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Wood Chemistry

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Decoupling processability of callose enriched aspen wood: the study of extractability of lignin and carbohydrates during autohydrolysis and kraft pulping

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Abstract: Development of transgenic lignocellulosic biomass with increased cell wall accessibility could benefit development of resource efficient biorefinery concepts. Transgenic callose enriched aspen, reported to show an increased cell wall mesoporosity including increased cellulose-lignin distance and improved accessibility to enzymatic hydrolysis, has been assessed for its behaviour in industrially relevant decoupling processes: autohydrolysis mediated extraction and kraft pulping (not performed in sequence). Under mild autohydrolysis conditions (130 °C, initial 20 min of extraction) and during initial mild phase of kraft pulping (10–20 min) this modification indeed led to a modestly but consistently enhanced extractability of wood components of slightly higher molar mass. These results point out the potential of the callose enriched wood for new prospects of mild resource efficient biorefining.

Keywords: callose enriched aspen; autohydrolysis extraction; kraft pulping; cell wall porosity; extractability

1 Introduction

Selective, resource efficient decoupling of wood tissue, cell wall in particular, and recovery of individual components with structure control is a pre-requisite for development of sustainable bio-material concepts. In that respect, hydrothermal extractions and alkaline pulping are established processing technologies that can offer a platform for a large scale biorefining provided they can accomplish sufficiently precise and efficient primary separation of the wood components.

Alkaline pulping is in a large scale performed by the action of OH^- and HS^- penetrating the cell walls and accomplishing fragmentation of lignin with consecutive solubilization and diffusion of the formed fragments through the wood tissue out into the surrounding liquor (Gellerstedt et al. 2009; Gustafsson et al. 1983; Mattsson et al. 2017). This process is to different extents accompanied by undesired degradation and co-extraction of hemicelluloses and their degradation products. While the degradation contributes to the consumption of cooking chemicals (primarily when dominated by peeling and formation of isosaccharinic acid) and a lower yield, the loss of hemicelluloses, in general, compromises the recovery of potentially attractive compounds that could be used for bio-based materials and chemicals. Therefore, often, a pre-extraction step preceding the kraft cooking that would enable recovery of hemicellulose is desired (Garrote et al. 1999; Jedvert et al. 2012; Krogell et al. 2013; Rissanen et al. 2014a,b; Wojtasz-Mucha et al. 2017, 2020). Most commonly, such a pre-extraction is autocatalyzed by dissociation of acid groups on hemicelluloses leading to acidification and, thus, acid-catalysed partial depolymerization of their backbone (at elevated temperature) promoting the subsequent solubilization and transport out from the wood tissue.

Yet, while feasible with large scale processing, the precision of these separation technologies is strongly dependent on accessibility of the wood tissue and detailed knowledge about structural motifs governing their outcome. If properly related to processability behaviour, specific genetically introduced

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structural changes to wood tissue, cell wall in particular, may provide an insight into the structure-processability relationship and open up for tailoring of wood biomass for specific processing steps. For instance, an introduction of new polysaccharide structures into the cell wall architecture might decisively affect behaviour of this complex matrix during decoupling actions, whether alkaline delignification or extraction through autohydrolysis.

Recently, a genetically modified callose-enriched *Arabidopsis* was developed. Callose is a β-1,3 glucan, just like cellulose synthesized by plasma membrane-located glucan synthase complexes. It is commonly deposited under stress to control the cell wall permeability and prevent penetration of the pathogens and the loss of cellular water (Houston et al. 2016; Wang et al. 2021). At the sites of fungal attack, callose is, for instance, suggested to reinforce the cell wall by permeating the cellulose microfibrils (Eggert et al. 2014) and is also important for cell reproduction being deposited between the primary cell wall and the plasma membrane in order to prevent fusion of the cells (Gidley and Nishinari 2009; Stone 2009). Interestingly, cellulose-callose mixtures have shown higher elasticity (lower Young's modulus) and ductility compared to cellulose alone (Abou-Saleh et al. 2018). Recently, a transgenic approach was taken to introduce callose into the secondary cell wall in *Arabidopsis thaliana* and hybrid aspen *P. tremula x tremuloides* plant species (Bourdon et al. 2023). In the various transgenic *Populus* lines the amount of callose in wood ranged between 1 and 10 % of glycosidic linkages. Based on the solid-state NMR analysis, callose appears to act as a spacer between cellulose macrofibril aggregates and partially between cellulose and lignin. Consequently, callose was shown to increase the cell wall porosity. This could potentially influence accessibility of the cell wall components to the processing agents and reduce the mass transport resistance enhancing thereby the kinetics of decoupling and recovery of individual wood components, while also reducing the consumption of active chemical agents.

These analyses now raise the question of callose impact on the decoupling processability of the obtained wood in a biorefining context. Could an enhanced production of callose in a cell wall promote extractability of carbohydrates and lignin from this most abundant source of bio-building blocks? Here, this possibility is explored by subjecting the callose enriched aspen wood to a mild hydrothermal extraction and kraft pulping, respectively and following its response in terms of amounts, extraction kinetics and properties of extracted carbohydrates and lignin. A flow-through set-up was chosen for both types of processing, as it allows for treatment of relatively small amounts of wood powder (where mass transfer phenomena can be considered limited to the cell wall level) with easy adjustment of processing conditions while obtaining time-resolved information through continuous sampling of extracted material (Figure 1). Given the transgenic aspen material with two different levels of callose content, understanding of their processing behaviour in relation to unmodified wood would provide a valuable insight into the structure-processability interplay, instrumental for further tailoring of sustainable bio-material concepts.

2 Materials and methods

2.1 Materials

Experiments were performed on two genetically modified lines (GM11 & GM17, Sainsbury Laboratory, University of Cambridge, UK) of *P. tremula x tremuloides* against the same unmodified *P. tremula x tremuloides* reference tree line (wild type, WT). GM11 contains slightly more callose than GM17 (1.2 % of the glycosidic linkages in the greenhouse grown material in GM11 vs. 0.8 % in GM17), and therefore is expected to display a stronger difference to the wild-type non-transgenic control line (Bourdon et al. 2023). Samples were wet-debarked by hand, dried, cut into ~4 cm pieces and milled in a Wiley mill equipped with a <1 mm sieve. Lower branches of each tree were used; multiple branches were combined in milling (within the sample group) for an appropriate sample size. The wood meals were double-sieved through a 1.0 mm meshed test sieve. Prior to every cook the dry content of the wood meal was measured after overnight drying at 105 °C.

Due to the young age of the analysed species, greenhouse grown trees of the first year (Bourdon et al. 2023), the pith tissues were present in the analysed material.

2.2 Decoupling processing

2.2.1 Hot water extraction in a flow-through reactor

The continuous autohydrolysis process was conducted in a small flow-through reactor, presented in Figure 1. The reaction column (300 × 7.8 mm ID, SS), secured with 2.0 µm frit caps, was connected to coiled flow lines at the inlet and outlet for the instant heating of reaction liquor and cooling of effusing samples, respectively. The continuous flow of liquors was sustained by a high-pressure pump, while the relief valve maintained high pressure throughout the system and prevented vapour formation.

Approximately 4 g of the wood meal was carefully packed in the flow-through reactor column to avoid lumps and plugs, for an even flow of water. Prior to the reaction, around 3–4 free column volumes of water were pumped through the reactor (r.t.; 5 ml/min), to obtain good

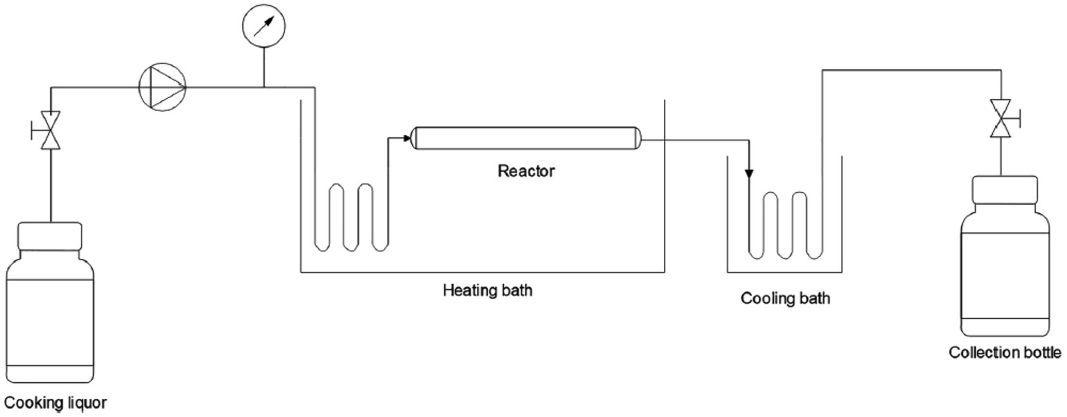

Figure 1: Schematic diagram of the small flow-through reactor. Adapted from Wojtasz-Mucha (2020).

impregnation of the wood samples. Directly after impregnation, the column was immersed in an oil bath. The reaction temperatures of consecutive runs were set to 130, 150 and 170 °C for 120 min, with a continuous flow of water set to 5 ml/min. During the cook, seven liquor samples were collected in total, at time intervals of 0–10, 10–20, 20–40, 40–60, 60–80, 80–100 and 100–120 min, respectively. Following the last collection time-point, the water flow was cut, and the reaction column was immediately transferred to a cooling bath (10 min). Afterwards, the pulp was removed from the column, homogenised with 300 ml of deionized water and filtered with additional washing. Extractions at each temperature were performed in duplicates.

2.2.2 Kraft pulping in a flow-through reactor

The cooking liquor of aqueous Na_2S and NaOH, with constant concentrations of hydroxide and hydrosulfide ions equal to 0.26 mol/kg, was prepared from ~1.0 mol/kg stock. The exact composition of the stock solution was determined with stepwise titration (‘ABC titration’) using SI Analytics TritroLine 7000 auto titrator and 1.0 M HCl as titrant.

Approximately 4 g of reference wood (WT) and 2.5 g of genetically altered wood (GM11 & GM17) meal was carefully packed in the column of the flow-through reactor, to avoid lumps and plugs for an even flow of the processing medium. During the kraft cooking, the genetically modified wood species (GM11 & GM17) showed vast tendency to swell up once the temperature reach ~140 °C (20–40 min cooking time point); the swelling caused clogging of the reactor column, overpressuring the system, and releasing the security valve (*i.e.:* termination of cooking sequence). For that reason, the load of genetically modified wood was reduced, to avoid the disturbance of cooking process. The intensified swelling compensated for the reduced wood load without risk of changing mass transfer properties.

Prior to the treatment, around 3–4 free column volumes of cooking liquor were pumped through the reactor (r.t.), to obtain good impregnation of the samples. Directly after impregnation, the column was immersed in an oil bath heated to 115 °C; at the same time, the temperature was increased to 160 °C and kept constant until the end of the process. The temperature profile of kraft cooking is presented in Figure 2.

The flow rate of cooking liquor was changed over the collection of samples according to Table 1. The adjustment of the flow rate aimed to compensate for the change in OH^- concentration during the initial phase of the kraft process, where the alkali consumption is relatively fast. It has been shown that the level of $[OH^-]$ decreases by ~20 % during the first 10 min of reaction (outlet *vs* inlet volume), equalizing after 15 min of cooking (Bogren et al. 2009). Following the last

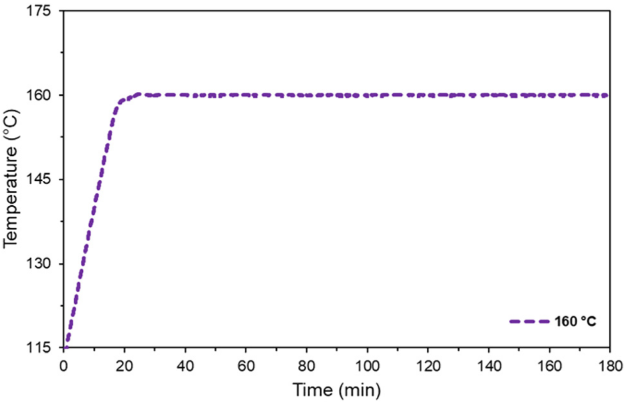

Figure 2: Temperature profile of the kraft cooking experiment.

Table 1: Summary of flow rates and sampling ranges during the kraft cooking.

Flow rate time range (min)		Flow rate (ml/min)	Fraction no.	Sampling range (min)
Cooking cycle	0–12	10.0	1	0–10
	12–22	8.0	2	10–20
	22–40	2.5	3	20–40
	40–60	2.5	4	40–60
	60–90	2.5	5	60–80
	90–120	2.5	6	90–120
	120–150	2.5	7	120–150
	150–180	2.5	8	150–180
Cooling cycle	180–190	1.0		

collection time-point, the water flow was cut, and the reaction column was immediately transferred to a cooling bath (10 min). Afterwards, the pulp was removed from the column, homogenised with 300 ml of deionized water and filtered with additional washing. Selected runs were performed in duplicates.

2.3 Analytical work

The experimental approach for characterisation of wood samples and products of wood autohydrolysis (liquid fractions & pulp) is summarised in Figure 3.

2.3.1 Pre-analysis treatment of samples

Wood pulp: the filter cake of wood pulp was dried for a minimum of 24 h at 105 °C and weighed. The dry material was used in further analysis.
Autohydrolysis fractions: each of the fractions was evaporated in a rotor evaporator (40 °C/72 mbar), frozen (pre-freezing at −20 °C for approx. 2 h + LN_2 freezing) and lyophilised during a continuous freeze-drying process (−82 °C/0.01–0.07 mbar) for 5–7 days.

2.3.2 Acid hydrolysis procedure

Samples of reference wood meal, autohydrolysis pulps and freeze-dried liquors were subjected to a complete acid

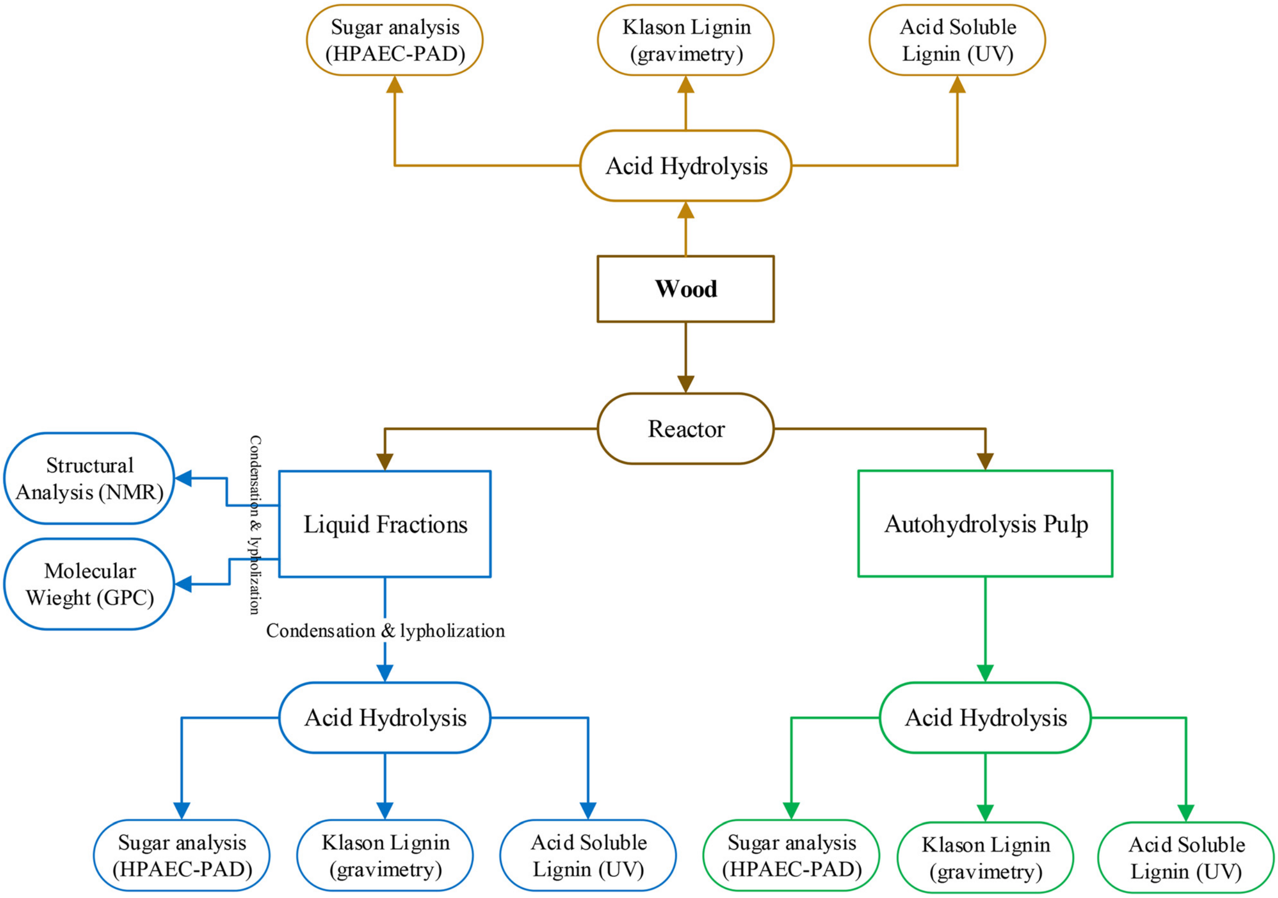

Figure 3: Outline of the experimental work, starting with raw material, through the cooking in a flow-through reactor, and finally analytical methods for characterisation.

hydrolysis using 72 % sulphuric acid. Approx. 30 mg of dried material weighed in 50 ml beakers was treated with 0.45 ml of sulphuric acid (72 % w/w, Thermo Scientific), carefully stirred, and evacuated for 15 min. Afterwards, samples were incubated at 30 °C for 1 h (20 min intervals stirring), followed by dilution with 12.6 g of water (approx. acid concentration: 4 %). The diluted mixtures, secured with aluminium lids, were incubated in a pre-heated autoclave at 125 °C for 1 h, after which, the solutions were pre-cooled (~80 °C) and filtered through glass microfiber filters (Cytiva Whatman™ Binder-Free Glass Microfiber Filters, Grade GF/A) with hot water washing.

2.3.3 Klason lignin content

Acid hydrolysis of wood-originated samples (wood meal, pulps, freeze-dries) break the bonds between lignin and polysaccharides. In low pH, the freed lignin (referred to as Klason lignin) precipitates (Gellerstedt et al. 2009) and is separated from the solution of sugars *via* the above-mentioned filtration. Therefore, the glass microfiber filters with solid residues were collected, dried at 105 °C for at least 15 h, and gravimetrically measured to determine the amount of Klason lignin in the analysed material (Theander and Westerlund 1986).

2.3.4 Carbohydrates analysis

The polysaccharides were degraded to mono-sugars, upon action of acid. The monosaccharides ended in filtrate that was diluted (1:50), spiked with fucose as injection control standard (8 mg/ml per sample) and submitted to analysis.

Monosaccharide standard mixtures (Fuc – fucose, Arb – arabinose, Glc – glucose, Gal – galactose, Xyl – xylose and Man – mannose) were prepared at a series of concentrations in relation 1: 4: 8: 16: 24 for calibration purposes. Each analytical sample was filtered through a 0.22 µm syringe filter (PTFE, Fisher Scientific), prior to injection.

The analysis was performed using a Dionex ICS-5000 Ion Chromatography System (HPAEC, Dionex Corporation, CA, USA). The analytical compartment was composed of an Eluent Organizer with Dual Pump Channel, Detector Module, Autosampler, and Pulsed Amperometric Detector (PAD) with a disposable gold working electrode. Separation was carried out using a CarboPac™ PA1 analytical column (2 × 250 mm) protected by a similarly packed guard column (2 × 50 mm, Thermo Fisher Scientific,) at 30 °C. The mobile phase was delivered at 0.26 ml/min, with a multi-step gradient flow, mobile phase A, 200 mM NaOH and mobile phase B, 200 mM NaOH + 170 mM NaAc. The injection volume was set to 10 µl. The chromatograms were recorded with Chromeleon software v. 7. 1.0.898. (Thermo Fisher Scientific).

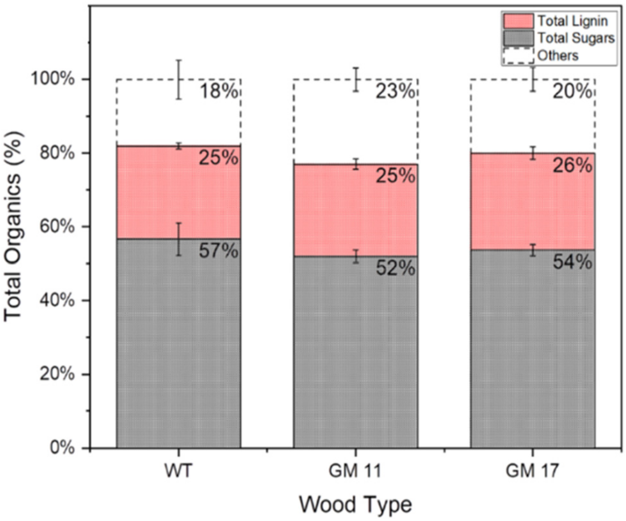

Figure 4: Composition of the wood samples prior to treatment. The components were calculated to the dry mass of sample, estimated after overnight drying at 105 °C. Total lignin represents Klason and ASL lignin; total sugars represent detected monomers (Ara, Gal, Glc, Xyl, Man); 'others' account for not analysed extractables (*i.e.:* ash, acetyl groups, rhamnose, sugar acids and unidentified matter). WT, wild type wood; GM11, genetically modified wood type 1; GM17, genetically modified wood type 2. Error bars represent standard deviation of duplicate runs.

Two corrections were introduced in calculations of monomeric units in raw samples: (1) correction to the hydrolysis yield, *i.e.:* Arb: 0.93, Gal: 0.93, Glc: 0.92, Xyl: 0.79, Man: 0.90; based on the Wojtasz-Mucha (2020); (2) correction to the anhydrous character of units in carbohydrate chains, *i.e.:* 0.88 for pentoses and 0.90 for hexoses.

2.3.5 Acid-soluble lignin content

The hydrolytic filtrate (dilution 1: 50) was used to determine the fraction of lignin soluble in acid solutions (ASL), based on the absorbance measured at a wavelength of 205 nm. The measurements were carried out using Specord 205 spectrophotometer (Analytic Jena), with data collected in WinASPECT Software v. 2.3.10, against an aqueous reference solution containing the same amount of fucose and sulfuric acid as in analytical samples. The calculations were based on equation (1), where A is absorbance measured at 205 nm, df stands for dilution factor, b is cell path length (cm) and a is absorptivity (here 110 l/g*cm) (Ehrman et al. 1998).

$$\mathrm{ASL}\left(\frac{\mathrm{g}}{\mathrm{L}}\right)=\frac{A\times df}{b\times a} \tag{1}$$

2.3.6 Molar mass distribution analysis

The molar mass distribution (MMD) and weight average molar mass were measured for hydrolytic fractions of maximum extraction yield (mg/g wood) at a particular temperature, *i.e.:* 130 °C: 0–10 min, 150 °C: 20–40 min, 170 °C: 10–20 min. The freeze-dried samples (10 mg) were initially dissolved in 1 ml DMSO/LiBr and left overnight. These solutions were further diluted to a concentration of 0.24 mg/ml, filtered through a 0.2 μm syringe filter (GHP, Fisher Scientific) and submitted for analysis.

The analysis was performed using size exclusion chromatography PL- GPX 50 Plus Integrated GPC system, equipped with two PolarGel-M columns (300 × 7.5 mm) protected by a similarly packed (50 × 7.5 mm), and connected refractive index (RI) and ultraviolet (UV, 280 nm) detectors (Polymer Laboratories, Varian Inc.). The mobile phase of DMSO containing 10 mM of LiBr was pumped at a flow rate of 0.5 ml/min and a temperature of 50 °C. Samples were run in duplicates, with a relative standard deviation (RSD) of 0.43 %. The instrument was calibrated with 10 pullulan polysaccharide standards with molar mass ranging from 180 to 708,000 Da and data analysis was carried out using Cirrus GPC Software v. 3.2.

2.3.7 Structural analysis

The range of freeze-dried extracts (130 °C: 100–120 min, 150 °C: 40–60 min, 170 °C: 0–10 min), was submitted to NMR analysis (Swedish NMR Centre). The choice of samples was made to show the widest spectrum of possible structural changes over time and in a range of temperatures, additionally considering the extraction yield. The dried samples were dissolved in DMSO-d_6 (~30 mg/0.70 mL DMSO-d_6). The heteronuclear single quantum coherence spectroscopy (^{1}H^{13}C HSQC) spectra were acquired with the 'hsqcedetgpsisp 2.3' sequence., 0.13 s acquisition time for ^{1}H, and 5.3 μs for ^{13}C, the interscan delay 3 s. The ^{1}JC–H coupling constant was set to 45 Hz. Each spectrum was recorded for 4.5 h in 800 MHz with a Bruker Avance III HD 18.8 T spectrometer at 25 °C. The ^{1}H-spectra were recorded at a 30° pulse angle, 6 s pulse delay, 1024 scans and 2.04 s acquisition time.

3 Results

Composition of the untreated wood samples, summarised in Figure 4., suggests nearly neglectable differences between the total lignin, i.e.: 25 %, 25 % and 26 %, and total carbohydrates, i.e.: 57 %, 52 % and 54 % contents for WT, GM11 and GM17 wood, respectively; values fitting very well the literature span (Borovkova et al. 2022; Studer et al. 2011; Wang et

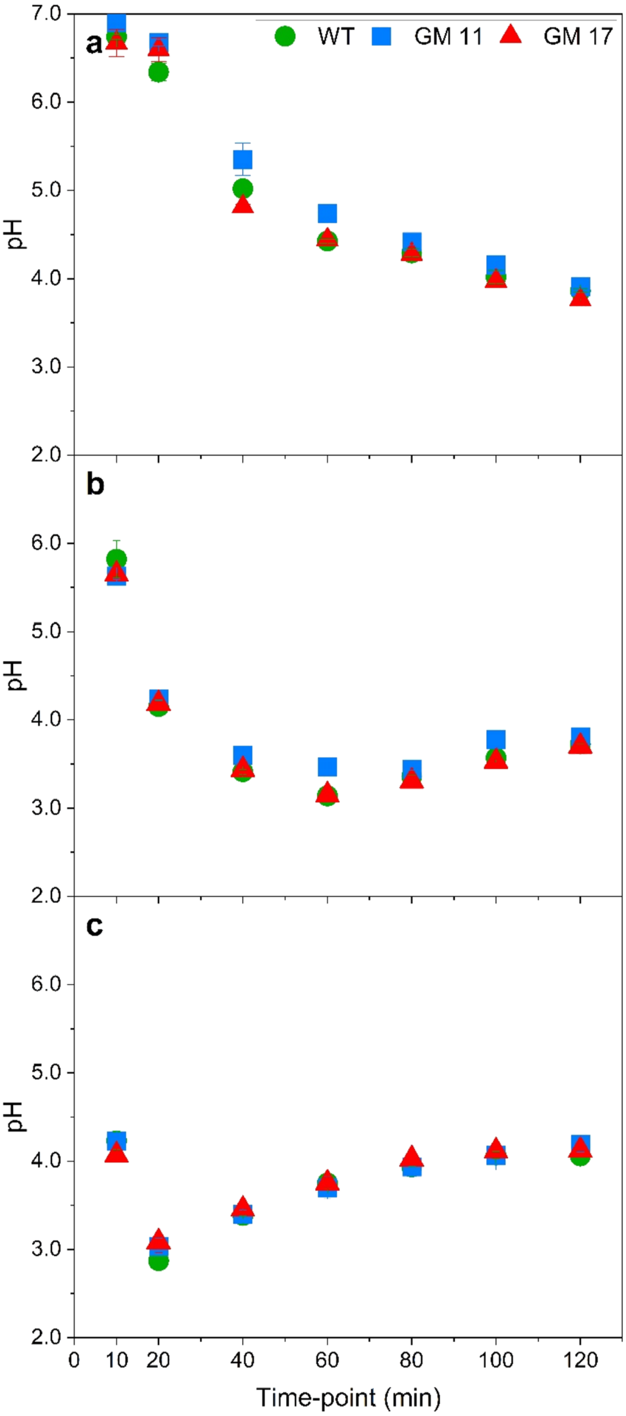

Figure 5: Change of pH of hydrolytic fractions straight after the collection at (a) 130 °C, (b) 150 °C and (c) 170 °C. WT, wild type wood; GM11, genetically modified wood type 1; GM17, genetically modified wood type 2. Error bars represent standard deviation of duplicated runs.

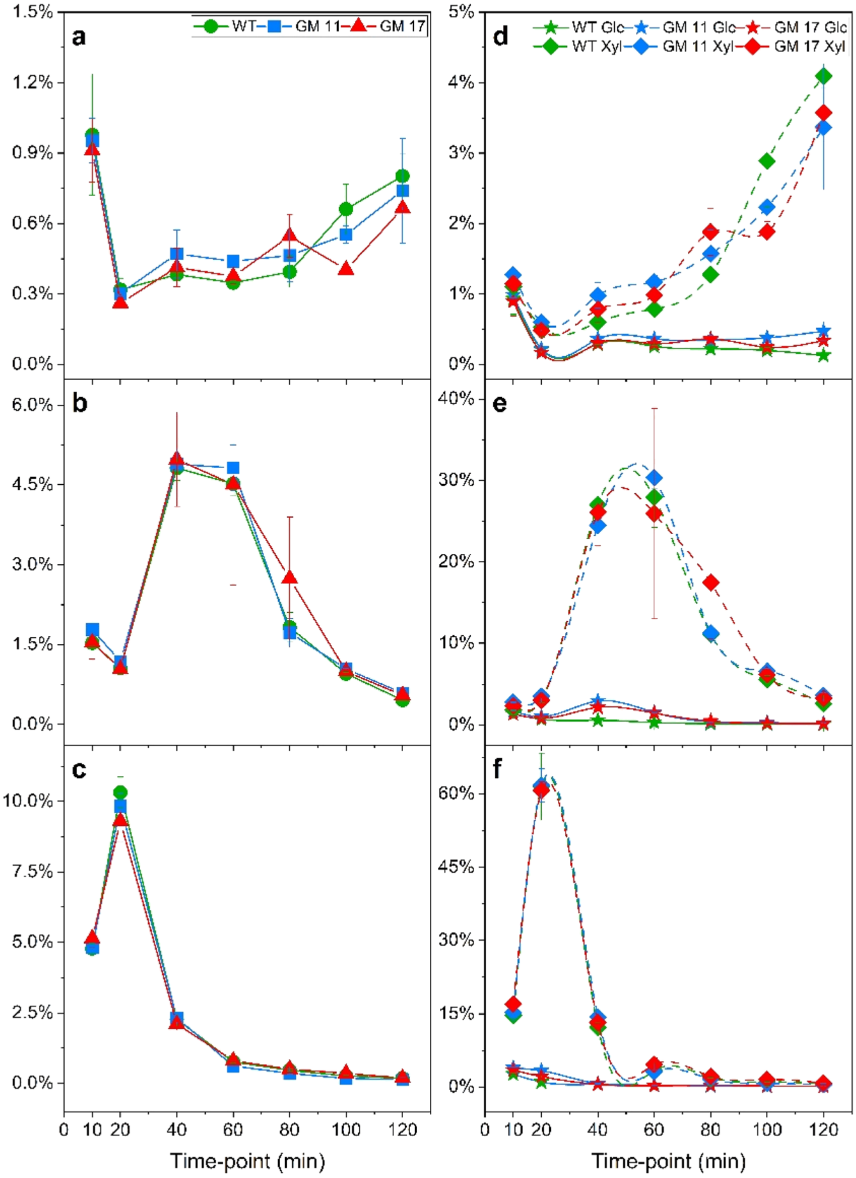

Figure 6: Carbohydrates content extracted in hydrolytic fractions over time, calculated as % analysed wood at (a) 130 °C, (b) 150 °C and (c) 170 °C and glucose and xylose content calculated as % of total glucose and xylose content in the analysed wood at (d) 130 °C, (e) 150 °C and (f) 170 °C. WT, wild type wood; GM11, genetically modified wood type 1; GM17, genetically modified wood type 2.

al. 2018). On the other hand, the values for unanalysed material (ash content, acetyl groups, rhamnose, sugar acids and extractives), i.e.: 18 %, 23 % and 20 % for WT, GM11 and GM17, respectively, seem to be on the high end reflecting also shortcomings of the Klason methodology for hardwood as well as the young age of analysed specimens. 2D HSQC NMR confirmed presence of callose (1–3 glucan) in the modified wood (Supplementary Figures S1–S5). These results are in line with the elevated level of callose in the line GM11 as compared to GM17 (Bourdon et al. 2023).

3.1 Autohydrolysis behaviour

Acidification of the wood tissues and extraction medium is the chemical engine behind the autohydrolysis leading to a partial acid hydrolysis of the polysaccharides necessary to enable their diffusion through the cell wall and final extraction. Observed pH values of the hydrolytic extracts reflect a combined effect of deprotonation of the hemicellulose acids, cleavage of their acetate groups and diffusion of the formed acids, including degradation products and polysaccharide bound acid groups (promoted by acid hydrolysis of the polysaccharides back bones) into the extraction liquor (Figure 5). The process is highly temperature dependent which is reflected in pH-profiles for the three studied temperatures. At 130 °C, the acidification is relatively slow and progresses from pH 6.7–6.9 to 3.8–3.9 relatively monotonously over the entire extraction time. At 150 °C, the process is faster (starting at 5.7–5.8) and reaches a pH minimum at 3.1–3.5 after approximately 60 min (as the maximum extraction of the acid carrying structures is reached) turning up again (as the wood

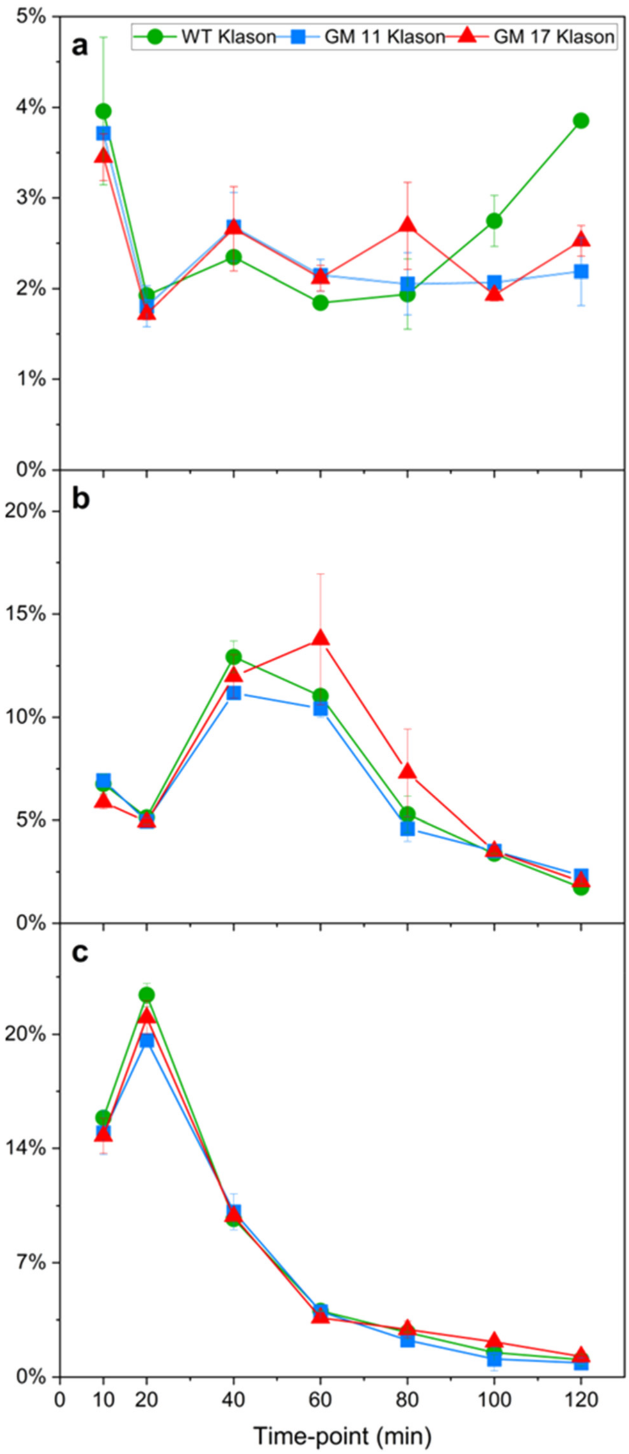

Figure 7: The change of lignin amount extracted in hydrolytic fractions, calculated to mass of analysed wood at (a) 130 °C, (b) 150 °C and (c) 170 °C.

gradually gets depleted on the acid material) to finally reach 3.7–3.8 at the end of the extraction.

Interestingly, in both cases GM11 seems to provide a more modest acidification in the middle of the extraction process (20–60 min), pointing out either a lower initial content of acid groups in GM11 or a slower extraction of the acid carrying hemicelluloses at these temperatures. Consequently, a somewhat lower extraction yields could be expected from GM11 at these time points, but the reason for this modest acidification remains to be identified. Increase of the temperature to 170 °C enhances further the acidification leading to an even deeper pH minimum (2.9–3.1) at a shorter processing time (10–20 min), reflecting an even more intense depletion of the acid carrying structures in the cell wall. However, at these harsh conditions, the differences between the studied wood types are largely erased.

3.1.1 Kinetics of lignin and carbohydrate extraction

Contrary to the implications of the pH analysis (a relatively modest pH decrease, Figure 5) predicting a lower extraction at mild extraction conditions, GM11 showed comparable extraction of carbohydrates with the other two types at 130 °C after 20–40 and 40–60 min (Figure 6). This extraction at a comparably lower acidity might indicate improved accessibility and/or reduced mass transport resistance in the cell wall of GM11 that would allow extraction of longer carbohydrate chains without the need of acid hydrolysis promoting the diffusion. Indeed, as will be shown below, the molar mass distribution of the material extracted from GM11 is shifted towards higher molar masses compared both to the control wood and GM17. Given the relatively modest drop in pH the extracted xylan from GM11 is probably also more acetylated calling for a thorough investigation of the hemicellulose substitution patterns during processing in the upcoming studies. The other modified wood type (GM17) showed also a modest but consistently higher extraction of carbohydrates in this interval compared to the control wood. While the differences between the wood types get more difficult to identify at harsher conditions (at 150 °C and especially at 170 °C) it is striking that the two modified types (at 130 °C–20–60 min and at 150 °C in the interval 20–60 min) consistently show comparable extraction of glucose with the control wood despite the relatively modest pH decrease they give rise to (Figure 5). The question is whether this release originates from the callose content of these samples, getting extracted first at higher temperatures.

In line with this, at mild conditions (at 130 °C allowing observation of the material differences) lignin release from the modified wood types is somewhat higher in the time interval 40–60 min (Figure 7a). While the extraction from GM17 is uncertain (large error bars), the release of lignin from GM11 decreases compared to the control wood towards the end of the extraction at 130 °C. This might be indicative of an initially enhanced extraction of lignin (possibly a

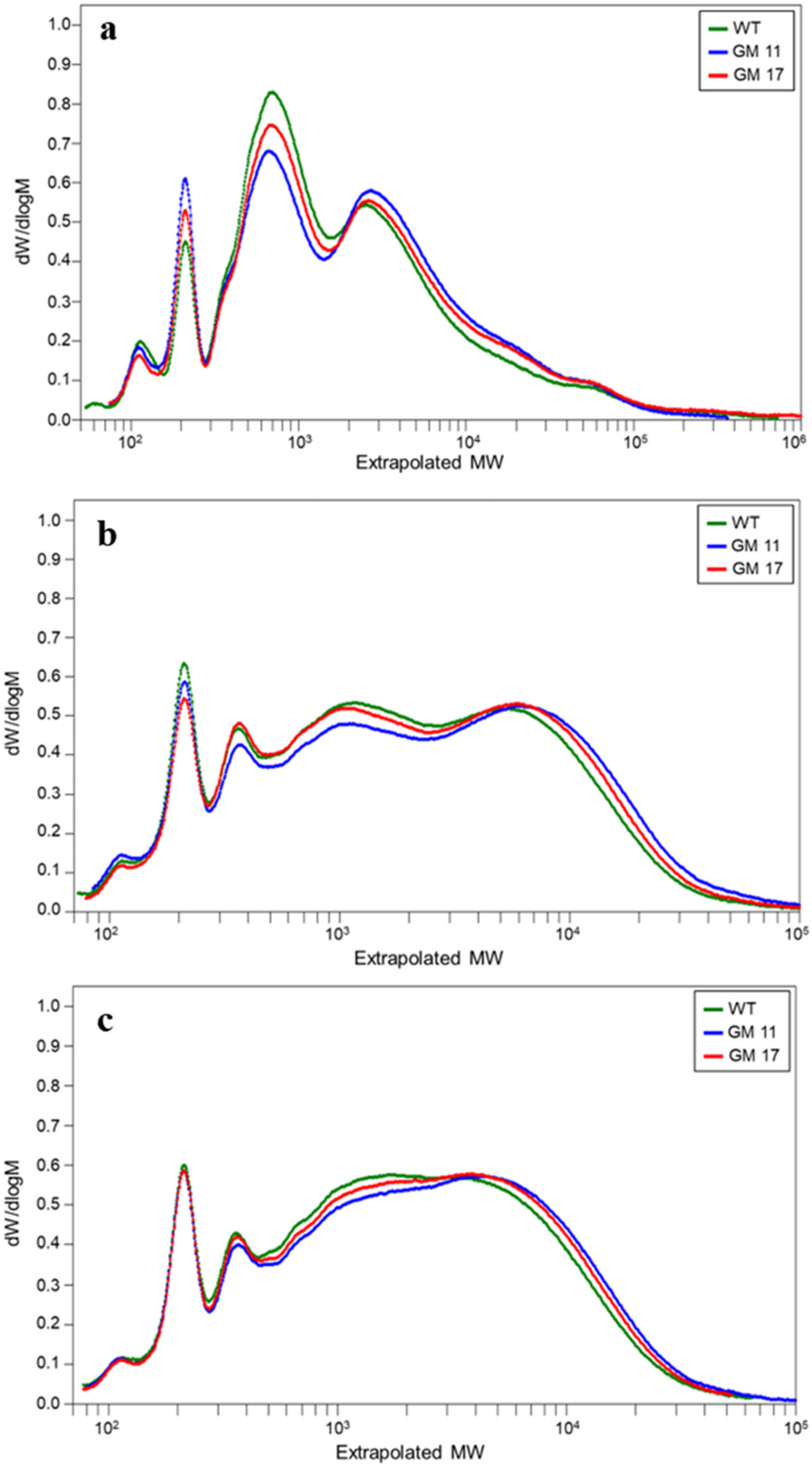

Figure 8: Molar mass distribution of material in hydrolytic fractions at maximum extraction yield (mg/g wood) at (a) 130 °C: 0–10 min, (b) 150 °C: 20–40 min and (c) 170 °C: 10–20 min. The samples were analysed after freeze-drying in duplicates. WT, wild type wood; GM11 and GM17, genetically modified wood types.

relatively easily extractable lignin) from the modified types followed by a decreasing release when this lignin gets depleted. The effect is visible also at 150 °C, but earlier on during the treatment (10–20 min) probably due to the enhanced lignin release at a higher temperature. It is possible that extraction of this lignin in the control wood experiences a stronger mass transport resistance requiring longer times and/or higher temperatures.

3.1.2 Molar mass distributions of the extracts

Indeed, molar mass distributions of the extracts show a consistent shift towards higher molar masses for the two modified wood types (Figure 8). GM11 shows the strongest shift followed by GM17.

The extraction of higher molar mass material from GM11 is in agreement with the observed lower acidification during autohydrolysis of this wood type and might point out towards improved mass transport of xylan chains (as these elute as a relatively high-molecular material, before being degraded). It is possible that a more efficient extraction of xylan retards its deacetylation and, thus, its contribution to acidification. Our previous studies of autohydrolysis showed a rather slow deacetylation of xylan (Wojtasz-Mucha 2020), proceeding possibly more efficiently within the cell wall due to a relatively higher local concentration of ions promoting hydrolysis of the acetates.

If the diffusion of the acetylated xylan out from the cell wall is fast, deacetylation and consequently acidification will be retarded. Importantly, in this case, it might be indicative of a lower mass transport resistance within the cell walls of the genetically modified wood types, which is pronounced in GM11. Thus, these results are in line with an earlier observation of enhanced porosity of the callose rich wood (Bourdon et al. 2023).

3.2 Kraft pulping behaviour

In line with the above observed behaviour of the modified wood lines, these showed a lower retention of carbohydrates in pulps after being subjected to an alkaline kraft delignification: 76 %, 69 % and 67 % of the initial carbohydrates was retained in pulps obtained from WT, GM11 and GM17, respectively. A lower content of glucose in the modified wood pulps (Figure 9) indicates degradation of cellulose possibly associated with enhanced accessibility in the modified wood cell walls.

Interestingly, during these studies, the genetically modified wood samples showed also a vast tendency to swell up once the temperature reached ~140 °C (at 20–40 min cooking time), necessitating a reduction of the material amount in order to prevent clogging of the reactor column. This extensive swelling in alkaline medium, not observed with the control wood, additionally points out towards an enhanced accessibility/porosity of the modified wood structure and is consistent with the previously observed callose improved water absorption and mesoporosity of the transgenic poplar cell wall (Bourdon et al. 2023).

Likewise, the lignin extraction profiles during delignification (Figure 10) indicated enhanced lignin extraction from GM11 in the very initial phase of the process and a slight shift of the delignification maxima towards shorter processing times for the modified woods, but could not provide any clear insights in the delignification rate differences.

Comparison of the molar mass distributions for the lignin extracted early on in the cook (0–10 min), shows indeed an enhanced extraction of a relatively high-molecular lignin (5,000) from GM17 and a slight shift towards higher molar mass of extracted lignin for the modified wood types (in the region approximately 7,000–30,000), GM11 in particular (in line with the observations above). Increased porosity of the callose enriched wood might, thus, in the very early delignification phase promote extraction of

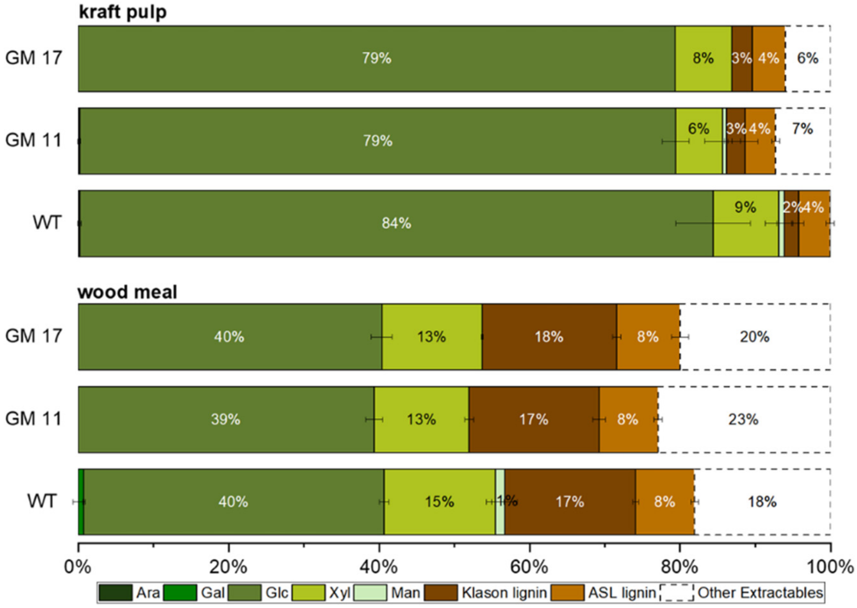

Figure 9: Chemical composition of kraft wood pulp versus reference wood. The components were calculated for the dry mass of the sample, estimated after overnight drying at 105 °C. 'Others' account for not analysed (i.e.: ash, acetyl groups, rhamnose, sugar acids and extractives) extractables. WT, wild type wood; GM11, genetically modified wood type 1; GM17, genetically modified wood type 2.

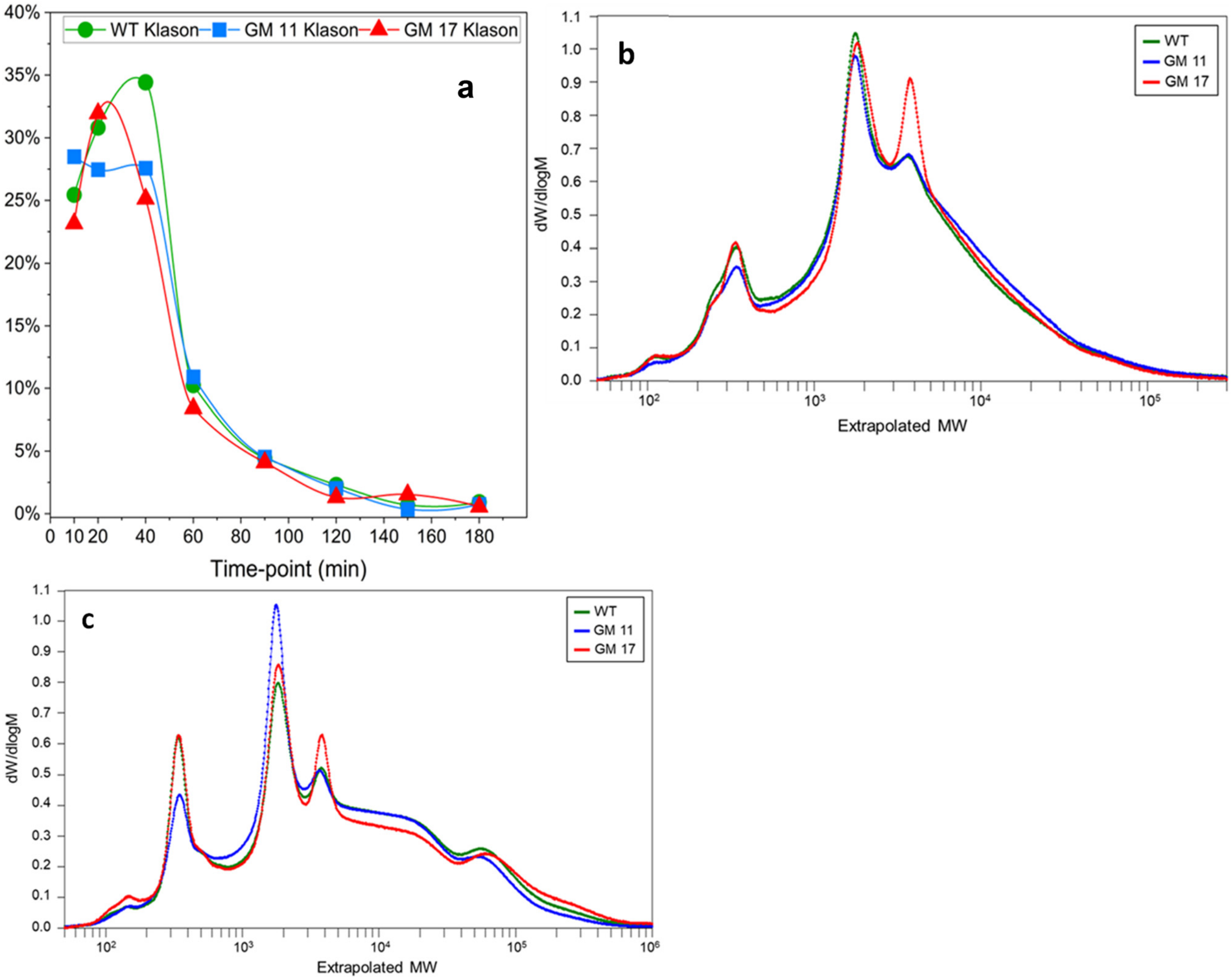

Figure 10: Lignin extraction during kraft pulping. (a) Kinetics of Klason lignin extracted in black liquors calculated as % of total Klason lignin in analysed wood. (b) Molar mass distribution of acid precipitated lignin from black liquor samples extracted at 0 – 10 min and (c) 20 – 40 min of kraft cooking process. Samples were analysed with a GPC-UV instrument in duplicated runs. WT – wild type wood, GM 11 and GM 17 – genetically modified wood types 1 and 2.

higher molar mass lignin, evident as modest but clear shift towards higher molar masses in GM11 and GM17 lignin. As the delignification progresses, this initial porosity difference becomes less influential (due to both depolymerization reactions and extensive porosity changes), as observed in the fraction taken out 20–40 min. While additional investigations are needed, this observation on the initial delignification aligns with the above indicated increased accessibility of the wood matrix (cf. retention of glucose in the obtained pulps).

4 Concluding remarks

Transgenic callose enriched aspen wood lines showed under mild autohydrolysis conditions (130 °C, during initial 20 min of extraction) and weaker acidification effects, a comparable extraction of wood components (primarily xylan) to the reference wood. Given the relatively weaker acidification accompanying these mild extractions of the transgenic wood along with the slightly higher molar mass of the obtained extracts, it is likely that the presence of callose (anticipated to act as a cell wall spacer increasing the cellulose-lignin and cellulose macrofibril distance), indeed provides an increased accessibility of the cell wall matrix, influential under mild conditions and early on in the process. Accordingly, the transgenic wood lines also show an enhanced extraction of slightly higher molar mass lignin in the very initial phase of kraft pulping (corresponding to the heating-up phase) along with a lower retention of carbohydrates in pulps upon completed delignification: 76 %, 69 % and 67 % of the initial carbohydrates was retained in pulps obtained from WT, GM11 and GM17, respectively. While both kraft pulping and autohydrolysis extraction investigations here show very modest impact of the callose impaired porosity on the processing under relatively harsh conditions (i.e. autohydrolysis at T > 130 °C, and kraft pulping after the initial 10–20 min), they clearly point out an enhanced initial susceptibility of the transgenic material to both acid and alkaline processing, promoting recovery of higher molas mass wood components, possibly due to enhanced accessibility of the cell wall matrix. The trend correlating with the content of introduced callose. In light of the increasingly important development of mild stepwise decoupling technologies that would ensure a good atom economy of biorefining and improved resource efficiency in biomass conversions, this type of material becomes particularly interesting. It calls for further investigations of mild biorefining routes that would benefit from the enhanced accessibility, opening up for new prospects of selective stepwise decoupling.

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