Sodium Periodate Mediated Oxidative Extraction of Polysaccharide-derived Structures from Wood Biomass: Influence of Reaction Time and Oxidation Conditions in Birch and Spruce

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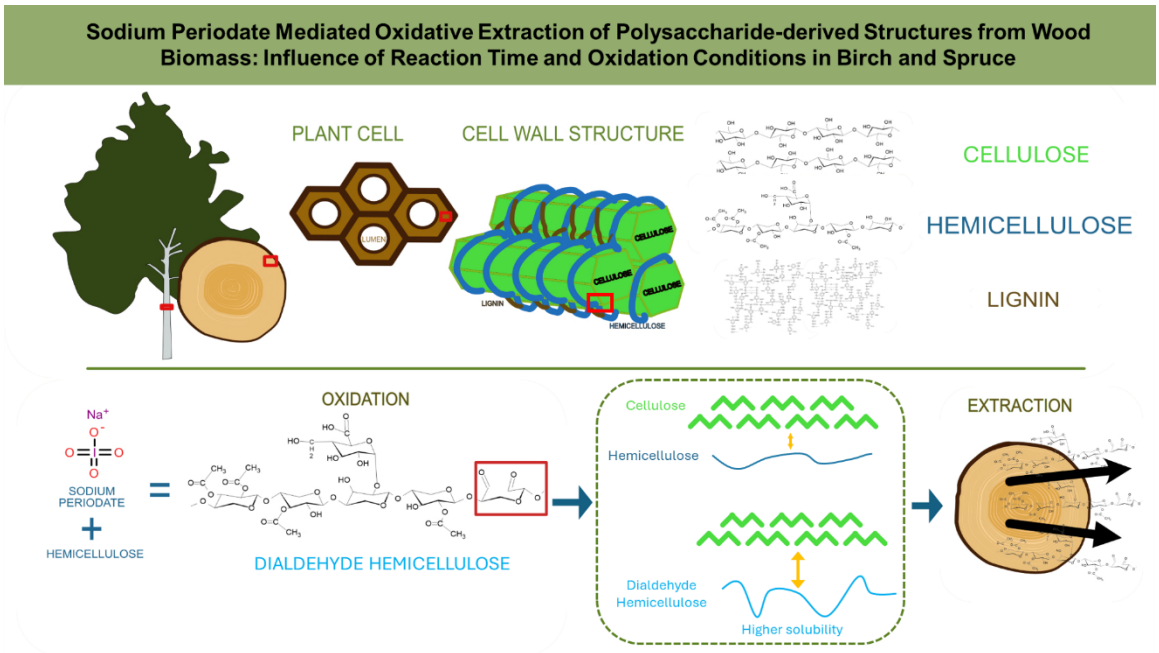
GRAPHICAL ABSTRACT



Sodium Periodate Mediated Oxidative Extraction of Polysaccharide-derived Structures from Wood Biomass: Influence of Reaction Time and Oxidation Conditions in Birch and Spruce

Lucas K. Azambuja,[a,b,*] and Merima Hasani [a,b]

Sodium periodate ($NaIO_4$) oxidation was used as a direct method to recover soluble wood components, primarily hemicelluloses, from untreated birch and spruce biomass. Through monitoring the reaction over time and at different oxidant-to-biomass ratios, the study elucidated the relationship between periodate consumption, aldehyde formation, and polysaccharide solubilization. A rapid initial uptake of the oxidant led to aldehyde generation and the extraction of hemicellulosic fractions, mainly xylan-rich in birch and glucomannan-rich in spruce followed by a decreasing (detectable) oxidizing effect likely due to further conversions of introduced aldehydes. Fourier transform infrared and heteronuclear single quantum correlation nuclear magnetic resonance analyses confirmed structural modifications consistent with monosaccharide ring opening and further conversions of created aldehydes. Size-exclusion chromatography revealed the expected accompanying chain cleavage of the affected structures. Together, these findings demonstrate both the potential and complexity of using periodate oxidation for simultaneous extraction and functionalization of wood polysaccharides, emphasizing the need for proper optimization.

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Keywords: Oxidation; Periodate; Extraction; Carbohydrates

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INTRODUCTION

Lignocellulose, a complex biomaterial composed of plant cell walls, offers substantial potential for sustainable development in bio-based product manufacturing, making it an excellent alternative to petrochemistry (Ojo 2023). It is mainly composed of cellulose, hemicellulose, lignin, and extractives, varying in composition according to the source. For example, hardwoods such as birch are composed primarily of 40 to 50% cellulose, 25 to 35% hemicellulose, and 20 to 25% lignin. Otherwise, softwood, such as spruce, is composed of 40 to 45% cellulose, 20 to 30% hemicellulose, and 25 to 30% lignin (Räisänen and Athanassiadis 2013; Okolie *et al.* 2021; de Freitas *et al.* 2022).

Cellulose forms long, linear chains of glucose that assemble into highly ordered, semi-crystalline microfibrils, making it insoluble and resistant to degradation. In contrast, hemicelluloses consist of shorter, branched chains of mixed monosaccharides with irregular substitutions that disrupt crystallinity and increase solubility. Their amorphous nature and chemical diversity make them much more reactive (Mudgil and Barak 2019).

In birch, one of the industrially most important hardwoods, hemicelluloses constitute about 15 to 30% of the wood mass and consist mainly of O-acetyl-(4-O-methylglucurono)xylan. The backbone of this polymer is formed by β-(1→4)-linked D-xylopyranose residues, with, on average, one α-(1→2)-linked 4-O-methylglucuronic acid substituent per 10 to 20 xylose units (Teleman *et al.* 2002). In addition to xylan, it also contains a minor fraction (2 to 5% of the wood mass) of glucomannan, which is composed of β-(1→4)-linked D-glucopyranose and D-mannopyranose units (Rowell 2012).

In spruce, hemicelluloses represent about 20 to 30% of the wood mass and are dominated by galactoglucomannan (15 to 20% of the wood mass), a copolymer consisting of β-(1→4)-linked D-mannopyranose and D-glucopyranose residues, partially substituted with α-(1→6)-linked D-galactopyranose side chains and acetyl groups. Spruce hemicellulose also contains arabinoglucuronoxylan (7 to 10% of the wood mass), composed of a β-(1→4)-linked D-xylopyranose backbone carrying α-(1→2)-linked 4-O-methylglucuronic acid and α-(1→3)-linked L-arabinofuranose substituents (Rowell 2012). These structural differences influence the chemical and physical properties of hemicelluloses (de Freitas *et al.* 2022) and drive the specific applications of each polymer, shaping how wood biomass is utilized across different sectors (FAO 2024). In the global context, while the pulp and paper industry globally leads the conversion of wood biomass into both traditional and novel products, recent research points out several other valuable applications for lignocellulose biomass in drug delivery systems (Sun *et al.* 2013; Chandra *et al.* 2022; Ainani *et al.* 2024), tissue engineering (Chandra *et al.* 2022), wound healing (Chandra *et al.* 2022), food (Abik *et al.* 2023; Mathura *et al.* 2024), packaging (Rao *et al.* 2023), and many others (Naidu *et al.* 2018). There also has been interest in new routes for hemicellulose extraction and functionalization (Lu *et al.* 2021). To unlock these advanced applications, however, efficient fractionation of wood components and recovery methods are essential (Lu *et al.* 2021).

Common methods to separate hemicelluloses, the most sensitive and underutilized constitutive polymers in lignocellulosics, rely on their partial depolymerization, promoting their solubility, diffusion, and finally recovery (Wojtasz-Mucha *et al.* 2017). Employing oxidative methods in this context could potentially provide both necessary solubility enhancement - whether through shortening of the chain or conformational change. For instance, some previous studies point out increased chain flexibility upon oxidation (Börjesson *et al.* 2018), and the introduction of oxidized functionalities that can be attractive from an application point of view.

Among these still unexplored extraction approaches, sodium periodate ($NaIO_4$) stands out as a particularly attractive reagent due to its selectivity (towards structural motives carrying vicinal OH-groups with suitable orientation) and well-documented behavior through extensive research work, including the oxidation of different types of hemicelluloses, pectin, and cellulose (Nypelö *et al.* 2021). $NaIO_4$ oxidizes free vicinal OH groups of polysaccharides to dialdehydes while breaking the C-C bonds carrying these alcohols and leading to opening of the monosaccharide rings (*e.g.*, pyranose rings are opened through oxidation of the C2 and C3 carbons and cleavage of the C2-C3 bond). The reaction requires orientation of the vicinal alcohols in equatorial-equatorial or axial-equatorial position. While introduction of aldehyde functionalities into polysaccharides enhances their chemical versatility (Amer *et al.* 2016; Nypelö *et al.* 2021; Palasingh *et al.* 2022), it is also associated with numerous side-reactions of the created aldehydes including formation of intra- and inter-chain hemiacetal bonds with hydroxy groups, formation of hemialdals or hydrates with water, as well as depolymerizing reactions (Fliri *et al.* 2023a;

Sasaki and Kosma 2025; Zhu *et al.* 2025). The latter have been shown to mainly go through beta elimination (Sasaki and Kosma 2025). Moreover, in the context of employing $NaIO_4$ in direct extractions from wood material its oxidative effects on the total polysaccharide content of wood must be taken into account including also the otherwise less extractable cellulose.

While previous research has primarily focused on post-extraction oxidation for structure modification, to the authors' knowledge, there has been no work exploring a direct utilization of periodate oxidation of wood biomass for the purpose of extracting oxidized structures without prior treatment. Here, this study aimed to explore this method as a way to prompt the extraction of polysaccharide structures (Amer *et al.* 2016; Chemin *et al.* 2016) by investigating how the extraction yield, aldehyde introduction, composition, and molar mass of the extracted material depend on the applied process conditions and type of biomass (here, birch, and spruce).

EXPERIMENTAL

Material

Wood chips of birch (B) a hardwood and spruce (S) a softwood, obtained from industrially cut logs, were screened to be free of bark and knots. The chips were then milled separately with a knife mill and sieved into particles passing a 1-mm mesh. Hydroxylamine hydrochloride (H_2N-OH ·HCl), potassium iodide (KI), sulfuric acid (H_2SO_4, 72%), sodium thiosulfate ($Na_2S_2O_3$), sodium hydroxide (NaOH), and sodium borohydride ($NaBH_4$) were purchased from Sigma-Aldrich. $NaIO_4$ was purchased from ThermoScientific, and the ethylene glycol and dimethyl sulphoxide (DMSO) were purchased from VWR.

Oxidation as a Function of Time

Birch biomass powder and $NaIO_4$ were mixed in a mass ratio of 1:2 ($NaIO_4$: biomass) and suspended in deionized water at a solid-to-liquid ratio of 1:100 (w/v). The reaction was conducted in a 1-L reactor placed in a dark room to prevent light-induced side reactions, with continuous stirring provided by a mechanical impeller to ensure homogeneous mixing. Aliquots of 70 mL were withdrawn from the reactor in time intervals of 2, 5, 10, 15, 20, 40, 60, 120, 180, and 240 min. After each sampling, the removed volume was immediately replaced with an equal amount of deionized water to maintain a constant reaction volume. Each aliquot was subsequently divided into four portions: 10 mL for carbohydrate composition analysis, quenched with ethylene glycol; 20 mL for aldehyde content titration; 20 mL for residual periodate quantification; and 20 mL subjected to reduction with sodium borohydride (10:1 $NaBH_4$:$NaIO_4$ molar ratio) in a sodium phosphate buffer medium (pH between 6.8 and 8), followed by dialysis using a 0.5 kDa molecular weight cut-off membrane (Spectrum Laboratories, USA) at a sample-to-water ratio of 1:20. The dialyzed fraction was then used for yield determination and molecular weight distribution analysis by gel permeation chromatography (GPC, PL-GPC-50, Polymer Laboratories, Varian Inc., Agilent, Santa Clara, CA, USA).

Oxidation as a Function of Oxidant Charge

$NaIO_4$ and birch or spruce biomass were combined at varying mass ratios of [0:1], [1:4], [1:2], and [1:1] and suspended in deionized water at a solid-to-liquid ratio of 1:100 (w/v). The mixtures were stirred continuously with a mechanical impeller for 1 h in a dark

room to prevent light-induced side reactions. After the reaction period, each mixture was filtered using a Whatman® fiberglass microfilter to separate the solid and liquid phases. The solid residues retained on the filter were dried and subsequently analyzed for carbohydrate content and aldehyde and periodate content. GPC analyses were performed for $NaIO_4$:biomass mass ratios investigated [1:1], [1:2], and [1:4], in order to evaluate the molar mass distributions of the extracts under each oxidation condition. In contrast, Heteronuclear Single Quantum Correlation NMR (HSQC) analyses were conducted only for the [0:1] and [1:2] $NaIO_4$:birch mass ratios, which were selected as representative samples of the unoxidized reference and a moderately oxidized system, respectively.

Determination of Aldehyde Content

The aldehyde content was determined following a method adapted from Zhao and Hendrik (1991a). A 10-mL aliquot of the reaction liquor was diluted with 50 mL of deionized water, followed by the addition of 1 mL of 1 M $H_2N–OH·HCl$ solution. The mixture was stirred for 40 min to allow the formation of oximes. After the reaction, the liberated HCl was titrated with 0.05 M sodium hydroxide (NaOH) using an automatic titrator (SI Analytics, San Diego, CA, USA). The endpoint of the titration was set at pH 4.

Measurement of Periodate Consumption

The determination of residual periodate was performed based on the method described by Babor *et al.* (1973). A 10-mL aliquot of the reaction liquor was mixed with 1 mL of 1 M H_2SO_4 and 1 mL of 1 M KI, and the mixture was stirred for 1 h to allow complete reaction. The liberated iodine was then titrated with 0.1 M $Na_2S_2O_3$ using a manual burette. Titration continued until the solution turned colorless, indicating the endpoint. A starch solution was used as an indicator to enhance the visual detection of the endpoint.

Carboxylic Acid Determination

The method was adapted from a method to quantify carboxyl groups in pulp (Barbosa *et al.* 2013) using an autotitrator equipped with a pH electrode (SI Analytics, San Diego, CA, USA). An aliquot of 25 mL reaction liquor was used directly for titration under magnetic stirring (300 rpm). Residual periodate was quenched prior to analysis using ethylene glycol. The titration was performed with standardized 0.01 M NaOH, starting from an initial pH of approximately 4 and proceeding until the solution reached pH 8.30, corresponding to the phenolphthalein equivalence point where all carboxyl groups were neutralized. The autotitrator operated in dynamic dosing mode, automatically reducing the NaOH addition step size near the endpoint to ensure accurate pH stabilization.

Yield

To determine the extraction yield, 20 mL of each sample was collected and dialyzed using a 0.5 kDa molecular weight cut-off membrane (Spectrum Laboratories, Rancho Rodriguez, USA) at a sample-to-water ratio of 1:20. The dialysis water was exchanged five times. After dialysis, the samples were frozen and subsequently freeze-dried for 24 h. The yield was calculated using Eq. 1, where m_f is the weight (g) of the container after freeze-drying, m_0 is the initial container weight (g), V_t is the total reaction volume (mL), V is the aliquot volume (mL) subjected to freeze-drying, and m_t is the total mass (g) of biomass used in the reaction. To account for residual oxidant, an additional titration was performed on 1 mg of the freeze-dried sample to determine any excess periodate that was not removed

during dialysis. The measured amount of residual periodate was subtracted from the calculated yield.

$$Yield\ (\%)\ = \frac{(m_f - m_0) \times V_t}{V \times m_t} \quad (1)$$

Fourier Transformation Infrared Spectroscopy

Samples obtained under condition B [1:2] were oxidized for 20, 40, and 60 min and analysed by FTIR. In order to enable comparison with the NMR investigations of the 60 min samples (reduced with $NaBH_4$ to allow for solubility in D_2O, see the sample preparation procedure for NMR below), these samples were also subjected to $NaBH_4$ reduction. After the oxidation, the reaction mixtures were filtered, and the filtrates were collected, adjusted in volume, concentrated, reduced with $NaBH_4$ (the procedure described above), dialyzed, and subsequently freeze-dried. The solid residues were thoroughly washed with deionized water, oven-dried at 50 °C, and finally freeze-dried. For Fourier-transform infrared (FTIR) analysis, 1 mg of each dried sample was homogenized with 120 mg of spectroscopic-grade KBr and pressed into pellets. The FTIR spectra were recorded in transmission mode using a Frontier FTIR spectrophotometer (PerkinElmer Frontier, Waltham, MA, USA) over the range of 4000 to 650 cm^{-1}, with a spectral resolution of 4 cm^{-1}. For each sample, 16 scans were collected and averaged to improve the signal-to-noise ratio. All measurements were performed at room temperature. For comparison, an additional sample prepared under condition B [0:1] with a reaction time of 60 min was analyzed, along with untreated birch powder used as a reference material.

Liquor Compositional Analysis Sample Preparation

The extracted polysaccharides were analyzed using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD, Waltham, MA, USA) as described below (Solid residues compositional analysis). For sample preparation, 10 mL of the aliquots were mixed with ethylene glycol to quench the reaction. Acid hydrolysis was performed by adding 0.01 mL of 72% (w/w) H_2SO_4 to each sample, followed by autoclaving at 125 °C for 1 h. After cooling, 1.25 mL of 0.2 M fucose was added as an internal standard, and the solution was diluted to a final volume of 25 mL. Prior to analysis, 2 mL of each sample was filtered through 0.2-µm PTFE filters.

Solid Residues Compositional Analysis

For determination of the composition of the solid residues, the material was subjected to total acid hydrolysis following the procedure described above (Liquor compositional analysis sample preparation). Carbohydrates were quantified using the filtrate solutions produced after acid hydrolysis. The samples were analyzed using anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD, Dionex ICS-5000, Thermo Fisher Scientific, Waltham, USA). The system was equipped with a gold reference electrode and Dionex CarboPac PA1 columns (a 2 × 50 mm^2 guard column and a 2 × 250 mm^2 analytical column). The elution (dual pump: 0.26 mL/min and 0.13 mL/min) took place at 30 °C for 25 min using H_2O/200 mM NaOH, followed by a washing step of 13 min using 200 mM NaOH/200 mM NaOH + 170 mM sodium acetate. The injection volume was 10 µL. Data processing was performed using the Chromeleon software version 7.1. The detected amounts of sugar monomers were corrected for the yield loss after acid hydrolysis (Wojtasz-Mucha *et al.* 2017) and expressed as anhydro sugars (Janson 1974).

Klason lignin content was determined after acid hydrolysis, following the procedure described by the National Renewable Energy Laboratory (Sluiter *et al.* 2008).

Carbonyl Content in the Pulp

The total amount of carbonyl groups was determined using a method based on Zhao and Heindel (1991) and described by Martinsson *et al.* (2022). A never-dried pulp sample equivalent to 0.5 g of oven-dried pulp was dispersed in 100 mL of deionized water adjusted to a pH of 4. After 10 min, the suspension was filtered, and the sample was washed with 100 mL of deionized water at a pH of 4. The pulp was then transferred to a beaker containing 25 mL of 0.25 M hydroxylamine hydrochloride solution, also adjusted to pH 4. The mixture was placed on a shaking table for 2 h, after which it was filtered. The filtrate was titrated back to pH 4 using 0.01 M NaOH. The pulp was subsequently washed with deionized water and dried in an oven at 105 °C for 24 h to determine the oven-dry mass. The total carbonyl content was calculated by determining the volume of NaOH consumed during the titration, relative to the oven-dry weight of the pulp.

Gel Permeation Chromatography

After freezing and drying, a solid sample of 1 mg of each condition was analyzed in the mobile phase of DMSO overnight, diluted to 0.25 mg/mL, and filtered through a 0.2-µm filter. The molecular weight distribution (MWD) of the samples was measured using gel permeation chromatography (PL-HPC 50 Plus Integrated GPC system (Pl-GPC-50, Polymer Laboratories, Varian Inc., Agilent, Santa Clara, CA, USA)). The system used two 300 × 7.5 mm^2 PolarGel-M columns and one 50 × 7.5 mm^2 PolarGel-M guard column. Dimethyl sulphoxide (DMSO) with 10 mM LiBr was used as the mobile phase, and the flow rate was 0.5 mL/min at 50 °C. Detection was made using an RI detector. The detector was calibrated with 10 Pullulan standards ranging from 0.180 to 708 kDa (Varian PL2090-0100, Varian Inc., Agilent, Santa Clara, CA, USA).

Nuclear Magnetic Resonance Spectroscopy

After freezing and drying, a solid sample of 1 mg was dissolved in 1 mL of deuterium oxide (D_2O). The products generated by the oxidation of the biomass were analyzed with HSQC. The spectra were recorded at 25 °C using a Bruker Avance III HD (Rheinstetten, Germany) with a 3-mm TXO cold probe, operating at 600 MHz for ^{1}H and ^{13}C. The pulse program used was hsqcedetgpsisp2.3, with acquisition times of 96 ms and 6 ms, respectively. The FID sizes were 3072 and 512 points, respectively. The experiment consisted of 16 scans with a 1 s relaxation time, resulting in an experimental time of 3 h and 48 min. The supernatant was then used for analysis in 3-mm tubes. Data analysis was performed in Bruker TopSpin 4.1.4 software.

RESULTS AND DISCUSSION

Oxidation as a Function of Time

The selection of concentrations used in this work was based on a range of previously reported studies. For instance, Chemin *et al.* (2016) oxidized xylan using a sodium periodate-to-xylan mol ratio of 0.05 to 1, while Amer *et al.* (2016) employed 20 g of xylan with 35 g of suspended $NaIO_4$. However, most of the available references have focused on delignified materials or have not accounted for the complex matrix of whole

wood. Therefore, considering these and other studies, together with results from the preliminary experiments, the mass ratio of 1:2 ($NaIO_4$: biomass) was selected as a starting point for investigating the effect of oxidation time applied.

During the periodate oxidation of birch wood, the consumption of periodate exhibited a rapid initial phase, with approximately 40% of the oxidant being consumed within the first 20 min, increasing to 70% after 240 min (Fig. 1a). This partial and time-dependent consumption is consistent with the behavior reported by Chemin *et al.* (2016) for xylan, where incomplete oxidation was observed. For pure xylan, this observation was tentatively attributed, by Palasingh *et al.* (2022), Zhao and Heindel (1991), and Zhu *et al.* (2025), to the formation of inter- and intra-chain hemiacetal crosslinks, which can shield vicinal hydroxyls from further periodate attack, effectively "protecting" a portion of the polysaccharide from oxidation, as observed by Gomez *et al.* (2007), Amer *et al.* (2016), and Chemin *et al.* (2016). In wood material, on the other hand, both crosslinking reactions and the poor accessibility of the polysaccharide structures in general may contribute to a relatively slow consumption of the reagent.

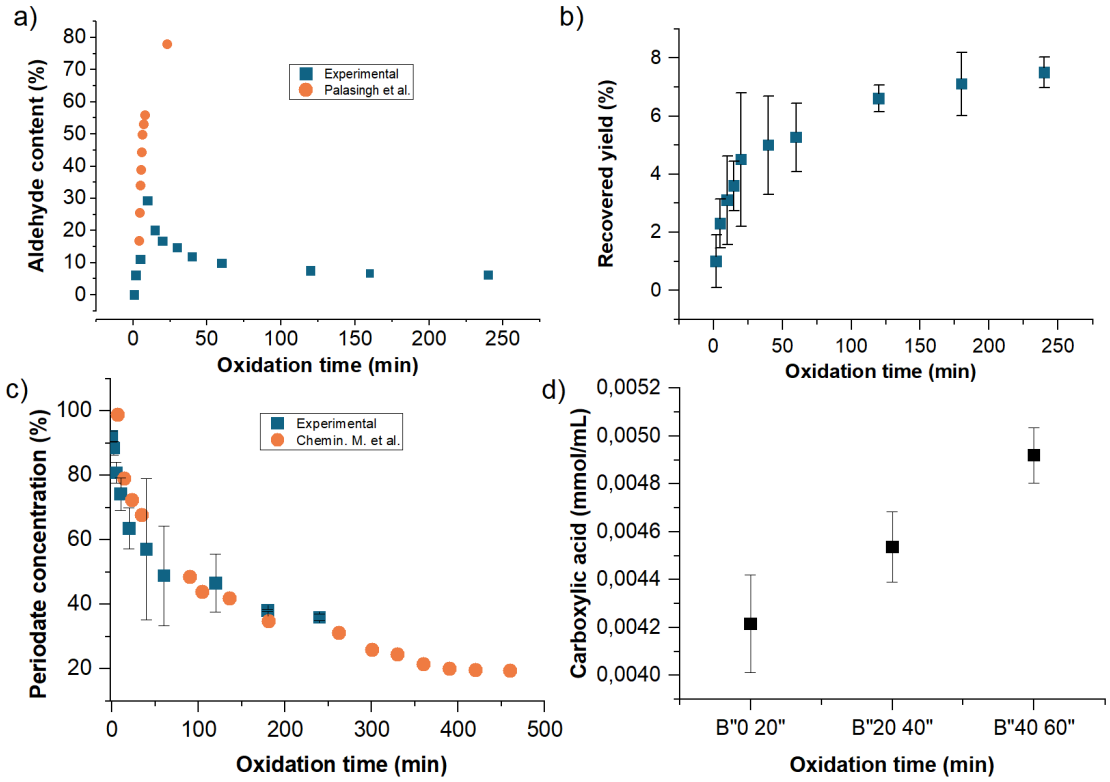

Fig. 1. a) Aldehyde concentration detected in the extracted material (as % of the total dry mass over time compared to that obtained by Palasingh C. *et al.* (2022) b) yield of material extracted over the studied oxidation time; c) Periodate concentration (as % of total oxidizing liquor) during oxidation of birch, mass ratio 1:2 ($NaIO_4$: biomass) compared to the results obtained by Chemin M. *et al.* (2016); and d) carboxylic acid amounts measured in extracted material over time for the same $NaIO_4$: biomass mass ratio

The yield of extracted material (Fig. 1b) increased over time, reflecting partial solubilization. At the same time, the generation of aldehydes in this material (as quantified by hydroxylamine titration (Fig. 1c)), rose rapidly within the first 5 min, corresponding to the early periodate consumption. This increase was likely due to the formation of dialdehyde units along the hemicellulose backbone, which may enhance chain flexibility and solubility, as observed by Börjesson *et al.* (2018),Nishiyama *et al.* (2003), and Nypelö *et al.* (2021), but can also be associated to depolymerizing side-reactions promoting solubility. However, after this initial increase, the aldehyde concentration declined despite the continued presence of periodate. This was likely due to above-mentioned crosslinking and accessibility issues hampering the oxidation and/or due to overoxidation to carboxylic acids, as noted by Amer *et al.* (2016), Pandeirada *et al.* (2022), and Zhu *et al.* (2025). Indeed, a progressive increase in carboxylic acid content could be observed during the oxidation of birch powder with $NaIO_4$, using the Janson (1974) method, indicating that the oxidation proceeds beyond the initial formation of dialdehyde Fig. 1d.

Compositional analysis of the extracts (Fig. 2) in comparison to the yield of extracted material over time (Fig. 2), points out a consistent carbohydrate conversion (most likely through oxidation) throughout the extraction process. Within the first 2 to 5 min, approximately 50% of the extracted carbohydrates were converted and undetectable as monosugars. After 60 min, only about 10% of the initially detectable carbohydrates still existed in their underivatized forms. Beyond 240 min, the concentration stabilized, with more than 7% of the carbohydrates detectable. Glucose and xylose, which represented *ca* 78% of the recovered carbohydrates after 2 min of extraction, were both consumed approximately to 90% after 60 min and 95% after 240 min. Arabinose, which represented 12% of the recovered carbohydrates after 2 min of extraction, was consumed to 85% and 92% after 180 min and 240 min. These findings align with the results reported by Pandeirada *et al.* (2022), who, despite using varied concentrations of hemicelluloses from different biomass sources, also observed a clear and comparable trend in carbohydrate conversion over time.

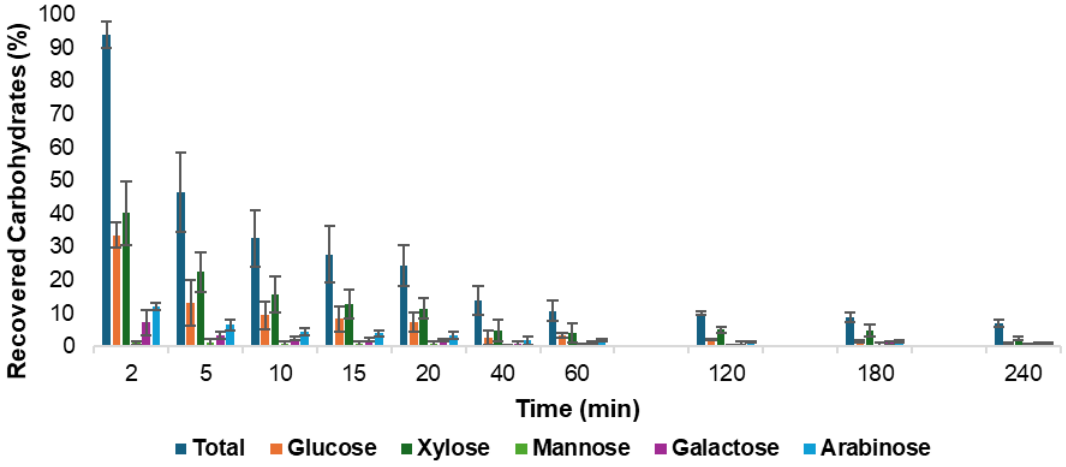

Fig. 2. a) Carbohydrate concentration (%) in the dry material of the liquor extracts over time in a birch treated with 1:2 ($NaIO_4$: biomass) mass ratio; Total concentration of detected carbohydrates in the extracted material is shown in dark blue

Glucose typically accounts for no more than 5% of the hemicellulose fraction in birch, which is predominantly composed of xylan (Roger 2005). In the present work it

constituted an important fraction of the extracted material, indicating a contribution from oxidative effects on cellulose. The latter can be associated with the mechanical milling the wood material was subjected to, thereby increasing cellulose accessibility and susceptibility to oxidative and degrading chemistry of periodate. The milling step employed here facilitates, thus, likely subsequent oxidative or hydrolytic reactions on cellulose by exposing otherwise less accessible cellulose regions (Piras *et al.* 2019), which (apart from a contribution from glucomannan and a probably minor presence of starch) could explain a significant presence of glucose in the extracts.

Further insights into the structure and composition of the obtained extracts were attempted by investigating molar mass distribution (GPC) and HSQC NMR spectra. However, as solubility of the obtained extracts was too poor to allow for this type of analytics, the obtained material was reduced with $NaBH_4$, which enabled dissolution in DMSO/LiBr.

The size exclusion chromatograms (Fig. 3) of $NaBH_4$-reduced extracts show a bimodal distribution with a distinct, relatively narrow low-molecular fraction (corresponding to oligomer structures of DP ~5 to 11) and a broader, less abundant higher molecular fraction.

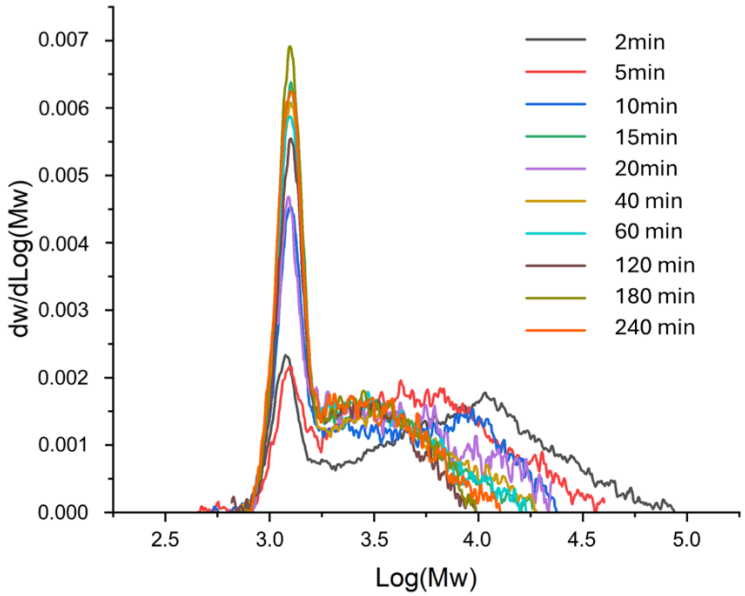

Fig. 3. Molar mass distribution of the extracted and reduced materials obtained after different oxidation times (0 to 240 min) while treated with the ratio 1:2 ($NaIO_4$: biomass)

It is worth mentioning that the former increases over oxidation time, retaining rather constant molar mass accompanied with a simultaneous shift of the latter towards lower molar masses. This behavior is consistent with the previously observed depolymerization during periodate oxidation by Pandeirada *et al.* (2022), who observed that periodate oxidation generates distinct oligomers formed at branching positions within the polysaccharide. These branch points inhibit continued cleavage, resulting in stable fragments of characteristic size. For birch wood xylan, Pandeirada *et al.* observed release of oligomers of DP 2-20 even at room temperature. Fliri *et al.* (2023) confirmed that depolymerization begins early during periodate oxidation, even at low to moderate degrees of oxidation. Chetouani *et al.* (2017) reported formation of a low-molecular fraction during

prolonged periodate oxidation of pectin. In the current study, the biomass extracts comprised rather complex mixtures of oxidized poly- and oligosaccharides, where the alkaline reduction associated with beta-elimination likely additionally affected molar masses of the extracted carbohydrates Thus, while it is possible that the observed low molecular fraction indeed is characteristic of the main polysaccharide degrading during this treatment (possibly xylan, *cf.* Fig. 2.), further investigations are required. Particularly interesting would be those focusing on revealing characteristic residual oligosaccharide from the periodate degradation of native xylan.

The HSQC spectrum of the reference water-extract (with no oxidation involved, sample B[0:1]) displays resonances characteristic of water-soluble polysaccharide fragments together with signals of phenolic compounds. A dense cluster of cross-peaks in the region δH 3.2 to 4.5 / δC ~60 to 105 ppm corresponds to carbohydrate signals, including anomeric protons at δH 4.5 to 5.2 ppm with δC ~95 to 105 ppm and ring protons H2–H5 at δH 3.2 to 4.2 ppm with δC ~60 to 78 ppm. These features are consistent with soluble oligosaccharides leached at room temperature (Teleman *et al.* 2002). Furthermore, a signal at δH 2.0 to 2.2 / δC ~20 ppm corresponds to acetyl methyl groups (O-acetylated xylan fragments), indicating partial solubilization of hemicellulose even under ambient extraction conditions (Teleman *et al.* 2002). Resonances in the aromatic region (δH 6.0 to 8.0 / δC 100 to 140 ppm) provide strong evidence for the presence of phenolic compounds, such as tannins and other polyphenols, which are abundant among water-soluble birch extractives (Liimatainen *et al.* 2012). Additional weak cross-peaks in the aliphatic region (δH 1.0 to 1.5 / δC 12 to 32 ppm) likely arise from minor quantities of water-soluble extractives (Paiva *et al.* 2016).

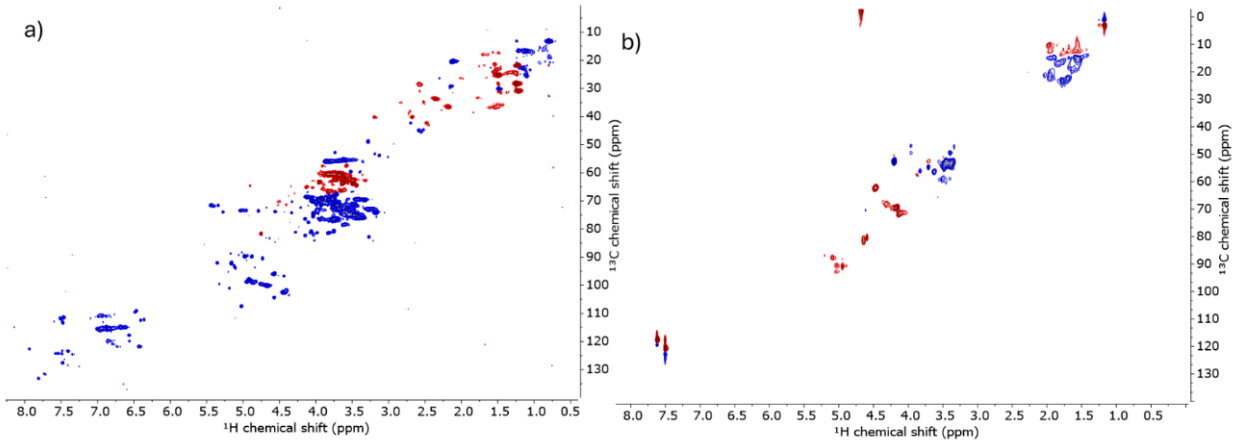

Fig. 4. ^{1}H-^{13}C HQSC NMR spectra of birch for a) reference water-extracted sample for 60 min and b) periodate extracted sample after treatment for 60 min, 1:2 ratio ($NaIO_4$: biomass), Red and blue contours represent signals with positive and negative phases, respectively, corresponding to CH/CH_3 and CH_2 groups in the multiplicity-edited HSQC experiment.

In Fig. 4b, the HSQC spectrum of the periodate extract from the 1:2 ($NaIO_4$: biomass) sample treated for 60 min shows a mixture of signals originating from oxidized and unoxidized carbohydrate structures. In addition to the above-mentioned signals of the unoxidized sugars, a distinct cross-peak at δH ~4.8 / δC ~90 ppm is attributed to hemiacetal or anomeric-like carbons adjacent to C affected by oxidation (Kim *et al.* 2000; Tangthuam *et al.* 2020). Weak resonances at δH 1.2 to 1.5 / δC 14 to 30 ppm indicate aliphatic methylene chains, which may originate from oxidative degradation products (Paiva *et al.*

2016). Additional peaks at δH 2.0 to 2.2 / δC ~20 ppm confirm the presence of acetyl substituents in birch xylan, demonstrating retention of O-acetylation following oxidation (Teleman *et al.* 2002), although it remains unclear to what extent the O-acetylation is preserved.

The complementary FTIR analysis of the liquors obtained after birch oxidation and subsequent reduction (Fig. 5a) revealed several characteristic bands associated with structural changes in the polysaccharide matrix. According to previous studies on oxidized polysaccharides (Tangthuam *et al.* 2020), the band near 1637 to 1650 cm^{-1} corresponds mainly to the bending vibration of absorbed or bound water (H–O–H), while the contribution at 1610 cm^{-1} is assigned to the asymmetric stretching of carboxylate groups (COO^-). The persistence of the 1610 cm^{-1} signal after $NaBH_4$ reduction indicates that carboxyl groups, formed during oxidative cleavage of polysaccharide chains, remain unaffected by the reducing agent, which selectively reduces aldehydes but not acids. In contrast, the absorption band at 1740 to 1750 cm^{-1} indicative of hemicellulose acetates could be observed only in the reference (water) extract, probably as the acetates were hydrolyzed off during the $NaBH_4$ reduction of the periodate extracted samples. In the solid residue (not subjected to the reduction) these bands could be observed both after water- and periodate-extraction (Fig. 5b).

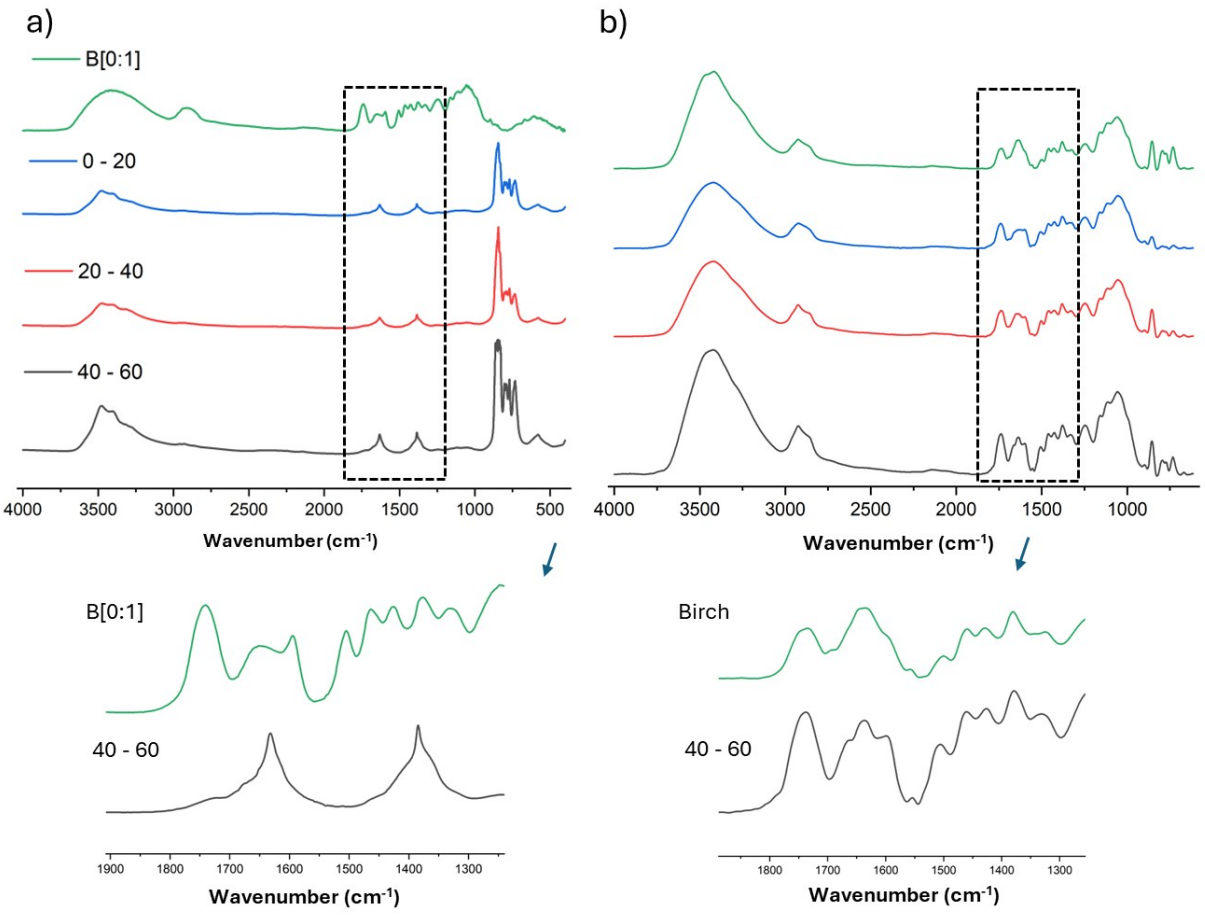

Fig. 5. FTIR spectra of a) liquor samples extracted with only water (B[0:1] and periodate $NaIO_4$:biomass ratio 1:2 for different treatment times (0 to 60 min), followed by $NaBH_4$ reduction; b) the remaining solid fractions

This loss of acetate groups accompanying $NaBH_4$ reduction (albeit performed under buffered, near-neutral conditions) needs to be further investigated and might be associated with side reactions of $NaBH_4$, especially considering lower stability of this reagent at relatively low pH, but also considering the possibility of additional activation in the post-oxidation reaction mixtures. .

In the 1430 to 1380 cm^{-1} region, the observed bands are attributed to C–H stretching and bending modes of methylene groups ($–CH_2–$) (Ge *et al.* 2016). Their increased relative intensity following reduction is consistent with the conversion of aldehyde groups to primary alcohols ($–CH_2OH$), confirming the reduction of dialdehyde intermediates. A diffuse band near 850 to 890 cm^{-1} is assigned to C–C or C–O skeletal vibrations of the pyranose ring and to the presence of hemiacetal and hydrated forms, characteristic of periodate-oxidized carbohydrates (Kim *et al.* 2000).

The Impact of Periodate Concentration and Type of Biomass

To investigate the impact of biomass and periodate charge on the extraction procedure, birch and spruce were treated with different periodate concentration for 1 h – the length of the treatment that would, according to the results showed above, allow for relatively high extraction yields and aldehyde generation (Fig. 1b), while the degradation reactions were still quite limited (Fig. 3). In Fig. 6a, the consumption of periodate after 60 min of oxidation of birch and spruce is presented for different concentrations. In all cases, more than 50% of the oxidant was consumed within this period. As shown in Fig. 6b, the extraction yield calculated relative to the initial biomass input increased with higher periodate dosages, indicating a positive correlation between oxidant loading and recovery. Aldehyde formation and recovered yield are also reported in Fig. 6b.

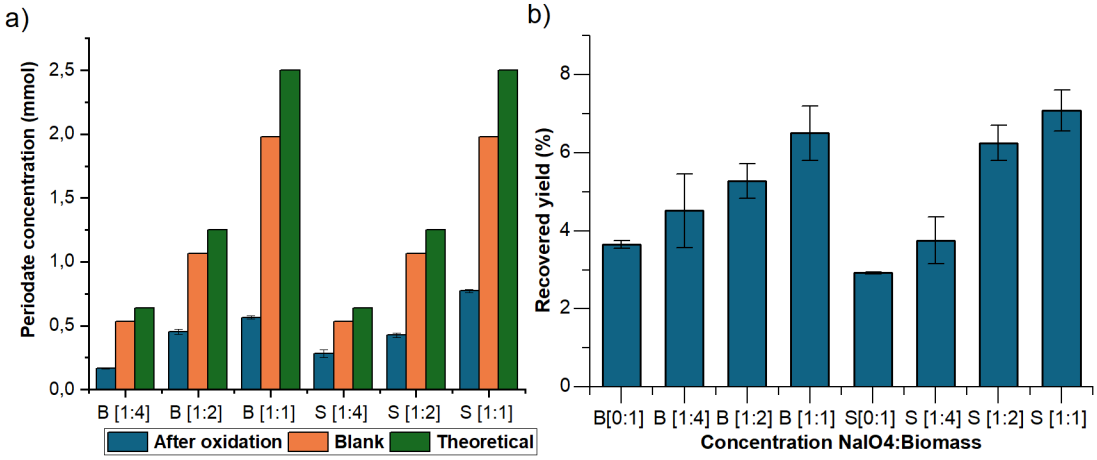

Fig. 6. a) Periodate consumption during oxidation birch (B samples) and spruce (S samples) for different oxidant charges ($NaIO_4$: biomass mass ratios), b) Yield of material recovered for different oxidation charges

Although attributing the oxidation effects to individual monosugars is difficult in this work, Fig. 7a indicates extracted monosugars that are resistant to periodate oxidation. Xylose in birch seems to be affected only to a relatively low extent by oxidation, as xylose levels in extracts only modestly vary with periodate concentration, even when comparing the water to periodate extraction. This is especially prominent in comparison with the quite

drastic decrease in glucose concentration as the periodate charge is increased. One possible explanation for these different behaviors might be the relatively high level of acetylation of the birch xylan (Teleman *et al.* 2002), preventing oxidation by blocking access to the vicinal alcohols (in C2 and C3). Glucose, on the other hand (in cellulose and glucomannan), is unsubstituted, allowing for more extensive oxidation through reaction of available vicinal alcohols (Teleman *et al.* 2002). Their concentrations decrease with increasing periodate loading, dropping from 37% to 12% and 7% at the B[1:2] ratio, and further to 5% and 3% at the B[1:1] ratio, respectively, when compared with the B[0:1] reference sample.

In spruce, where the hemicelluloses are primarily composed of galactoglucomannan (galactose, glucose, and mannose, with acetylations on mannose) and some xylan carrying 4OMe-glucuronic acid (and no acetylations), lower amounts of unoxidized sugars could be detected. This possibly indicated a higher degree of oxidation, which might be associated with a higher content of unsubstituted hexoses (even though some positions in mannose are substituted by galactose or acetates) and pentoses (non-acetylated xylan) with available vicinal hydroxy groups. After oxidation, the concentrations of galactose, glucose, and mannose were reduced to 6%, 8%, and 5% at the S[1:2] ratio, and to 4%, 3%, and 3% at the S[1:1] ratio, respectively, compared with the S[0:1] reference sample.

In agreement with the decreasing amounts of non-oxidized monosugars, the formation of aldehydes in extracts increased with the increasing periodate charge (Fig. 7b). However, the aldehyde concentration did not consistently follow decreases in monosugar content, especially not at elevated periodate levels, where it decreased despite the continued decrease of the amount of detected monosugars (indicating their oxidation). As discussed earlier, this behavior suggests that excessive oxidation promotes multiple side routes, such as degradation of aldehydes (as discussed by Pandeirada *et al.* 2022), crosslinking reactions reducing their accessibility to the titrating agent (Fliri *et al.* 2023) and overoxidation of aldehydes to carboxylic acids, as shown in Fig. 1d and observed by Zhu *et al.* (2025). Therefore, the measured aldehyde content may not increase proportionally with the periodate dosage or reaction time, as some aldehydes are lost or inaccessible.

Carbohydrate composition of the residual solid material reflected these extraction trends, showing a decrease in carbohydrate content for both birch and spruce as periodate charge is increased (Fig. 7c). For birch, the overall monosaccharide loss was 0%, 1.6%, 3.1%, and 3.3% at the B[0:1], B[1:4], B[1:2], and B[1:1] ratios, respectively. The largest relative decrease was observed for mannose, galactose, and arabinose; however, these sugars together accounted for only ~ 3% of the total dry mass, thereby limiting their overall impact. In contrast, glucose and xylose showed a smaller relative decrease, but because they represented ~44% of the dry mass, their extraction contributed importantly to the total carbohydrate loss.

For spruce, the overall monosaccharide loss was higher: 4.4%, 4.7%, 5.2%, and 7.7% at the S[0:1], S[1:4], S[1:2], and S[1:1] ratios, respectively. The largest relative decrease (≈34%) occurred in galactose and mannose, which together constituted ~8% of the dry mass, but major contribution to the extracts came from the more abundant glucose.

Even aldehyde generation in the solid material followed the same trend (Fig. 7d) confirming that the oxidation affected not only the extracted materials but also the solid residue. These aldehydes arose both from the oxidation of exposed vicinal diols in the glucose units of cellulose and from modifications of hemicelluloses retained in the structure. Just as observed in the corresponding liquors, aldehyde content increased up to

the [1:2] $NaIO_4$:biomass ratio, after which a further increase in oxidant dosage resulted in a decline in measurable aldehyde concentration due to the above discussed side-reactions leading to inaccessibility or removal of aldehydes. Moreover, the amount of detected Klason lignin in the solid residue increased with increasing periodate charge (Fig. 8). This probably indicates the increasing introduction of oxidized groups to carbohydrates that during the total acid hydrolysis (involved in Klason lignin determination) contributed to formation of condensed insoluble (crosslinked) structures detected in the solid Klason lignin fraction (pseudo lignin) (Sannigrahi *et al.* 2011; Chen *et al.* 2024), and reported in previous studies in the field (Amer *et al.* 2016; Chemin *et al.* 2016; Pandeirada *et al.* 2022; Zhu *et al.* 2025).

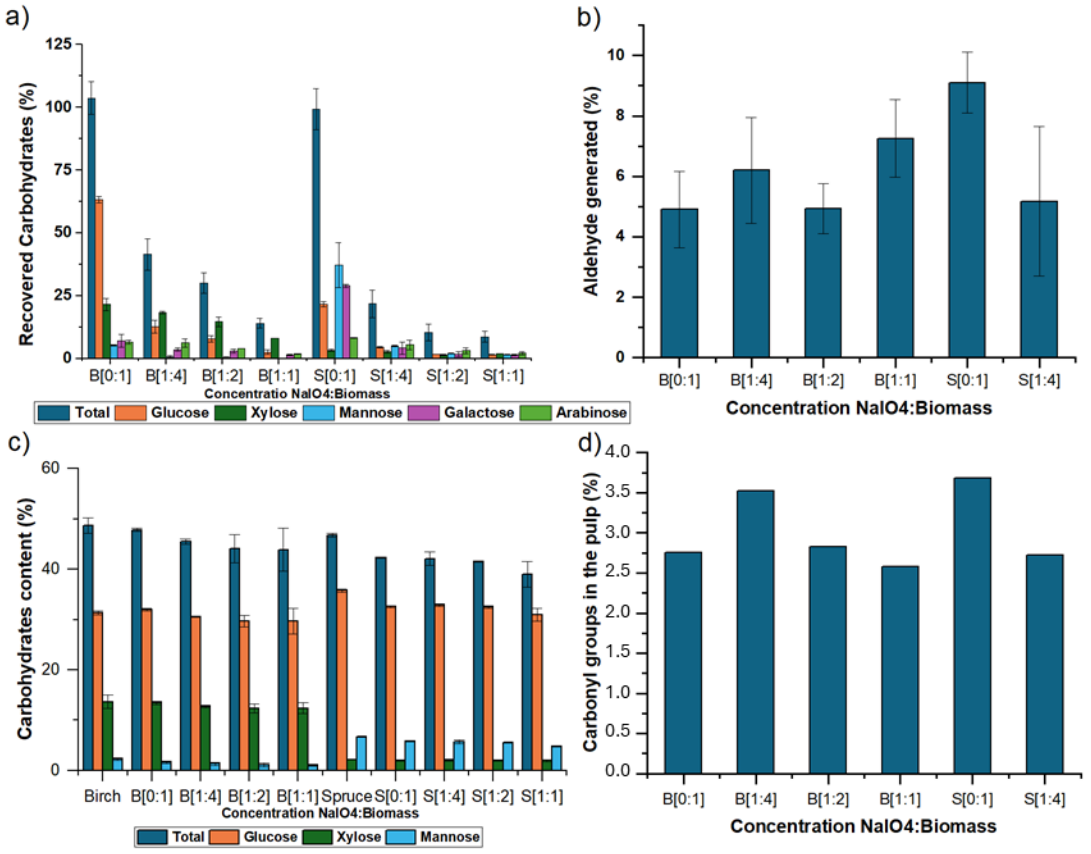

Fig. 7. a) Recovered extracted carbohydrates as % of the dry total extracted material for different periodate : biomass mass ratios after 60 min, for birch and spruce; b) Aldehyde generation as % of the total dry mass of the liquor extracted; c) Amount of carbohydrates in solid material recovered after extraction as % of the total dry mass of detected carbohydrates for different periodate: biomass mass ratios after 60 min, for birch and spruce, total amount of extracted carbohydrates as % of the total dry mass is shown in dark blue; d) Aldehyde concentration detected in the extracted material (as % of the analyzed dry pulp)

Hemicellulose oxidative reactivity to periodate evidently differs between hardwoods and softwoods due to variations in composition and probably also cell wall architecture (which was not investigated in this work). In birch, the dominant hemicellulose is O-acetyl-4-O-methylglucuronoxylan, which is rather heavily acetylated (on average 4 acetates per 10 xylose units (Teleman *et al.* 2002)) with less vicinal diols accessible to periodate oxidation (Nypelö *et al.* 2021; Yoshimi *et al.* 2024). In contrast, spruce

hemicelluloses are primarily galactoglucomannans, which are comparably less substituted (with a typical Man:Glc:Gal ratio in Norway spruce being 3.5 to 4.5:1:0.5 to 1.1 and 2 to 3 acetates per 10 mannose units) (Lundqvist *et al.* 2002; Willför *et al.* 2003) and as such might offer higher amount of vicinal diols available for oxidation (Palasingh *et al.* 2022).

Figure 8 shows that after oxidation, Klasson lignin levels in the remaining solid material had increased. Sannigrahi *et al.* (2011) mentioned the formation of pseudo-lignin by the combination of carbohydrate and lignin degradation products to be responsible for the increased Klason lignin content in biomass pretreated under acidic conditions. Sodium periodate oxidizes wood polysaccharides leading also to a generation of various degradation products (Sannigrahi *et al.* 2011) that under the strongly acidic conditions used in Klason lignin analysis, can undergo condensation reactions to form acid-insoluble material, enriching in the Klason lignin fraction despite not originating from native lignin. Such carbohydrate-derived degradation products are commonly referred to as pseudo-lignin. As a result, the Klason method may overestimate the actual lignin content when pseudo-lignin is present, particularly following oxidative pretreatments.

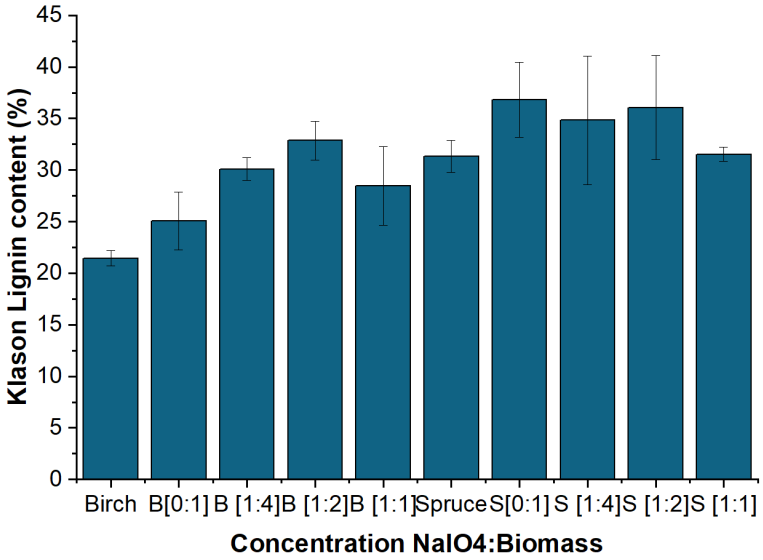

Fig. 8. Klason lignin concentration detected in the solid residue expressed as % of the dry content for different periodate charges after 60 min, for birch and spruce biomass

The influence of the various periodate oxidation conditions on the molecular weight (M_w) distribution of polysaccharides was studied by GPC (Supplementary Fig. S1). All aliquots contained structures within a degree of polymerization in agreement with other studies (Chemin *et al.* 2016; Pandeirada *et al.* 2022). Still in agreement with Pandeirada *et al.* (2022) and Fliri *et al.* (2023), the increase in periodate concentration extends the depolymerization.

CONCLUSIONS

1. Oxidation can be directly applied to untreated birch and spruce biomass, demonstrating that pretreatment is not required to achieve effective extraction and

functionalization. Periodate oxidation considerably enhances the solubilization of carbohydrate-rich fractions, resulting in higher recovery yields compared to untreated materials.

2. The oxidation process is characterized by rapid oxidant consumption, aldehyde formation followed by their conversion into hemiacetal crosslinks and hydrates, and a decrease in molar mass of the solubilized polymers, consistent with chain scission and depolymerization. The solid residue also undergoes oxidation, as indicated by increased aldehyde content and pseudo-lignin formation during Klason lignin analysis.
3. Higher oxidant loadings improve extraction efficiency but simultaneously reduce detectable aldehyde content due to crosslinking, degradation, and overoxidation. These findings highlight the importance of optimizing oxidation conditions to balance extraction yield and chemical functionality of both soluble and solid fractions.

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APPENDIX

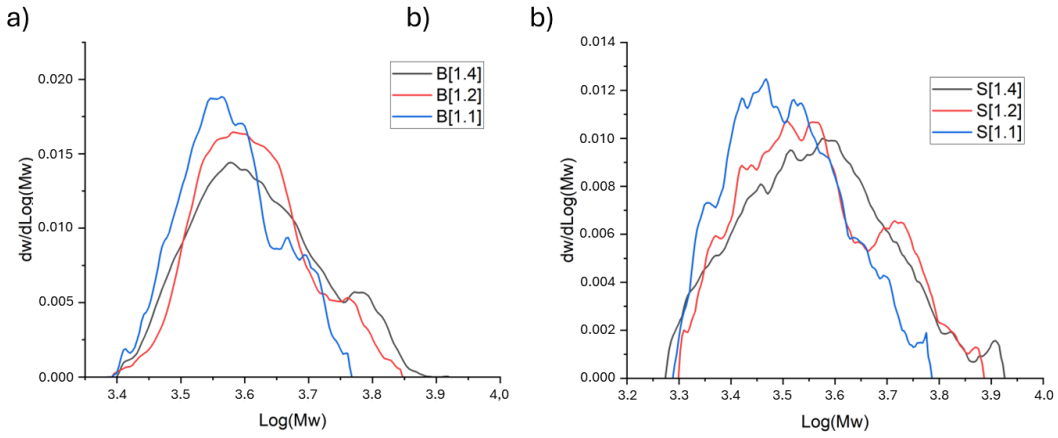

Fig. S1. Influence of periodate concentration on molecular weight after oxidation in birch, b) Influence of periodate concentration on molecular weight after oxidation in spruce