreatment method for bioethanol production from floodplain meadow hay," p. 8.
- 470 [22] M. Barbanera, E. Lascaro, D. Foschini, F. Cotana, and C. Buratti, "Optimization of
471 bioethanol production from steam exploded hornbeam wood (*Ostrya carpinifolia*) by enzymatic
472 hydrolysis," *Renewable Energy*, vol. 124, pp. 136–143, Aug. 2018, doi:
473 10.1016/j.renene.2017.07.022.
- 474 [23] W. G. Glasser, W. E. Kaar, R. K. Jain, and J. E. Sealey, "Isolation options for non-cellulosic

heteropolysaccharides (HetPS),” *Cellulose*, vol. 7, no. 3, pp. 299–317, Sep. 2000, doi: 10.1023/A:1009277009836.

[24] H. Krawczyk, T. Persson, A. Andersson, and A.-S. Jönsson, “Isolation of hemicelluloses from barley husks,” *Food and Bioproducts Processing*, vol. 86, no. 1, pp. 31–36, Mar. 2008, doi: 10.1016/j.fbp.2007.10.018.

[25] M. Chadni, N. Grimi, O. Bals, I. Ziegler-Devin, and N. Brosse, “Steam explosion process for the selective extraction of hemicelluloses polymers from spruce sawdust,” *Industrial Crops and Products*, vol. 141, p. 111757, Dec. 2019, doi: 10.1016/j.indcrop.2019.111757.

[26] M. Palm and G. Zacchi, “Extraction of Hemicellulosic Oligosaccharides from Spruce Using Microwave Oven or Steam Treatment,” *Biomacromolecules*, vol. 4, no. 3, pp. 617–623, May 2003, doi: 10.1021/bm020112d.

[27] A. W. Mohammad, C. Y. Ng, Y. P. Lim, and G. H. Ng, “Ultrafiltration in Food Processing Industry: Review on Application, Membrane Fouling, and Fouling Control,” *Food Bioprocess Technol*, vol. 5, no. 4, pp. 1143–1156, May 2012, doi: 10.1007/s11947-012-0806-9.

[28] T. Persson and A.-S. Jönsson, “Isolation of hemicelluloses by ultrafiltration of thermomechanical pulp mill process water—Influence of operating conditions,” *Chemical Engineering Research and Design*, vol. 88, no. 12, pp. 1548–1554, Dec. 2010, doi: 10.1016/j.cherd.2010.04.002.

[29] A. Maartens, E. P. Jacobs, and P. Swart, “UF of pulp and paper effluent: membrane fouling-prevention and cleaning,” *Journal of Membrane Science*, vol. 209, no. 1, pp. 81–92, Nov. 2002, doi: 10.1016/S0376-7388(02)00266-1.

[30] T. Persson, A.-S. Jönsson, and G. Zacchi, “Fractionation of hemicelluloses by membrane filtration: 14th European Biomass Conference,” *Proceedings of the 14th European Biomass Conference*, pp. 1693–1696, 2005.

[31] A.-S. Jönsson, A.-K. Nordin, and O. Wallberg, “Concentration and purification of lignin in hardwood kraft pulping liquor by ultrafiltration and nanofiltration,” *Chemical Engineering Research and Design*, vol. 86, no. 11, pp. 1271–1280, Nov. 2008, doi: 10.1016/j.cherd.2008.06.003.

[32] M. Mänttari *et al.*, “Improvement of ultrafiltration performance by oxidation treatment in the recovery of galactoglucomannan from wood autohydrolyzate,” *Separation and Purification Technology*, vol. 149, pp. 428–436, Jul. 2015, doi: 10.1016/j.seppur.2015.06.001.

[33] V. Oriez, J. Peydecastaing, and P.-Y. Pontalier, “Separation of sugarcane bagasse mild alkaline extract components by ultrafiltration – Membrane screening and effect of filtration parameters,” *Process Biochemistry*, vol. 78, pp. 91–99, Mar. 2019, doi: 10.1016/j.procbio.2019.01.006.

[34] B. Al-Rudainy, M. Galbe, and O. Wallberg, “From lab-scale to on-site pilot trials for the recovery of hemicellulose by ultrafiltration: Experimental and theoretical evaluations,” *Separation and Purification Technology*, vol. 250, p. 117187, Nov. 2020, doi: 10.1016/j.seppur.2020.117187.

[35] R. Nitzsche, A. Gröngroft, I. Goj, and M. Kraume, “Ultrafiltration of Beechwood Hydrolysate for Concentrating Hemicellulose Sugars and Removal of Lignin—Parameter Estimation Using Statistical Methods and Multiobjective Optimization,” *Ind. Eng. Chem. Res.*, vol. 59, no. 16, pp. 7875–7887, Apr. 2020, doi: 10.1021/acs.iecr.0c00487.

[36] “Présentation du Secteur Forestier au Gabon (1).pdf.”

[37] E. Mouguala Moukagni, I. Ziegler-Devin, R. Safou-Tchima, and N. Brosse, “Extraction of acetylated glucuronoxylans and glucomannans from Okoume (*Aucoumea klaineana* Pierre) sapwood and heartwood by steam explosion,” *Industrial Crops and Products*, vol. 166, p. 113466, Aug. 2021, doi: 10.1016/j.indcrop.2021.113466.

[38] E. Simangunsong, I. Ziegler-Devin, L. Chrusciel, P. Girods, N. J. Wistara, and N. Brosse, “Steam Explosion of Beech Wood: Effect of the Particle Size on the Xylans Recovery,” *Waste Biomass Valor*, vol. 11, no. 2, pp. 625–633, Feb. 2020, doi: 10.1007/s12649-018-0522-4.

[39] S. Y. Lin and C. W. Dence, Eds., *Methods in Lignin Chemistry*. Berlin, Heidelberg: Springer, 1992. doi: 10.1007/978-3-642-74065-7.

- 527 [40] A. Andersson, T. Persson, H. Stålbrand, and A.-S. Jönsson, "Comparison of diafiltration and
528 size-exclusion chromatography to recover hemicelluloses from process water from
529 thermomechanical pulping of spruce," *Applied Biochemistry and Biotechnology*, vol. 136, p. 13,
530 2007.
- 531 [41] T. Sauvageon, J.-M. Lavoie, C. Segovia, and N. Brosse, "Toward the cottonization of hemp
532 fibers by steam explosion – Part 1: defibration and morphological characterization," *Textile
533 Research Journal*, vol. 88, no. 9, pp. 1047–1055, May 2018, doi: 10.1177/0040517517697644.
- 534 [42] Y.-H. Feng *et al.*, "Characteristics and environmentally friendly extraction of cellulose
535 nanofibrils from sugarcane bagasse," *Industrial Crops and Products*, vol. 111, pp. 285–291, Jan.
536 2018, doi: 10.1016/j.indcrop.2017.10.041.
- 537 [43] L. Desharnais, F. Du, and N. Brosse, "Optimization of Galactoglucomannans and Acidic
538 Arabinans Recovery in Softwood," *Ind. Eng. Chem. Res.*, vol. 50, no. 24, pp. 14217–14220, Dec.
539 2011, doi: 10.1021/ie202273d.
- 540 [44] R. Sun and J. Tomkinson, "Fractionation and Characterization of Water-soluble
541 Hemicelluloses and Lignin from Steam-exploded Birchwood," *International Journal of Polymeric
542 Materials*, vol. 45, no. 1–2, pp. 1–19, Jan. 2000, doi: 10.1080/00914030008034866.
- 543 [45] D. Morais de Carvalho *et al.*, "Isolation and characterization of acetylated
544 glucuronoarabinoxylan from sugarcane bagasse and straw," *Carbohydrate Polymers*, vol. 156, pp.
545 223–234, Jan. 2017, doi: 10.1016/j.carbpol.2016.09.022.
- 546 [46] A. Gil-Ramirez, D. M. Salas-Veizaga, C. Grey, E. N. Karlsson, I. Rodriguez-Meizoso, and
547 J. A. Linares-Pastén, "Integrated process for sequential extraction of saponins, xylan and cellulose
548 from quinoa stalks (*Chenopodium quinoa* Willd.)," *Industrial Crops and Products*, vol. 121, pp.
549 54–65, Oct. 2018, doi: 10.1016/j.indcrop.2018.04.074.
- 550 [47] N. Giummarella and M. Lawoko, "Structural Insights on Recalcitrance during Hydrothermal
551 Hemicellulose Extraction from Wood," *ACS Sustainable Chem. Eng.*, vol. 5, no. 6, pp. 5156–5165,
552 Jun. 2017, doi: 10.1021/acssuschemeng.7b00511.
- 553 [48] S. N. Sun, T. Q. Yuan, M. F. Li, X. F. Cao, F. Xu, and Q. Y. Liu, "STRUCTURAL
554 CHARACTERIZATION OF HEMICELLULOSES FROM BAMBOO CULMS
555 (*NEOSINOCALAMUS AFFINIS*)," p. 12.
- 556 [49] F. Peng, J.-L. Ren, F. Xu, J. Bian, P. Peng, and R.-C. Sun, "Fractionation of Alkali-
557 Solubilized Hemicelluloses from Delignified *Populus gansuensis* : Structure and Properties," *J.
558 Agric. Food Chem.*, vol. 58, no. 9, pp. 5743–5750, May 2010, doi: 10.1021/jf1003368.
- 559 [50] P.-P. Yue *et al.*, "Changes of Chemical Composition and Hemicelluloses Structure in
560 Differently Aged Bamboo (*Neosinocalamus affinis*) Culms," *J. Agric. Food Chem.*, vol. 66, no.
561 35, pp. 9199–9208, Sep. 2018, doi: 10.1021/acs.jafc.8b03516.
- 562 [51] V. Steinmetz, M. Villain-Gambier, A. Klem, F. Gambier, S. Dumarçay, and D. Trebouet,
563 "Unveiling TMP Process Water Potential As an Industrial Sourcing of Valuable Lignin–
564 Carbohydrate Complexes toward Zero-Waste Biorefineries," *ACS Sustainable Chem. Eng.*, vol. 7,
565 no. 6, pp. 6390–6400, Mar. 2019, doi: 10.1021/acssuschemeng.9b00181.
- 566 [52] Y. Zhang *et al.*, "Valorization of Lignin–Carbohydrate Complexes from Hydrolysates of
567 Norway Spruce: Efficient Separation, Structural Characterization, and Antioxidant Activity," *ACS
568 Sustainable Chem. Eng.*, vol. 7, no. 1, pp. 1447–1456, Jan. 2019, doi:
569 10.1021/acssuschemeng.8b05142.