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Extraction of acetylated glucuronoxylans and glucomannans from Okoume (*Aucoumea klaineana* Pierre) sapwood and heartwood by steam explosion

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Abstract

Aucoumea klaineana Pierre (okoume) is a tropical hardwood of industrial importance, used for veneers and plywood production. Various conditions of Steam Explosion (SE) treatment (temperature, residence time, pH) have been applied on okoume co-products, sapwood (SW) and heartwood (HW) for the extraction of polymeric hemicelluloses. Conventional extractions with alkali or dimethyl sulfoxide (DMSO) were also performed from delignified SW and HW for comparison. The hemicelluloses extracts have been characterized by HSQC-NMR, FTIR, size exclusion chromatography. The acetyl contents have been determined by HPLC after saponification and from HSQC spectra. Using basic impregnation prior to SE (NaOH-SE), high molecular mass glucuronoxylans (GX, Mw = 65-100 KDa) were recovered in 3-6 % yields. A neutral impregnation (H₂O-SE) produced O2 and/or O3 acetylated GX and glucomannans (GM), preserving the acetyl moieties in a greater extent compared to the mild DMSO extraction (% Ac ≈ 6.6-8.4% and 1.0-4.1 % w/w respectively). This study provides insights on the composition of SW and HW okoume with a view to further innovative processing and valorization including the preparation of films with advanced properties for food packaging.

Key words: *Aucoumea klaineana* Pierre; steam explosion; acetylated glucomannans; acetylated glucuronoxylans; sapwood; heartwood

1. Introduction

The economic viability of the biorefineries depends of the optimal valorization of plant resources including the utilization of non-cellulosic constituents. Hemicelluloses are among the main components of wood and constitute a vast, heterogeneous and complex family of polysaccharides, containing diverse sugars and uronic acids (Ishii et al., 2001, Chadni et al., 2019). Hardwood hemicellulosic fraction is mainly composed of glucuronoxylans ($\approx$ 15-30% of wood) which have a backbone of β -1,4-linked xylose residues substituted with α -linked 4-*O*-Me- β -D-glucopyranosyl uronic acid on *O*-2 of xylosyl residue. Hardwood can also contain a minor amount of glucomannans ($\approx$ 4% of wood) which have a backbone of β -1,4-linked glucopyranosyl / mannopyranosyl units in a linear or slightly branched chain. These polysaccharides are usually diversely acetylated on the sugar backbone (Willfor et al. 2005). Unlike cellulose, which has been used industrially for a very long time, applications involving polymeric hemicelluloses are rare and the potential of hemicelluloses is still very little exploited. However, due to their chemical structure and high molar masses, hemicelluloses can be used to produce promising materials for various applications. One of the most studied applications for polymeric hemicelluloses is the design of films with advanced properties (e.g. biodegradability, edibility, barrier effect) for food packaging (Chadni et al., 2020, Zhao et al., 2020).

Various processes have been proposed for the recovery of hemicellulosic polymers from lignocellulosic matrix including alkali extraction of xylans and/or glucuronoxylans from agricultural co-products and liquid hot water extraction (Zhao et al. 2020). It has also been reported that galactoglucomannans can be recovered from the water stream of thermomechanical pulping of softwood using membrane technologies (Kishani et al., 2018, Willfor et al, 2003). The recovery of acetylated hemicelluloses could be of great interest for biological and material applications since acetylation degree affects the accessibility of polysaccharides to microbes, their solubility in water (Michalak et al. 2018, Voepel et al. 2009) and the physico-chemical properties of hemicelluloses-based materials (e.g. barrier properties of films, Kishani et al., 2018).

However, the recovery of acetylated and high molecular mass hemicelluloses from wood with a minimum of degradation is still challenging. In fact, the currently applied methods usually rely on partly modified and deacetylated polysaccharides. For characterization

purpose slightly modified and acetylated hemicelluloses can be recovered from holocellulose by DMSO extraction (Evtuguin et al. 2003, Marques et al. 2010).

Steam explosion (SE) is one of the most promising processes for biomass fractionation and pretreatment. SE is a thermo-mechanico-chemical treatment involving a steaming step followed by an explosive decompression provoking a partial breakdown of the lignocellulosic complex (Espirito Santo et al., 2020, Marques et al., 2020, Inseemeeesak and Areeprasert, 2020). This process is commonly used for increasing the accessibility of cellulose to enzymatic hydrolysis and has been developed, even at commercial stage, for cellulosic bioethanol production. More marginally, SE has also been described for high molecular mass hemicelluloses extraction from lignocellulosic biomass. Compared to a hot water extraction, it has been demonstrated by Wojtasz-Mucha et al. (2017) that SE provoked a more effective hemicelluloses extraction due to the advective mass transport during the pressure release step (explosion). Krawczyk et al. (2008) reported the isolation of arabinoxylans from barley husks by SE after water impregnation and ultra-filtration. In our group, we studied the extraction of arabinoxylans and galactoglucomannans from spruce by SE (Chadni et al., 2019, 2020). Extraction selectivity as a function of the pH of the SE treatment was demonstrated: for a basic impregnation arabinoglucuronoglycans were recovered in relatively high yields whereas for water impregnation highly acetylated galactoglucomannans were isolated. It was concluded that SE could constitute an effective technique for the recovery of slightly modified and acetylated hemicelluloses from wood of application interest (e.g. food packaging).

Aucoumea klaineana Pierre (okoume) is a fast-growing hardwood species of great industrial importance in Central-Africa. Okoume is primarily used for veneers and plywood production. In Gabon, the okoume wood industry produces approximately $1.5 \cdot 10^6 \text{ m}^3$ of transformed wood and 750 000 m^3 of waste per year currently not properly used. In wood stem, heartwood (HW) is the inner part characterized by its high mechanical properties and sapwood (SP) the outer part with a higher porosity and moisture content. Due to these properties, okoume SW and HW have been considered as low value coproducts, excluded from industrial transformation such as trenching, veneers and plywood production (Ngwa Obame et al., 2019). There are relatively few publications concerning the comparison between sapwood (SW)/heartwood (HW) composition. Unlike HW, SW is comprised of living cells and allows the water transport and nutrients storage, characterized by a higher

porosity and permeability. Willford et al. reported in 2005 the amount and composition of sugars in SW and HW of 11 industrially important hardwood species from temperate areas (Willford et al. 2005). More recently, extractives content of bark, SW and HW of okoume were described (Engozogho et al., 2015) but to the best of our knowledge, the sugars composition of SW /HW of tropical species has never been investigated. However, in a context of an optimal valorization of bioresources, a better knowledge of the chemical composition of the different parts of industrial wood, including co-products, is needed.

In previous papers, we have described organosolv and SE pretreatment of okoume SW under various conditions for lignin extraction and characterization but also for ethanol production (Ngwa Obame et al., 2019, Safou-Tchiama et al. 2016). The goal and the novelty of the present work are twofold: (1) to analyze the hemicelluloses composition of HW and SW of a tropical hardwood species and (2) to study the recovery of polymeric hemicelluloses from hardwood during SE pretreatment for further utilizations. A detailed characterization of acetylated hemicelluloses was carried out by NMR and chromatographic methods in order to better understand the parameters impacting the structure of the extracted polysaccharides, to anticipate their future applications and facilitating further processing.

2. Material and methods

2.1. Raw material

The samples used in this study were collected from an Okoume (*Aucoumea klaineana* Pierre) log in the Nzamaligué natural forest of Gabon by the “Société Equatoriale d'Exploitation Forestière SEEF”. The Okoume SW comes from slab waste, while the internal HW from the peeling core co-products.

2.2. Conventional hemicelluloses extraction with NaOH and DMSO

Sawdust from Okoume HW and SW (< 0.16 mm) was Soxhlet extracted using toluene / ethanol / methanol (4:1:1 v/v/v) (Safou-Tchiama et al. 2017). The extracted sawdust was dried at 105°C and stored in a plastic bag at room temperature before use. Extract-free sawdust was depectinized with a 1% (w/w) of ammonium oxalate solution (2 h at 80°C) and then dried at 40°C. Delignification was carried out in a water bath under reflux at 70°C by dispersing 1.5 g of depectinized sawdust in 125 mL of deionized water. Subsequently, 1 g of sodium chlorite and 1 mL of glacial acetic acid were added every 2 h until complete bleaching of the biomass. The delignified residue was carefully washed with deionized water

and filtered under vacuum. The white fibrous solid residue (holocellulose) was collected and dried at 40 °C. Alkaline extraction was carried out under magnetic stirring at 70 °C with 1 g of dry holocellulose in 50 mL of 2% (w/w) of NaOH. After 2 hrs, the liquid phase was recovered. The residue was extracted again with 50 mL of 2% NaOH for 2 hrs. Both liquid phases were acidified with 20% (w/w) of acetic acid to a pH between 5.5 and 6. The hemicelluloses precipitated by the addition of 3 volumes of ethanol were recovered by centrifugation at 4000 rpm for 10 min at room temperature. The recovered hemicelluloses were dried at 40°C and stored in plastic jars before analysis. The hemicelluloses yields were calculated as mass percentage of dry wood. The DMSO extraction of hemicelluloses was performed on holocellulose (1:25 solid/liquid) under magnetic stirring for 15 hrs at 70°C. Three volumes of ethanol were added. The hemicelluloses were recovered by centrifugation at 4000 rpm for 10 min and drying at 40°C. Yields of hemicelluloses were calculated as mass percentage of dry wood.

2.3. Extraction of hemicelluloses by steam explosion

Extraction of hemicelluloses by SE has been carried out by adapting the protocol described by Chadni et al. 2019 Sawdust from Okoume HW and SW of about 2 mm size was first dried at 105 °C overnight. Then 50 g of biomass were impregnated for 15 hrs in 1 M aqueous NaOH (solid/liquid ratio 1:7, NaOH-SE) or in water (solid/liquid ratio 1:7, H₂O-SE) before SE (Fig. 1).

The impregnated sawdust was then filtered and placed directly into a 2 L SE reactor. The sawdust was treated by injection of saturated steam at various temperatures and residence times (see Table 1), then a pneumatic ball valve was opened to abruptly drop the pressure and transport the biomass to the explosion chamber. The steam explosions were set at temperatures between 170 °C and 210°C and a residence time of 5 min to 10 min. The severity factor for each experiment was calculated by correlating temperature and retention time using the following equation (Overend and Chornet, 1987):

$$S = \log [t_s \cdot \exp\left(\frac{T - 100}{14.75}\right)]$$

Eq. 1
156 t_s is the residence time (min) and T is the temperature (°C).

After SE pre-treatment, the hemicelluloses-rich liquid fraction was separated from the treated wood by filtration. For NaOH-SE, the pH was adjusted between 5.5 and 6 with H₂SO₄ 72% (w/v). The liquid fraction was concentrated using a rotary evaporator until a volume of

≈ 100 mL. The hemicelluloses were precipitated with 4 volumes of ethanol, then separated by centrifugation at 40,000 rpm for 10 min. The supernatant was gently removed and the solid (composed of polymeric hemicelluloses) was retained for the dialysis step. The dialysis was performed for 3 days by filling a dialysis tube (cut-off of 2.5 kDa) with the hemicelluloses solubilized in ultrapure water. Finally, the dialysate consisting of hemicelluloses was freeze-dried.

Fig.1

2.4. Measurement of monosaccharides and uronic acids by HPEAC-PAD

The processing and analysis of the chemical composition of the extracts was performed using the National Renewable Energy Laboratory (NREL/TP-510-42618) standard. Monosaccharide and uronic acid concentrations were quantified by high performance anion exchange chromatography coupled with a pulsed amperometric detector (HPEAC-PAD: Dionex ICS-3000). The separation was carried out on a CarboPac PA20 (Dionex) column at a temperature of 35 °C and with an isocratic eluent flow rate of 0.4 mL.min⁻¹, with the following composition: 99.2% ultrapure water / 0.8% of 250 mM NaOH (0–20 min) ; 75% ultrapure water / 20% of 250 mM NaOH / 5% 1 M of CH₃COONa - 20 mM NaOH (20–37 min) and 40% of ultrapure water / 20% 250 mM of NaOH / 40% of 1 M CH₃COONa - 20 mM of NaOH (37–45 min) The separation was performed within 45 minutes. Calibration was performed with seven calibration points. This method allowed the simultaneous identification of the following sugars: galactose (Gal), glucose (Glc), mannose (Man), rhamnose (Rha), xylose (Xyl), arabinose (Ara), and glucuronic acid GlcA and galacturonic acid (GalA).

2.5. Determination of acetic acid by high performance liquid chromatography (HPLC)

The acetic acid content of hemicelluloses was determined by adapting the protocol of Yuan et al (2006). 1000 µL of the oligosaccharide solution (10 mg in 5 mL of water)) were saponified with NaOH (1250 µL at 0.4 M) for 2 hours in the dark, at room temperature, in a 5 mL volumetric flask. The reaction was stopped by addition of H₃PO₄ at 0.4 M. This solution was analyzed by high performance liquid chromatography coupled with a UV detector (Shimadzu Prominence) using a Rezex™ RHM-Monosaccharide H⁺ (8%) column with sulfuric acid (H₂SO₄ - 5.10⁻³ M) as the mobile phase with an isocratic flow rate of 0.8 mL.min⁻¹ and a temperature of 60°C; the wavelength for detecting acetic acid was 210 nm. An external calibration with 6 calibration points was performed.

2.6. Steric Exclusion Chromatography

Prior to the analysis, 5 mg of hemicelluloses were dissolved in 2.25 mL of 0.25 M NaNO₃. Molecular weight distributions of hemicelluloses extracts were determined by size exclusion chromatography coupled with a refractive index detector (Shimadzu Prominence). Two columns coupled in series were used (Phenomenex GFC-P2000, 3000) to separate hemicelluloses. The elution temperature was 35°C and the eluent consisted of 0.25 M NaNO₃ with a flow rate of 0.7 mL.min⁻¹. Pullulan standards of known molar masses were used for calibration.

2.7. Fourier transform infrared spectroscopy

The hemicelluloses obtained were subjected to Fourier Transform Infrared Spectroscopy (FTIR) analysis. The infrared spectra were performed using a Thermo Scientific Nicolet 7600 IR-FT spectrometer over a frequency range between 4000 cm⁻¹ and 650 cm⁻¹.

2.8. HSQC 2D NMR and calculation of the Degree of Acetylation (DS_{Ac})

The 2-dimensional nuclear magnetic resonance (NMR) analyses of heteronuclear singular quantum coherence (HSQC) were performed on a 400 MHz Bruker instrument with a standard Bruker program. The hemicelluloses from the steam explosion and those extracted by NaOH from the bleached wood were dissolved in 99.7% deuterium oxide (D₂O), while the hemicelluloses extracted by DMSO from the bleached wood were dissolved in 99.9% dimethyl sulfoxide d₆ (DMSO-d₆). 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.

The calculation of DS_{Ac} Xyl_p and DS_{Ac} Man_p are given below in Eq. 2 and 3.

$$\text{Eq 2.} \quad \text{DS(Ac) Xyl}_p = \frac{\frac{[\text{Ac}]^a}{[\text{Man} + \text{Xyl}]^b}}{1 + \frac{[\text{Man Ac}]^c}{[\text{Xyl AC}]^c}}$$

$$\text{Eq 3.} \quad \text{anp} = \frac{[\text{Ac}]^a}{[\text{Man} + \text{Xyl}]^b} - \text{DSAc Xyl}_p$$

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

3. Results and discussion

3.1. Composition

The compositions of okoume sapwood (SW) and heartwood (HW) used in this study and given in the Table 2 are in accordance with previous studies reported by Safou -Tchiama et al. (2007). In the present study, a comparable amount of Klason lignin and glucose and a substantially lower proportion of xylose and mannose was found.

Table 2

The polymeric hemicelluloses were recovered after SE of okoume SW and HW from the liquid stream of the process by ethanolic precipitation and dialysis. Two different impregnation conditions of wood sawdust prior to SE were examined (neutral and alkali impregnation) and three different experimental conditions (170°C-10 min, 190°C-8 min, 210°C-5 min) in order to examine the influence of the treatment severity on the extract yields and compositions. In this study the severity parameters (S) which combine temperature and duration of the SE treatment have been used (see Material and Methods section and Table 1). The condition sets used in this study were selected considering previously described works (Chadni et al. 2019). For comparison purposes, hemicelluloses were also recovered from okoume SW and HW holocellulose (after bleaching) with two commonly used methods with DMSO and with sodium hydroxide. It was reported that alkali solutions allow the extraction of hemicelluloses from bleached pulps with high yields especially for xylans. Alkaline treatment can cleave ester groups of lignocellulosic complexes in the cell wall and causes the swelling of cellulose facilitating the dissolution of hemicelluloses. On the other hand, DMSO extraction is used for the recovery of slightly modified hemicelluloses, close to their native structures but with lower yields (Evtuguin et al. 2003). Naturally acetylated hemicelluloses have been isolated using this procedure.

3.2. Hemicelluloses yields

Fig. 2 gives the hemicelluloses yields recovered from SW and HW after precipitation and dialysis for all the experimental conditions used in this study. As expected, from bleached pulps, higher yields were observed for alkali extractions compared to DMSO method. It is well known that unlike DMSO, alkali treatment can cleave ether and ester linkages between polysaccharides and lignin and effectively releases hemicellulose. The hemicellulose yields recovered by SE from non-bleached SW and HW are closed to those previously reported for spruce and are between 2% and 6% w/w (Chadni et al. 2019). For the reasons mentioned

above, a basic impregnation of wood prior to SE has led to higher yields. However, in these latter conditions and in accordance with previous works, an increase of the SE severity led to a decrease of hemicelluloses yields due to degradation reactions such as peeling and alkaline hydrolysis (Chadni et al. 2019, 2020).

Fig.2

3.3. Sugar compositions and molecular masses

The composition of the hemicelluloses isolated has been established in terms of monomeric sugars by ionic chromatography after a controlled acidic hydrolysis (see experimental section). The quantification of 4-*O*-MeGlcU being not possible by our chromatographic method, its content was estimated by HSQC NMR (see section 3.5).

The average compositions in sugars corresponding to the 8 extraction conditions applied to SW and HW are given in Table 1. The weight average molecular masses M_w of the polysaccharides, assessed by water-based sized exclusion chromatography, are given in Table 1. Number average (M_n) molecular weights and polydispersities are given in Supplementary Data section S1. Whatever the experimental parameters and starting materiel (SW or HW), for all the extractions involving alkaline conditions (classical alkali extraction or SE after basic impregnation), hemicellulosic extracts were of high molecular weights ($M_w \approx 60 - 100$ kDa) and mainly composed of xylose with a relatively high proportion of 4-*O*-MeGlcU suggesting the recovery of polymeric glucuronoxylans (ratio 4-*O*-MeGlcU / Xyl ≈ 0.35). Xylose was the predominant sugar accounting for 65% to 70% of total amount of hemicellulosic sugars. It has been reported that in hardwood about 60% of non-cellulosic sugars was xylose (Willfor et al. 2005).

Interestingly, it appears from Table 1 that glucuronoxylans of higher molecular masses were recovered with NaOH-SE extraction compared to classical alkali extraction performed from bleached pulp. This observation is in accordance with previous works, the structure of the recovered hemicelluloses from SE being strongly influenced by the rapid pressure release step which open the wood structure and limit the chemical degradations (Wojtasz-Mucha et al. 2017).

The compositions of the extracts isolated with DMSO displayed a very different pattern. A lower proportion of 4-*O*-MeGlcU was observed (ratio 4-*O*-MeGlcU / Xyl $\approx 0.05-0.08$).

Relatively high contents in mannose and glucose suggested the co-extraction of

glucomannans (GM). A difference in composition was clearly observed between SW and HW, with a higher glucomannan content in SP. For H₂O-SE extraction, similar trends have been observed with higher GM co-extraction. The relatively high GM content in SW was confirmed from H₂O-SE extracts. GM have been reported to be the second most abundant hemicellulose in hardwood (Sjöström, 1993). In addition, Willford et al. reported a relatively high GM content in hardwood SW with varying proportions depending on the species. Mannose accounted for about 10% of the sugars in *Populus deltoides* SW against only 1 to 2% in *Acacia crassicaarpa* SP (Willford et al. 2005). As expected the molecular masses of hemicelluloses extracted with DMSO and water-SE were significantly lower. DMSO method solubilizes relatively low molecular mass polysaccharide fragments. Teleman et al. 2003 extracted GM from birch and aspen wood with DP $\approx$ 16. Chadni et al. extracted galactoglucomannans from spruce by H₂O-SE in the same range of severity, producing polysaccharides with comparable molecular masses ($\approx$ 20 kDa) (Chadni et al. 2019, 2020). The relatively low molecular masses obtained using these conditions are justified by the well-known acid-catalyzed hydrolysis of glycosidic linkages in hemicellulose chains.

Table 3

3.4. FTIR characterization

FT-IR spectra of SE hemicelluloses from HW (H₂O-SE and NaOH-SE) are presented in Figure 3. FT-IR spectra of the other hemicellulosic fractions isolated in this study are given in Supplementary Data section S2. In H₂O-SE and DMSO extracts, the band at 1730 cm⁻¹ is due to carbonyl groups stretching indicating that the hemicellulosic fractions isolated in neutral conditions are acetylated. This observation was confirmed by the presence of high-intensity band at 1244 cm⁻¹ assigned to C-O deformation bonds of ester functions (Rowley et al., 2013). The bands at 1508 cm⁻¹ (δ_{C-C}) and 1460 cm⁻¹ (δ_{C-H} of CH₃-O groups of S and G units) suggest the presence of a high amount of residual lignin in hemicelluloses extracted in basic medium (Sun et al., 2012; Lv et al., 2013). It can also be observed that the band at 895 cm⁻¹, assigned to C¹-H bonds of β -(1-4)-Xylp chain (Sun et al., 2016), is stronger in H₂O-SE. This is in accordance with the higher xylose contents detected by HPAE-PAD in the H₂O-SE extracts (section 3.3).

Fig. 3

3.5. NMR characterization and acetylation degree

The sugar compositions of the hemicellulosic fractions were confirmed by a 2D NMR study ^1H - ^{13}C HSQC. The spectra were in good accordance with published data of GX and GM. For HW, the main signals were assigned to sugar residues contained in GX (4-*O*-MeGlcUp and Xylp) (Evtuguin et al. 2003, Teleman et al. 2003, Zhao et al. 2017). In SW spectra additional cross-linked signals of lower intensities and assigned to Manp and Glcp were detected attesting the presence of GM (Campestrini et al., 2013, Martínez-Abad et al. 2018). HSQC NMR spectra of hemicellulosic fractions are given in Fig. 4 and in Supplementary Materials section S3. The $^1\text{H}/^{13}\text{C}$ cross peaks assignments are given in Table 3.

Fig. 4.

Table 3

In accordance with the FT-IR data, NMR data showed that hemicelluloses recovered with DMSO and H₂O-SE are all acetylated. This was confirmed by ^1H NMR intense signals at ^1H at ≈ 2.0 - 2.2 ppm assigned to CH_3CO group (Supplementary Materials section S4). It has been shown that the presence of acetylated sugar residues in the polysaccharides backbone results in a large number of possible monosaccharide combinations and NMR cross-peaks signals, complicating the spectra considerably (Michalak et al. 2018). In alkali conditions, deacetylated polysaccharides were recovered because of a further deacetylation by saponification reactions.

For DMSO and H₂O-SE hemicelluloses fractions, the acetylation degree was determined by HPLC after saponification (Table 1). The $^{13}\text{C}/^1\text{H}$ HSQC NMR correlations also helped to determine distribution patterns of *O*-acetyl groups in GM and GX backbone. Evtuguin et al. (2003) determined the $^{13}\text{C}/^1\text{H}$ correlations of acylated heteroxylans backbone by homonuclear (TOCSY) and heteronuclear (HSQC) NMR experiments. Campestrini et al. (2013) characterized acetylated GM by NMR and other techniques. More recently, lignin carbohydrate complexes (LCC) extracted from birch hardwood have been analyzed by 2D NMR (HSQC and TOCSY). Martínez-Abad et al. (2018) described the assignment of the main $^{13}\text{C}/^1\text{H}$ HSQC signals of partially acylated GX and GM in the LCC complex by HSQC NMR. In this study, the ^1H and ^{13}C chemical shifts for the [*O*-Ac(1→2/3)- β -D-Xylp] and [*O*-Ac(1→2/3)- β -D-Manp] are in good agreement with the values reported earlier. Based on previous studies and using the data from NMR, HPLC and HPEA-PAD, a semi-quantification of *O*-2, *O*-3

and *O*-2,3 acetyl units in xylan and mannan backbones was performed (Giummarella et al., 2017).

The results regarding the acetylation pattern are gathered in Table 4. In DMSO, very different acetylation patterns were observed for SW and HW. Interestingly, SW hemicelluloses are more highly acetylated than HW (4.1% and 1.0% respectively). A comparable trend was observed for the water-SE extracts with significantly higher acetyl contents (6.2% and 8.4% for HW and SW respectively). It has been previously reported that hemicelluloses isolated from hardwood SW exhibited higher degree of branching and lower xylose content (Xiao et al., 2020) but, to our knowledge, a comparison of the acetyl content of SW and HW hemicelluloses has never been investigated. The higher acetylation degree of SW hemicelluloses reported in the present study could be one of the factors contributing to the higher porosity and water permeability of SW (Ramachandriya et al., 2014). In okoume SP, comparable results were obtained for DMSO and H₂O-SE in terms of acetylation pattern. Both GX and GM are *O*2 and *O*3 acetylated, the degree of acetylation being slightly higher for GM compared to GX ($DS_{Ac} Manp \approx 0.25$ and $DS_{Ac} Xylp = 0.20$ respectively). For HW, a significantly higher acetylation degree was also found for H₂O-SE compared to DMSO, GX being acetylated at position *O*2 and/or *O*3 ($DS_{Ac} Xylp = 0.26$). These results are close to those previously obtained from spruce and the recovery of galactoglucomannans (GGM) by H₂O-SE with $DS_{Ac} \approx 0.27 - 0.35$, very similar to the values reported for native Ac-GGM Chadni et al. 2019, 2020. Thus, these results confirm that SE allowed to extract highly acetylated and slightly modified polymeric hemicelluloses. The acetylation degree of hemicelluloses strongly affecting the physico-chemical properties of the resulting materials (e.g. barrier properties of films, Kishani et al., 2018), these experimental results could help to develop an extraction route from okoume waste wood for the design of films and materials with controlled properties.

Table 4

4. Conclusion

Steam explosion (SE) pretreatment was assessed for the extraction of high molecular weight hemicelluloses directly from okoume SW and HW and compared to traditional extraction methods with DMSO and alkali solution performed from bleached wood. The SE parameters which include a short steaming duration and a mechanical defibration limit the

polysaccharide hydrolytic breakdown and enhance the efficiency of the subsequent extraction. The recovery of high molecular weight GX was performed using a SE treatment after basic impregnation. On the other hand, after a water pre-soaking, SE allowed the recovery of a mixture of highly O2 and/or O3 acetylated GX and GM. It clearly appeared that H₂O-SE preserved the acetyl moieties in a greater extent compared to the mild DMSO extraction. The results obtained in our study showed that (1) SE can be employed for the extraction of complex polysaccharides from wood for characterization purposes, (2) okoume wood hemicelluloses are primarily composed of highly acetylated GX with a relatively high amount of acetylated GM in the SW. The impact of this work could involve better recovery of waste from the okoume industry in Gabon with a view to the production of hemicellulose-based materials.

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Table 1. Composition of sugars, acetic acid (% of dry mass of hemicelluloses molar mass of hemicelluloses) and average (Mw in KDa) of hemicelluloses

Treatment		Impr ^a	S ^o ^b	Xyl ^c	GIUOMe ^d	Man ^c	Glc ^c	Ara ^c	Gal ^c	Rha ^c	GalA ^c	GlcA ^c	Ac ^e	Mw ^f
NaOH ^e	SW			43.1 ± 1.0		n.a	1.7 ± 0.1	2.4 ± 0.1	1.7 ± 0.1	1.1 ± 0.0	2.9 ± 0.1	0.4 ± 0.0	-	64
	HW			34.1 ± 2.6	11.1	n.a	0.1 ± 0.1	0.3 ± 0.0	1.4 ± 0.1	0.9 ± 0.0	1.6 ± 0.2	0.3 ± 0.0	-	56
DMSO ^g	SW			16.0 ± 0.8	1.3	17.0 ± 0.6	27.4 ± 1.6	0.7 ± 0.0	1.5 ± 0.0	0.5 ± 0.0	1.1 ± 0.1	0.4 ± 0.0	4.1	29
	HW			44.3 ± 0.3	2.2	11.5 ± 0.4	11.0 ± 0.2	0.2 ± 0.0	0.9 ± 0.0	0.7 ± 0.1	0.9 ± 0.0	0.3 ± 0.0	1.0	24
Steam explosion ^h	SW	H ₂ O	3.1	47.7 ± 1.7	2.4	11.3 ± 0.8	9.3 ± 0.3	0.5 ± 0.0	5.4 ± 0.2	4.6 ± 0.1	5.4 ± 0.2	9.3 ± 0.2	8.4	9
		H ₂ O	3.6	44.5 ± 1.5		5.6 ± 0.2	25.7 ± 1.0	0.4 ± 0.0	4.4 ± 0.2	0.9 ± 0.1	0.9 ± 0.1	0.2 ± 0.0	8.1	26
		H ₂ O	3.9	29.4 ± 0.1		4.9 ± 0.0	34.3 ± 0.2	0.1 ± 0.0	3.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.1 ± 0.0	8.0	21
	HW	H ₂ O	3.1	54.9 ± 11.7	1.1	2.6 ± 2.6	1.2 ± 0.8	2.2 ± 2.0	10.1 ± 5.5	1.9 ± 1.7	6.8 ± 0.2	0.8 ± 0.0	6.2	10
		H ₂ O	3.6	44.7 ± 2.1		2.3 ± 0.2	1.3 ± 0.1	0.1 ± 0.0	3.2 ± 0.2	0.1 ± 0.1	1.5 ± 0.2	0.2 ± 0.0	7.7	11
		H ₂ O	3.9	64.4 ± 1.9		4.2 ± 0.2	3.1 ± 0.1	0.1 ± 0.0	5.6 ± 0.2	1.5 ± 0.0	2.0 ± 0.1	0.3 ± 0.0	6.6	6
		NaOH	3.1	30.7 ± 1.1	10.1	n.a	0.8 ± 0.0	1.4 ± 0.1	1.7 ± 0.1	1.4 ± 0.1	0.6 ± 0.0	0.4 ± 0.1	-	97
		NaOH	3.6	24.1 ± 1.3		0.2 ± 0.1	11.8 ± 0.7	1.7 ± 0.1	3.0 ± 0.2	1.7 ± 0.1	0.4 ± 0.0	0.2 ± 0.0	-	104
		NaOH	3.9	30.4 ± 0.1		0.2 ± 0.0	5.6 ± 0.0	1.6 ± 0.0	1.7 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	-	86
	HW	NaOH	3.1	44.1 ± 1.0	15.9	n.a	0.3 ± 0.0	0.4 ± 0.0	1.0 ± 0.0	0.9 ± 0.0	0.8 ± 0.0	0.3 ± 0.0	-	64
		NaOH	3.6	29.0 ± 5.4		0.1 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	1.4 ± 0.3	0.5 ± 0.1	0.5 ± 0.1	0.1 ± 0.0	-	69
		NaOH	3.9	40.1 ± 6.3		0.1 ± 0.0	0.2 ± 0.0	0.4 ± 0.1	1.2 ± 0.2	0.4 ± 0.1	0.3 ± 0.0	0.1 ± 0.0	-	70

^aImpregnation conditions prior to SE; ^b Severity factor of SE; ^cXylose, mannose, glucose, arabinose, galactose, Rhamnose, galacturonic and glucuronic acid %, determined after hydrolysis by HPEAC-PAD; ^destimation by HSQC-NMR; ^e acetic acid quantified by HPLC after saponification; ^f determined by SEC; ^gextraction from holocellulose; ^h extraction from wood by SE

Table 2. Chemical composition (% of dry wood) of raw wood.

	SW ^a	HW ^b
Extracts ^a	2.36 ± 0.31	2.00 ± 0.41
Klason lignin	29.68 ± 1.58	28.02 ± 0.37
Fuc	0.02 ± 0.00	0.00 ± 0.00
Rha	0,27 ± 0.04	0.24 ± 0.05
Ara	0.52 ± 0.04	0.11 ± 0.01
Gal	0.68 ± 0.01	0.56 ± 0.02
Glc	50.73 ± 0.15	54.19 ± 1.66
Xyl	11.34 ± 0.04	10.16 ± 2.41
Man	2.23 ± 0.03	2.09 ± 0.15
GalA	0.49 ± 0.28	0.64 ± 0.04
GlcA	0.13 ± 0.04	0.08 ± 0.07

Means of three independent analyses. ^aSapwood; ^bHeartwood; ^cToluene-ethanol-methanol extracts (see Material & methods section)

Table 3. Chemical shifts (ppm) of ¹H NMR spectra

Impregnation		Structural Units	Attributions						
			1	2	3	4	5eq	5ax	6
SW	NaOH	Xyl (isol.)	4.31	3.28	3.55			3.40	
		Xyl (Xyl-Ac)	4.42	3.23				3.35	
		αAra-3Xyl				4.27			
		MeGlcA		3.51	3.73	3.16	4.31		
	H ₂ O	Xyl (isol.)	4.31	3.8	3.57	3.79		3.40	
		Xyl (Xyl-Ac)	4.41	3.23	3.55	3.76		3.39	
		Xyl-3Ac	4.58	3.47	4.98	4.93			
		Xyl-2Ac		4.57	3.62	3.86			
		Xyl-2,3Ac			5.12	4.07			
		Xyl-2Ac-2MeGlcA			5.02				
		αAra-3Xyl	5.36			4.26			
		Xyl-2,3Ara	5.22						
		Man (Man-Ac)		4.03	3.65				
		Man-2Ac	4.88	5.44					
		Man-3Ac		4.17					
		MeGlcA	5.29	3.57	3.72			4.31	
HW	NaOH	Xyl (isol.)	4.41	3.28				3.37	
		Xyl(Xyl-Ac)	4.42	3.23	3.53			3.36	
		αAra-3Xyl				4.26			
		Xyl-2,3Ara	5.23						
	H ₂ O	MeGlcA		3.57	3.73	3.16		4.31	
		Xyl (isol.)	4.41	3.28	3.55	3.79		3.40	
		Xyl(Xyl-Ac)	4.42	3.23	3.54	3.76		3	
		Xyl-3Ac	4.56	3.49	5.0	4.93			
		Xyl-2Ac		4.57	3.62	3.88			
		Xyl-2,3Ac			5.12	4.04			
		Xyl-2Ac-2MeGlcA			5.01				
		αAra-3Xyl				4.27			
		Xyl-2,3Ara	5.23						
		Man (Man-Ac)		4.03	3.65				
		Man-2Ac	4.90						
		Man-3Ac		4.17					
		MeGlcA		3.57	3.72	3.16	4.31		

Xyl (isol.): non-acetylated Xylp unit in the skeleton isolated from another acetylated unit; Xyl (Xyl-Ac): non-acetylated Xylp unit linked with an acetylated neighbor; Xyl-3Ac: 3-*O*-acetylated Xylp unit; Xyl-2Ac: 2-*O*-acetylated Xylp unit; Xyl-2,3Ac: 2,3-di-*O*-acetylated Xylp unit; Xyl-3Ac-2GlcA: Xylp unit carrying a 2-*O*- and 3-*O*-acetylated MeGlcA; αAra-3Xyl: Ara terminal *O*-3 bonded to a monosubstituted Xylp unit; MeGlcA: *O*-2 bonded MeGlcA terminal residue; αAra-3Xyl: Ara terminal *O*-3 bonded to a monosubstituted Xylp unit; MeGlcA: *O*-2 bonded MeGlcA terminal residue to monosubstituted Xylp and also 3-*O*-acetylated.

Table 4. Acetylation degree of hemicelluloses

		Ac% ^a	DS _{Ac Manp} ^b	NMR $\delta C/\delta H$ ppm	DS _{Ac Xylp} ^c	NMR $\delta C/\delta H$ ppm
DMSO	SW	4.1	0,24	O-2 71.85/5.30 O-3-	0,18	O-2 73.85/4.52 O-3 75.45/4.82
	HW	1.0	0,02	O-2 - O-3 73.36/4.96	0,04	O-2 70.25/4.50 O-3 75.45/4.82
H ₂ O-SE	SW	8.4	0,25	O-2 73.66/5.28 O-3 75.47/5.19	0,20	O-2 71.02/4.50 O-2,3 72.00/4.84 O-3 77.13/4.64
	HW	6.2	0,08	O-2 73.13/5.25 O-3-	0,26	O-2 72.55/4.50 O-2,3 72.00/4.84 O-3 77.13/4.64

^aAc: Acetic acid (% of dry mass of hemicelluloses after saponification); ^bDS_{Ac Manp}: Degree of substitution of mannose residue with acetyl groups; ^cDS_{AcXylp}: Degree of substitution of xylose residue with acetyl groups.

Figure 1. Experimental procedures for hemicelluloses extraction from okoume sawdust

NaOH SW
NaOH HW

Okoume
holocellulose

DMSO SW
DMSO HW

NaOH
ext.
Bleachin

DMSO
extraction

Okou
sawdust

Steam Explosion
logR° = 3.06, 3.55 or 3.94
H₂O or NaOH impregnation

NaOH-SE SW
NaOH-SE HW
H₂O-SE SW
H₂O-SE HW

Figure 2. Polymeric hemicelluloses yields (% of dry mass of the initial wood)

Figure 3. FT-IR Spectra of SE-extracted hemicelluloses (H₂O-SE HW and NaOH-SE HW, S = 3.1)

Fig. 4 2D HSQC NMR Spectral analysis of SE extracted hemicelluloses.^a

^aX: (1→4) β -D-Xylopyranosyl ; U: 4-O-methyl- α -D-glucuronic acid ; M: (1→4) β -D-Mannosyl ;

G: (1 →4) β -D- Glucosyl ; Ac: acetyl.