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Characterization of galactoglucomannan extracted from spruce (*Picea abies*) by heat-fractionation at different conditions

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Abstract

Water-soluble hemicelluloses were extracted from spruce chips by microwave heat-fractionation. The galactoglucomannan (GGM) extraction was evaluated on the basis of weight-average molecular weight (M_W), yield and carbohydrate composition of the GGM. The M_W was determined by size-exclusion chromatography with column calibration using off-line MALDI-MS analysis, and determination of mannan content in the fractions collected. Water impregnated spruce chips were heat-fractionated at three different temperatures (180, 190, and 200 °C). The spruce chips were also impregnated in NaOH solutions of different concentrations, and then heat-fractionated at 190 °C for 5 min. The highest mannan yield (78% based on the amount in the raw material) was obtained from water impregnated spruce chips heat-fractionated at 190 °C for 5 min (M_W of 3800). The highest M_W (14,000) was obtained from impregnation with 2% NaOH (190 °C, 5 min), but the yield of mannan was very low (3%). Impregnation with 0.025% NaOH and heat-fractionation at 190 °C for 5 min resulted in extraction of GGM with M_W of 9500 and a mannan yield of 31%. When the spruce chips were impregnated with ≤0.05% NaOH an *O*-acetyl-galactoglucomannan was extracted, whereas when higher NaOH charges were used in the impregnation, the extracted GGM lacked acetyl groups.

Keywords: Spruce (*Picea abies*); Hemicellulose; *O*-acetyl-galactoglucomannan; Microwave oven; Size-exclusion chromatography; MALDI-MS

1. Introduction

Hemicelluloses are complex polysaccharides found in plant cell walls. They are closely associated with cellulose and lignin in woody tissues. In softwoods (e.g. spruce and pine), the dominating hemicelluloses are *O*-acetyl-galactoglucomannan (~20 w/w% of the dry wood) and arabino-4-*O*-methyl-glucuronoxylan (5–10 w/w% of the dry wood) (Sjöström, 1993; Timell, 1967). Softwood *O*-acetyl-galactoglucomannan has been reported to have an approximate degree of polymerization, DP, between 100 and 150 (Timell, 1967), equivalent to a molecular weight around 16,000–24,000. It has a backbone of β-(1 → 4)-D-Man*p* and β-(1 → 4)-D-Glc*p* residues with α-(1 → 6)-D-Gal*p* and *O*-acetyl side-groups (Lindberg, Rosell, & Svensson, 1973; Timell, 1967). It is found primarily in the secondary cell wall layer of softwood fibers (Meier, 1985). Other types of heteromannans, such as galactomannan, are present in the endosperm of many plants as storage polysaccharides (Meier & Reid, 1982). Some of the galactomannans from leguminous plants are commercially important as thickeners in the food industry (Rol, 1973).

In recent years, the interest in hemicellulose as a renewable resource has grown. Xylans have been shown to be a potential raw material for different applications, e.g. for production of cationic biopolymers (Ebringerová, Hromádková, Kacuráková, & Antal, 1994), hydrogels (Gabrielli, Gatenholm, Glasser, Jain, & Kenne, 2000) and long-chain alkyl ester derivatives (Sun, Fang, Tomkinson, Geng, & Liu, 2001). Galactoglucomannan (GGM) is much less studied in this respect. Analytical isolation of GGM from several different species has been reported, i.e. from Kiwi fruit (Schroder et al., 2001), from the lichens *Cladonia substellata* and *Cladonia ibitipocae* (Woranovicz, Pinto, Gorin, & Iacomini, 1999), from *Populus monilifera* H (Kubacková, Karácsonyi, & Bilisics, 1992). One study (Capek et al., 2000) has been reported where GGM from *Picea abies* was isolated with alkali extraction. However, this GGM lacked acetyl side-groups, since the acetyl groups are sensitive to cleavage under alkaline conditions (Lai, 1991). In our previous study (Lundqvist et al., 2002), a microwave oven was used to heat-fractionate the hemi-

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Table 1
Conditions for the heat-fractionation in microwave oven

Impregnation medium	Concentration (%)[a]	Temperature (°C)	Time (min)	Designation of filtrate obtained
Water		180	5	–
		190	5	F-0
		200	2	–
NaOH	0.010	190	5	F-0.010
	0.020	190	5	F-0.020
	0.025	190	5	F-0.025
	0.05	190	5	F-0.05
	0.1	190	5	F-0.1
	0.5	190	5	F-0.5
	1	190	5	F-1
	2	190	5	F-2

[a] % w/v liquid.

celluloses in spruce chips. Prior to the heat-fractionation, the spruce chips were impregnated in different media. The yield of mannan at different conditions (variation in impregnation media, temperature and residence time) was investigated. Further size-fractionation was made with size-exclusion chromatography (SEC) in order to isolate *O*-acetyl-galactoglucomannan. The carbohydrate content and the carbohydrate structure of two fractions, obtained by SEC of filtrate from water impregnated spruce chips heat-fractionated at 200 °C for 2 min, was investigated in detail with NMR. The yield of mannan (poly- and oligosaccharides) in the filtrate after the heat-fractionation was 35% and the two fractions analyzed had a DP of ~20 and 11 (weight-average molecular weight (M_W) ~2500 and ~1600), respectively.

The formation of a GGM-based hydrogel has been reported (Söderqvist Lindblad, Ranucci, & Albertsson, 2001) using GGM from water impregnated spruce chips heat-fractionated according to the conditions presented in our previous report (Lundqvist et al., 2002). In the current work, heat-fractionation of spruce for the extraction of GGM was studied in more detail. The goal was to find conditions where a high yield and high M_W of the polymer is obtained, because such polymers would be more useful as a raw material. However, the presence and content of side-groups (acetyl and galactosyl) also influence the properties of polysaccharides and potentially their usefulness as a raw material. Therefore in the evaluation of the heat-fractionation conditions, we determined not only the yield and the M_W, but also the molar composition of the GGM. Thus the main focus of the current work has been on the effect of heat-fractionation, temperature and pH on the yield, size and composition of the extracted GGM.

2. Material and methods

2.1. Heat-fractionation

The substrate was spruce (*P. abies*) obtained as chips from the Harry Nilsson sawmill (Hästveda, Sweden). The spruce chips were milled and then impregnated (soaked) with water and NaOH. After impregnation, 9.1 g (dry weight) of the wood material in 100 ml water was heat treated at a predetermined temperature and residence time in a microwave oven (MLS-1200 Mega Microwave workstation from Milestone) (Junel, 1999; Lundqvist et al., 2002). After heat treatment the insoluble material (mainly cellulose and lignin) was removed by filtration (Acrodisc® Syringer Filter, 0.2 μm, PALL Gelman Laboratory, Ann Arbor, MI, USA) to yield a filtrate. The water-soluble oligomers and polymers in the filtrate were further size-fractionated by SEC essentially as described elsewhere (Lundqvist et al., 2002) and in Section 2.2.1. The conditions for impregnation and heat-fractionation (%NaOH, temperature and residence time) are displayed in Table 1.

2.2. Size-exclusion chromatography

2.2.1. Molecular weight distribution

After filtration of the solution obtained from the heat-fractionation the filtrate was loaded on a SEC system (FPLC, Pharmacia Biotech, Uppsala, Sweden) with refractive index (RI)—(Erma-Inc., Tokyo, Japan) and ultra violet (UV)—(Pharmacia Biotech, 280 nm) detectors. The preparative and analytical separations were performed using gel filtration media from Pharmacia Biotech (Uppsala, Sweden). In the SEC system, three columns were connected in series, (i) a precolumn Superdex 75 (HR 5/5), (ii) a Superdex 75 (HR 10/30), and (iii) a Superdex 200 (HR 10/30) column, (Pharmacia Biotech, Uppsala, Sweden) (Lundqvist et al., 2002). The molecular weight distribution was determined using MALDI-TOF-MS analysis of the fractions collected with SEC. Samples of 500 μl were loaded on the columns and water was used as mobile phase at a flow rate of 0.5 ml/min at room temperature. Acetone was used to determine the total volume of the columns (47 ml).

2.2.2. Collection of fractions for MALDI-TOF-MS analysis and acid hydrolysis

Fractions obtained by SEC of the filtrate from spruce chips water impregnated and heat-fractionated at 200 °C for 2 min and at 180 °C for 5 min (fractions 1–10) were collected for further investigation. The filtrate (25 mg dry weight material in 500 μl water) was loaded on the SEC and the same fractions from 10 different separations were pooled and lyophilized.

2.3. Analytical procedures

2.3.1. Total carbohydrate and lignin content analysis of the raw material

The carbohydrate monomer composition of the spruce chips was analyzed as described previously (Lundqvist et al., 2002). Acid hydrolysis (Hägglund, 1951) was used and the monomers were then analyzed by high performance liquid chromatography (HPLC) (Shimadzu, Kyoto, Japan) with an Aminex HPX-87P column (BIO-RAD, Hercules, CA, USA) at 80 °C with RI-detection (Erma-Inc.) giving monomeric sugar unit composition. The elution was performed using water. The flow rate was 0.4 ml/min and injection volume 20 μl. Lignin determination was performed according to Hägglund (1951).

2.3.2. Analysis of the sugar components in the filtrates

The filtrate after heat-fractionation (1 ml) was diluted in 0.5 M H_2SO_4 up to 2 ml and then hydrolyzed in an autoclave for 4 h at 120 °C (Lai, 1991; Wright & Power, 1987). For the determination of monosaccharides and sugar monomer components of oligo- and polysaccharides (i.e. monomeric sugars after acid hydrolysis) of filtrates and the fractions from SEC, a high performance anionic exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (DIONEX, Sunnyvale, CA, USA) was used. The HPAEC-PAD consisted of an AS50 autosampler, a GP40 gradient pump and Carbo Pac Pa10 guard and analytical column and an ED40 electrochemical detector. The flow rate using water elution was 1 ml/min and the injection volume 10 μl. A post-column pump (DIONEX, Sunnyvale, CA, USA) with 200 mM NaOH as eluent was used to enable detection of the sugars. D-mannose, D-xylose, D-glucose, D-galactose, and L-arabinose (Fluka Chemie AG, Buchs, Switzerland) were used as standards for the analysis.

The amount of extracted GGM in the filtrates after heat-fractionation was estimated by analyzing the mannose content in the filtrates before and after acid hydrolysis. The total amount (i.e. mass) of mannose obtained in the filtrates after hydrolysis of oligo- and polysaccharides is denoted m_H, the mannose amount in the original filtrate is denoted m_F and the total mannose amount obtained from raw material analysis (Section 2.3.1) is denoted m_T. The yield of mannan (oligo- and polysaccharides containing mannose), expressed in % of the total amount in the raw material (Section 2.3.1) was calculated as $(m_H - m_F)/m_T$. (Junel, 1999). The same procedure was used for the determination of the xylan yield (oligo- and polysaccharides).

2.3.3. Determination of acetyl content

The filtrate after heat-fractionation was dissolved in 1% NaOH solution to remove the *O*-acetyl moieties of the acetylated GGM. It was treated for 12 h at room temperature. The acetic acid was analyzed using HPLC (Pharmacia, Uppsala Sweden) with Aminex HPX-87H column (BIO-RAD, Hercules, CA, USA) at 65 °C with a RI-detector (Erma-Inc.) (Kaar, Cool, Merriman, & Brink, 1991). The elution was performed using 0.005 M H_2SO_4. The flow rate was 0.6 ml/min and the injection volume 100 μl. Acetic acid (Merck, Darmstadt, Germany) was used as standard. The D-galactosyl side-groups were determined using acid hydrolysis followed by HPLC analysis (Section 2.3.2).

2.3.4. MALDI-MS

The MALDI analyses were performed according to Jacobs, Lundqvist, Stålbrand, Tjerneld, and Dahlman (2001), using a Hewlett-Packard G2025 A MALDI-TOF mass spectrometer equipped with a linear detector, employing 1–5 μJ energy pulses of the UV (337 nm) laser beam. Both positive and negative spectra, representing the sums of 20–50 laser shots, were recorded. The hemicellulose samples were dissolved in water to obtain a concentration of 3 mg/ml. The matrix solution (20 mg/ml) was then mixed with the hemicellulose solution at a ratio of 1:4 (v/v). The mesa surface of the MALDI probe employed was coated with a perfluorosulfonated ionomer (Nafion) film according to a procedure described previously (Jacobs & Dahlman, 2001b).

Approximately 0.5 μl of the sample/matrix mixture was applied to the MALDI probe, after which the solvent was removed by evaporation. The peak-average molar mass (M_p) of each hemicellulose fraction was determined as the molar mass at the peak intensity maximum of the MALDI-MS spectrum. The weight- and number-average molar masses (M_W and M_n) for the peaks observed in the MALDI-MS spectra were calculated by the Hewlett-Packard G2025 MALDI-TOF software.

2.3.5. Molecular mass calibration of the SEC system

Fractions from the outlet of the SEC system were collected (Section 2.2.2). The M_p values for the mannan in the fractions were determined by MALDI-MS analysis (Section 2.3.4). For each fraction, the logarithm of the M_p was represented graphically as a function of the elution volume from the SEC system. Analysis of this relationship by least-squares linear regression yielded a regression coefficient of 0.998 for the two filtrates from water impregnated spruce chips heat-fractionated at 180 °C for 5 min and at 200 °C for 2 min. This linear relationship between log M_p (mannan) and elution volume was subsequently employed to determine the M_W (Section 2.3.6) of

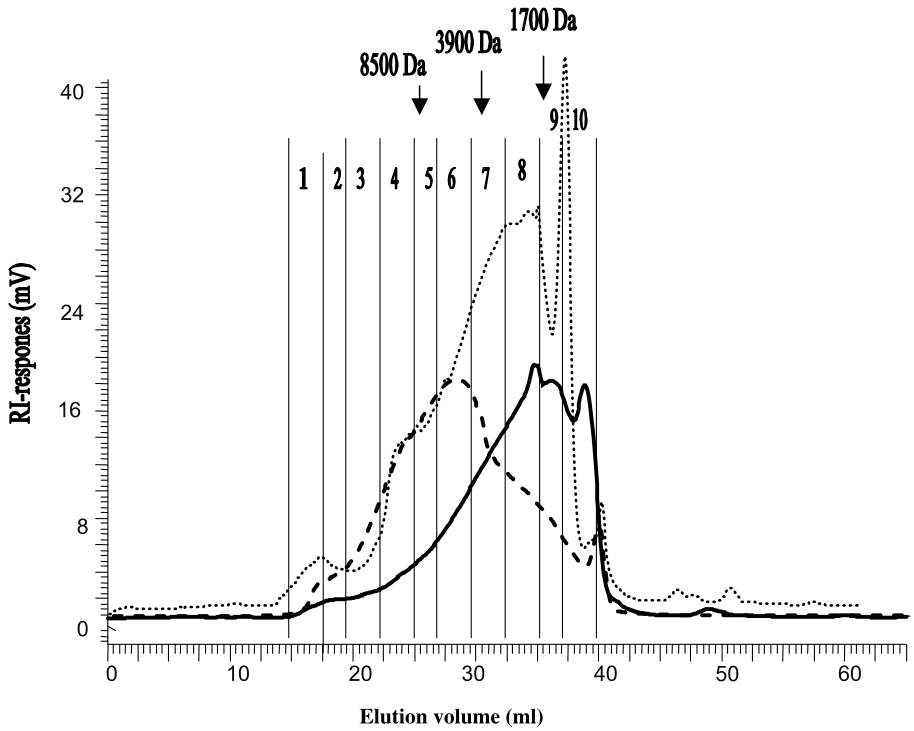

Fig. 1. Elution profile of water-soluble material from microwave oven treated spruce chips after SEC using Superdex 75 and 200 columns. The spruce chips have been heat-fractionated at 190 °C for 5 min impregnated with water (dotted line), at 200 °C for 2 min (solid line) and at 180 °C for 5 min (hatched line). RI-detection is shown; UV-absorbance (280 nm) was mainly detected at elution volumes of 15–23 ml; sharp UV-peaks were detected at 27–28 and 34–36 ml. The applied sample volume was 500 μl. The arrows mark the elution volumes from fractions (5 (M_p 8500), 7 (3900) and 9 (1700)) of galactoglucomannan analyzed with MALDI-MS. Monomeric sugars eluted at 39–40 ml.

the mannan in the filtrates from heat-fractionated from spruce chips.

2.3.6. Weight-average molecular weight

Eq. (1) (Mourey & Coll, 1995) is generally used (described below) for the calculation of M_W of polymers from SEC-chromatograms.

$$M_W = \frac{\sum_{i=1}^{N} (c_i M_i)}{\sum_{i=1}^{N} c_i} \quad (1)$$

where M_i, the molecular weight at elution volume v_i, c_i, the concentration at elution volume v_i, and i is the fraction number.

Mourey and Coll (1995) used printed chromatograms (essentially RI-detector output) for determination of the concentration. Due to the complexity of the sample in the present study, a value for the M_W of the mannan part estimated from a chromatogram plotted from the detector response would not be accurate. Therefore, the mannan concentration in each fraction (Section 2.3.2) was determined and used in Eq. (1). The M-values used were the M_p-values (Section 2.3.4) that were determined for each fraction (10 fractions at 2.5 ml each) using the calibration curve described in Section 2.3.5.

3. Results and discussion

In our previous study (Lundqvist et al., 2002), we made an initial screening of heat-fractionation conditions. An evaluation was made on the basis of the yield of dissolved mannan after heat-fractionation and the weight-average molecular weight (M_W) of the obtained dissolved lignin-free carbohydrates (i.e. mainly hemicellulose). The highest yield (75%) of polymeric and oligomeric mannan (% extracted water-soluble mannan after heat-fractionation of spruce chips) was obtained with water impregnation and heat-fractionation at 200 °C for 5 min. The yields (% mannan oligo- and polysaccharides) were lower with treatments for 2 min (35%), 10 min (48%) and 20 min (21%). Estimated from SEC-chromatograms using Dextran M_W-standards, the M_W increased moderately with shorter residence times (Lundqvist et al., 2002). It is likely that milder heat-fractionation conditions not only in terms of shorter residence time, but also in terms of lower temperature should result in higher M_W of the extracted GGM. In the current work, filtrates from water impregnated spruce chips heat-fractionated at 180 °C for 5 min and at 190 °C for 5 min were investigated. Furthermore, in order to be able to use the SEC-procedure for isolation of high M_W GGM it is essential to have limited overlap with fractions containing other polysaccharides. Therefore, as a first step, the

Table 2
The peak-average molar mass (M_p) of the mannan analyzed with MALDI-MS and the amount of mannan in the fraction from 180 °C/5 min and 200 °C/2 min impregnated in water

Fraction	Mannan			
	180 °C/5 min		200 °C/2 min	
	M_p	Conc. (mg/ml)	M_p	Conc. (mg/ml)
1		0.13		0
2		1.2		0
3		6.4		0.30
4	11,500	17	11,300	2.9
5	8500	31	8300	6.6
6	5500	41	5500	16
7	3900	31	3900	34
8	2600	15	2500	40
9	1700	11	1800	41
10		0		12

distribution of GGM and arabino-4-*O*-methylglucuronoxylan (AGX) in SEC-chromatograms was analyzed.

3.1. Composition of the raw material

In all experiments described herein, spruce chips were used as the starting material. First, a total carbohydrate analysis was made on the spruce chips to determine the monomeric sugar content (Section 2.3.1). The contents of mannose units were 13.7%, xylose units 5.9%, glucose units 46.1%, galactose units 2.7%, arabinose units 3% and lignin (Klason Lignin) 28.5% of dry unextracted spruce wood. 4-*O*-methylglucuronic acid was not analyzed.

3.2. The distribution of mannan and xylan

From the previous work (Lundqvist et al., 2002), it is apparent that both xylan-based and mannan-based oligo- and polysaccharides are solubilized by heat-fractionation of spruce chips. To investigate the possibility of isolation and further characterization of high M_W GGM using SEC the distribution of these polysaccharides and AGX after SEC was determined. The filtrates from spruce chips (impregnated in water) heat-fractionated at 180 °C for 5 min and at 200 °C for 2 min were applied on the SEC column. All the fractions from SEC-separation of these two filtrates (chromatogram shown in Fig. 1) were analyzed with MALDI-MS to determine a more accurate molecular weight compared to the use of dextran standards, as in our previous study (Lundqvist et al., 2002). With MALDI-MS analysis it was also possible to distinguish between xylan-based and mannan-based polysaccharides. Table 2 shows how the mannan distributes with SEC for the filtrates from water impregnated spruce chips heat-fractionated at 200 °C for 2 min and 180 °C for 5 min. The mannan is distributed in fractions 4–9 for both 180 °C/5 min and 200 °C/2 min. The xylan has a lower molecular weight (500–3500) than the mannan, but elutes earlier (fractions 1–4). This separation is likely to be due to the negative charge of the 4-*O*-methylglucuronic acid attached to the xylan, as discussed before (Jacobs et al., 2001). This charge has previously been used in separation of mannan and xylan with anion-exchange chromatography (Dahlman, Jacobs, Liljenberg, & Olsson, 2000) and with analytical SEC (Jacobs & Dahlman, 2001a; Jacobs et al., 2001). The big difference in elution volume for AGX and GGM of the same molecular weight demonstrates the need for proper M_W-standards for accurate determination of M_W of polysaccharides using SEC.

3.3. Determination of the M_W for the GGM part of the polysaccharides

To obtain a good description of the GGM in the filtrate, the M_W had to be determined. For this approach, we needed to develop (i) a more accurate M_W determination for GGM using SEC; (ii) a means to distinguish the mannan content from other material in each SEC fraction. The first criterion was met by peak-average molar mass (M_p)-determination of the GGM in SEC-fractions using off-line MALDI-MS as described earlier (Section 3.2) and the use of these values to calibrate the SEC-column (Section 3.3.1). The second criterion was met by determination of the mannan content in the SEC-fractions using acid hydrolysis and HPLC (Section 3.3.2).

3.3.1. Improved M_W-determination of GGM using SEC

In the previous work (Lundqvist et al., 2002), the M_W of extracted polysaccharides was estimated from SEC-chromatogram using dextran standards. Due to the uncertainty with dextran standards, a more accurate method for determination of the M_W of the mannan was developed. To determine the M_W with the SEC, the relationship between log M_p of GGM in the filtrate (determined by MALDI-MS) and the elution volume obtained by SEC (Jacobs & Dahlman, 2001a; Jacobs et al., 2001) was plotted. Fractions 4–9 from SEC (Fig. 1) were analyzed with MALDI-MS in order to obtain calibration points to make a mannan-specific calibration of the SEC elution volume measurements. The values of the M_p in Table 2 for the filtrate 180 °C/5 min and 200 °C/2 min were used for the calibration curves (Fig. 2). The two calibration curves agreed very well with each other, and they were therefore used to calculate the M_W for the mannan in other filtrates from different heat-fractionation conditions.

3.3.2. M_W of the mannan in filtrates obtained at 180, 190, and 200 °C

In order to characterize the extracted GGM from water impregnated spruce chips obtained at a lower heat-fractionation temperature than 200 °C, the filtrates from the conditions 180 °C, 5 min and 190 °C, 5 min were studied. The yield of mannan in the filtrate heat-fractionated

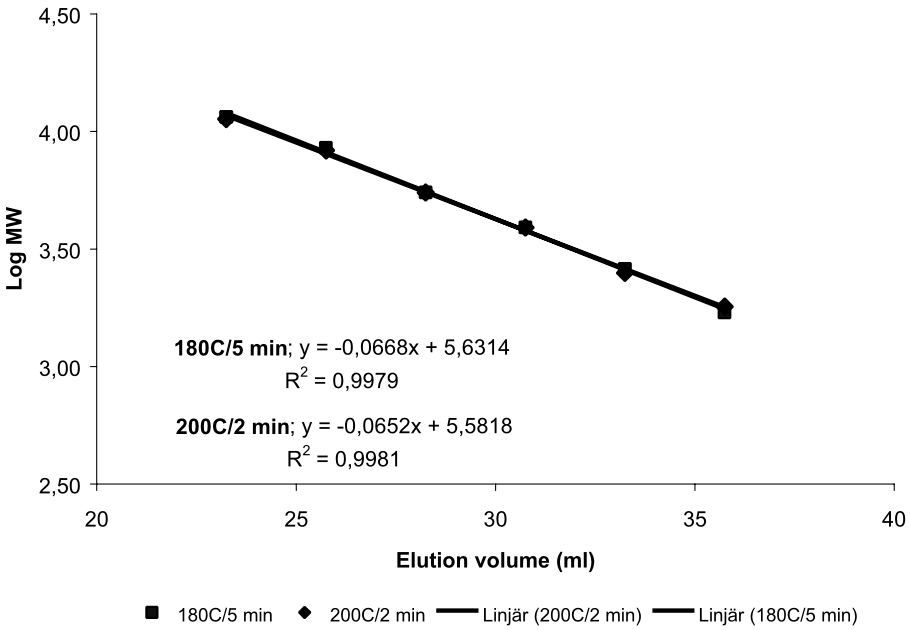

Fig. 2. Relationship between log M_p (determined by MALDI-MS) and elution volume obtained with SEC using Superdex 75 and 200 columns. The mannan from filtrates heat-fractionated at 200 °C for 5 min and at 180 °C for 5 min was compared. The linear relationships and regression coefficients (obtained by least-squares linear regression analysis) of both samples are also shown.

at 180 °C/5 min was 32%. This was similar to that from 200 °C/2 min impregnated in water (35%, Lundqvist et al., 2002) but showed a shift of the SEC-chromatogram toward higher M_W (Fig. 1) and had a higher amount of mannan in earlier eluted fractions (Table 2). The filtrate heat-fractionated at 190 °C/5 min had twice the yield (78%) compared to 180 °C/5 min and 200 °C/2 min, but had a M_W that appeared to be between the two (Fig. 1). The yield of mannan was slightly higher than the highest previously obtained (200 °C/5 min) (Lundqvist et al., 2002).

In order to determine the M_W of mannan in the filtrates as explained earlier (Section 2.3.6), acid hydrolysis was used for the determination of the mannan content in each fraction. The M_p of the mannan in each fraction was estimated using the SEC calibration curve based on GGM fractions for which the M_p had been determined, as described in Section 3.3.1. The M_W for the mannan in the filtrate from 200 °C/2 min was 3300 (the fractions analyzed obtained from the SEC run shown in Fig. 1). For the mannan in the filtrate from 180 °C/5 min and 190 °C/5 min the M_W was 6500 and 3800, respectively. The higher yield of mannan in the filtrate heat-fractionated at 190 °C/5 min (78%) compared with 200 °C/2 min (35%) and 180 °C/5 min (32%) made it interesting for further investigation.

Table 3
Relative contents of monosaccharide residues in the filtrates impregnated at different concentration of NaOH and heat-fractionation at 190 °C for 5 min. The carbohydrate monomer content bound in oligo- and polysaccharides was determined after acid hydrolysis by HPLC. The acetyl content was determined with HPLC after release with alkali (1% NaOH). For details see Section 2. At a NaOH concentration of ≥0.1% the amount acetate was below the detection limit

Filtrate designation	% NaOH	pH (after impreg.)	pH (after heat frac.)	Yield mannan, %	Molar ratio					
					Man	Glu	Gal	Ac	Xyl	Ara
F-0	0	5.3	3.4	78	1	0.3	0.1	0.05[a]	0.3	0.1
F-0.010	0.010	7.1	3.6	36	1	0.3	0.1	0.39[a]	0.2	0
F-0.020	0.020	7.2	4.0	29	1	0.3	0.1	0.39[a]	0.2	0
F-0.025	0.025	8.3	4.2	31	1	0.2	0.1	0.54[a]	0.3	0.02
F-0.05	0.05	9.0	4.6	16	1	0.3	0.1	0.19[a]	0.6	0.4
F-0.1	0.1	10.1	5.1	1.5	1	0.4	0.4	n.d.	1.9	1.8
F-0.5	0.5	12.2	6.9	2.0	1	0.7	0.4	n.d.	1.1	0.2
F-1	1	12.3	11.7	2.2	1	0.7	0.4	n.d.	1.1	0.3
F-2	2	12.3	12.7	3.0	1	0.6	0.4	n.d.	3.4	1.0

n.d., not detected.

[a] Corresponding to DS 0.04 (F-0), 0.31 (F-0.010), 0.31 (F-0.020), 0.45 (F-0.025), 0.15 (F-0.05), assuming that all *O*-acetyl moieties were originating from the galactoglucomannan.

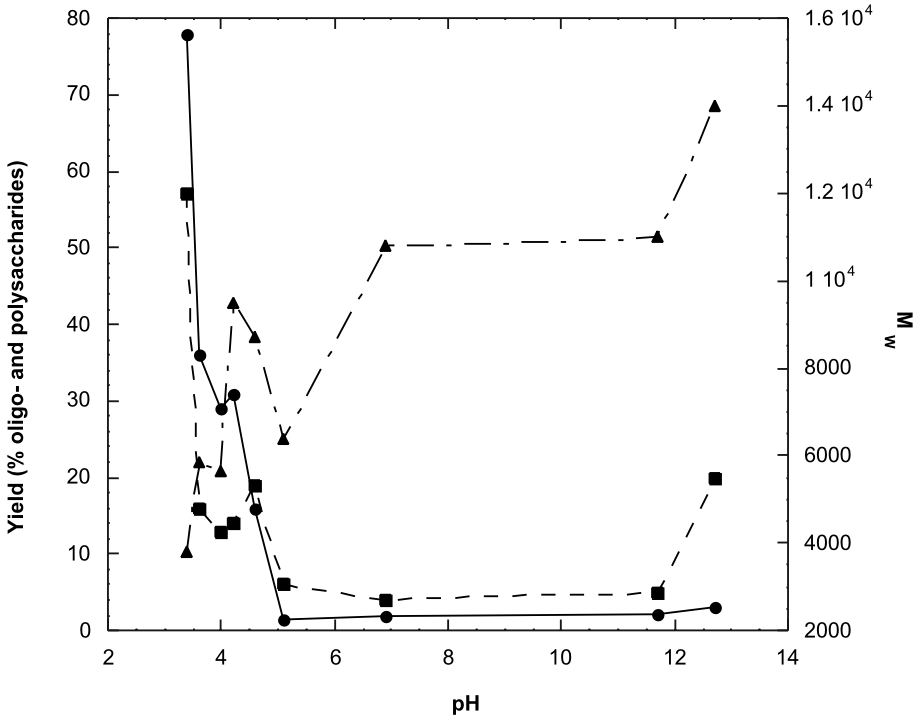

Fig. 3. Analysis of filtrates obtained from heat-fractionation of spruce chips (190 °C, 5 min) in a microwave oven impregnated at different pH. The yields of mannan (•) and xylan (■) and the M_W of mannan (▲) are shown. The yield and the M_W were determined as described in Sections 2.3.2 and 2.3.6, respectively. The filtrates were F-0 (pH 3.4 after heat-fractionation), F-0.010 (pH 3.6), F-0.020 (pH 4.0), F-0.025 (pH 4.2), F-0.05 (pH 4.6), F-0.1 (pH 5.1), F-0.5 (pH 6.9), F-1 (pH 11.6) and F-2 (pH 12.7).

3.4. The influence of pH on the acetylated GGM polymer in the filtrate

Previously we have shown that the impregnation medium influences the yield and the M_W of extracted material (Lundqvist et al., 2002). Here, we further study the effect using fixed heat-fractionation conditions (190 °C/5 min) but varying the NaOH concentration during impregnation, as the pH value during the heat-fractionation was likely the most important parameter. Therefore the pH before and after heat-fractionation was measured. The starting point for the pH screening was the filtrate from spruce chips heat-fractionated at 190 °C for 5 min impregnated in water (Filtrate-designation, F-0). Filtrates obtained from spruce chips impregnated with the following %-values of NaOH were also investigated (Table 1) 0.010 (F-0.010), 0.020 (F-0.020), 0.025 (F-0.025), 0.05 (F-0.05), 0.1 (F-0.1), 0.5 (F-0.5), 1 (F-1) and 2% NaOH (F-2). The yield, M_W, and pH before and after heat-fractionation are shown in Table 3.

Fig. 3 shows how the pH influences the yield and the M_W of the mannan in the filtrates extracted from spruce chips heat-fractionated at 190 °C for 5 min. The M_W was determined as described in Section 3.3. The pH values given are those measured in the filtrates (after heat-fractionation). The yield of mannan decreased with increasing pH. For the filtrates F-0, F-0.010, F-0.020, F-0.025 and F-0.05, the yields of mannan were 78, 36, 29, 31, and 16%, respectively (Fig. 3). When using a NaOH concentration of $\geq 0.1\%$ the yield was very low ($<3\%$). The M_W of the mannan was generally increased with pH, except for F-0.1 where a lower M_W (6400) was obtained than for lower NaOH concentrations. For F-0, F-0.010, F-0.020, F-0.025 and F-0.05 the M_W for the mannan was 3800, 5900, 5700, 9500, and 8700, respectively. Using NaOH concentrations of $\geq 0.5\%$, the M_W was $>10{,}000$. Furthermore, Fig. 3 also indicates that the xylan generally follows the same tendency as mannan. At a NaOH concentration of $\geq 0.1\%$ (pH 5) there are less than 10% of the xylan (poly- and oligosaccharides) left in the filtrate.

Comparing the SEC-chromatograms (Fig. 4) for the filtrates F-0.025 and F-0.05 a shift against higher M_W could be seen compared to the filtrate F-0. The chromatograms are consistent with the results from the calculated M_W: an increase of M_W with increased pH.

3.5. The pH influence on the composition of galactoglucomannan and xylan

As described in Section 3.4 the pH during heat-fractionation influences the yield and the M_W of the GGM. However, it is also likely to influence the structure (i.e. side-group contents) of the polymers. The monomeric composition of oligo- and polysaccharides in heat-fractionated filtrates was determined. The carbohydrate content was determined using acid hydrolysis followed by HPLC analysis. The acetyl content was determined using deacetylation with alkali (1%

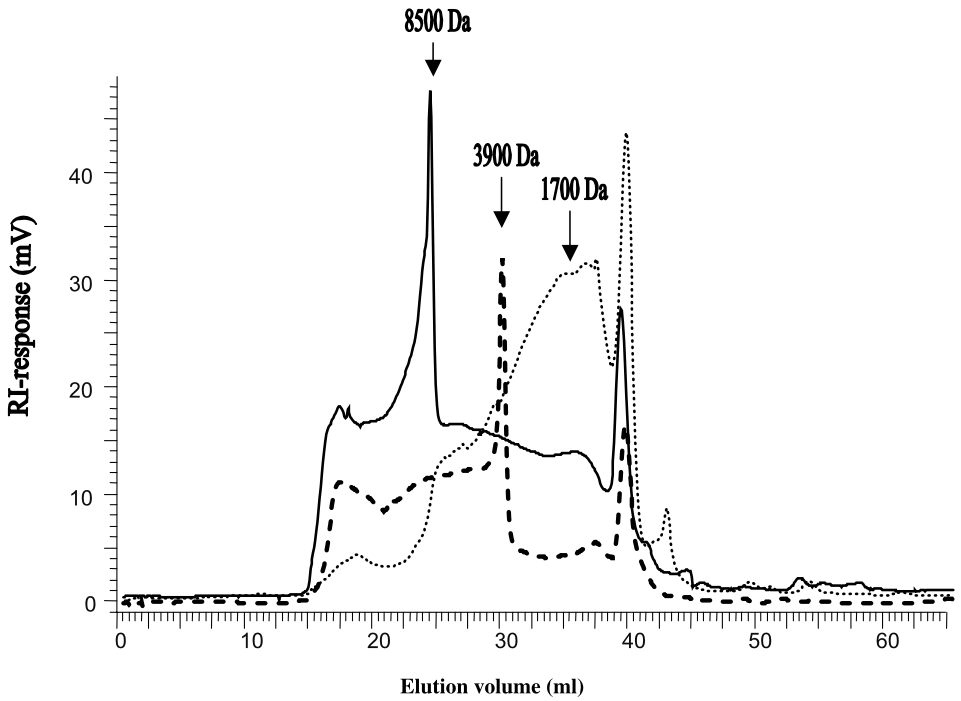

Fig. 4. Elution profile of water-soluble material from microwave oven treated spruce chips after SEC using Superdex 75 and 200 columns. The filtrates compared were F-0 (dotted line), F-0.025 (solid line) and F-0.05 (hatched line). RI-detection is shown. The sharp peaks at elution volumes of 23–25 and 30–32 ml coincide with sharp peaks UV-absorption at 280 nm. The applied sample volume was 500 μl. The arrows mark the elution volumes from fractions (5 (M_p 8500), 7 (3900) and 9 (1700)) of galactoglucomannan analyzed with MALDI-MS. Monomeric sugars eluted at 39–40 ml.

NaOH) and HPLC analysis (Kaar et al., 1991). First, a validation of the determination of acetyl content was made by analyzing the GGM (200 °C/2 min, water) previously isolated (Lundqvist et al., 2002) by the use of the method above. The value obtained here was a degree of substitution (DS) of 0.22 compared to the previously obtained value which was determined with NMR (DS 0.28).

Table 3 presents the monomeric composition for the filtrates obtained by impregnation of spruce chips at 0–2% NaOH and heat-fractionated at 190 °C for 5 min. With a NaOH concentration of ≥0.1% no *O*-acetyl groups bound to oligomers or polymers could be detected. Thus, all the *O*-acetyl side-groups are likely to have been cleaved from the GGM. Earlier reported values of DS (*O*-acetyl side-groups) were 0.36 for pine glucomannan (Lindberg et al., 1973) and DS 0.32 for spruce GGM (Tenkanen, Puls, Rättö, & Viikari, 1993). In these cases, the isolation was done with dimethyl sulphoxide (DMSO) followed by water extraction, and by the organocell process, respectively.

For the D-galactosyl units it appears that a smaller amount is attached to GGM when a lower pH has been used in the heat-fractionation. For F-0, the mannosyl/galactosyl ratio was 1:0.1. Also for F-0.010, F-0.020, F-0.025 and F-0.05 the molar ratios were 1:0.1 (Table 3). At NaOH concentrations of ≥0.1% the molar ratio was increased to 1:0.4. The D-glucosyl ratio was increased at higher alkali additions, thus our results indicate that the pH during heat-fractionation influences the structure of the GGM, which in turn may influence the yield.

The side-groups appear to be sensitive to pH. In the literature, it is described that the *O*-acetyl side-groups on GGM are sensitive to cleavage at neutral pH and alkaline conditions and also that both *O*-acetyl- and D-galactosyl side-groups are sensitive to acid conditions (Hamilton, Partlow, & Thompson, 1960; Lai, 1991; Maloney, Chapman, & Baker, 1985; Timell, 1962). Deacetylation is likely to lower the solubility and cause the precipitation of the GGM. Impregnation of spruce chips with 0.1% NaOH or higher alkali charges is perhaps not suitable for extractions of water-soluble GGM. The best conditions for the heat-fractionation of spruce with a microwave oven for extraction of GGM was found at a temperature of 190 °C for 5 min and impregnation with 0.025% NaOH. At these conditions, the M_W of the mannan was 9500 and the yield was 31%. However, the M_W obtained was only approximately half of the value of native *O*-acetyl-galactoglucomannan (Timell, 1967).

4. Conclusions

The weight-average molar mass, M_W, the extraction yield (% oligo- and polysaccharides) and the monomer composition are three important parameters used here to investigate the water extraction of GGM from spruce chips using microwave heat-fractionation. Temperature, residence time and pH during the heat-fractionation are variables which influence these parameters. The highest

extraction yield of mannan (78%) was obtained from water impregnated spruce chips heat-fractionated at 190 °C for 5 min. Under these conditions, some degradation of the polymers occurred and the M_W of the extracted mannan was 3800. The highest M_W (14,000) was obtained from 2% NaOH impregnated spruce chips heat-fractionated at 190 °C for 5 min, but where the yield mannan was very low (3%). The main water-soluble polymers that were extracted at NaOH charges of ≤ 0.05 and $\geq 0.1\%$ were *O*-acetyl-galactoglucomannan and GGM, respectively.

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