MILD STEAM EXPLOSION AND CHEMICAL PRE-TREATMENT OF NORWAY SPRUCE

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The aim of this work is to open up the structure of wood while retaining a large amount of hemicelluloses, in particular (galacto)glucomannans. The effects of pre-treatments on wood meal from spruce (*Picea abies*) with a reducing agent ($NaBH_4$) combined with steam explosion at very mild conditions were investigated. The effects of steam explosion at 160 °C were studied for various residence times (5 to 35 min) on both water-impregnated wood meal and samples pre-treated with $NaBH_4$. The findings showed that pre-treatment with sodium borohydride stabilized the reducing end-groups of glucomannans and that the treatment was effective both during mild steam explosion, for both long and short residence times, as well as during subsequent treatment in alkali. Extraction experiments at different pH and temperatures showed that the main part of the hemicelluloses still remained in the wood residue after treatment. The molecular weight distributions of the extracted material from the liquors indicated that there were broad molecular distributions and that the molecular weight averages were between 3 and 6 kDa.

Keywords: Mild steam explosion; Wood separation; Spruce; (galacto)Glucomannan; $NaBH_4$

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INTRODUCTION

The material efficiency of a kraft pulp mill producing bleached pulp is about 40 to 45%, and the product contains mainly cellulose. The wood, however, also contains other biopolymers; mainly lignin, glucomannans, and xylans (Sjöström 1993). Until now, the main parts of these polymers have been degraded during kraft cooking and have been used as fuel in the recovery boiler. A modern kraft pulp mill has a reasonably large energy surplus, which in many cases has been used to produce electrical power (Sixta 2006). An alternative to producing electrical power would be to use the dissolved polymers in order to produce different types of material compounds of potentially high economic value.

An increase in interest in the biorefinery concept has greatly opened up research on utilizing all the components of wood, of which spruce is a resource with interesting potential. A greater awareness of environmental issues has also led to a growing interest in wood-based products (Persson 2009). Bioethanol production and the production of high-value products and specialty chemicals from wood components, such as lignins and hemicelluloses, are now major research areas (Ragauskas et al. 2006). Xylans and glucomannans with retained high molecular weight are however interesting also for other

applications. (Galacto)glucomannans could for example be used in abrasion-resistant clothing, for pharmaceutical purposes, in specialty papers, or for antibacterial bandages by modifying cellulose surfaces (Willför et al. 2008, Alonso-Sande et al. 2009). Emulsion stabilization in the food, health, and cosmetic industries are other examples. Oligomeric hemicelluloses prepared by steam explosion via size exclusion chromatography have been shown to be easily purified and modified for the production of hydrogels (Söderqvist-Lindblad et al. 2001; Edlund and Albertsson 2008). Both glucomannans and xylans are also considered as potential barriers in bioplastic films (Gatenholm et al. 2004; Zhu et al. 2011; Mikkonen et al. 2010). In order for a biorefinery process to benefit from energy and material efficiency, it is important to be able to selectively fractionate the different components. This would provide the potential to produce high value, low volume products parallel to low value, high volume fuels/products (Bozell 2010).

During alkaline treatment, the carbohydrate components in wood are subjected to degradation through the hydrolysis of acetyl groups, the dissolution of low weight carbohydrates, peeling reactions, and alkaline hydrolysis (Sjöström 1993). Hemicelluloses are degraded to a greater extent than cellulose. Xylans in softwood are more stable than glucomannans since they have alkali-stabilizing arabinose and 4-O-methyl glucoronic acid side-groups, and the yield of xylan is further increased by the fact that xylan has the ability to re-sorb onto fibres (Yllner and Enström 1956). Galactoglucomannans, which are the principal hemicelluloses in spruce (Fengel and Wegener 1989; Sjöström 1993; Timell 1967), have a high solubility and are currently most often degraded in various processes and thereby not used to their full potential. Thus, glucomannan is the component degraded most extensively, and the peeling reactions take place at moderate temperatures, significant for glucomannan already below 100 °C (Wigell et al. 2007). The peeling reaction starts at the reducing end-group of a carbohydrate polymer and one monomer at a time is rearranged and cleaved off. The reaction continues until a competitive stopping reaction occurs, primarily by the rearrangement of the reducing end-group (Franzon and Samuelson 1957).

When exposed to acidic treatments, glucomannans and other carbohydrates are subjected to acidic hydrolysis, which leads to breakage of glycosidic bonds. During thermal treatments, dehydration reactions of polysaccharides often occur as well, and these reactions are acid-catalyzed. The result is the formation of anhydro sugars with intramolecular glycosidic linkages. Because these bonds can easily be hydrolyzed, further degradation products, such as furfurals and uronic acids, may also be formed. Oxidation reactions are also common in acidic media, and these reactions lead to the conversion of hydroxyl groups and reducing end-groups into aldehyde-, keto-, and carboxyl groups (Fengel and Wegener 1989). Measurements of pulps after pre-hydrolysis and kraft cooking have shown that a pre-treatment at pH 2.5 and a temperature of 100 °C does not negatively affect the pulp yield and viscosity, while an increase to a temperature of 120 °C leads to a significantly lower pulp yield (Brelid 2002). Studies on the stabilization of glucomannan before sulfite pulping have shown that cleavage of ester bonds and the deacetylation of the glucomannan lead to molecules that are more resistant to acid hydrolysis. This can be achieved by a prolonged impregnation at low temperature or by a slightly alkaline precook. Stabilization cannot, however, be accomplished by weak acidic conditions in the precook (Annergren and Rydholm 1960; Annergren et al. 1961). The

reason for stabilization seems to be that deacetylated glucomannan fragments are more linear and therefore have the ability to sorb to the wood cellulose.

Due to these degradation reactions, some form of stabilization of the galactoglucomannans is necessary in order to be able to extract and isolate glucomannans from wood. The stabilization of polysaccharides during alkaline degradation can be achieved by the elimination of the aldehyde function of the end-groups. It is well known that borohydrides reduce the carbonyl groups in pulp (Meller 1953), giving the pulp an increased resistance to alkali (Richtzenhain et al. 1954; Miyake 1967) and reducing its brightness reversion (Giertz and McPherson 1956). An addition of 1% (on wood) of $NaBH_4$ can increase the pulp yield by 10% and the amount of retained glucomannan is increased significantly (Hartler 1959). Another approach to stabilizing the glucomannans is to oxidize the end-groups into carboxyl groups or other stable derivatives (Sjöström 1993).

In order to be able to extract galactoglucomannans effectively from spruce, the wood structure needs to be more accessible for chemicals and, more importantly, for enzymes. Steam explosion (STEX) is a technique whereby wood chips or other types of biomass are treated with high pressure steam followed by a rapid release to lower pressure. The liquid inside the wood chips expands, leading to a rupture of the wood structure. The cell components become somewhat hydrolyzed by the acetic acid that is released from the wood (Glasser and Wright 1998; Overend and Chornet 1987). Depending mainly on temperature and residence time, steam explosion treatment could result in anything from introducing small cracks in the wood structure to total defibrillation (Tanahashi 1990), and the method could, consequently, be used for several purposes.

The concept of steam explosion was introduced in two patents in 1932 and 1928. The former patent describes experiments on pine with saturated steam at 218 to 262 °C for 0.5 to 5 seconds, which was rapidly discharged, and the pine was then subjected to aqueous extraction and fermentation. The latter patent describes the means by which steam explosion could be performed on an industrial scale, and the same basic approach has been used later in the production of Masonite boards. Steam explosion has been suggested as an environmentally friendly pulping process (Kokta and Ahmed 1998) as well as a pre-treatment method for biomass intended for chemical production or enzyme treatment in, for example, ethanol production. An acidic catalyst such as SO_2 is often used in order to aid in the decomposition of the lignocellulosic biomass in these cases (Li et al. 2009; Shimizu et al. 1998; Grethlein and Converse 1991; Mabee et al. 2006). Steam explosion as a pre-treatment method is similar to pre-treatments with diluted acids in so far as they both increase the pore volume of the wood by removing hemicelluloses (Grethlein and Converse 1991; Carrasco et al. 1994)

Two important factors that govern the effect of steam explosions are temperature and residence time. It has been a general observation that it is possible to trade these factors and achieve equivalent results (Overend and Chornet 1987). This concept implicitly includes the assumptions that the overall kinetics follow first-law concentration dependence and that the rate constant has an Arrhenius-type dependence on temperature, although the apparent activation energy may itself be a function of temperature. Time and temperature have, thus, been combined into a single (severity) factor for describing

processes in general, similar to the H-factor in kraft pulping (Heitz et al. 1991). Although time, temperature, and pH are important parameters when it comes to the severity of steam explosion treatments, other factors such as wood source, chip size, and moisture content also affect the results and need to be taken into consideration when optimizing a steam explosion process. The effects of some of these parameters have been examined (Cullis et al. 2004) and it was found that the "relative severity" decreased with increasing chip size and increased initial moisture content (30% compared to 12%). Thus, thicker chips of higher initial moisture content in the chips reduced the harshness of the steam explosion treatment.

The effect of steam explosion on the structure and appearance of wood has been studied, for example, on sub-alpine fir (Zhang and Cai 2006) and on aspen wood chips (Kallavus and Gravitis 2009). By using light microscopy and electron microscopy (SEM and TEM), fractures could be observed, especially at bordered pit pairs and in pits between ray and parenchyma cells and in earlywood tracheids of the sub-alpine fir. In the study of aspen wood chips, it was concluded that vessels and ray cells worked in opposite ways; vessels accelerated the separation process while ray cells retarded it. It was also shown that lignin was redistributed both on the inner and outer surfaces of the cell wall as well as inside the cell wall.

The idea of using steam explosion as a pre-treatment method to utilize all wood components was previously presented in a study where hardwood samples were subjected to steam explosion at 180 to 230 °C for 1 to 20 minutes (Shimizu et al. 1998). In that study, hemicelluloses became extractable with hot water after steaming, and lignin was degraded by the cleavage of α- and β-aryl ether linkages. The wood components were fractionated by successive extraction with water and 90% dioxane.

In order to utilize glucomannans, they need to be extracted and isolated from the wood material. Numerous studies on extraction methods for different hemicelluloses have been conducted throughout the years; early investigations were made on delignified wood (holocellulose), and the hemicelluloses were extracted in successive steps with different types and concentrations of alkali, depending on the wood source (Fengel and Wegener 1989; Sjöström 1993). Examples of methods and results have been summarized in a review article by Timell (1967), and many of these methods still dominate current research. Glucomannans can be extracted by the use of barium hydroxide, which forms an insoluble complex with mannans and glucomannans (Meier 1958a). Organic solvents, such as dioxane, DMF, DMSO, and alcohols have also been used in different studies (McPherson 1958; Bennani et al. 1991). The extraction of galactoglucomannans from spruce wood has recently been achieved with pressurized hot water (Song et al. 2008). The study shows that the pH profile needs to be controlled in order to reach a high yield of high molecular weight hemicelluloses and that the yield forwood chips is significantly lower than the yield for ground wood. An extraction method with aqueous extraction liquors at acidic, near-neutral, and alkaline conditions was used in another recent study (Perez et al. 2011), where the impact of extraction conditions and wood species on chemical composition were investigated. It was concluded that for spruce, extraction at near-neutral conditions yielded glucomannan-rich fractions with low residual lignin content. Sulfuric acid resulted in similar behavior, while acetic acid at low concentrations acted as an organic solvent and led to the presence of high amounts of residual lignin.

From the above it can be concluded that steam explosion opens up the structure of wood, but if the conditions are too harsh there will also be a substantial degradation of the wood polymers, especially the hemicelluloses.

The aim of this study was to examine the potential of using mild steam explosion, as an industrially relevant technique, as a pre-treatment step in a process for the fractionation of spruce wood into its main components; cellulose, lignin, and hemicelluloses. The prime focus of this study is on the behavior of glucomannans and the possibility to stabilize these biopolymers. To investigate the stability of the wood polymers after steam explosion, various extraction tests were carried out. Furthermore, since the focus in this study is on the effects and chemical reactions in the fiber wall, wood meal was chosen in order to avoid mass transport phenomena between different fibers.

EXPERIMENTAL

Materials

Wood meal from Norway spruce (*Picea abies*) was used as the raw material. Preparation was made by grinding industrially cut wood chips in a Wiley mill (< 1 mm).

Initial Impregnation Experiments

In the first part of the study, different pre-treatment methods with various impregnation liquors were performed on spruce wood meal. This was done in order to obtain reference data for untreated wood meal. The samples were subjected to the alkaline and acidic impregnation chemicals sodium hydroxide (NaOH, 0.75 M, pH 13.9, 25 °C) and sulfuric acid (H_2SO_4, 0.005 M, pH 2.0, 25 °C). Some experiments were conducted in which the samples were pre-treated with a reducing agent before being subjected to the alkaline or acidic treatment. For these tests, sodium borohydride was chosen as a model substance (Wigell et al. 2007). All experiments were carried out in steel autoclaves with a volume of about 1.2 liters. Samples of wood meal were used to minimize mass transport phenomena, such as penetration and diffusion, which make it easier to study chemical reactions and the kinetics of these reactions.

The reducing pre-treatment with sodium borohydride was performed by putting the wood meal in beakers with an aqueous $NaBH_4$ solution (7 w/w% on wood) with a liquor-to-wood ratio of 7:1. The mixture was left for 4 to 5 days in order to achieve as complete a reaction as possible. The beakers were evacuated several times during the treatment by subjecting the samples to vacuum in an exsiccator so as to improve impregnation. Afterwards, the wood meal was thoroughly washed with deionized water at room temperature.

The wood meal samples were mixed with impregnation liquor in autoclaves with a high liquor-to-wood ratio (100:1) to maintain a virtually constant concentration of the impregnation chemical. The autoclaves were evacuated for 5 min and were then pressurized for 5 min with 5 bars of nitrogen gas. The pressure was then released, and the autoclaves were put in a pre-heated polyethylene glycol bath. The temperatures applied were 90, 110, and 130 °C, which corresponds to 87, 108, and 127 °C inside the

autoclaves when the equilibrium temperature was reached (see Fig. 1). The treatment times were 10, 30, 60, and 120 minutes, and as can be seen in Fig. 1, 10 minutes represents the conditions near the end ofthe heating up period. The wood meal was filtered off and analyzed for Klason lignin, acid-soluble lignin, and carbohydrate contents; the analytical methods are described in more detail below. In addition, near infrared (NIR) spectroscopy measure-ments were performed and correlated with the results from the chemical analyses with the use of multivariate data analysis.

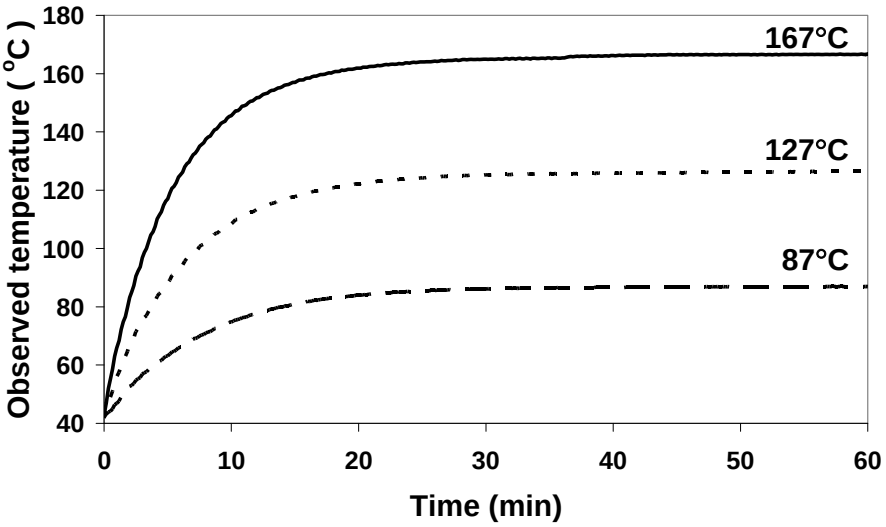

Fig. 1. Temperature profiles inside autoclaves at oil bath temperatures 90, 130, and 170 °C. The temperature inside the autoclaves was monitored using a 175-T3 logger with a Teflon thermocouple to attain correct temperature development inside the autoclaves (Ribe et al. 2010).

Mild Steam Explosion

The mild steam explosion experiments were performed in lab scale equipment comprising a modified steel autoclave. The autoclave had a volume of about 1.2 liters, and for each experiment 50 g of sample was run. The lid of the autoclave had an inlet for steam and a temperature measurement device, and the pressure was released by opening the vent to the outlet in the middle of the lid (see Fig 2). The experiments were performed in a batch steam explosion apparatus, where the biomass was exploded inside the reactor. In order to better maintain the temperature inside the autoclave, it was put in an insulated outer beaker.

Experiments on water-impregnated wood meal were performed, and the effects of different residence times during mild steam explosion were investigated. All of these experiments were executed at the same steam explosion temperature, 160 °C. Impreg-nation with water was done with a liquor-to-wood ratio that corresponded to that of fully impregnated wood chips, namely 2.3:1 (115 g water: 50 g o.d. wood). Impregnation was also performed in steel autoclaves. In order to make sure that all of the wood material was penetrated with liquor, 5 min evacuation followed by pressurizing for 5 min with 5 bars of nitrogen gas was done twice, and the system then was left overnight under pressure. Residence times of 5, 10, 15, 20, 25, 30, and 35 minutes were investigated in the steam explosion equipment. After steam explosion treatment, the material was washed with 5 liters of deionized water at room temperature.

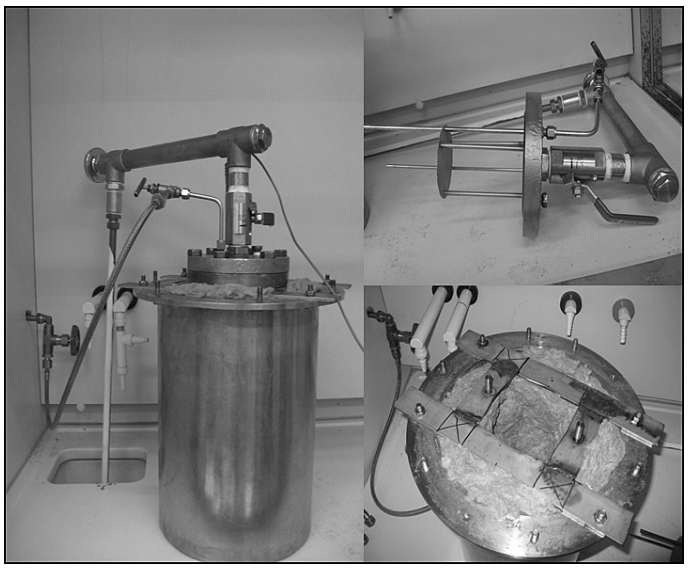

Fig. 2. Steam explosion equipment for lab-scale experiments. The lid had an inlet of steam, a temperature measurement device, and a larger vent used for release of pressure. The autoclave was put in an insulated outer beaker to more easily maintain the desired temperature.

Additionally, a series with $NaBH_4$ pre-treated wood meal was conducted. The samples were prepared in the same way as in the previous chemical pre-treatment experiments. Following the treatment they were subjected to steam explosion after maintaining a temperature of 160 °C for 5, 10, 20, and 30 min. Afterwards, the samples were analyzed for Klason lignin content and sugar content, mostly to investigate the extent of autohydrolysis.

Extractions

Aqueous solutions at different pH levels were chosen as the extraction liquors because of their simplicity and industrial relevance and were applied after mild steam explosion.

Extraction experiments with high liquor-to-wood ratio

One test series was done on wood meal samples that were steam-exploded after maintaining 160 °C for 25 minutes, and the samples were subsequently subjected to extraction in liquors of varying pH levels - pH 2.5, pH 4.5, and pH 8. In these experiments, 15 g o. d. wood meal was used during the steam explosion treatment, and 10 g (o. d.) of this material was transferred to the subsequent extraction. The steam-exploded wood meal was put in autoclaves with the extraction liquors of varying pH. The liquor-to-wood ratio was 100:1 in order to maintain a virtually constant concentration of extraction liquor. The autoclaves were then put in a pre-heated polyethylene glycol bath at a temperature of 130 °C, which corresponds to 127 °C at steady temperature conditions, which were reached after approximately 15 minutes (see Fig. 1). The total residence time was one or two hours (see the different experimental conditions in Table 1). After extraction, the samples were filtered off and displacement washed with 5 liters of deionized water at room temperature. The samples were then analyzed for

carbohydrate, Klason lignin, and acid-soluble lignin contents. Both water-impregnated and $NaBH_4$ pre-treated wood meal samples were examined.

Table 1. Different Conditions During Extraction with High Liquor-to-Wood Ratio

Experimental Condition	pH	Temperature (°C)	Time (h)
1	8	130	1
2	4.5	130	1
3	4.5	130	2
4	2.5	130	1

Extraction experiments with low liquor-to-wood ratio

A few experiments were also conducted on larger quantities of wood material and at a lower liquor-to-wood ratio (10:1) in order to obtain liquor with higher concentrations of extracted hemicelluloses. Both water-impregnated and $NaBH_4$ pre-treated samples were investigated. As in the first experiments, the samples were first treated with steam explosion at 160 °C for 25 minutes. 50 g of o. d. wood meal was steam-exploded and 40 g of o. d. meal was transferred to extraction. The three different conditions for the extraction experiments can be seen in Table 2.

Table 2. Different Conditions During Extraction with Low Liquor-to-Wood Ratio

Experimental Condition	pH	Temperature (°C)	Time (h)
1	2.5	160	1
2	11	110	1
3	11	130	1

The wood material was afterwards washed and analyzed as described above. The extraction liquors were neutralized to pH 7 and were put in a rotary evaporator (water bath at 60 °C, vacuum pump) and then freeze-dried (FreeZone, Triad™, Freeze Dry System, Labconco Corp.) to remove all water. Some of the solid freeze-dried materials were then dissolved in DMSO with the addition of 10 mM LiBr to a concentration of 5 mg/mL, and the resulting solutions were then run in a Gel Permeation Chromatograph (GPC) in order to measure molecular weights and molecular weight distributions. The solid residues were also analyzed for carbohydrate content by performing a total hydrolysis followed by HPLC measurements, similar to the method described for determining Klason lignin.

Analytical Methods

Klason lignin

Klason lignin is defined as the residual material after the samples have been hydrolyzed with 72% sulfuric acid. The method used in this paper is based on the procedure presented by Theander and Westerlund (1986).

200 mg of (o. d.) sample was weighed, and 3 mL of 72% H_2SO_4 was added for each sample. The samples were then evacuated for 15 min and put in a 30 °C water bath for one hour, during which time the samples were stirred at least three times. 84 g of distilled water was added to the samples, and they were put in an autoclave at 125 °C for one hour. Next, the solid residue was filtered off and the filtrates were put in 100 mL round flasks. 9 mL (2000 mg/g) of fucose was added to each round flask, and the samples

were diluted up to 100 mL. Fucose was used as an internal standard for the HPLC measurements (40 mg/L). In order to be able to obtain good accuracy for both major and minor monomeric sugars, a ten times weaker solution was prepared by diluting the concentrated sample. The concentrated and diluted solutions were filtered through a 0.45 μm PVDF filter prior to injection to the HPLC. The weak solution was also used for UV measurement to calculate the acid soluble lignin content.

Acid soluble lignin

The acid soluble lignin content was calculated in relation to the absorbance value measured with UV at a wavelength of 205 nm on a Specord 205, Analytikjena. The content was calculated assuming an absorptivity constant of 110 $dm^3g^{-1}cm^{-1}$ (Lin and Dence 1992).

Carbohydrate analysis

Sugar analysis was performed on a Varian Pro-Star HPLC with AutoSampler Model 410. An Electrochemical Detector, Varian Star 9080, was used for detection. Dionex columns; pre-column CarboPac™ PA1 (2x50 mm) and main column CarboPac™ PA1 (2x250 mm) and Dionex Isocratic Pump IP20 were used. The software used was Star Chromatography Workstation, System Control version 5.50 by Varian. A flow rate of 2 mL/min was applied for all samples. The amounts of sugars analyzed were corrected for acid hydrolysis yield (Janson 1974). The acid hydrolysis yield varies for different sugars and the values used were taken from experimental results presented by Wigell (2007).

The amounts of cellulose, galactoglucomannan, and xylan were calculated from the carbohydrate analysis by using the assumptions and corrections described in the Appendix. The standard deviations of the analyses were estimated to be 0.88% for Klason lignin, 0.25% for xylan, and 0.22% for glucomannan, based on four different measurements of untreated wood meal.

Molecular weight determination

Molecular weight was measured on a PL-GPC 50 Plus, Integrated GPC System, by Polymer Laboratories (A Varian Inc. Company) with both RI and UV detection. The system was equipped with two PolarGel-M (300 x 7.5 mm) columns and a PolarGel-M Guard column (50 x 7.5 mm). The mobile phase was DMSO with the addition of 10 mMLiBr and the samples were injected using a PL-AS RT GPC Autosampler. The samples were evaluated with the software Cirrus GPC version 3.2. Ten different molecular weights of pullulan (708, 375, 200, 107, 47.1, 21.1, 11.1, 5.9, 0.667, and 0.180 kDa) were used for calibration.

Near infrared spectroscopy

The NIR experiments were performed on Foss NIR Systems 6500 with a Rapid-Content™ Analyzer in the wavelength range 400 to 2500 nm, i.e. both in the visible and near infrared range, detected by silica and PbS detectors. There were 32 scans per sample and the software used was Vision 2.51. The results were analyzed using multivariate analysis in the software Simca-P+ version 12.0.1.0 by Umetrics.

RESULTS AND DISCUSSION

Initial Impregnation Experiments

Water-impregnated samples and wood meal pre-treated with $NaBH_4$ were subjected to alkaline treatment (0.75 M NaOH) at 90 °C, 110 °C, and 130 °C for different duration times. Considerable differences were found only for the glucomannans. The degradation of glucomannan decreased significantly for the samples pre-treated with $NaBH_4$, which is shown in Fig. 3, where the glucomannan content (% on wood) is compared with the pure alkaline samples. For samples treated at, for example, 110 °C for 60 min, the remaining amount of glucomannan was 7.4% (on wood) for the water-impregnated sample and 15.9% for the $NaBH_4$ pre-treated sample.

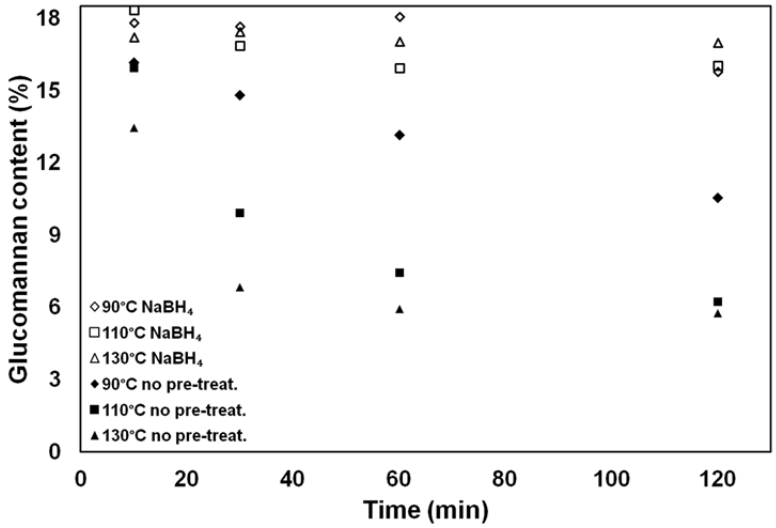

Fig. 3. Glucomannan content (% on wood) after alkaline treatment. Untreated samples are compared with $NaBH_4$ pre-treated samples, the differences between the different temperatures and residence times are also shown.

Sodium borohydride treatment of softwood will probably also lead to other effects, since its reducing abilities will most likely influence other parts of the chemical structures of hemicelluloses and lignin. These effects were, however, not studied here, since the main objective was to stabilize the (galacto)glucomannans. The results shown in Fig. 3 were the basis for using pre-treatment with $NaBH_4$ when the mild steam explosion experiments were continued. The results from the alkaline experiments also showed that the Klason lignin content decreased somewhat for the samples treated at higher temperatures and for longer times (130 °C and 120 min). The decrease was, however, moderate (lignin content ranged from 28.0 to 21.9% on wood). The cellulose content for these tests was relatively constant. This is in agreement with what can be found in the literature (Wigell et al. 2007). The samples that were pre-treated with sodium borohydride and then with alkali had values for Klason lignin and cellulose content that were similar to the samples exposed to alkaline extraction without pre-treatment.

Samples were also treated at acidic pH, and the treatment resulted in nearly stable values for the cellulose and Klason lignin content. They ranged between 26.2 and 27.8%

(on wood) for Klason lignin and 37.4 and 39.2% (on wood) for cellulose. The hemicelluloses were degraded to a greater extent, and the decrease was greater with increased temperature and time. This was obvious for both xylan and glucomannan, and even more so for arabinose, where the concentration after acidic treatment at harsh conditions was virtually zero. The glucomannan content (% on wood) is shown in Fig. 4 for the different temperatures and retention times after acidic treatment, and it can be seen that the degradation was more severe for the samples treated at 130 °C than at other temperatures. The overall yield was, however, generally higher and the degradation of glucomannan was not as severe as during the corresponding conditions for the alkaline treatments.

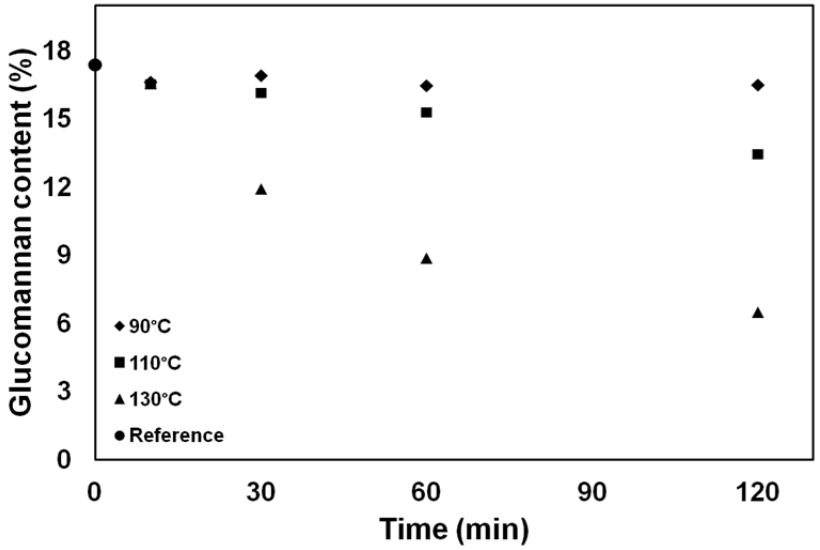

Fig. 4. Glucomannan content (% on wood) after acidic treatment for the three different temperatures and after different residence times. The samples were only water-impregnated before the acidic treatment.

Mild Steam Explosion

During mild steam explosion, acidic groups from the wood are released and acetic acid is formed. The slightly acidic environment leads to acidic hydrolysis of the hemicelluloses, so-called autohydrolysis. It should, therefore, be kept in mind that there are both mechanical as well as chemical effects when steam explosion is used on wood. The full potential of the mechanical treatment is obtained as soon as the wood is heated up to the target temperature, which takes between 5 and 10 min, calculated using an approximation of Fourier's Law of heat conduction, before the temperature is reached inside the wood chips. At this point, the minimum influence of the acid hydrolysis is reached, and consequently, after this point, acid hydrolysis had an additional influence on the wood. However, the major wood components were almost unaffected by the steam explosion treatment at 160 °C, which can be seen in Fig. 5. The exception was glucomannans, which were degraded to some extent, especially after longer duration times. After the experiments it was possible to recover 76 to 88% of the solid material. The remaining solids were lost during dismantling of the equipment. The chemical analyses of the recovered material had detection levels between 90 and 95%.

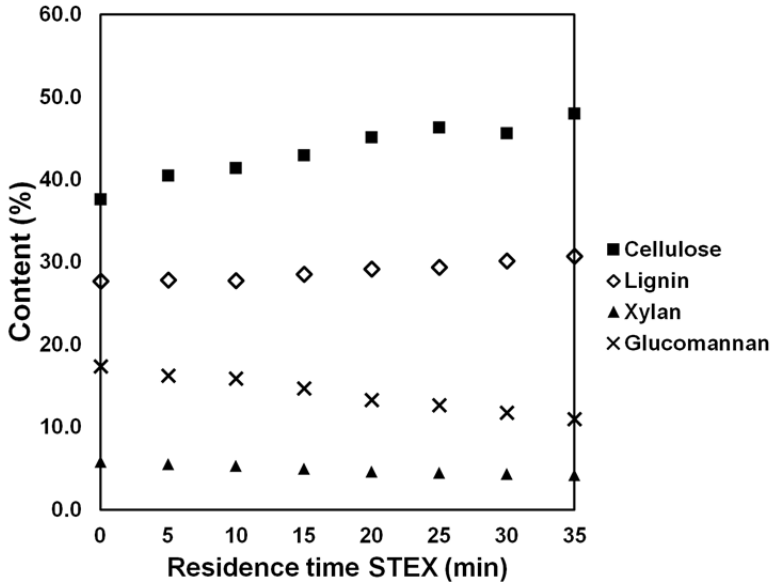

Fig. 5. The content of the major wood components (% in wood after STEX) after mild steam explosion at 160 °C for different residence times

Figure 6 shows the results for the degradation of the hemicelluloses from mild steam explosion experiments. The degradation was most apparent for mannose and arabinose. The degradation of arabinose was most likely due to acidic hydrolysis and the fact that arabionse side-chains of the arabinoglucuronoxylans are cleaved off. The backbone of xylans seemed to be quite resistant to the conditions during steam explosion.

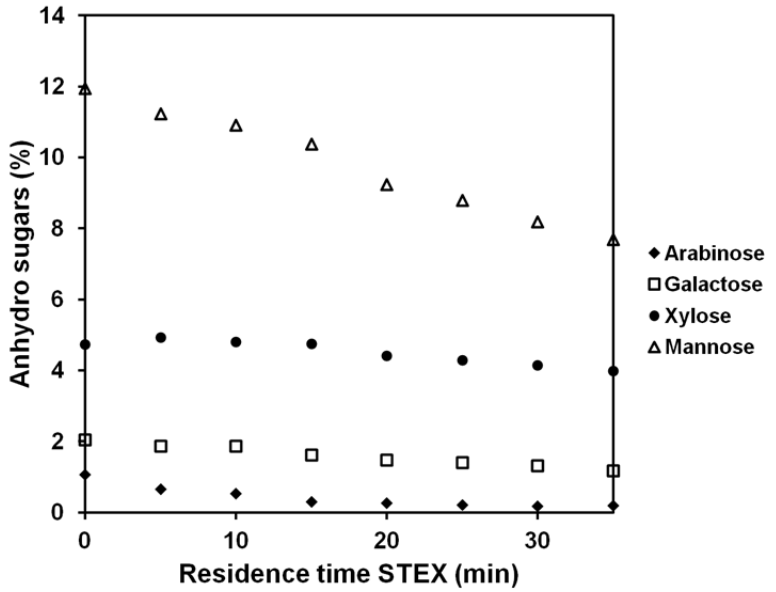

Fig. 6. Degradation of hemicelluloses during mild steam explosion (160 °C) of wood meal at different residence times, the amount of anhydro sugars in the samples after mild steam explosion are shown (in % on sample) for the different duration times

It is also clear that glucomannans were more degraded when the residence time was increased. The amount of acetyl groups in the wood residue have not been measured in this study, but it is reasonable to assume that the degree of deacetylation was quite high. The degradation of hemicelluloses begins almost immediately during the mild steam explosion treatment, and since damage to the hemicelluloses and fibers is undesirable, the conditions need to be as mild as possible. On the other hand, chemicals and enzymes require sufficient access to the wood structure, and therefore, conditions should be optimized.

Figure 7 is a comparison between the glucomannan content in the wood meal samples pre-treated with $NaBH_4$ and the glucomannan content in the corresponding water-impregnated samples. It can clearly be seen that the pre-treatment method with sodium borohydride, resulting in stabilization of the end-groups of glucomannan, was quite effective, even against autohydrolysis. After the initial decrease, the level of maintained glucomannan remained almost constant even for longer residence times. For this reason, the duration time for steam explosion was chosen to be 25 min in the subsequent extraction experiments. The stabilizing effect of the $NaBH_4$ pre-treatment is interesting, as it might be an effect of both the change in the reducing end-group of the glucomannans, an effect of pH stabilization (the pH is around 9 during the pre-treatment), or a combination of both. Some deacetylation due to the slightly alkaline conditions during the $NaBH_4$ pre-treatment may also produce a more linear glucomannan, which have a higher affinity to the cellulose, and therefore are retained in the wood material (Annergren et al. 1961). It can also be seen that there is a slight decrease in glucomannan content after as soon as 5 min, and the degradation becomes more severe for longer residence times. The degradation of hemicellulose is not in the same range as chemical treatments at conditions corresponding to chemical pulping (Wigell et al. 2007).

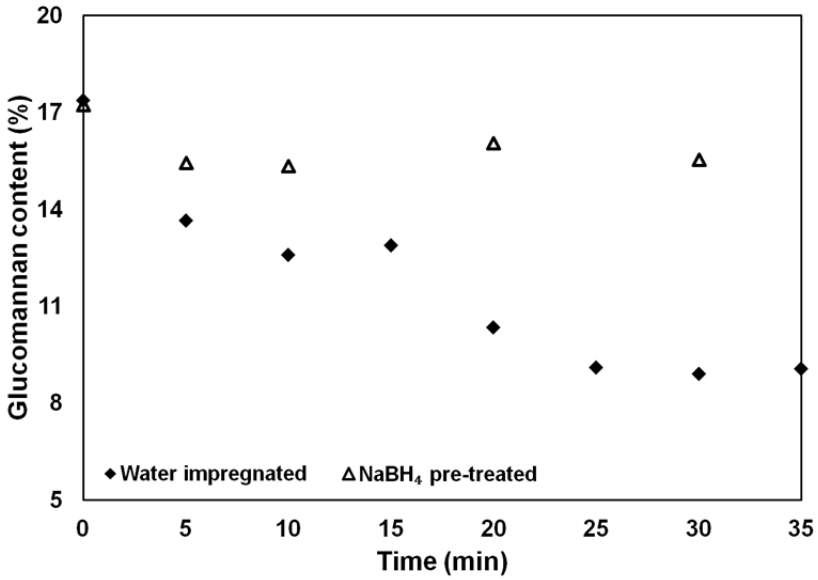

Fig. 7. The glucomannan content (% on wood) after mild steam explosion (160 °C) for different residence times; water-impregnated samples are compared with $NaBH_4$ pre-treated samples. Note that the y-axis starts at 5%.

Extractions

Extraction experiments after steam explosion at 160 °C for 25 min were performed on wood meal in order to investigate the stability of the wood polymers after mild steam explosion, and also to evaluate methods for isolating glucomannans from the wood. Some experiments were conducted with a high liquor-to-wood ratio (100:1) to maintain the same chemical concentration throughout the extraction. Liquors at different pH levels (pH 2.5, pH 4.5, and pH 8) were used as extraction liquors. Both water-impregnated samples and $NaBH_4$ pre-treated samples were steam exploded and then subjected to subsequent extraction. The results of the analysis of solid residues after extraction are shown in Fig. 8. The different sets of bars represent the different experimental conditions (see Table 1).

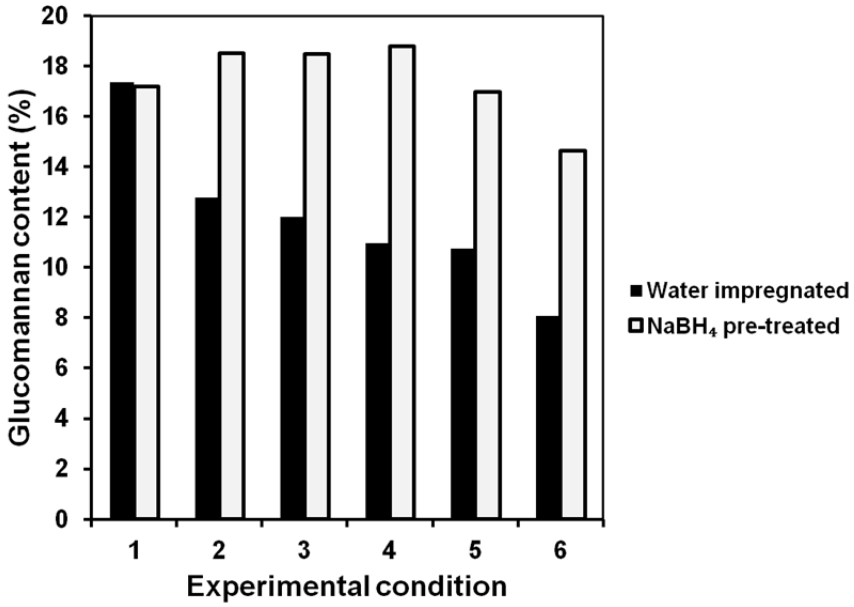

Fig. 8. Glucomannan content (% on wood) in the solid residues for 1. Original raw material, 2. Mild steam explosion only, 3. After STEX + extraction at pH 8, one hour, 4. After STEX + extraction at pH 4.5, one hour, 5. After STEX + extraction at pH 4.5, two hours, and 6. After STEX + extraction at pH 2.5, one hour.

The effect of pre-treatment with $NaBH_4$ can also be seen in this series of experiments, because the amount of remaining glucomannan in the pre-treated samples was almost the same as in the original raw material. Measurements of lignin and sugar content after steam explosion (but before extractions) showed that for the water-impregnated samples, the amount of glucomannan was in the range of 12.7 to 12.9%, and for the samples pre-treated with $NaBH_4$ it was in the range of 18.4 to 18.7% (as percentages of wood). This is in accordance with the results from the first wood meal experiments, and the narrow intervals indicate that the samples had the same composition before being subjected to extraction, and thus the pre-treatment had good reproducibility. The other sugars as well as the lignin content were more similar between the two batches and were almost constant after extraction.

Acidic extraction had a greater effect than pH closer to neutral conditions, and it is likely that a method for the extraction of glucomannan requires quite harsh conditions or prolonged extraction times.

Extractions with low liquor-to-wood ratio

In order to obtain liquors with higher concentrations of extracted carbohydrates, a few experiments were performed with more wood meal and a lower liquor-to-wood ratio. The different experimental conditions during these extractions can be found in Table 2. The remaining wood was analyzed in the same manner as in the previous extraction experiments. The results from the extractions performed at pH 11 showed, as before, that the $NaBH_4$ pre-treated samples resulted in larger amounts of remaining glucomannan than the water-impregnated samples. There was, however, no significant difference between the two temperatures (110 °C and 130°C); neither for the water-impregnated samples (glucomannan content of 12.3% and 12.4% on wood) nor for the $NaBH_4$ pre-treated samples (glucomannan content of 18.9% and 19.4% on wood).

The materials in the extraction liquors were analyzed in GPC after evaporation at 60 °C, freeze-drying, and dissolution in DMSO/LiBr. The results showed that for the sodium borohydride pre-treated sample extracted at pH 2.5, the extracted material was only in the range of monomers (M_w 160 to 600 Da, polydispersity between 1.02 and 2.37 for the three peaks detected), which indicates that these extraction conditions were too severe to extract hemicelluloses of polymeric molecular weight (see Fig 9).

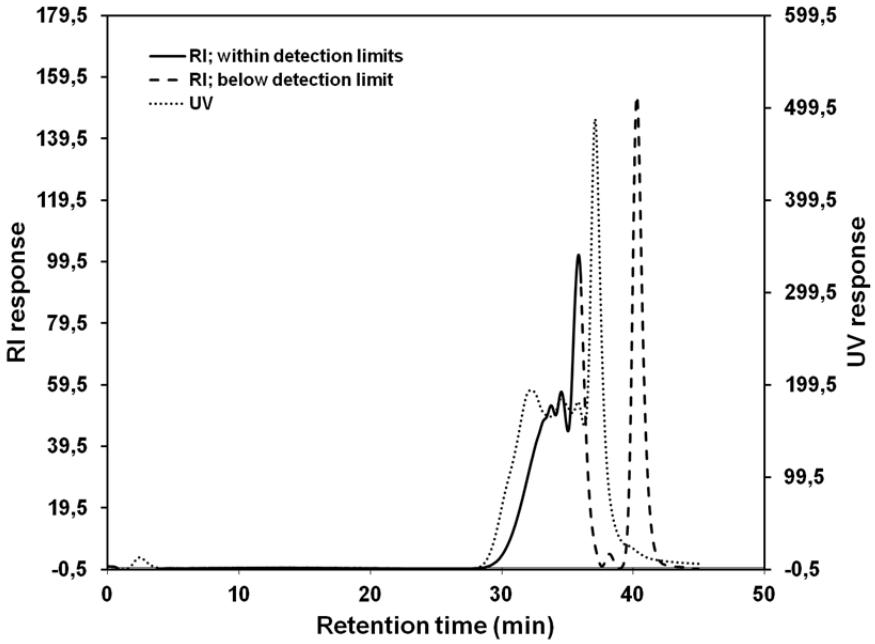

Fig. 9. Chromatogram for the extraction liquor from the water-impregnated sample extracted at pH 2.5 and 160 °C, where both RI and UV responses are displayed

For the samples treated at pH 11 (both at 110 °C and 130 °C), the molecular weight ranged between 3300 and 5700 Da, which corresponds to a chain length of about 20 to 30 sugar units. The molecular weights and polydispersities are presented in Table 3. Figure 10 shows the GPC results from the extraction liquor from the water-impregnated sample extracted at pH 11 and 110 °C. All the figures have been baseline corrected.

Table 3. The Molecular Weight Averages and Polydispersities for the Samples Extracted at pH 11, 1 Hour

Extraction Condition	Mw (Da)	Mn (Da)	PD
pH 11, 110 °C, water	5688	908	6.26
pH 11, 110 °C, $NaBH_4$	3310	577	5.74
pH 11, 130 °C, $NaBH_4$	5426	1909	2.84

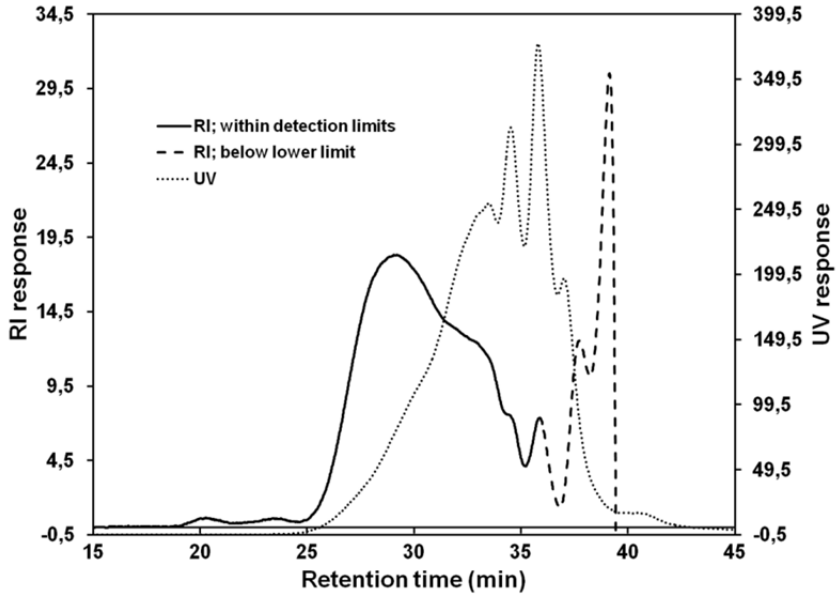

Fig. 10. Chromatogram for the extraction liquor from the water-impregnated sample extracted at pH 11 and 110 °C; both RI and UV responses are displayed.

Figures 11 and 12 show the results from the $NaBH_4$ pre-treated samples extracted at pH 11 at 110 °C and 130 °C. The distribution of molecular weight was very broad for the analyzed samples and it is therefore reasonable to assume that there are several different molecules of various sizes in these samples. It is, however, difficult to distinguish whether the larger molecules originate from lignin or from polysaccharides.

The extracted materials from the liquors were also analyzed for carbohydrate and Klason lignin content. There were uncertainties, because only a degree of the total sample content could be explained by the analyses, and the detection levels also differed between the samples (45-73%). The results for the samples extracted at pH 11 are compared in Tables 4 through 6.

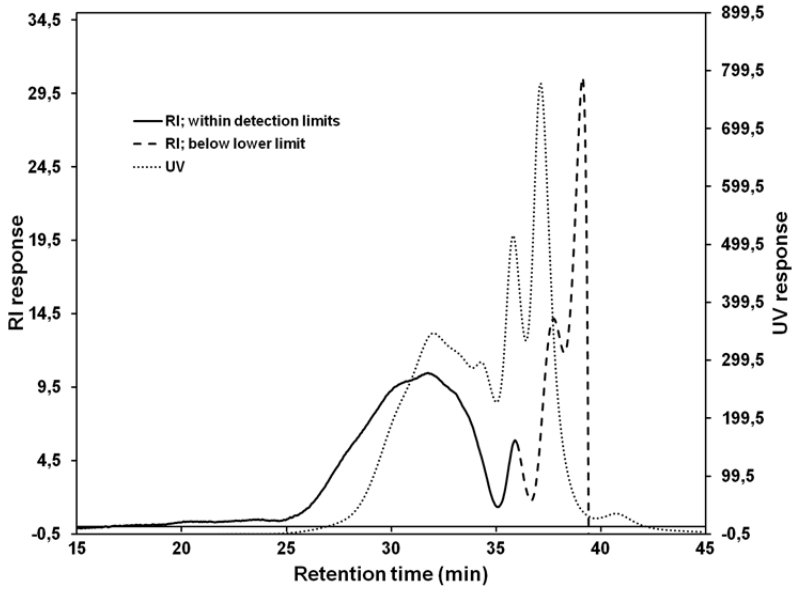

Fig. 11. Chromatogram for the extraction liquor from the $NaBH_4$ pre-treated sample extracted at pH 11 and 110 °C; both RI and UV responses are displayed.

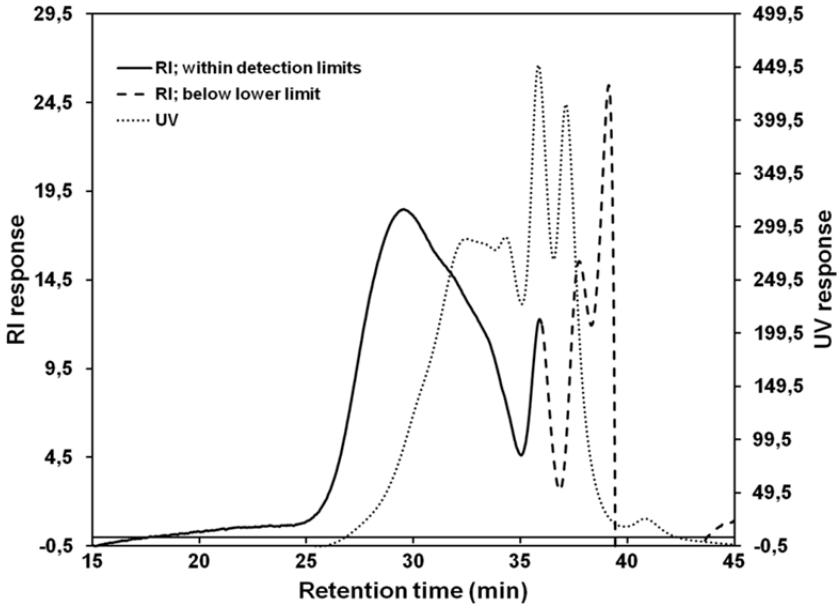

Fig. 12. Chromatograms for the extraction liquor from the $NaBH_4$ pre-treated sample extracted at pH 11 and 130 °C; both RI and UV responses are displayed.

Table 4. Comparison of the Amount Extracted Components for Water-Impregnated Samples Extracted at pH 11, 110 °C

Water-Impregnated Samples	Glucomannan (% on Sample)	Xylan (% on Sample)	KlasonLignin (% on Sample)
Untreated Wood Meal	17.4	5.8	27.9
Wood Meal After STEX	12.8	4.6	29.9
Wood Meal After Extraction	12.6	4.4	29.7
Extraction Liquor	20.8	6.2	9.4

Table 5. Comparison of the Amount Extracted Components for $NaBH_4$ Pre-Treated Samples Extracted at pH 11, 110 °C

$NaBH_4$Pre-TreatedSamples	Glucomannan (% on Sample)	Xylan (% on Sample)	KlasonLignin (% on Sample)
Only Pre-Treatment	17.2	5.8	27.5
Wood Meal After STEX	19.2	5.7	27.2
Wood Meal After Extraction	18.9	5.4	26.7
Extraction Liquor	4.7	5.7	12.7

Table 6. Comparison of the Amount Extracted Components for $NaBH_4$ Pre-Treated Samples Extracted at pH 11, 130 °C

$NaBH_4$Pre-TreatedSamples	Glucomannan (% on Sample)	Xylan (% on Sample)	KlasonLignin (% on Sample)
Only Pre-Treatment	17.2	5.8	27.5
Wood Meal After STEX	19.9	5.7	28.2
Wood Meal After Extraction	19.4	5.2	28.2
Extraction Liquor	7.5	15.0	9.0

The results indicated that for the water-impregnated sample, the amount of (galacto)glucomannans was significantly higher than for the sodium borohydride pre-treated samples, which is reasonable, since a larger amount was shown to remain in the solid residue for these samples. It is also interesting that the amount of xylan in the extracted material from the 130°C experiment seemed to be very large and that the molecular weight in this case was also higher than in the 110 °C experiment. A higher temperature is perhaps required in order to dissolve larger fragments of xylan.

Evaluation of NIR Method and Results

NIR spectroscopy is a simple, fast method for a broad range of wavelengths. The differences between the spectra of the samples are small but become enlarged when baseline correction methods are applied to the spectra, e.g. derivatives or other scaling filters. These differences can give relevant information with respect to the chemical variations of the samples. All the wood meal samples from the initial chemical pre-treatment studies were measured both the visible and near infrared range, and the results were transferred to the software Simca-P; the spectra of the different samples can be found in the Appendix. Both PCA (Principal Component Analysis) and PLS (Partial Least Squares) models were applied (Naes et al., 2002). Only the spectra were considered in the PCA models, whereas the spectra in the PLS models were compared with the reference data from the Klason and sugar analyses. The second derivative was used as a spectral filter with a quadratic polynomial order with 13 points in each sub-model (Savitzky and Golay 1964). Since the instrument switches detector in the shift between the wavelengths in the visible electromagnetic spectrum and the near infrared region, i.e. wavelengths between 1090 and 1110 nm, these signals were also removed.

The PCA models showed that the spectra from samples treated with the same liquors (alkaline, acidic, and $NaBH_4$ pre-treated samples) could be divided into three distinct groups, i.e. the different treatments led to diverse chemical reactions that could be distinguished (see Fig. 13). The samples did not group at all according to the

temperatures and residence times, which could be because the samples contained the same chemical components even though the concentrations differed.

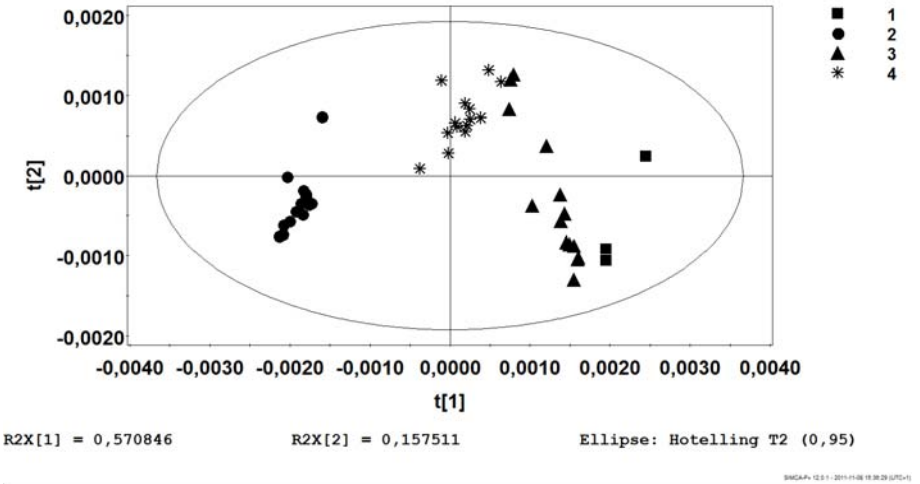

Fig. 13. The alkaline, acidic, and sodium borohydride pre-treated samples divided into three distinct groups in the PCAmodel. They are represented in the figure by different markers; 1. Reference, 2. Alkaline treatment, 3. Acidic treatment, and 4. $NaBH_4$ pre-treated samples.

The observed values in the PLS models are compared with the predictions; this can be used to make a calibration curve for future samples by simply measuring a spectrum. These models varied significantly for the different wood components; some showed many and significant correlations, while others could not be used for making reliable conclusions, although this could perhaps be due to the small ranges in concentration for these components in the samples. The glucomannan content, for example, which differed a lot among the samples, showed good agreement between observed and predicted values (see Fig. 14), while the model for cellulose content was not significant.

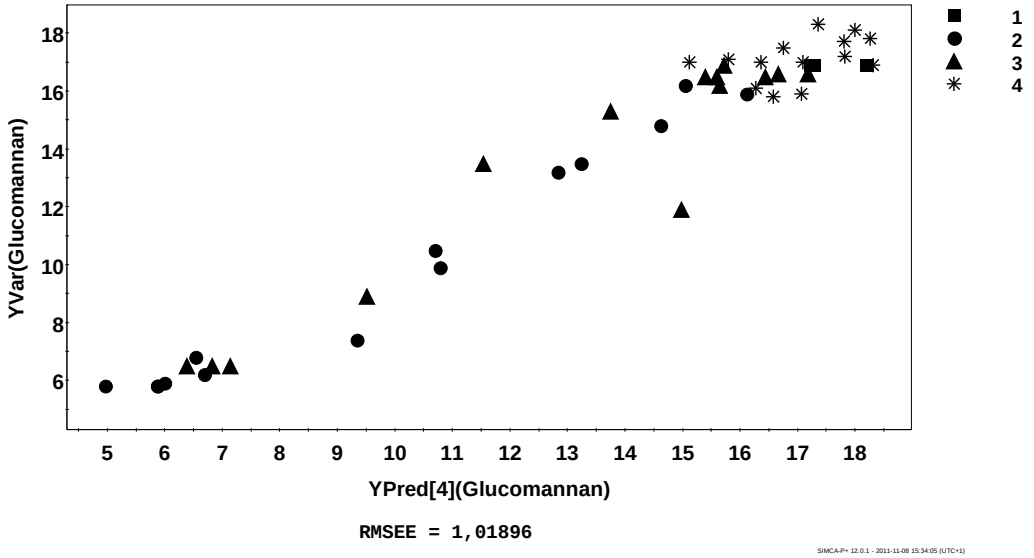

Fig. 14. Observed vs. predicted values for the glucomannan content in a PLS model based on four significant components. The different treatments are represented in the figure according to: 1. Reference, 2. Alkaline treatment, 3. Acidic treatment, and 4. $NaBH_4$ pre-treated samples.

CONCLUSIONS

1. Mild steam explosion has both a mechanical and a chemical effect on wood structure, which influences reactivity during subsequent leaching. Mild steam explosion could possibly be used as a pre-treatment method before chemical and enzymatic treatments in order to separate wood components.
2. Glucomannan is stabilized both during mild steam explosion as well as during chemical treatments by using $NaBH_4$ in a pre-treatment step, especially at subsequent alkaline conditions.
3. Harsh conditions during steam explosion or extraction lead to a high degree of hemicellulose degradation. It is therefore important to aim for as mild methods as possible and/or to stabilize the hemicelluloses prior to extraction. In order to obtain higher yields of extracted material, an optimization of the extraction conditions must be considered.
4. NIR spectroscopy could be applied to a well-characterized material to see the chemical effects of different treatments by performing simple measurements. It is important that the chemical composition of the samples differ sufficiently in order to obtain significant PLS models.

ACKNOWLEDGMENTS

The project is part of the “From wood chips to material components” theme at the Wallenberg Wood Science Center (WWSC), and the separated components from this project will later be used in other research projects at the WWSC with the overall goal of developing new materials from trees. The Knut and Alice Wallenberg Foundation is gratefully acknowledged for its financial support.

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APPENDIX

Carbohydrate Analysis

The contents of cellulose, galactoglucomannan, and xylan were calculated after carbohydrate analysis by using the following assumptions/corrections:

The amounts of sugars analyzed were corrected for the acid hydrolysis yield. Anhydro sugars were calculated from sugar monomers by the withdrawal of water (multiplication by 0.88 in the case of pentosans and by 0.90 in the case of hexosans). Glucomannan was calculated as the sum of galactan, mannan, and part of the glucan. The molar ratio between the mannose and the glucose in galactoglucomannan was assumed to be 3.5:1 (Meier 1958b). All galactan measured is included in galactoglucomannan. Acetyl groups are, however, not included. Xylan was calculated as the sum of xylan and arabinan. All arabinan measured is included in the xylan. Cellulose was calculated as the content of glucan after withdrawal for the contribution of glucan to galactoglucomannan.

$$\text{Cellulose} = \text{Glucose} - (1/3.5) * \text{Mannose}$$

$$\text{Galactoglucomannan} = \text{Galactose} + (1 + (1/3.5)) * \text{Mannose}$$

$$\text{Xylan} = \text{Xylose} + \text{Arabinose}$$

The analyses were summed up into a mass balance with the assumption that the carbohydrates were divided into cellulose, galactoglucomannan, and xylan, which were calculated as described above.

NIR Spectra

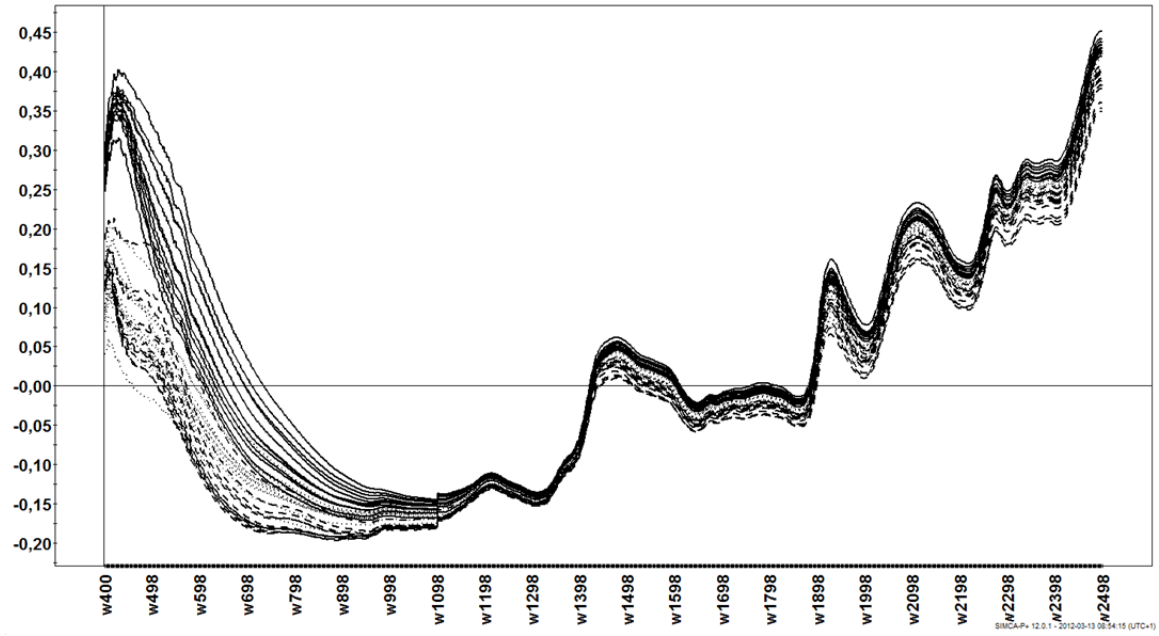

Fig. 15. The figure shows the NIR spectra, with wavelengths on the x-axis. The solid black lines represent the samples treated at alkaline conditions, long dashed lines represent the samples treated at acidic conditions and the dotted lines are the spectra from the samples that were pre-treated with sodium borohydride.

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