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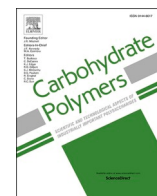
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# O<sub>2</sub> as initiator of autocatalytic degradation of hemicelluloses and monosaccharides in hydrothermal treatment of spruce

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## ABSTRACT

The influence of oxygen (0–50 bar) on the molar mass and composition of hemicelluloses after hydrothermal treatment of spruce chips was studied in a batch reactor setup at 130 °C–160 °C. Purified galactoglucomannan was studied as a reference. The dissolved oxygen enhanced significantly the depolymerization of hemicelluloses from over 15,000 g/mol to 180 g/mol (monomers) as well as promoted acids formation from the monosaccharides. About 2–3 times faster depolymerization was observed already with rather low amounts of oxygen (2–8 bar), while the kinetics of depolymerization was significantly slower in inert atmosphere. The decrease in pH from neutral to as low as 2.5 lead to an autocatalytic effect on the polysaccharide hydrolysis and the consecutive reactions of monosaccharides. The results emphasize the importance of performing hydrothermal treatment under inert atmosphere if large polymeric hemicelluloses are desired or if monosaccharides are targeted in hydrolysis of hemicelluloses. If autocatalysis begins, it is difficult to stop.

## 1. Introduction

Novel biorefinery concepts based on lignocellulosic biomass have been under increasing interest during the last decade and new high value added ways of utilizing this valuable resource are searched for continuously. Norway Spruce is a great source of lignocellulosic biomass and one of the major component in it is the hemicellulose galactoglucomannan (GGM) which has been under growing interest due to its properties and good availability (Mikkonen et al., 2009; Willför et al., 2008). A distribution of hemicelluloses with different molar masses is obtained from the fractionation of the wood (Gallina et al., 2018). The low molecular fractions of the hemicelluloses obtained from the extraction from wood are very suitable for the hydrolysis (Perez Nebreda et al., 2016; Perez Nebreda et al., 2018; Perez Nebreda et al., 2019a, 2019b; Rissanen et al., 2014a) and sugar monomers can be utilized in the chemicals industry as platform chemicals for producing fuels as well as more value added products such as sugar alcohols and sugar acids (Lu et al., 2021). The long-chained high molecular mass hemicelluloses can be utilized in numerous applications such as

pharmaceuticals, textiles, cosmetics, waste water cleaning, barriers as well as for alimentary, health and food products, which is increasing the demand for them provided that the properties are sufficient (Hromádková et al., 2013; Kardošová et al., 2004; Lozhechnikova et al., 2014; Peng & She, 2014; Wang et al., 2015). The average molar mass of the obtained extract is crucial for its further refinement.

Pressurized hot water extraction from lignocellulosic biomass typically results in obtaining hemicelluloses and some lignin into the aqueous phase (Anderez et al., 2018; Yedro et al., 2017). The controlled extraction of a hemicellulose-lignin extract and the influence of the extraction conditions on the properties of this mixture have obtained very little attention, however, new potential applications have emerged for these non-pure fractions (Pitkänen et al., 2018; Valoppi et al., 2019) in the form of emulsifiers for e.g. food and cosmetics. The main part of LCC is presumed to be formed by lignin and hemicellulose and the suggested types of covalent bonds between lignin and carbohydrates are mainly benzyl-ether, benzyl-ester, and phenyl glycoside bonds (Balakshin et al., 2007).

Pressurized hot water extraction is a rather safe and natural choice of

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solvent/method for e.g. alimentary and cosmetics applications considering cost of purification and regulatory issues. This fact limits the use of additives in the extraction process. Hemicelluloses molar mass depends on the extraction conditions used in the PHWE (Rissanen et al., 2014a). Under acidic conditions, glycosidic bonds in hemicelluloses and pectins are hydrolyzed at high temperatures. This occurs without any added chemicals or catalysts, mainly due to the release of acetyl groups and uronic acids from the hemicelluloses, which both contribute to the acidity in the mixture (Grénman et al., 2011). Extraction and consecutive partial hydrolysis of the hemicelluloses is very temperature dependent (Mäki-Arvela et al., 2011). The further degradation of the sugar monomers is significant if the extraction conditions become too harsh and process times too long. Extraction parameters should be selected carefully if the aim is to utilize the obtained hemicellulose without severe degradation (Rissanen et al., 2014b; Rissanen et al., 2015; Rissanen et al., 2016).

Hydrothermal processing is typically performed under air and oxygen is, thus, present in the reaction mixture. On the other hand, higher oxygen concentrations can be used e.g. in the removal of lignin compounds from residual solids, such as pulp, or in the process intensification of the fractionation process. The aim of the current study was to shed light on the qualitative and quantitative influence of oxygen on the properties of hemicellulose extracts during and after hydrothermal processing. The hypothesis was that the presence of molecular oxygen is very detrimental in hydrothermal processing of wood if large hemicelluloses or monosaccharides are targeted and that the influence is enhanced by autocatalysis. The study focused on spruce, however, it is reasonable to assume that the results are valid also for other biomass. In addition to extraction from wood chips, the hydrolysis of a well characterized purified spruce galactoglucomannan (GGM) was studied by subjecting it to the same conditions used in the extraction studies. The results were then compared to evaluate if the decrease in molar mass is purely due to polysaccharide depolymerization or if the other substances in the extracts such as lignin also contribute to the phenomenon.

## 2. Materials and methods

Chemicals were purchased from commercial sources if not stated otherwise and used without further purification. The hemicellulose GGM was extracted from Norway spruce (*Picea abies*) wood chips by pressurized hot water under reduced pressure in a pilot plant process by the company CH-Bioforce Oy. The high-molar mass hemicelluloses were precipitated from the obtained GGM concentrate by EtOH:H<sub>2</sub>O (75:25 v/v). The molar mass of the insoluble GGM fraction was  $M_w = 8000$  g/mol determined by HPSEC, and the monosaccharide composition of the precipitated sample was determined by gas chromatography after being subjected to acid methanolysis and silylation (Sundberg et al., 1996). The composition of the GGM was the following: arabinose 9.8 mg/g, rhamnose 2.3 mg/g, xylose 64.5 mg/g, mannose 576.4 mg/g, galactose 112.2 mg/g, glucose 165.4 mg/g, glucuronic acid 3.9 mg/g, 8.0, methyl glucuronic acid mg/g, 14.0 galacturonic acid mg/g. NMR spectra of the GGM can be seen in Figs. S1 and S2.

### 2.1. Experimental setups and procedure

Experiments were performed in a tailor made batch reactor with a total volume of 1 L and online monitoring of temperature and pressure. Additionally, the system included gas inlet and outlet, and the possibility to pre-heat the reaction mixture. The reactor was also equipped with a Ruston turbine and baffles. Moreover, online sample taking and internal cooling were mounted. The heating was provided by a heating jacket (Fig. S3). Each experiment contained 700 g of pure water and 40 g of wood material giving a liquid-solid ratio to 17.5. Argon, air and oxygen atmospheres at different pressures were used in the experiments, in order to vary the oxygen concentration in the liquid phase. Hydrogen atmosphere was also tested, however, the results were very similar to

**Table 1**

The experimental matrix.

Experiment	Atmosphere	Temperature [°C]	Pressure [bar]	Process time [min]	Chip size [mm]
1	Ar	130	20	240	0.5–1.25
2	O <sub>2</sub>	130	20	120	0.5–1.25
3	O <sub>2</sub>	130	50	120	0.5–1.25
4	Ar	140	20	160	0.5–1.25
5	O <sub>2</sub>	140	20	80	0.5–1.25
6	O <sub>2</sub>	140	50	80	0.5–1.25
7	O <sub>2</sub>	140	50	80	0.5–1.25
8	Ar	150	20	60	0.5–1.25
9	H <sub>2</sub>	150	20	60	0.5–1.25
10	O <sub>2</sub>	150	50	40	0.5–1.25
11	O <sub>2</sub>	150	20	40	0.5–1.25
12	Ar	160	20	120	0.5–1.25
13	O <sub>2</sub>	160	20	120	0.5–1.25
14	O <sub>2</sub>	160	2	120	0.5–1.25
15	O <sub>2</sub>	160	50	120	0.5–1.25
16	Air	160	Ambient	120	0.5–1.25
17	O <sub>2</sub>	160	8	120	0.5–1.25
18	O <sub>2</sub>	150	20	240	GGM- solution
19	O <sub>2</sub>	140	20	480	GGM- solution
20	O <sub>2</sub>	130	20	720	GGM- solution
21	Ar	150	20	240	GGM- solution

the ones obtained with Argon, which is why inert and oxygen atmospheres were focused on.

The wood material used in this study was Spruce sapwood (*Picea abies*), obtained from South-West Finland. Before the experiments, the wood material was debarked and the heartwood, knots and compression wood were removed. Pure sapwood was cut to smaller pieces, dried one week under intense ventilation and then ground to smaller chips. After chipping, different size fractions were obtained by sieving. The 0.5–1.25 mm fraction was used in the experiments. The residual water content of the chips was determined by using freeze drying and oven drying.

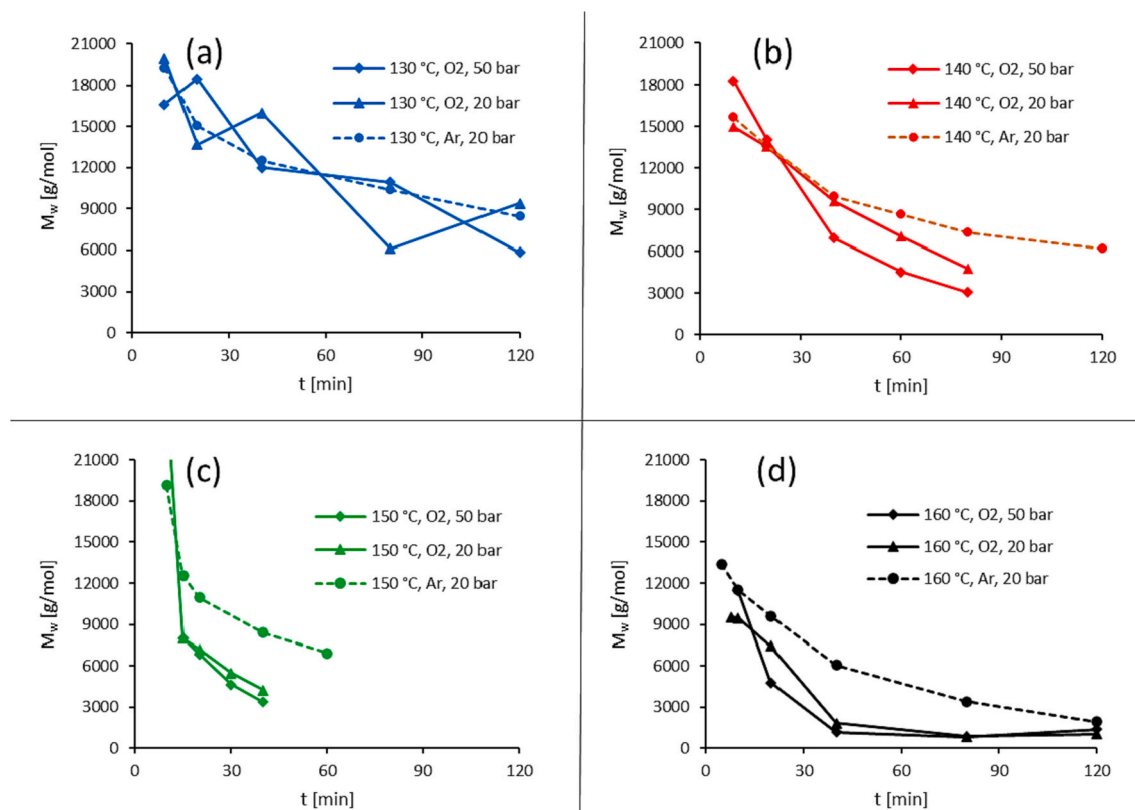
Prior to an experiment the reactor was loaded and then pressurized with the desired gas and kept under intense stirring after which, the pressure was decreased and increased again. This cycle was repeated for five times in order to flush out other volatile compounds from the liquid phase and to saturate the solution. Before the experiments, the pressure was adjusted to the desired value and after sampling it was adjusted again to the same level than before sampling. In every experiment, the extraction was considered to begin when the mixture achieved 100 °C. Increasing the temperature from 100 °C to the desired process temperature took about 4–6 min.

The same solid-liquid ratio (1:17.5) was used in all the experiments. The process parameters varied in the experiments were temperature, pressure, type of atmosphere and process time according to Table 1. The volume of the liquid samples were kept to a minimum to avoid significant changes in the solid to liquid ratio however, it was taken into account in the calculations. After sampling, all the samples were quickly cooled down and frozen at –18 °C.

The end of the experiments, quenching was used with build-in cooling system to decrease the temperature under 100 °C within a few tens of seconds. The liquid and solid end-products were stored at –18 °C.

The total dissolved solids (TDS) was determined from all the liquid phase samples by weighing about 6 g of the solution and placing it in the oven at 93 °C for 24 h and after that to 104 °C for 4 h, after which the mass of the remaining dry matter was determined. The amount of total dissolved solids was then calculated by dividing the mass of the dry substance by the mass of the weighed liquid.

In the temperature range 130–160 °C, the solubility of oxygen in water increases with pressure and temperature (Tromans, 1998, 2000).



**Fig. 1.** Molar mass of macromolecules during extraction from wood as a function of time under different atmospheres and pressures at 130 °C (a), 140 °C (b), 150 °C (c) and 160 °C (d).

According to Tromans, accurate values of oxygen concentrations in aqua can be calculated according to Eq. (1), where  $T$  is temperature,  $P_{O_2}$  is the oxygen pressure and  $c_{aq}$  is the molality of oxygen. Results at different temperatures and pressures are shown in Table S1 and Fig. S4.

$$c_{aq} = P_{O_2} \exp \left( \frac{0.046T^2 + 203.35T \ln \left( \frac{T}{298} \right) - (299.378 + 0.092T)(T - 298) - 20.591 \times 10^3}{(8.3144)T} \right) \quad (1)$$

## 2.2. Molar mass analysis

The molar mass determination of the liquid phase (without preparation) was performed with a HPSEC-MALLS system (miniDAWN, Wyatt Technology, Santa Barbara, CA) with RI-detector (Shimadzu Corp., Tokyo, Japan). A combination of two columns (2 × Ultrahydrogel™ linear 7.8 × 300 mm, Waters, Milford, MA) was used in series. NaNO<sub>3</sub> (0.1 M) was used as eluent with a flowrate of 0.5 cm<sup>3</sup> min<sup>-1</sup>. Residual solid particles which could be present in the samples were removed by using nylon syringe filters (25 mm, w/0.2 μm nylon membrane, VWR). All samples were concentrated to about 3 g/L and the injection volume of 200 μL was applied to the system. The dn/dc value was set to 0.150 cm<sup>3</sup> g<sup>-1</sup> and the overall data analysis was performed by Astra software (Wyatt Technology) (Rissanen et al., 2014a; Rissanen, Grénman, Willför, et al., 2014; Xu et al., 2008; Xu et al., 2009). The results obtained with HPLC were supported by data from UV adsorption and GC analysis.

## 2.3. Sample preparation for NMR- and GC-MS analysis

Extracted hemicelluloses: 250 mL of the hemicellulose solutions (Table 1. Entry 8–11) were extracted four times with 100 mL MTBE. The

MTBE-phase was dried with NaSO<sub>4</sub> and concentrated under vacuum before submitted to silylation and analysis with GC-MS. The water-phase was concentrated in a rotary evaporator and finally freeze dried before submitted to NMR-analysis.

Hemicellulose degradation studies: Two samples from the reaction (Table 1. Entry 18) after 160 min (30 mL) and 240 min (130 mL) was collected and extracted five times with 10 mL EtOAc in the first sample, and 20 mL EtOAc in the second sample. The EtOAc-phase was dried with NaSO<sub>4</sub> and concentrated under vacuum before submitted to silylation and analysis with GC-MS. The water-phase was concentrated in a rotary evaporator and finally freeze dried before submitted to NMR-analysis.

## 2.4. NMR-analysis

All the NMR experiments were performed at 298 K in DMSO-*d*<sub>6</sub> on an AVANCE III spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany) operating at 500.13 MHz for <sup>1</sup>H and 125.77 MHz for <sup>13</sup>C. HSQC experiments used the Bruker's pulse program "hsqcetdgpsisp2.3" for multiplicity edited with a spectral width of 8012 Hz (from -3.3–12.7

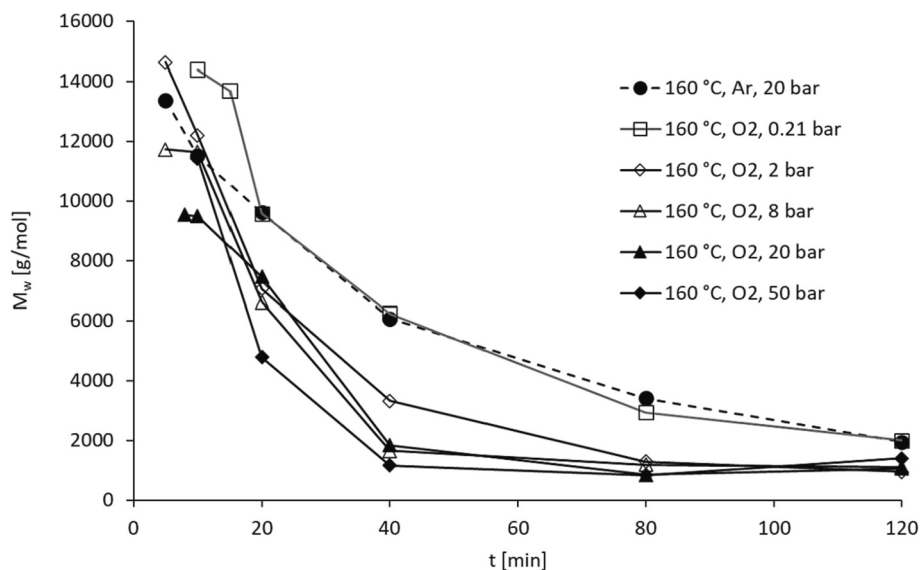


Fig. 2. Molar mass of macromolecules during extraction from wood as a function of time under different atmospheres and pressures at 160 °C.

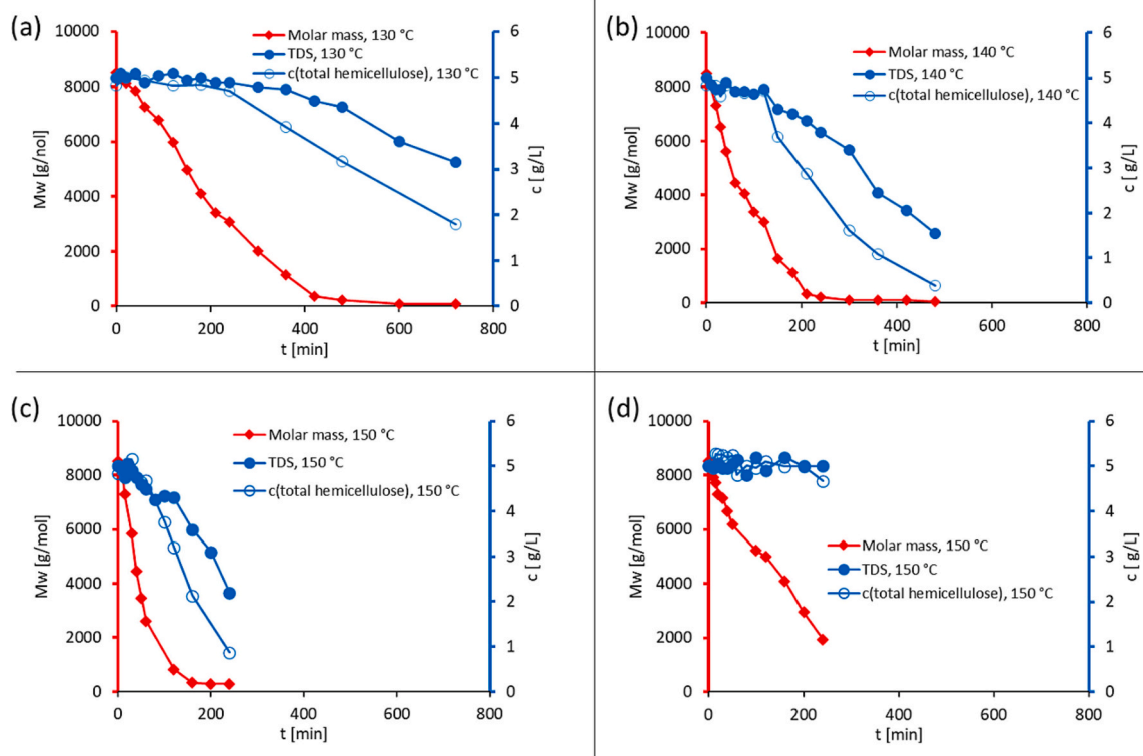


Fig. 3. TDS, total hemicellulosic sugars and molar mass of purified GGM as a function of time at 20 bar oxygen atmosphere at 130 °C (a), 140 °C (b) and 150 °C (c), compared to reference at 20 bar of argon atmosphere at 150 °C (d).

ppm) and 20,750 Hz (from  $-7.5$ – $157.5$  ppm) for the  $^1\text{H}$ - and  $^{13}\text{C}$ -dimensions. The residual solvent peak was used as the internal reference  $\delta\text{H}/\delta\text{C}$  ( $2.50/39.52$  ppm).

## 2.5. GC–MS analysis

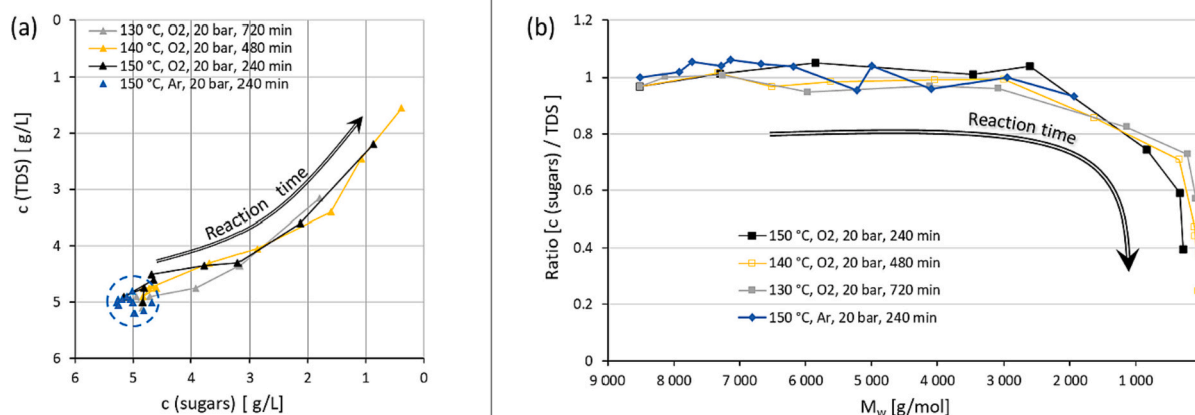
GC-EIMS analyses were performed on an Agilent Technologies 7890A GC-system equipped with a 5975C EIMS-detector and an Agilent J&W HP-5ms GC Column ( $30\text{ m} \times 0.25\text{ mm}$ ,  $0.25\text{ }\mu\text{m}$  film) (Agilent Technologies Inc., Santa Clara, CA, USA). The samples were silylated

using hexamethyldisilazane-chlorotrimethylsilane in pyridine, prior to analyses.

## 3. Results and discussion

### 3.1. Influence of temperature and oxygen concentration on hemicelluloses during extraction from wood

When the atmosphere was kept inert but the temperature was varied between  $130$  °C– $160$  °C, the thermally induced acid catalyzed hydrolysis



**Fig. 4.** Total dissolved solids as a function of hemicellulosic sugars (a) and the ratio of hemicellulosic sugars and total dissolved solids as a function of molar mass (b) at 20 bar oxygen atmosphere at 130 °C, 140 °C and 150 °C and 20 bar of argon atmosphere at 150 °C. The starting material was purified GGM.

occurred as depicted in Fig. 1. The temperature dependence of the depolymerization rate was quite strong and a substantial difference was noticed between the different temperatures during the extraction. When the molar mass remained about 7000 g/mol at 130 °C it decreased to around 2000 g/mol in 120 min at 160 °C. The same dependence continued when oxygen was introduced, however, the depolymerization rate was even faster. Moreover, the differences between the degradation rates at different temperatures were larger under oxygen atmosphere compared to inert atmosphere indicating that the activation energy of the overall reactions taking place is higher (Fig. S5).

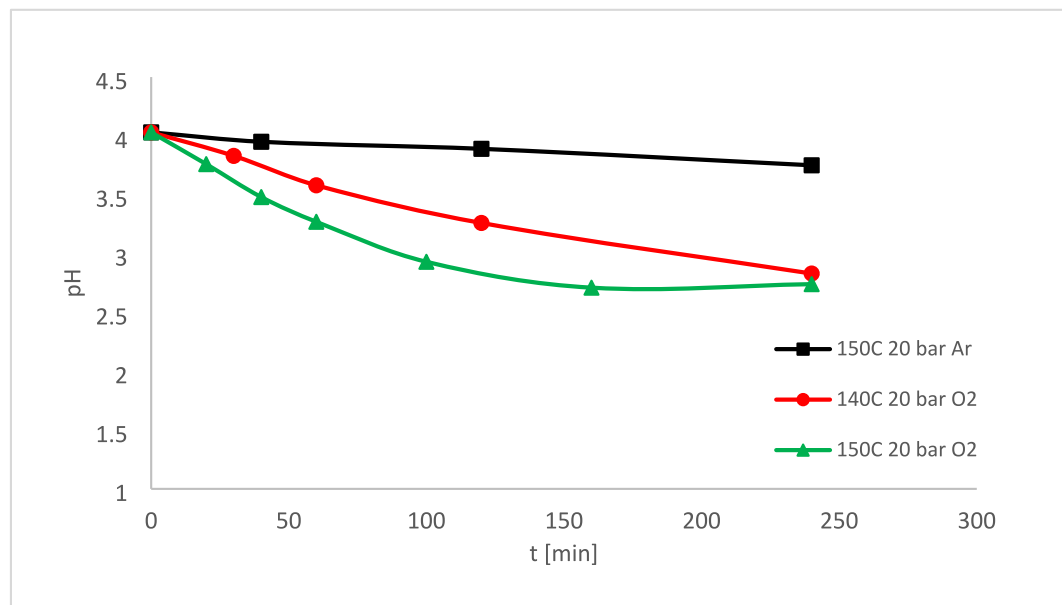
The change in molar mass as a function of time in experiments performed at 130 °C and with different oxygen pressures is depicted in Fig. 1a. The molar masses decreased during the experiments from somewhat below 20,000 g/mol to just above 5000 g/mol. There was no significant difference in the results obtained with increased oxygen concentration compared to the inert atmosphere. At 140 °C, the influence of oxygen on the molar masses became apparent (Fig. 1b). The increase in the oxygen concentration increased the rate of decrease in the average molar masses. The same phenomena was even faster at 150 °C, where the average molar mass dropped to under 10,000 g/mol in 15 min of extraction (Fig. 1c) and further to between 3000 and 5000

g/mol in 40 min. With the argon atmosphere, the molar masses remained several thousand g/mol higher in otherwise identical reaction conditions. At 160 °C, both the rate of depolymerization as well as the difference between inert and oxygen atmosphere increased compared to the results obtained at lower temperatures. After 40 min of extraction, the molar mass under argon remained over 6000 g/mol, while at high oxygen concentrations it decreased to the practical lower detection limit of the analysis method, which is about 1000 g/mol (Figs. 1d & 2).

### 3.2. Purified GGM hydrolysis kinetics

Purified galactoglucomannan was subjected to 20 bars of oxygen at 130 °C, 140 °C and 150 °C. Fig. 3 display the evolution of total dissolved solids (TDS), the concentration of hemicellulosic sugars and molar mass as a function of time at the selected temperatures. As a reference, an experiment under inert atmosphere by using 20 bars of argon at 150 °C was performed (Fig. S5).

Hydrolysis of GGM at 130 °C (Fig. 3a) shows clearly how GGM molecules were cleaved to smaller oligosaccharides virtually from the beginning of the process. Within 400 min, the molar mass had decreased from 11,000 g/mol to 1000 g/mol. However, the concentration of



**Fig. 5.** The pH as a function of time during depolymerization of purified GGM under argon and oxygen atmosphere.

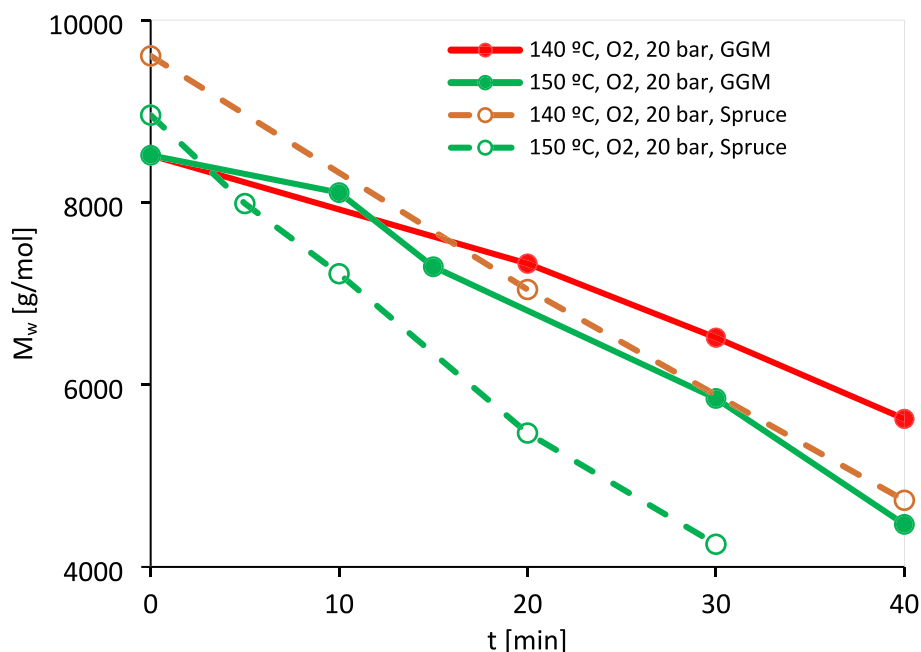


Fig. 6. Molar mass as a function of time for purified GGM (solid symbols) and GGM during extraction from spruce.

hemicellulosic sugars remained very stable, which shows that the degradation of the sugar monomers to furfurals is negligible. After 4 h, degradation of sugars increased while the TDS still remained high indicating the degradation of sugars to other liquid phase products. After 6 h of extraction, the TDS started to decrease and after 8 h only 60 % of the TDS remained. The decrease of the TDS was due to degradation of the sugars to volatile compounds.

The observations made at 140 °C and 150 °C are similar, except that the kinetics of each reaction was faster i.e. the degradation of hemicelluloses, sugars, and finally sugar degradation products was faster, as displayed in Fig. 3b & c.

The results of total dissolved solids showed clearly that the amount of dissolved mass in the liquid phase decreased under the oxidative environment when the reaction continued and that it could be mainly explained by the gaseous degradation products which released out from the reactor in connection with sampling. Some very minor amounts of precipitated degradation products were also observed, but compared to the gaseous degradation products, the amounts were negligible.

Under oxygen atmosphere at 150 °C the molar mass was already 50 % lower after about 40 min of experiment, so the rate of depolymerization was significantly faster than at 130 °C and 140 °C. The TDS and the total sugar concentration started to decrease within 20 min experiment. After 2.5 h, 50 % of the sugars had been degraded and after 4 h the TDS was 50 % lower compared to the initial concentration.

Under 20 bars inert argon atmosphere at 150 °C, a significantly slower rate of cleavage of GGM fractions was determined (3d) compared to under oxidizing conditions at the same temperature (3a). In an inert environment, no decomposition of monosaccharides occurred, nor did the conversion of total solids to gaseous reaction products (3d).

Overall, temperature has a clear effect on the rate of depolymerization of GGM, which can be observed as a reduction in the average molecular weight under constant oxygen atmosphere. The degradation rate of the monosugars leading to the loss of total dissolved solids due to the gaseous degradation products is also visible in Fig. 3. If lignin does not react in inert atmosphere under relatively low temperature, but the hemicellulose chain is cleaved, it could provide a route to tailor lignin-carbohydrate complexes structure selectively.

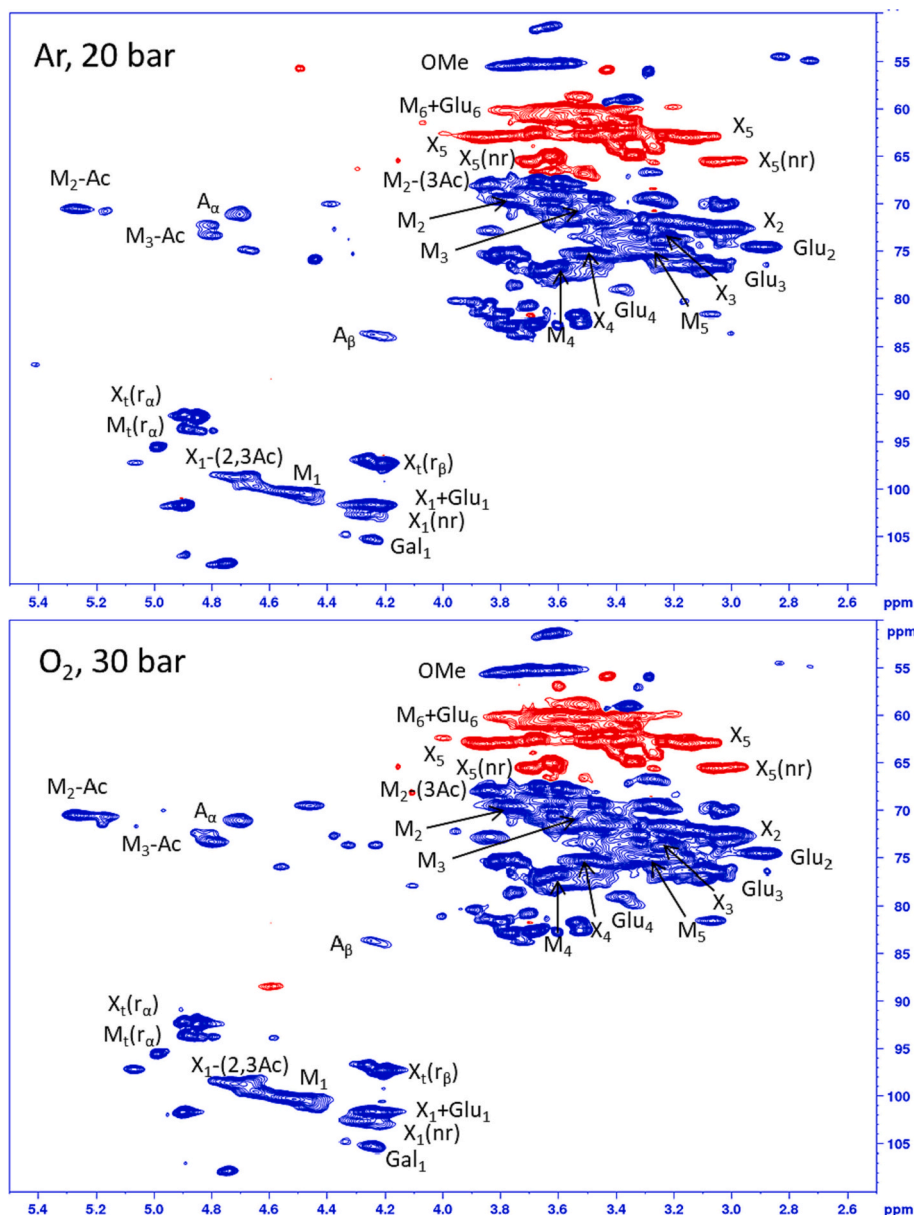
The evolution of the ratio between TDS and sugar concentration under 20 bar oxygen at 130–150 °C (Fig. 4a) show that the sugar

concentration decreased more rapidly than the TDS compared to inert environment at 150 °C which remains unchanged. Additionally, it displays that the reactions occurred identically at the different temperatures, under oxidizing conditions, even though the kinetics vary. The experiments showed also that the ratio of sugars and total dissolved solids stayed nearly constant ( $\approx 1$ ) until the average molar mass ( $M_w$ ) decreased to between 3500 and 2500 g/mol (Fig. S7). After this stage the monosugars started to degrade significantly under oxygen atmosphere. Fig. 4b displays how the ratio of the sugar concentration and TDS varied with molar mass. The results clearly show that sugar degradation began, when free oxygen exist and when the GGM had been hydrolyzed to a molar mass of about 3000 g/mol and that severe degradation occurred after that point.

### 3.2.1. Behaviour of pH during hydrolysis of purified GGM

The pH decreased during the hemicelluloses hydrolysis. Fig. 5 depicts the pH as a function of time from when the desired reaction temperature was reached in the experiments (point 0). The pH remained at a high level when the hydrolysis occurred under argon atmosphere while it decreased substantially when the depolymerization was performed in the presence of molecular oxygen. The decrease in pH is due to the oxidation and possibly dehydration of the sugars to organic acids. The hydrolysis and dehydration reactions are catalyzed by acids, which leads to an autocatalysis in the reaction mixture. Moreover, the reaction rates increase with temperature, which is also observed in Fig. 3. The results emphasize the importance of performing PHWE under inert atmosphere if large polymeric hemicelluloses are desired and performing the hydrolysis of polysaccharides under inert atmosphere if monosaccharides are targeted. Already low concentrations of oxygen encountered when working under ambient atmosphere should be avoided.

The decrease in pH corresponds well with the formation of organic acid from the sugars. For example, if the highest temperature 150 °C is considered, about 4.5 g/L of sugars is further reacted after the experiment. The major sugar present in GGM is mannose, which has a molar mass of about 180 g/mol, which correspond to a concentration of 0.025 mol/L. If all of the mannose would be converted to mannonic acid, the theoretical pH would be about 2.5. The observed pH is 2.74, which is logical as some further degradation to gaseous compound occurred and additionally, galactonic and gluconic acid are weaker acids than



**Fig. 7.** Multiplicity edited HSQC of the carbohydrate correlation signals, CH/CH<sub>3</sub> shown as positive (blue) and CH<sub>2</sub> as negative (red). 20 bar argon atmosphere sample (above) and 30 bar oxygen atmosphere sample (below).

mannonic acid. Formation of some gaseous degradation products was demonstrated by an increase in pressure in a four hour experiment with 5 g/L GGM-solution without sampling (Fig. S6).

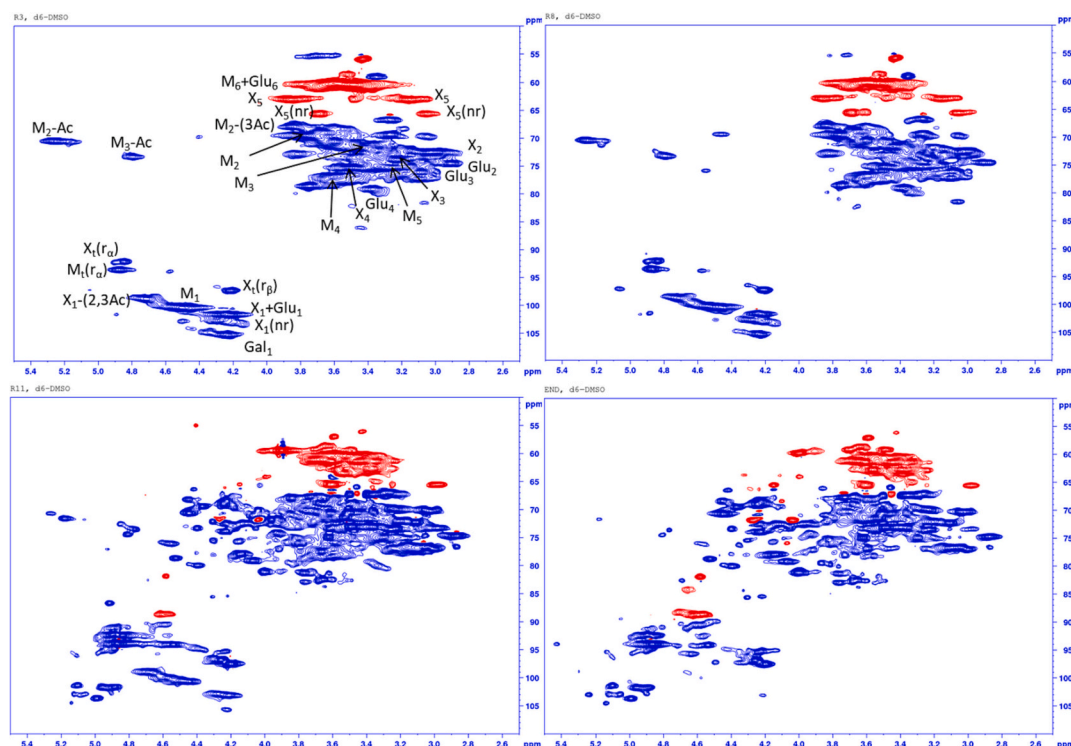
### 3.3. Comparison between hydrolysis kinetics of wood extract and purified GGM

As the hydrolysis kinetics of hemicelluloses often depends on the degree of depolymerization (DP) (Perez Nebreda et al., 2019a, 2019b), the comparison of the hydrolysis rate of the hemicelluloses (GGM) during extraction from wood with the results obtained with purified GGM is not straight forward. In order to obtain comparable data points, the authors used data on the development of molar mass obtained with 20 bar oxygen at 140 °C (Fig. 1b) from 40 min forward and 150 °C (Fig. 1c) from 15 min forward, as the molar mass at these points correspond closely to the molar mass of the purified GGM. The comparison of the hydrolysis rates is depicted in Fig. 6. It can be observed, that the hydrolysis rates are rather similar, which is very logical

considering that both are GGM extracted from spruce, one in “raw” and the other one in purified form. However, it can be noticed that the hydrolysis rate of the hemicellulose during extraction is slightly faster, which is due to the lower pH in the spruce extract solution. For example, at the time point 40 min, the extract had a pH of 3.0 at 140 °C and about 2.85 at 150 °C, while the pH in the purified GGM solutions was about 3.5 or higher as displayed in Fig. 5. The difference in pH is rather understandable as the purified GGM was precipitated from the extraction solution, so some of the acidity resulting from the extraction stayed in solution and did not remain with the purified product. However, the results do not support the hypothesis that other compounds in the extraction solution originating from e.g. lignin, would have a very significant effect on the hydrolysis rate in other means than contributing to the pH.

### 3.4. Qualitative analysis by NMR spectroscopy and GC-MS

To study the influence oxygen atmosphere has on the hemicellulose



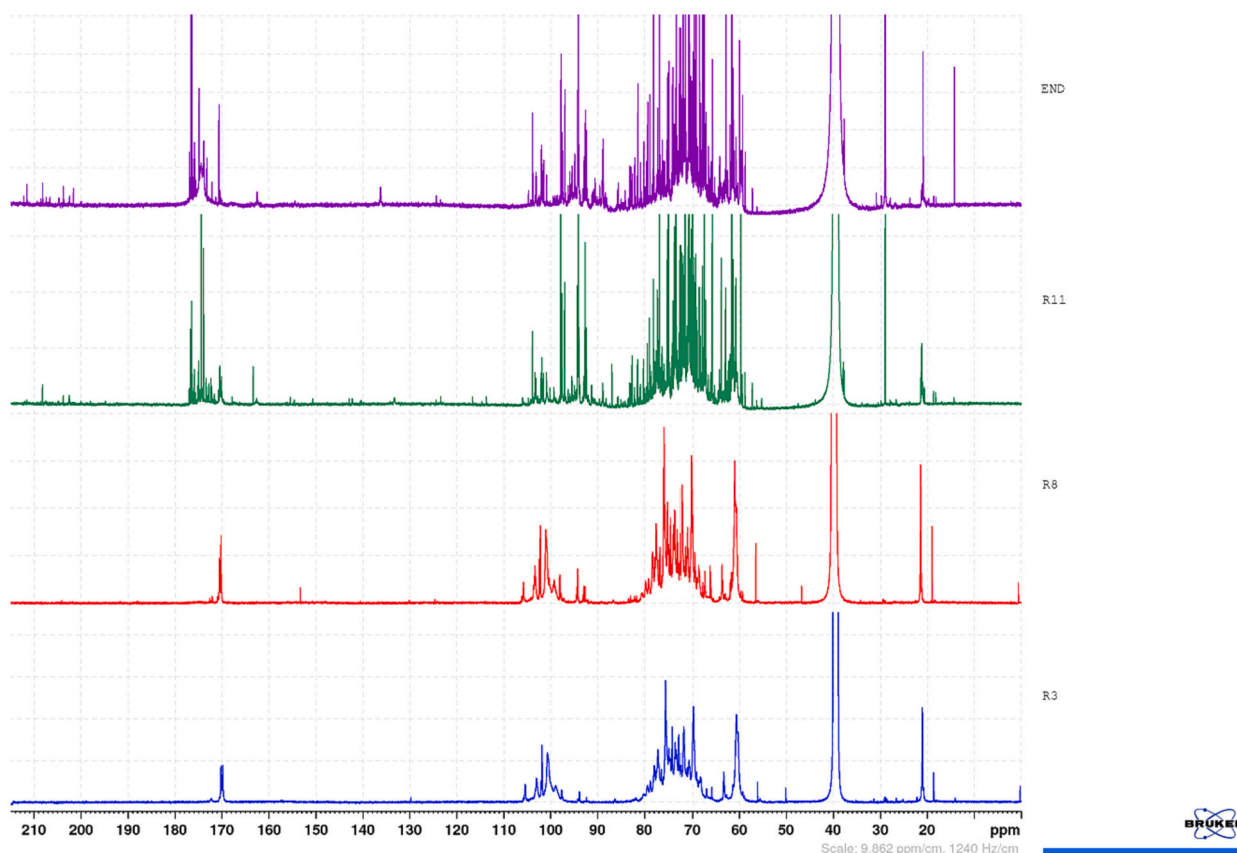
**Fig. 8.** Multiplicity edited HSQC of the carbohydrate correlation signals, CH/CH<sub>3</sub> shown as positive (blue) and CH<sub>2</sub> as negative (red). Top left: 130 °C, 20 bar O<sub>2</sub>, 40 min; top right: 130 °C, 20 bar O<sub>2</sub>, 180 min; bottom left: 150 °C, 20 bar O<sub>2</sub>, 160 min and bottom right: 150 °C, 20 bar O<sub>2</sub>, 240 min.

structure during hot water extraction four samples with different atmospheres were isolated and analyzed (Table 1. Entry 8–11). The wood material was extracted at 150 °C in argon-, hydrogen or oxygen atmosphere, all wood samples were extracted at 20 bars except one sample that was extracted at 50 bar oxygen. As the degradation was faster under oxygen atmosphere the process time were 40 min for the samples under oxygen atmosphere and 60 min for the samples under argon- and hydrogen atmosphere. The samples were extracted by MTBE to remove low molar weight degradation fragments from the extraction process. The samples contained approximately 5 wt% MTBE-extractives and these were analyzed by GC–MS (Figs. S8–S11). The MTBE-extractives obtained from the argon- and hydrogen samples were similar and contained mainly structures derived from monolignols and small amount of furfural derivatives. The MTBE-extractives obtained from the two oxygen samples were near identical and contained a higher degree of oxidized lignol structures and dicarboxylic acids. The hemicellulose fractions were analyzed by <sup>13</sup>C NMR (Fig. S12) and 2D HSQC (Figs. S13–S20). From the <sup>13</sup>C NMR spectrum it can be seen that there are only small differences between the hemicellulose samples, a small increase of carbonyl/carboxyl peak at 174.7 ppm in the spectrum from the oxygen samples compared to the argon and hydrogen samples. The partial HSQC spectrum of the argon sample and the oxygen 30 bar sample can be seen in Fig. 7. While the majority of the peaks remain the same there are some differences between the spectrums and there can be quantitative differences as well. Characteristic aromatic signals from lignin units can be detected from both <sup>13</sup>C NMR (~105–155 ppm) and 2D HSQC at spectrum approximately  $\delta_C/\delta_H$  (105–125/6.30–7.10). The cross correlation peaks from the traditional lignin linkage  $\beta$ -O-4 (A) are also observed in the 2D HSQC spectrum (see Fig. 7). Whether these lignin fragments are covalently bonded to the hemicellulose structure as LCC or as free units is not entirely clear. There are no clear signals from lignin-carbohydrate ester or benzyl ether linkages, however, some of the correlation peaks in the anomeric region ( $\delta_H/\delta_C$  4.90/101.7 ppm) could potentially be from phenyl glucoside bonds. (Balakshin et al., 2007) To investigate whether lignin are enriched based on the molecular size in

some part of the hemicelluloses two isolated samples, the sample extracted at 20 bars oxygen- and the one in 20 bars hydrogen atmosphere, were fractionated from a EtOH:H<sub>2</sub>O (80:20 v/v) solution. The precipitated insoluble hemicelluloses had higher molecular weight than the hemicelluloses in the soluble fraction, with a cut off at  $M_w$  of ~2000 g/mol. In both samples, the EtOH insoluble fraction contained mainly hemicelluloses while EtOH soluble fraction contained hemicelluloses with considerable amounts of lignin in it (Figs. S21–S24). As the low molar mass monomers had been removed from the samples by MTBE extraction prior to the H<sub>2</sub>O:EtOH fractionation, it can be concluded that the remaining lignin are either small lignin fragments or lignin/carbohydrate glycosides with a molar mass of approximately 1000–2000 g/mol. Hemicellulose from the hydrogen atmosphere had a higher insoluble to soluble wt% ratio (45:55) compared the sample from the oxygen atmosphere (25:75), which is consistent with the other data that oxygen atmosphere more readily degrades the hemicelluloses.

### 3.5. Hemicellulose degradation studies

To investigate the hemicellulose degradation, pure polymeric spruce hemicellulose was subjected to similar conditions as the hot water extractions of the wood chips under oxygen atmosphere. The conditions the samples were subjected to were: 130 °C, 20 bar O<sub>2</sub> (Table 1. Entry 20) samples were taken after 40 min and 180 min; and at 150 °C, 20 bar O<sub>2</sub> (Table 1. Entry 18) after 160 min and 240 min. From the milder condition at 130 °C (Table 1. Entry 20) it can be seen from Fig. 3a. that the sample taken after 40 min still retained its molar mass, TDS and concentration total hemicelluloses. The sample taken after 180 min retains TDS and concentration total hemicelluloses but has a reduced molar mass. The samples that were taken from the harsher 150 °C (Table 1. Entry 18) and prolong reaction time can be seen from Fig. 3c. The sample taken after 160 min has a greatly reduced Molar mass, moderately reduced concentration total hemicelluloses and slightly reduced TDS, and the final sample taken after 240 min has all parameters greatly reduced. The main differences in the HSQC spectrum (Fig. 8)



**Fig. 9.**  $^{13}\text{C}$  NMR spectrum (from bottom to top): (130 °C, 20 bar  $\text{O}_2$ , 40 min); (130 °C, 20 bar  $\text{O}_2$ , 180 min); (150 °C, 20 bar  $\text{O}_2$ , 160 min) and (150 °C, 20 bar  $\text{O}_2$ , 240 min).

between the samples taken after 40 min and 180 min in (Table 1. Entry 20) is that the intensity of the cross correlation peaks is higher in the signals assigned as anomeric reducing end ( $X_t(r_\alpha)$ ,  $X_t(r_\beta)$  and  $M_t(r_\alpha)$ ) compared to the normal anomeric signals ( $X_1$ ,  $M_1$ ,  $\text{Gal}_1$  and  $\text{Glu}_1$ ) in the sample taken after 180 min. This is to be expected as the hemicelluloses are being cleaved into smaller fragments which increase the amount of reducing ends. The samples taken after 160 min and 240 min from the harsher 150 °C (Table 1. Entry 18) were considerably more degraded than the samples taken at 130 °C (Fig. 8). Prior to analysis with NMR spectroscopy, the samples taken after 160 min and 240 min from the harsher 150 °C (Table 1. Entry 18), were extracted with EtOAc to remove low molar weight degradation products. The EtOAc-soluble compounds were analyzed by GC–MS (see Figs. S25–S26) and mainly dicarboxylic acid were detected in the EtOAc-soluble fraction from the samples taken after 160 min and dicarboxylic acids, carbohydrates and carbohydrate degradation products were detected in the EtOAc-soluble fraction from the sample taken after 240 min. The remaining hemicelluloses were heavily oxidized and deacetylated compared to the samples taken from the 130 °C reaction (Table 1. Entry 20) after 40 min and 180 min. This can be seen from the  $^{13}\text{C}$  NMR spectrum (Fig. 9) where the acetyl signals at 21.1 ppm and 170.0 ppm are reduced and carboxylic at  $\sim 174.5$  ppm and carboxylic peaks at above 200 ppm are increased. From the HSQC spectrum it is evident that the majority of the anomeric peaks are the reducing or non-reducing ends of the carbohydrates in the sample were taken from the harsher 150 °C (Table 1. Entry 18). There are also considerable amount of new peaks, most likely from oxidized degradation products. There are a cluster of new signals in the anomeric area, at approximately  $\delta\text{H}/\delta\text{C}$  (5.00/102.7 ppm), these signals are have similar chemical shifts to those of phenyl glucoside signals which could imply that the correlation peaks in the wood sample are from degradation products.

#### 4. Conclusions

The influence of oxygen atmosphere on the molar mass and composition of galactoglucomannan after subcritical extraction of spruce chips was studied in a semi-batch reactor setup. It can be concluded, that oxygen dissolved in the liquid phase significantly influences both the depolymerization of the hemicellulose as well as promotes acid formation from the monomers and the kinetics is enhanced with increased concentration and temperature. Moreover, the decrease in pH caused by the organic acids formed during extraction catalyzes the polysaccharide hydrolysis and also the dehydration and degradation of the sugars. This results in autocatalysis in the reaction mixture. The results show that the presence of molecular oxygen even in low concentrations is very negative if polysaccharides of large molar mass are desired from the extraction or even if monosaccharides are desired from the hydrolysis of polysaccharides. The autocatalysis increases significantly the significance of the oxygen as it is difficult to stop the degradation if it readily begins. The results have direct implications also for industrial scale hydrothermal treatment of biomass and polysaccharides from biomass.

#### CRediT authorship contribution statement

**Jussi V. Rissanen:** Data curation, Writing – original draft. **Lucas Lagerquist:** Data curation, Writing – original draft. **Kari Eränen:** Methodology. **Jarl Hemming:** Data curation. **Patrik Eklund:** Conceptualization, Methodology, Writing – review & editing. **Henrik Grönman:** Conceptualization, Supervision, Writing – review & editing, Methodology, Funding acquisition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.carbpol.2022.119740>.

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