DOI:10.1021/jf902930t

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Oxidation of Polysaccharides by Galactose Oxidase

Kirsti Parikka,*,† Ann-Sofie Leppänen,‡ Leena Pitkänen,† Markku Reunanen,‡ Stefan Willför,‡ and Maija Tenkanen†

†Department of Applied Chemistry and Microbiology, Faculty of Agriculture and Forestry, University of Helsinki, P.O. Box 27, FIN-00014 Helsinki, Finland and ‡Åbo Akademi University, Process Chemistry Centre, Laboratory of Wood and Paper Chemistry, Porthansgatan 3, FIN-20500 Turku/Åbo, Finland

Galactose oxidase was used as a catalyst to oxidize selectively the C-6 hydroxyls of terminal galactose to carbonyl groups. The polysaccharides studied included spruce galactoglucomannan, guar galactomannan, larch arabinogalactan, corn fiber arabinoxylan, and tamarind seed xyloglucan, with terminal galactose contents varying from 6% to 40%. A multienzyme system was used, with catalase and horseradish peroxidase to enhance the action of galactose oxidase. An analysis technique was developed for the quantification of the reactive aldehydes with GC-MS, utilizing $NaBD_4$ reduction and acidic methanolysis. The best oxidation degrees of terminal galactosyls were obtained with xyloglucan (85% of galactose) and spruce galactoglucomannan (65% of galactose). The highest oxidation degree based on total carbohydrates was achieved with guar gum (28%), which had the highest galactose content. The oxidation resulted in changes in the physicochemical properties of the polysaccharide solutions, and the changes observed varied between the polysaccharides. The clearest change was in tamarind xyloglucan, which formed a gel after the oxidation. After the oxidation, larger particles were present in the solution of spruce galactoglucomannan, but changes in its rheological properties were not observed.

KEYWORDS: Galactose oxidase; enzymatic oxidation; galactoglucomannan; galactomannan; arabinogalactan; arabinoxylan; xyloglucan

INTRODUCTION

Galactose oxidase (GO, EC 1.1.3.9) is a 65−68 kDa single copper metalloenzyme that selectively oxidizes primary alcohols to aldehydes. GO is secreted by the fungus *Fusarium* spp. (*1*, *2*). To improve the production of enzyme, the corresponding gene has been isolated and expressed in *Aspergillus nidulans* (*3*), *Pichia pastoris* (*4*), and *Escherichia coli* (*5*). The catalytic reaction of GO comprises oxidative and reductive half-reactions, using molecular oxygen as an electron acceptor and producing hydrogen peroxide. In the half-reactions, the enzyme alters between three different forms: active, inactive, and fully reduced. The active site of GO contains a tyrosine residue and a copper atom, and in the active form of GO the tyrosine is in the radical form and the copper atom at oxidation state +2 (*1*). The inactive form of GO can be oxidized to the active radical by peroxidases (*4*). A high concentration of H_2O_2 (produced in the oxidation) is reported to inactivate GO, and thus, the presence of catalase, breaking down H_2O_2, enhances the action of GO (*6*). We have recently studied the optimization of the reaction conditions for producing aldehydes from galactose derivatives utilizing a three-enzyme system (GO, catalase, horseradish peroxidase) and succeeded in minimizing the formation of side products from the reactive aldehydes (*7*).

The regioselectivity of GO is high for the hydroxyl group at C-6 of galactose. Also some other alcohols with a primary hydroxyl group, such as glycerol, salicyl alcohol, and xylitol, are reported to be substrates for GO (*8*−*10*). The C-6 oxidized galactose derivatives are valuable starting materials for further chemical conversions, and their production with GO is favorable compared to chemical catalysis: protective groups are not needed, the reactions are performed in aqueous solutions, and only molecular oxygen is required as an oxidant. Oxidized galactose (galactohexodialdose) and oxidized lactose are, for example, potential protein cross-linkers, demonstrated by reaction with butylamine and Amadori rearrangement of the product (*11*). Some previous reports on GO-catalyzed oxidation of galactose-containing polysaccharides exist, including the production of aldehyde derivatives of guar gum (*4*, *12*−*14*) and locust bean gum (*13*) galactomannans. GO has also been used in the oxidation of xyloglucan, but the reaction conditions or yield were not described (*15*). Applications for the oxidized products include use as a potential dry or wet strength additive in the paper industry (*4*) and further reactions, such as oxidation to carboxylic acid (*14*) or reductive amination (*13*). More commonly, the selective C-6 oxidative modification of polysaccharides has been done with 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), but TEMPO oxidation is less selective as it oxidizes all primary hydroxyls in polysaccharides. Moreover, TEMPO oxidation mainly produces carboxylic acids, and the degree of polymerization is often reduced (*16*, *17*).

We report here the selective oxidation of several galactose-containing polysaccharides utilizing a three-enzyme system (GO, catalase, and HRP). The polysaccharides studied include spruce

*To whom correspondence should be addressed. E-mail: Kirsti.Parikka@Helsinki.fi.

 Published on Web 12/11/2009

galactoglucomannan (*O*-acetyl galactoglucomannan, GGM), guar galactomannan (guar gum, GM), larch arabinogalactan (AG), corn arabinoxylan (corn fiber gum, AX), and tamarind seed xyloglucan (XG). The aims were to study the degree of oxidation and to investigate the effects of temperature, substrate concentration, ratio of enzyme to substrate, and structure of the polysaccharide on the quantity and quality of the product formed. The possible formation of side products (*7*) was also followed. The GO preparation produced by transgenic *Pichia pastoris*, which is feasible for industrial use, was used as a catalyst. During the study, a specific method was developed for the detection of the reactive aldehydes and quantification of the degree of oxidation with GC-MS after $NaBD_4$ reduction, depolymerization, and derivatization. The production of aldehyde derivatives of spruce GGM, larch AG, and corn AX is reported here for the first time.

MATERIALS AND METHODS

Enzymes and Substrates. Galactose oxidase was produced by *Pichia pastoris* carrying the gene encoding GO from *Fusarium* spp. (*4*). The GO preparation was a gift from Hercules (Barneweld, Netherlands), and it was used without further purification. Analysis by SDS-PAGE showed that it contained one major 65 kDa protein. The protein content was 2.0 mg/mL according to the Bradford assay (Quick Start Bradford, Bio-Rad, United Kingdom). As the activity of the GO preparation was not known, the reported specific activity of a similar preparation (26 U/mg) was used for the estimation of the GO amounts (*18*). Methyl α-D-galactopyranoside, guar gum galactomannan (GM, 14K0168), $NaBD_4$, horseradish peroxidase (HRP, P8250, Type II, 181 U/mg), and catalase (C30, from bovine liver, 22 000 U/mg) were purchased from Aldrich (St. Louis, MO). Larch arabinogalactan (AG, 20701), tamarind seed xyloglucan (XG, 00401), and endo-1,4-β-mannanase (00803, from *Aspergillus niger*, 42 U/mg) were purchased from Megazyme (Ireland). Corn fiber arabinoxylan (AX) was a gift from Dr. Madhav Yadav (U.S. Department of Agriculture, Wyndmoor, PA). Three different spruce galactoglucomannan (GGM) preparations were used in the experiments: (1) GGM isolated from the process waters of a Finnish mill producing TMP (thermomechanical pulp) (*19*); (2) GGM obtained by (1), dissolved in water (ca. 1.3% w/v), heated to 80 °C, and filtered through a glass microfiber filter (Whatman, cat. no. 1820 125), resulting in ca. 1% w/v solution, that was used directly in the oxidation; (3) GGM prepared with a large laboratory-scale method from spruce TMP (*20*). Enzymatically hydrolyzed GM of ca. 50 kDa molecular weight was prepared by incubating 0.6 mU of endo-1,4-β-mannanase/1 mg of GM (1% w/v solution in 0.1 M sodium acetate buffer, pH 5) for 4 h at 40 °C.

Oxidation of Polysaccharides. Polysaccharide (GGM, GM, AG, AX, or XG; in most experiments, 10 mg/mL) was stirred in Milli-Q water at 4 °C or RT for 1−12 h to ensure dissolution. Heating was not used for the dissolution, as it would have decreased the O_2 concentration of the solution. Enzymes were added (GO, catalase, HRP), and the mixture stirred for 48 h. The enzyme dosages were based on the previously optimized amounts and enzyme ratios using methyl α-D-galactopyranoside as a substrate (*7*). The amount of GO was related to the approximate amount of terminal galactose present in the polymer (0.052 U of GO/1 mg of galactose). The dosage of catalase was ca. 115 U/mg and HRP ca. 1.5 U/mg. For example, 1.3 U of GO, 2875 U of catalase, and 38 U of HRP were used in the oxidation of 250 mg of GGM containing ca. 25 mg of galactose (10% of total sugars, ca. 0.15 mmol). Reactions were followed by taking samples after 1, 2, 3, 4, 5, 24, and 48 h. The samples (500 μL) were placed in eppendorf tubes and incubated in a boiling water bath for 3 min to inactivate the enzymes.

Preparation of d_1-Methyl α-D-Galactopyranoside. Methyl α-D-galactopyranoside (0.5 mmol, 100 mg) was dissolved in water (7.5 mL). Enzymes were added (GO, 5.2 U; catalase, 11 200 U; and HRP, 45 U), and the mixture was stirred at RT for 24 h. The disappearance of starting material was ensured with thin layer chromatography (TLC) carried out on silica gel 60 plates (Merck) using a mixture of $MeOH/CH_2Cl_2/AcOH/H_2O$ (10:20:1:1) as eluent and a sample from the reaction directly applied to the TLC plate. The product was detected by dipping the plate in $EtOH/H_2SO_4$ and heating the plate at 100 °C for 10 min. After the oxidation, $NaBD_4$ (1.5 mmol, 62 mg) was added and the mixture was stirred overnight. The formation of the product was followed with the same TLC procedure. The solution was concentrated with a rotary evaporator to a volume of ca. 2 mL and run through a PD-10 desalting column (Amersham Biosciences) to remove salts using Milli-Q water as an eluent. After evaporation, the product was obtained in a 90% yield (91 mg). A sample (0.5 mg) was silylated with TMSCl/BSTFA/pyridine (1:100:100) at 60 °C for 30 min. Analysis with GC-MS showed that the product was pure with high isotopic purity.

Analysis of the Degree of Oxidation. The GC-MS method for following the oxidation was developed with a HP 6890-5973 GC-MSD instrument equipped with a HP-1 column. The method was calibrated using methyl α-D-galactopyranoside and d_1-methyl α-D-galactopyranoside, mixed in various ratios, as model compounds. After dissolution in pyridine (80 μL), the samples were silylated with hexamethyldisilane (HMDS) (150 μL) and trimethylsilyl chloride (TMSCl) (70 μL) and were kept at RT overnight. GC-MS analysis of the tetramethylsilane (TMS) derivatives was used to determine the different ratios of deuterated compounds in the samples. The ratios were verified from comparison of the abundance of the peaks at m/z 361 and m/z 362. The column temperature program was as follows: 80 °C (0.5 min)—8 °C/min—300 °C.

To the samples taken from the reaction mixture, $NaBD_4$ was added (3 mol equiv related to the maximum molar amount of galactose in the sample) and the solution stirred overnight. Alternatively, H_2O (1−2 mL) was added to the most viscous solutions (GM, XG), and the $NaBD_4$ reduction performed in a 10 mL flask. EtOH was added to obtain a ca. 1:3 H_2O/EtOH solution, which precipitated the polymers. The samples were centrifuged for 5 min at 13 400 rpm. The supernatant was discarded, and the polymer placed in a pear-shaped flask and dried. Acidic methanolysis was performed (*21*): HCl/MeOH (2 M, 1 mL) was added, the flask sealed, and the solution kept at 100 °C for 3 h. The samples were allowed to cool, neutralized with pyridine (50 mL), and diluted with MeOH (4 mL). For the GC-MS analysis, 800 μL of the obtained solution was placed in a Kimax tube, evaporated, silylated with 200 μL of TMSCl/BSTFA/pyridine (1:100:100) at 60 °C for 30 min, evaporated, heptane (1 mL) was added, and the sample filtered. GC-MS analysis of the samples was performed with a HP 6890 gas chromatograph connected to an Agilent 5973 Network Mass Selective Detector mass spectrometer equipped with a HP-5 column. The column temperature program was as follows: 150 °C—2 °C/min—220 °C (5 min).

Monitoring O_2 Consumption. The amount of dissolved oxygen in the reaction mixture was determined using a Mettler-Toledo MO128 oxygen meter in mg/L mode. Prior to the measurements, the meter was calibrated to 100% in a flask containing MQ water. The oxidations were performed in the absence of catalase. For example, 0.26 U of GO and 9 U of HRP/mg of galactose were added to a solution of ca. 0.6 mM galactose concentration. The measurement was conducted with magnetic stirring in a container which was full of the solution and sealed with Parafilm M (Pechiney Plastic Packaging, IL).

Analysis of the Physicochemical Properties. Dynamic light scattering experiments were conducted with a Brookhaven Instruments (Holtsville, NY) BI-200SM goniometer, a BI-TurboCorr digital auto/cross correlator, and a BI-CrossCorr detector, including two BI-DS1 detectors. The light source was a Sapphire 488-100 CDRH laser from Coherent GmbH, operating at the wavelength of 488 nm (vertically polarized), the power was adjusted to 50 mW, and the scattering angle was 140°. The temperature of the samples was controlled by means of a Lauda RC 6C water bath. The concentration of the samples was 10 mg/mL (1% w/v). The aqueous solutions were passed through 0.45 μm syringe filters (GHP Acrodisc 13, Pall Corp., Ann Arbor, MI) prior to measurements to remove dust particles. Pseudo cross-correlation functions of the scattered light intensity G2(t) were collected.

The intrinsic viscosities were determined using an Ubbelohde capillary in a water bath at 25 °C. The sample volume was 2.5 mL, and each compound was evaluated using sample concentrations of 0.2%, 0.4%, 0.6%, 0.8%, and 1% (w/v). The samples of the 0.2−0.8% concentrations were diluted from the 1% reaction concentration. All the samples were filtered with GHP 0.45 μm syringe filters. The measurements were repeated five times, and the averages of the time in which each sample passed the capillary were calculated. The extrapolation to zero of η_{sp}/c and $\ln(\eta_r)/c$ as a function of concentration gave the intrinsic viscosity.

Table 1. Approximate Carbohydrate Composition (*20*, *23*, *26*−*28*, *30*−*32*) and Molecular Weight of Spruce Galactoglucomannan (GGM), Guar Galactomannan (GM), Larch Arabinogalactan (AG), Corn Arabinoxylan (AX), and Tamarind Xyloglucan (XG)[a]

	Gal T	Gal	Man	Glc	Xyl	Ara	GlcA	M_w (kDa)
GGM	10		70	20				20
GM	40		60					2600
AG	24	60				15	1	40
AX	6			1	55	33	4	120
XG	16			48	36			470

[a] The ratios of the sugar units are presented as percentages of terminal galactose (Gal T), nonterminal galactose (Gal), mannose (Man), glucose (Glc), xylose (Xyl), arabinose (Ara), and glucuronic acid (GlcA).

Dynamic viscosity and oscillation measurements were performed for samples of 1% w/v concentration with a TA Instruments (New Castle, DE) AR 2000 controlled stress rheometer at 20 °C. The measuring geometry was a concentric cylinder with a conical end (DIN) rotor (radius, 14 mm; stator radius, 15 mm; immersed height, 42 mm; and gap, 5920 μm). The measurements were conducted in the linear area. Viscosity was measured against shear rates of 1−68 s^{-1}. The moduli G' and G'' were determined by oscillation measurements in the range of 0.1−60 Hz.

High performance size exclusion chromatography (HPSEC) analysis of the samples was carried out using water containing 0.1 M $NaNO_3$ as an eluent. Prior to HPSEC analysis, samples (1 mg/mL) were filtered with GHP 0.45 μm syringe filters. The HPSEC equipment consisted of an integrated autosampler and pump module (GPCmax, Viscotek Corp., Houston, TX), a combined light scattering and viscometric detector (270 Dual Detector, Viscotek Corp.), and a refractive index (RI) detector (VE 3580, Viscotek Corp.). Two linear type Shodex columns were used (OHpak SB-806 M HQ, 8 × 300 mm, exclusion limit 2 × 10^7, Showa Denko, Ogimachi, Japan) with a guard column (OHpak SB-6, 4.6 × 10 mm). The light scattering detector (λ_0 = 670 nm) included two scattering angles: 7° (low angle light scattering, LALS) and 90° (right angle light scattering, RALS). The flow rate was 1 mL/min, and injection volume 100 μL. All the M_w values were calculated using a dn/dc value of 0.15 mL/g (*22*), which varies only slightly between different polysaccharides in aqueous solution. The combined light scattering and viscometric detector and RI detector were calibrated with the pullulan narrow standard (M_w = 212 000 g/mol, Polymer Laboratories, Shropshire, U.K.). Intrinsic viscosities ([η]) were calculated from the viscosity signals obtained from the viscometric detector. The weight average molar mass (M_w), intrinsic viscosity ([η]), and hydrodynamic radius (R_h) were calculated using OmniSEC 4.5 software (Viscotek Corp.).

RESULTS

Structures of the Polysaccharides Studied. The polysaccharides studied had different galactose contents, and their structures varied from relatively simple to highly branched (**Table 1** and **Figure 1**). Mannans, spruce *O*-acetyl galactoglucomannan (GGM) and guar galactomannan (GM) contain 10% and ca. 40% terminal α-D-galactosyl residues, respectively, directly attached to C-6 of the mannosyl units of the backbone (*20*, *23*−*25*). Larch arabinogalactan (AG) contains 28% terminal β-D-galactosyls bound to the backbone via [→6)-β-D-Gal*p*-(1→] (*26*). Corn arabinoxylan (AX) is a highly branched galactoglucuronoarabinoxylan with side chains consisting of xylopyranosyls and arabinofuranosyls and ending in galactosyls (ca. 6% of the sugar units) (*27*−*29*). The galactoxyloglucan from tamarind seed (XG) has terminal β-D-galactosyl residues (16% of the sugar units) at xylose side chains [→2)-α-D-Xyl*p*-(1→] attached to C-6 of glucose (*30*). The molecular weights of the polysaccharides were determined by HPSEC (**Table 1**).

Analysis Method for the Degree of Oxidation. According to the preliminary experiments, it was clear that the commonly used analysis techniques prior to GC-MS analysis, such as acidic methanolysis for depolymerization (*21*), were not suitable for the oxidized polysaccharides, as the carbonyl groups were very reactive and were lost during the sample preparation. Another analysis method investigated was NMR, but accurate NMR analysis required samples of high concentrations which could not be prepared due to solubility problems of the products in suitable NMR solvents, such as dimethyl sulfoxide (DMSO). D_2O would have been the best solvent, but the otherwise conveniently observed proton or carbon chemical shifts of carbonyls were not present due to the hydrate formation of aldehydes in water (*7*). The chemical shifts of hydrates exist in the same area with those of anomeric protons and carbons, which complicated the analysis.

Thus, a new analysis method was developed for the detection and quantification of the aldehydes. The carbonyls formed were reduced back to the C-6 hydroxyls with $NaBD_4$ immediately after the oxidation. Thus, the oxidized galactosyls were labeled with one deuterium atom. As the reactive aldehyde was no longer present, acidic methanolysis could now be used for depolymerization, followed by silylation and GC-MS analysis.

During the development of the GC-MS method, and to ensure that the analysis technique was reliable, mixtures of silylated methyl α-D-galactopyranoside and d_1-methyl α-D-galactopyranoside were analyzed by GC-MS. The ratio of the peaks at m/z 361 and m/z 362 (**Figure 2A** and **B**) gave the correct ratio of the amounts of nondeuterated and deuterated methyl α-D-galactopyranoside in the mixture, showing that the method was reliable. This fragment was chosen, since it is the most abundant isolated fragment of the TMS derivative of methyl-aldohexoses still containing C-6 (*33*).

The following equation was used in the calculation of the percentage of the deuterated galactopyranoside present in the mixtures, that is, the oxidation degree of the products. In the equation, A = abundance of m/z 361, the amount of unoxidized galactose units, and B = abundance of m/z 362, the amount of oxidized galactosyls. First, the abundance of the $M+1$ peak of m/z 361 (0.33 × abundance of 361, see **Figure 2A**) was subtracted from m/z 362. The proportion of the remaining abundance of m/z 362 to the sum of abundances was the degree of oxidation. An example of the fragment in the mass spectrum of an oxidized polysaccharide is shown in **Figure 2C**.

$$\text{oxidation degree} = \frac{\left(B - \frac{A}{3}\right) \times 100\%}{A + B - \frac{A}{3}}$$

Oxygen Consumption. Testing the suitability of the five substrates for GO by its oxygen consumption in the absence of catalase showed that the best substrate was GM (**Figure 3**). The reaction rate was very slow in the case of AX and AG, where the oxygen concentrations remained almost static during the measurement, indicating that they were not good substrates for GO. The use of an excess amount of the enzymes was investigated with AX (1.04 U of GO and 36 U of HRP/mg of galactose) but did not accelerate the O_2 consumption significantly.

Oxidation of the Polysaccharides. The oxidation conditions optimized earlier with methyl α-D-galactopyranoside and raffinose (*7*) were now applied to the oxidation of the polysaccharides (**Scheme 1**), and spruce GGM was used in the preliminary experiments to confirm the reaction conditions and enzyme dosages suitable for the polymers. The dosage of GO was based on the approximate molar amount of galactose present in the polysaccharide and equaled the amount that was successfully used in the optimization of the oxidation with the mono- and

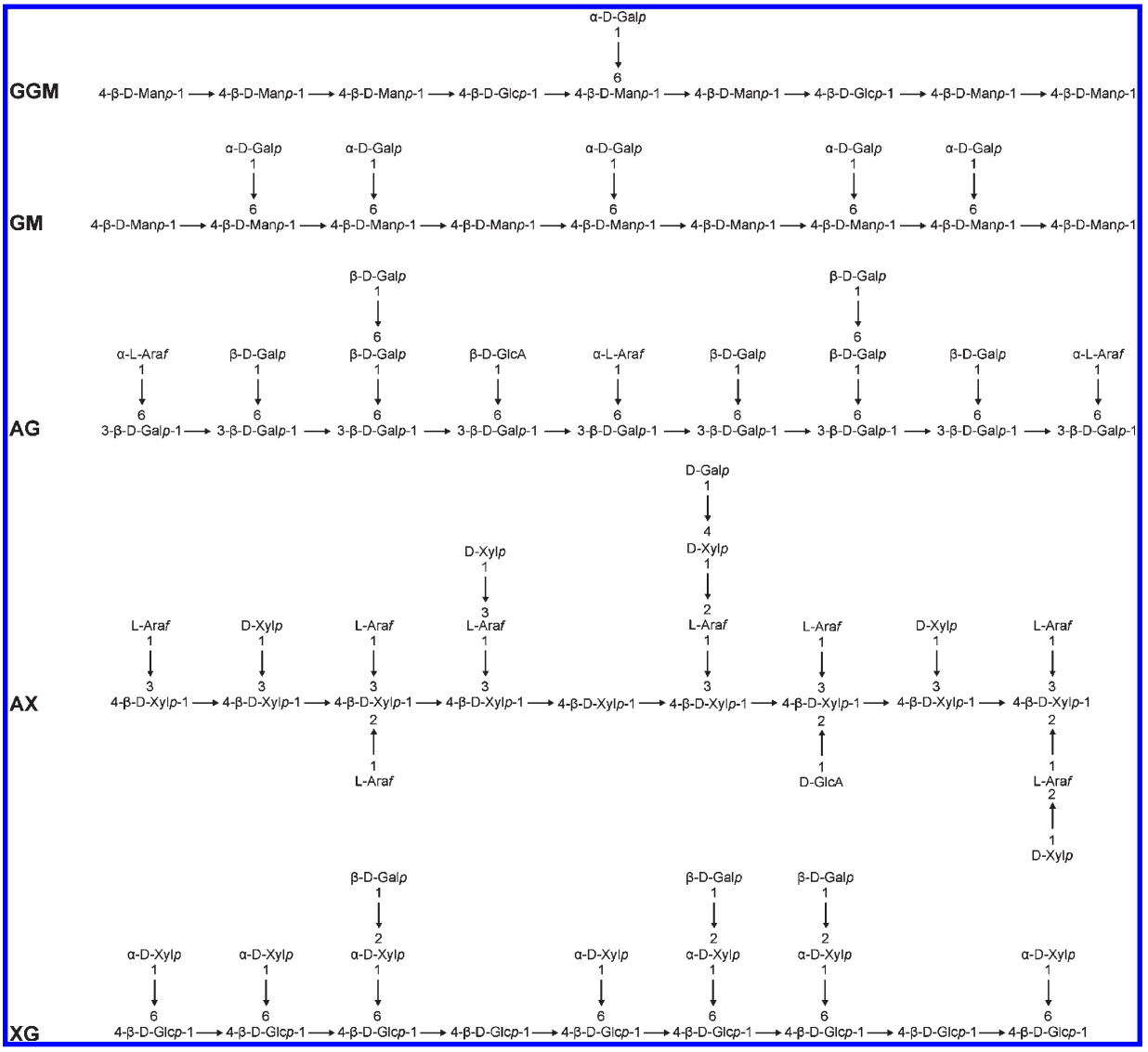


Figure 1. Structures of spruce galactoglucomannan (GGM), guar galactomannan (GM), larch arabinogalactan (AG), corn arabinoxylan (AX), and tamarind xyloglucan (XG) (*20*, *23*, *24*, *26*−*31*).

trisaccharide (0.052 U/mg ≈ 9.5 U/mmol). The dosages of catalase and HRP were ca. 115 and 1.5 U/mg, respectively. The presence of all the three enzymes was required for a successful reaction. The use of higher amounts of enzymes, such as 0.2 U/mg of GO, did not enhance the yield of the aldehydes. The possible presence of side products was investigated by comparing the GC chromatograms of the methanolyzed and silylated starting materials and products, which should be similar to each other after the $NaBD_4$ reduction of the oxidation product, and differences of only 0−5% in the areas of the peaks were observed. Galacturonic acid is a possible side product (*7*) and stable under acid methanolysis, thus being facile to detect by GC, but was not found.

The degree of oxidation of terminal galactose of the products (GGMox, GMox, AGox, AXox, XGox) varied from ca. 10% to ca. 85% after 48 h of reaction time (**Figure 4A**). The highest oxidation degrees were obtained with XG (ca. 85%) and GGM (ca. 65%). The oxidation degree of the polysaccharides, based on the content of oxidized galactosyls as a percentage of total carbohydrates, was best in the case of GMox that had the highest galactose content (16% for 1% w/v substrate concentration, **Figure 4B**). Calculated this way, the oxidation degree of AXox was very low, only 1.5%, due to the low galactose content of AX (6% of the sugar units).

The oxidation of GGM preparations was the focus of interest. The degree of oxidation of the preparations was significantly altered by the purification or isolation technique. The sample with the highest purity, obtained by a laboratory-scale method (*20*) involving purification by dialysis (GGM C, **Figure 5**), had the highest oxidation degree. The samples with a lower purity, that had been precipitated from TMP mill process water without further purification (*19*), or precipitated and then filtered, were converted to the aldehydes with a significantly lower degree of oxidation.

The Effect of Reaction Conditions on the Oxidation. The maximum substrate concentration used in the experiments was quite low (1% w/v), as the optimization reactions had shown dilute substrate concentrations were more reactive (*7*), and more concentrated solutions of GM and XG were found too viscous for the efficient stirring of the reaction mixture. The substrate concentration had a clear effect on product formation in the case of GM. The oxidation degree of GMox was higher, especially during the first 5 h, when the concentration was reduced from 1% to 0.25% w/v (**Figure 6**). A 0.1% w/v substrate concentration further increased the oxidation degree of GMox to ca. 70% of terminal galactose (28% of total sugar units; data not shown). The dilution of the other polysaccharide solutions did not affect

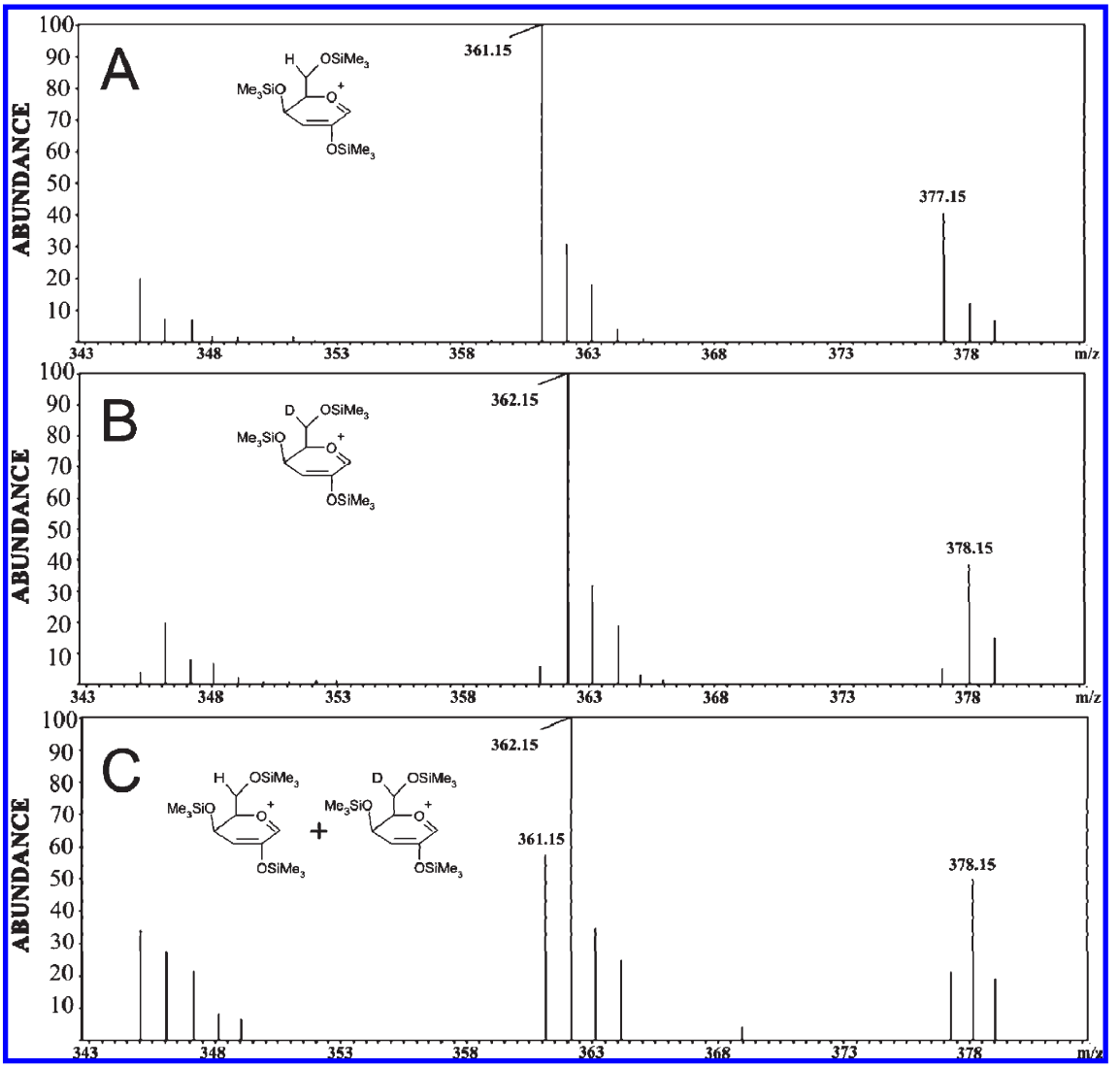


Figure 2. (**A**) *m*/*z* 361 fragment of silylated methyl α-D-galactopyranoside. (**B**) *m*/*z* fragment 362 of silylated d_1-methyl α-D-galactopyranoside. (**C**) Part of the mass spectrum of the mixture of deuterated and nondeuterated methyl α-D-galactopyranoside present in oxidized spruce galactoglucomannan after reduction with $NaBD_4$, acidic methanolysis, and silylation (ca. 60% oxidation degree of galactose).

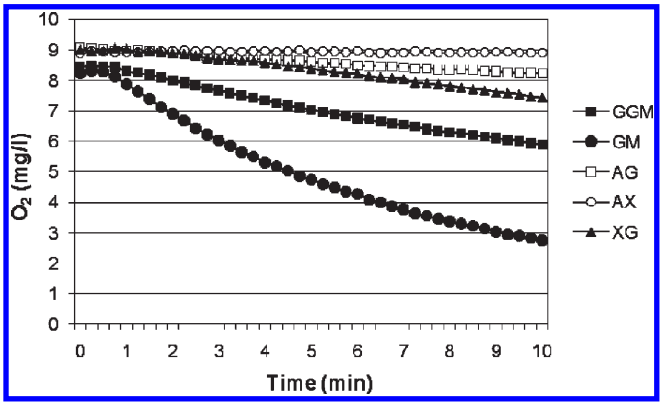
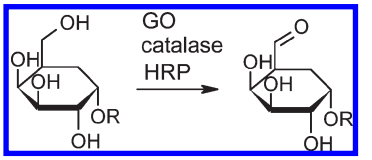


Figure 3. Consumption of O_2 in the galactose oxidase-catalyzed reaction in polysaccharide solutions of approximately 0.6 mM galactose concentration at RT. GGM = spruce galactoglucomannan, GM = guar galactomannan, AG = larch arabinogalactan, AX = corn arabinoxylan, XG = tamarind xyloglucan. Enzyme dosage: 0.26 U of galactose oxidase and 9 U of horseradish peroxidase/mg of galactose.

the oxidation degrees significantly. In the more dilute solutions of GMox and XGox (0.1−0.25% w/v), visible aggregates formed

Scheme 1. GO-Catalyzed Oxidation of the Galactose Units of Polysaccharides[a]



[a] GO = galactose oxidase, HRP = horseradish peroxidase, R = sugar unit in the polysaccharide.

soon after the reaction was finished, whereas 1% w/v solutions formed homogeneous gels.

When the effect of the reaction temperature on the reaction rate was studied at 4 °C and at RT, the oxidation degree of GGM, GM, and AX was ca. 5−10% lower during the first 3−5 h at 4 °C than at RT, and even 30% lower in the reaction of XG, but reached the same level as the reactions performed at RT after 48 h (data not shown). An exception was the reaction of AG, which proceeded at the same rate at both temperatures.

Physicochemical Properties of the Products. The products GMox and XGox were visibly very different from the starting materials, resembling firm, viscous gels (**Figure 7**). The products

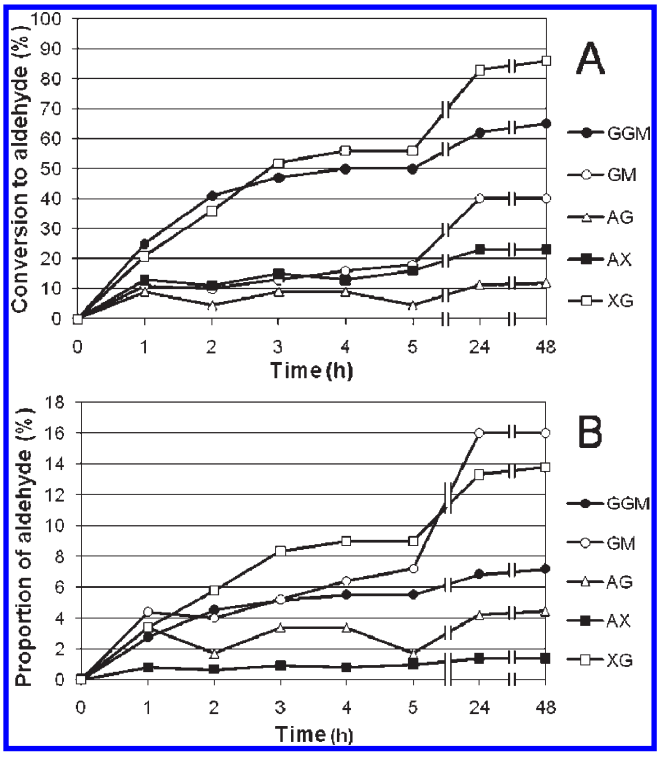


Figure 4. Oxidation degree of polysaccharides during 48 h oxidation at RT (**A**) based on the amount of oxidized terminal galactose compared to the amount of unoxidized terminal galactose and (**B**) based on the proportion of oxidized galactosyls to the total amount of carbohydrates in the polysaccharides. GGM = spruce galactoglucomannan, GM = guar galactomannan, AG = larch arabinogalactan, AX = corn arabinoxylan, XG = tamarind xyloglucan. Substrate concentration = 1% w/v. Reactions performed at RT. Enzyme dosage: ca. 0.052 U of galactose oxidase, 115 U of catalase, and 1.5 U of horseradish peroxidase/mg of galactose.

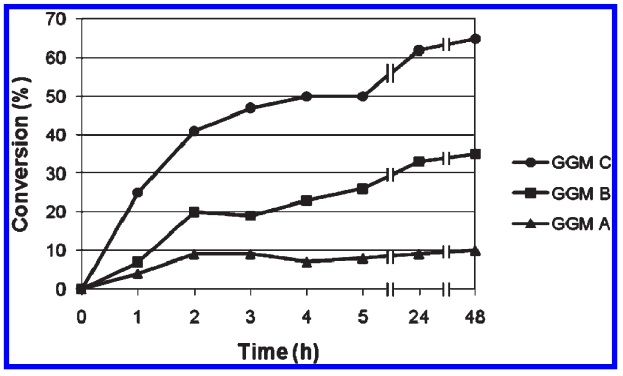


Figure 5. Oxidation of three preparations of spruce galactoglucomannan during 48 h oxidation at RT. GGM A, precipitated with EtOH from TMP (*19*); GGM B, GGM A filtered through a glass microfiber filter; GGM C, obtained by laboratory-scale isolation and purification method (*20*). Substrate concentration = 1% w/v. Reactions performed at RT. Enzyme dosage: ca. 0.052 U of galactose oxidase, 115 U of catalase, and 1.5 U of horseradish peroxidase/mg of galactose. Oxidation degrees based on the amount of oxidized galactose compared to the amount of unoxidized galactose.

GGMox, AGox, and AXox did not differ visibly from the starting materials, seeming to have low viscosity. The physicochemical properties of these two product types were investigated in separate ways. The gel consistence of GMox and XGox precluded analysis by dynamic light scattering (DLS), HPSEC,

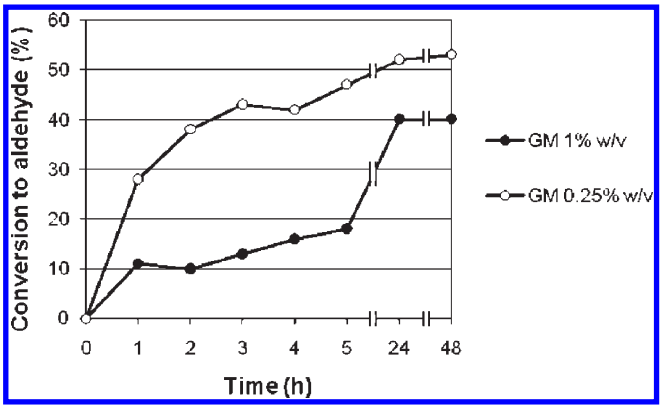


Figure 6. Comparison of the oxidation degrees of 1% and 0.25% w/v solutions of guar galactomannan (GM) during 48 h oxidation at RT. Enzyme dosage: ca. 0.052 U of galactose oxidase, 115 U of catalase, and 1.5 U of horseradish peroxidase/mg of galactose. Oxidation degrees based on the amount of oxidized galactose compared to the amount of unoxidized galactose.

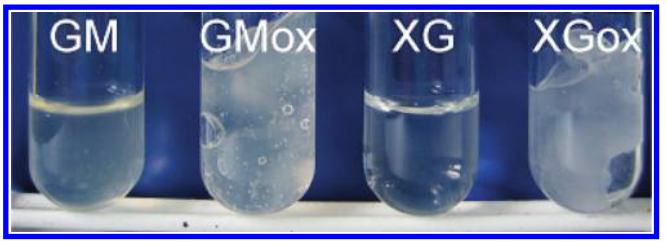


Figure 7. Starting materials and products (1% w/v solutions in H_2O). GM = guar galactomannan; GMox = oxidized GM, 40% oxidation degree; XG = tamarind seed xyloglucan; XGox = oxidized XG, 85% oxidation degree. Oxidation degrees based on the amount of oxidized terminal galactose compared to the amount of unoxidized terminal galactose.

and Ubbelohde capillary rheology; thus, these methods were used on GGM, AG, and AX. Viscosity of GM and XG was analyzed instead by controlled stress rheometry.

DLS experiments showed that the particle size distributions of GGMox and AGox differed from those of the starting materials (**Figure 8A** and **B**). Oxidation caused aggregation or interaction between polymer chains, such as hemiacetal bond formation between carbonyls and hydroxyl groups, especially in the case of the GGM samples (shift in the position of the first peak, ca. 10 nm). The second peak in the particle size distribution at the position of 100 nm could be due to aggregated particles, although the amount of these aggregates was probably very small. The samples were filtered through 0.45 μm filters prior to DLS measurements, and thus, the large particles, for example, having a diameter of 1 μm, were reformed in the solutions after the filtration. No significant change in the particle size distribution was found in the case of AX and AXox, attributable to the low oxidation degree of AXox (**Figure 8C**).

The HPSEC analysis confirmed the aggregation of oxidized GGM, seen as increased molar mass and as a shoulder in the light scattering signal (HPSEC chromatogram not shown). No evidence of aggregation, however, could be observed in the HPSEC analysis of AGox, even if the DLS measurement suggested the presence of aggregates. Due to aggregation of GGMox, its apparent M_w was higher than that of unoxidized GGM (35 and 20 kDa, respectively). Oxidation did not change the apparent M_w values of AG and AX from the respective original values of 42 and 120 kDa, respectively. The hydrodynamic radii (R_h)

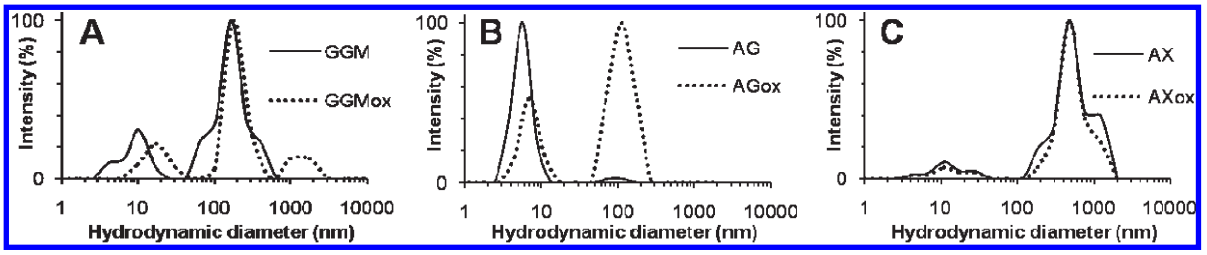


Figure 8. Hydrodynamic diameter of the nonmodified and oxidized polysaccharides (1% w/v solutions in H_2O filtrated with a 0.45 μm polypropylene filter). GGM = spruce galactoglucomannan; GGMox = oxidized GGM, 65% oxidation degree; AG = larch arabinogalactan; AGox = oxidized AG, 11% oxidation degree; AX = corn arabinoxylan; AXox = oxidized AX, 23% oxidation degree. Oxidation degrees based on the amount of oxidized terminal galactose compared to the amount of unoxidized terminal galactose.

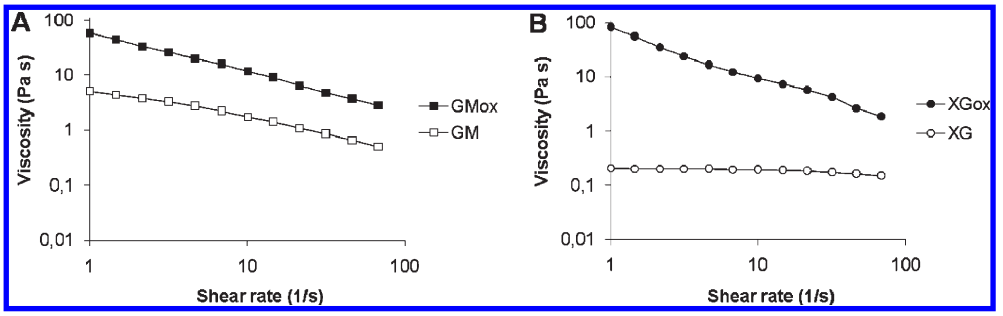


Figure 9. Dynamic viscosities of the nonmodified and oxidized GM (**A**) and XG (**B**) as a function of shear rate (1% w/v solutions in H_2O). GM = guar galactomannan; GMox = oxidized GM, 40% oxidation degree; XG = tamarind seed xyloglucan; XGox = oxidized XG, 85% oxidation degree. Oxidation degrees based on the amount of oxidized galactose compared to the amount of unoxidized galactose.

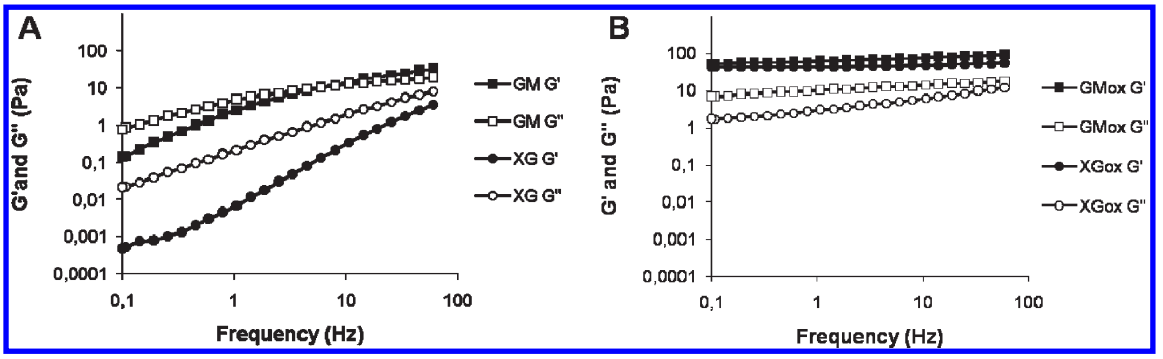


Figure 10. G' and G'' of the nonmodified (**A**) and oxidized (**B**) polysaccharides (1% w/v solutions in H_2O) as a function of frequency at 20 °C. GM = guar galactomannan; GMox = oxidized GM, 40% oxidation degree; XG = tamarind seed xyloglucan; XGox = oxidized xyloglucan, 85% oxidation degree. Oxidation degrees based on the amount of oxidized galactose compared to the amount of unoxidized galactose.

were in agreement with the results of the DLS measurements. The R_h value for GGMox was slightly higher than that for GGM (6.5 and 5.5 nm, respectively). Oxidation had no effect on the R_h values of AG and AX (R_h for AG and AGox 3 nm, and for AX and AXox 10 nm). According to both DLS and SEC experiments, no depolymerization had occurred in the GO-catalyzed oxidation. Also, to exclude the effect of the cross-links formed, the M_w of oxidized and then reduced GGM was analyzed by SEC and found to be similar to that of the starting material (20 kDa), which confirmed the oxidation did not induce degradation.

Oxidation had no significant effect on the rheological properties of GGM, AG, and AX as determined using an Ubbelohde capillary at 25 °C (series of 0.2−1% w/v solutions). The intrinsic viscosities were ca. 70, 8, and 100 mL/g for GGM and GGMox, AG and AGox, and AX and AXox, respectively. The intrinsic viscosities observed by HPSEC differed slightly from the values obtained with Ubbelohde equipment being ca. 60, 4, and 80 mL/g, respectively. Thus, the increase of the molecular weight in the oxidation of GGM did not affect viscosity.

The dynamic viscosities of the oxidized products of GM and XG (1% w/v solutions) were higher than those of the starting materials at all shear rates. Both GM and GMox thinned against shear at a similar rate (**Figure 9A**). The shear thinning of XGox was also strong, indicating the presence of large unstable entanglements in addition to other interactions, and differed from the behavior of XG, which did not thin (**Figure 9B**). The oscillation measurements suggested that the oxidized polysaccharides were gels, having higher G' than G'', no crossover points of the curves, and quite low variation related to the changing frequency (**Figure 10**). The rheological properties of XG changed especially strongly due to the oxidation.

GM and XG had the highest molecular weight and viscosity of the polysaccharides studied, and the oxidation clearly changed their rheology. The effects of the molecular weight and the viscosity on the oxidation degree and rheology of the products were investigated using enzymatically hydrolyzed GM with a molecular weight of ca. 50 kDa (GM_{50}). The M_w of GM_{50} was about one-fiftieth of that of the original GM and close to that of

GGM. The oxidation degree obtained in the oxidation of GM_{50} (1% w/v) was high, 78%, and the intrinsic viscosity of the solution was low, ca. 0.6 dL/g, according to HPSEC. The results were similar to the oxidation degree and rheological properties of GGMox and indicated that the viscosity and the M_w of the starting material had an effect on the product formation and properties.

DISCUSSION

The polysaccharides studied have important applications, either currently or potentially. Unmodified guar GM is widely used as a thickening or gelling agent, making food products appealing to the consumer and maintaining turbidity in soft drinks and juices, as well as for various other purposes (*23*). Guar GM has also been used as an additive in papermaking (*34*). Spruce GGM has been studied as a film-forming agent for the development of new, biopolymer-based packaging materials (*35*). Also, the addition of GGM has been found to enhance the emulsion formation and increase the emulsion stability in a model beverage system (*36*). Corn AX has been found to be an excellent emulsifying agent that might be used in beverages instead of gum arabic, especially in applications where low viscosity is beneficial (*28*,*37*). Tamarind XG is commonly used as a food additive in Japan (*32*). Larch AG has been studied as a potential dietary fiber because of its bioactive properties (*38*).

A few applications have been reported for one of the oxidized polysaccharides as well. In papermaking, the dry tensile strength of paper increased significantly following the addition of GMox (*4*). Another application for GMox was a potential polyelectrolyte obtained by the further chemical oxidation of GMox to a carboxylic acid derivative (*14*).

This kind of screening of the GO-catalyzed oxidation of polysaccharides of a wide structural variety has not been previously reported. Also, the production of aldehyde derivatives of spruce GGM, larch AG, and corn AX is described here for the first time. The previously reported GO-catalyzed reactions include the oxidation of GM (*4*, *12*−*14*). The reactions were performed in either buffer (*13*) or water (*14*). The dosage of enzymes varied a little (when mentioned), from ca. 4.5 (*14*) to 5.5 U/mg (*13*), and exceeded the amount we have found optimal. Previously, use of HRP has been reported in one case only (*4*) and was found necessary to obtain good yields of GMox (45% oxidation degree). However, the combination of GO and catalase without HRP was reported to give GMox with ca. 30% conversion, and the oxidation degree was determined after the further oxidation of the aldehyde to carboxylic acid (*14*).

A similar type of analysis method to that we report here has been used for the oxidation degree of GMox, but it required two reduction steps instead of one (*4*). Other analysis techniques that have been used in the determination of the oxidation degree are, for example, potentiometric, viscometric, or colorimetric (*14*). The technique utilizing $NaBD_4$ reduction and acidic methanolysis (*21*) was simple and reliable, as it was suitable for all the polysaccharides studied, and the results were easily interpreted and compared with each other.

An earlier optimization of the oxidation conditions showed that reaction temperatures higher than RT decreased the yield, attributed to the lower oxygen content of the warmer solutions (*7*), as the solubility of oxygen in water is inversely proportional to the temperature. Thus, in the present work, the reactions of the polysaccharides were investigated only at RT and at 4 °C. The oxygen content and consumption were measured at both temperatures, and they were in agreement with the obtained conversions. GO and HRP were used in the measurements without catalase, to avoid the production of O_2. For example, in a 1% w/v solution of GGM, higher O_2 concentrations were observed at 4 °C (ca. 11.3 mg/L) than at RT (ca. 8.5 mg/L) at the beginning of the reaction. After 10 min reaction, O_2 concentration had decreased only 1.4 mg/L at 4 °C, but 2.6 mg/L at RT, showing that O_2 consumption was clearly slower at the colder temperature, explaining the lower conversions during the first hours of the reactions at 4 °C. This did not confirm the results from the mono- and oligosaccharides that were used in the previous optimization (*7*), as their reactions had proceeded approximately at a same rate at both temperatures. Nevertheless, in the present work, there was no significant difference in the final yield after 48 h.

The structure of the polysaccharides might have an effect on the product formation depending, for example, on the conformation of the polymer in the solution, and on steric hindrance. The complex structure of AX and AG might explain the low oxidation degrees of AXox and AGox; the many side chains could even prevent GO from reaching the terminal galactosyls. However, there are side chains in XG as well, and XG was oxidized with a high oxidation degree. The compounds having the more simple structures (GGM and GM) seemed to have a higher oxidation degree than those with the more complex structures (AG, AX), but XG does not follow this pattern. AG was the only polysaccharide containing also nonterminal galactose, with its backbone consisting of β-D-(1→3)-Gal*p* units. The C-6 positions of the main chain units are mainly substituted by side chains (*26*), but some free C-6 hydroxyl groups of the nonterminal galactosyls might be available for the oxidation. The analysis method used did not allow the classification of the origin of the oxidized galactosyls, but based on the low degree of oxidation it seems that β-(1→3)-linked unsubstituted Gal*p* units in the backbone of AG were not good substrates for GO.

The molecular weights of the polysaccharides studied varied from 20−60 kDa for GGM (*20*, *31*) to the MDa range for GM (*23*). Comparing the mannans studied, the enzymatically hydrolyzed GM_{50} was quite similar to GGM, with the main difference being their different galactosyl content. The 78% oxidation degree of GM_{50}ox was relatively close to the oxidation degree of GGMox (65%). As the oxidation degree of GMox obtained in a diluted, and thus less viscose, 0.1% w/v solution was 70%, close to the oxidation degree of GM_{50}ox, a decrease of the viscosity of the GM solution might be the explanation for the increase of the oxidation degree. This, however, does not explain the low oxidation degrees of AG and AX, which both had low viscosity.

Significant variation in the degrees of oxidation was found in the different GGM batches. A possible reason for the different reactivity was the presence of minor amounts of impurities capable of inactivating the enzymes. The impurities capable of inhibiting GO, and left in the sample from the process water, could be phenolic compounds, such as lignins.

The rheological properties of GMox and XGox differed considerably from the properties of the starting materials, especially that of XG which changed dramatically due to the oxidation. The XG solution (1% w/v) did not show either shear thinning or gelling, whereas the XGox solution showed both of these properties. Oxidized GM and XG are thus interesting materials for further experiments and might have applications as gels, for example. The viscosities of the low molecular weight GGM and AG, and relatively low M_w AX, were low, and the oxidation did not change them, although clear changes were seen by DLS. In particular, GGMox formed aggregates, which was verified by the HPSEC analysis. Of these three polysaccharides, GGMox had the highest oxidation degree, which explained its clear aggregation compared to the others. The oxidation degree of

AX was very low and no changes were observed with any of the analytical techniques. The physical properties of GGMox, AGox, and AXox require further investigation. For example, their rheological properties could be investigated in higher concentrations, in which the rheological properties of unmodified GGM have been successfully studied (*19*). The reactive carbonyl groups make all the products promising starting materials for further chemical conversions.

To summarize, aldehyde derivatives of several structurally different polysaccharides were produced in varying oxidation degrees. Oxidized GGM, GM, and XG were obtained with a high oxidation degree. The oxidation degree of AG and AX remained low. The substrate concentration and viscosity of the solution had an effect on the product formation in the case of GM. The reaction rate was slower at a colder temperature, but the temperature did not have a significant effect on the final oxidation degree. A relationship between the structure and properties of the starting materials and the properties of the oxidized products was found: The oxidation of the low molecular weight polysaccharides that formed nonviscous solutions, that is, GGM, AG, and AX, did not change their rheology. In the case of the high molecular weight polysaccharides, GM and XG, a significant change was observed. The products GMox and XGox were highly viscous gels.

ACKNOWLEDGMENT

We thank Sybe Hartmans for supplying us with the *Pichia pastoris* galactose oxidase product. Madhav Yadav is thanked for donating corn fiber gum, Vladimir Aseyev for helping with dynamic light scattering measurements, and Sami Hietala for assistance with the measurement of dynamic viscosity. Patrik Eklund, Chunlin Xu, and Rainer Sjöholm are thanked for fruitful discussions.

LITERATURE CITED

(1) Whittaker, J. W. Free Radical Catalysis by Galactose Oxidase. *Chem. Rev*. **2003**, *103*, 2347–2363.

(2) Whittaker, J. W. The radical chemistry of galactose oxidase. *Arch. Biochem. Biophys*. **2005**, *433*, 227–239.

(3) Baron, A. J.; Stevens, C.; Wilmot, C.; Seneviratne, K. D.; Blakeley, V.; Dooley, D. M.; Phillips, S. E. V.; Knowles, P. F.; McPherson, M. J. Structure and mechanism of galactose oxidase. The free radical site. *J. Biol. Chem*. **1994**, *269*, 25095–25105.

(4) Hartmans, S.; De Vries, H. T.; Beijer, P.; Brady, R. L.; Hofbauer, M.; Haandrikman, A. J. Production of oxidized guar galactomannan and its applications in the paper industry. In *Hemicelluloses: Science and technology*; Gatenholm, P., Tenkanen, M., Eds.; American Chemical Society: Washington, DC, 2004; pp 360−371.

(5) Sun, L.; Petrounia, I. P.; Yagasaki, M.; Bandara, G.; Arnold, F. H. Expression and stabilization of galactose oxidase in Escherichia coli by directed evolution. *Protein Eng*. **2001**, *14*, 699–704.

(6) Cooper, J. A. D.; Smith, W.; Bacila, M.; Medina, H. Galactose oxidase from Polyporus circinatus. *J. Biol. Chem*. **1959**, *234*, 445–448.

(7) Parikka, K.; Tenkanen, M. Oxidation of methyl alpha-D-galactopyranoside by galactose oxidase: products formed and optimization of reaction conditions for production of aldehyde. *Carbohydr. Res*. **2009**, *344*, 14–20.

(8) Hamilton, G. A.; De Jersey, J.; Adolf, P. K. Galactose oxidase: The complexities of a simple enzyme. In *Oxidases and related redox systems*; King, T. E., Mason, H. S., Morrison, M., Eds.; University Park Press: Memphis, Baltimore, 1971; pp 103−124.

(9) Root, R. L.; Durrwachter, J. R.; Wong, C. H. Enzymic synthesis of unusual sugars: galactose oxidase-catalyzed stereospecific oxidation of polyols. *J. Am. Chem. Soc*. **1985**, *107*, 2997–2999.

(10) Siebum, A.; van Wijk, A.; Schoevaart, R.; Kieboom, T. Galactose oxidase and alcohol oxidase: Scope and limitations for the enzymatic synthesis of aldehydes. *J. Mol. Catal. B: Enzym*. **2006**, *41*, 141–145.

(11) van Wijk, A.; Siebum, A.; Schoevaart, R.; Kieboom, T. Enzymatically oxidized lactose and derivatives thereof as potential protein cross-linkers. *Carbohydr. Res*. **2006**, *341*, 2921–2926.

(12) Brady, R. L.; Cheng, H. N.; Haandrikman, A. J.; Kuo, P.; Moore, A.; Riehle, R. J.; Lapre, J. A.; Mcnabola, W. T.; Nguyen, T. T.; Haandrikman, A. J.; Devries, H. T. Reduced molecular weight galactomannans oxidized by galactose oxidase. PCT Int. Appl. **2002**, 2001-US24592; 2000-222869.

(13) Yalpani, M.; Hall, L. D. Some chemical and analytical aspects of polysaccharide modifications. II. A high-yielding, specific method for the chemical derivatization of galactose-containing polysaccharides: oxidation with galactose oxidase followed by reductive amination. *J. Polym. Sci., Polym. Chem. Ed*. **1982**, *20*, 3399–3420.

(14) Frollini, E.; Reed, W. F.; Milas, M.; Rinaudo, M. Polyelectrolytes from polysaccharides: selective oxidation of guar gum - a revisited reaction. *Carbohydr. Polym*. **1995**, *27*, 129–135.

(15) Urakawa, H.; Mimura, M.; Kajiwara, K. Diversity and versatility of plant seed xyloglucan. *Trends Glycosci. Glycotechnol*. **2002**, *14*, 355–376.

(16) Freitas, R. A.; Martin, S.; Paula, R. C.; Feitosa, J. P. A.; Sierakowski, M. Effect of the oxidation level on the thermogravimetric kinetics of an oxidized galactoxyloglucan from Hymenaea courbaril (Jatoba) seeds. *Thermochim. Acta* **2004**, *409*, 41–47.

(17) Sierakowski, M. R.; Milas, M.; Desbrieres, J.; Rinaudo, M. Specific modifications of galactomannans. *Carbohydr. Polym*. **2000**, *42*, 51–57.

(18) Schoevaart, R.; Kieboom, T. Galactose dialdehyde as potential protein cross-linker: proof of principle. *Carbohydr. Res*. **2002**, *337*, 899–904.

(19) Xu, C. Physicochemical properties of water-soluble spruce galactoglucomannans. Doctoral thesis, Åbo Akademi University, faculty of Technology, Turku, Finland. 2008.

(20) Willför, S.; Rehn, P.; Sundberg, A.; Sundberg, K.; Holmbom, B. Recovery of water-soluble acetylgalactoglucomannans from mechanical pulp of spruce. *Tappi J*. **2003**, *2*, 27–32.

(21) Sundberg, A.; Sundberg, K.; Lillandt, C.; Holmbom, B. Determination of hemicelluloses and pectins in wood and pulp fibers by acid methanolysis and gas chromatography. *Nord. Pulp Pap. Res. J*. **1996**, *11*, 216–219.

(22) Kapoor, V. P.; Milas, M.; Taravel, F. R.; Rinaudo, M. Rheological properties of seed galactomannan from *Cassia nodosa* buch.-hem. *Carbohydr. Polym*. **1994**, *25*, 79–84.

(23) Wielinga, W. C. Galactomannans. In *Handbook of Hydrocolloids*; Phillips, G. O., Williams, P. A., Eds.; Woodhead Publishing: Cambridge, UK, 2000; pp 137−154.

(24) Daas, P. J. H.; Schols, H. A.; de Jongh, H. H. J. On the galactosyl distribution of commercial galactomannans. *Carbohydr. Res*. **2000**, *329*, 609–619.

(25) Dea, I. C. M.; Morrison, A. Chemistry and interactions of seed galactomannans. *Adv. Carbohydr. Chem. Biochem*. **1975**, *31*, 241–312.

(26) Willför, S.; Sjöholm, R.; Laine, C.; Holmbom, B. Structural features of water-soluble arabinogalactans from Norway spruce and Scots pine heartwood. *Wood Sci. Technol*. **2002**, *36*, 101–110.

(27) Fredon, E.; Granet, R.; Zerrouki, R.; Krausz, P.; Saulnier, L.; Thibault, J. F.; Rosier, J.; Petit, C. Hydrophobic films from maize bran hemicelluloses. *Carbohydr. Polym*. **2002**, *49*, 1–12.

(28) Yadav, M. P.; Johnston, D. B.; Hicks, K. B. Structural Characterization of Corn Fiber Gums from Coarse and Fine Fiber and a Study of Their Emulsifying Properties. *J. Agric. Food Chem*. **2007**, *55*, 6366–6371.

(29) Fishman, M. L.; Doner, L. W.; Chau, H. K.; Hoagland, P. D. Characterization of hemicellulose B from corn fiber by high-performance size-exclusion chromatography with on-line molar mass and viscometric detection. *Int. J. Polym. Anal. Charact*. **2000**, *5*, 359–379.

(30) York, W. S.; Harvey, L. K.; Guillen, R.; Albersheim, P.; Darvill, A. G. The structure of plant cell walls. XXXVI. Structural analysis of tamarind seed xyloglucan oligosaccharides using beta-galactosidase digestion and spectroscopic methods. *Carbohydr. Res*. **1993**, *248*, 285–301.

(31) Willför, S.; Sjöholm, R.; Laine, C.; Roslund, M.; Hemming, J.; Holmbom, B. Characterisation of water-soluble galactoglucomannans from Norway spruce wood and thermomechanical pulp. *Carbohydr. Polym.* **2003**, *52*, 175–187.
(32) Nishinari, K.; Yamatoya, K.; Shirakawa, M. Xyloglucan. In *Handbook of Hydrocolloids*; Phillips, G. O., Williams, P. A., Eds.; Woodhead Publishing: Cambridge, UK, 2000; pp 247−266.
(33) Mejanelle, P.; Bleton, J.; Tchapla, A.; Goursaud, S. Carbohydrate Analysis by Modern Chromatography and Electrophoresis. In *Carbohydrate Analysis by Modern Chromatography and Electrophoresis*; El-Rassi, Z., Ed.; Elsevier Science B.V.: Amsterdam, The Netherlands, 2002; pp 845−902.
(34) Hannuksela, T., Holmbom, B. Sorption of mannans onto different fiber surfaces - an evolution of understanding. In *Hemicelluloses: Science and technology*; Gatenholm, P., Tenkanen, M., Eds.; American Chemical Society: Washington, DC, 2004; pp 222−235.
(35) Mikkonen, K. S.; Yadav, M. P.; Cooke, P.; Willför, S.; Hicks, K. B.; Tenkanen, M. Films from spruce galactoglucomannan blended with poly(vinylalcohol), corn arabinoxylan, and konjac glucomannan. *BioResources* **2008**, *3*, 178–191.
(36) Mikkonen, K. S.; Tenkanen, M.; Cooke, P.; Xu, C.; Rita, H.; Willför, S.; Holmbom, B.; Hicks, K. B.; Yadav, M. P. Mannans as stabilizers of oil-in-water beverage emulsions. *LWT—Food Sci. Technol.* **2009**, *42*, 849–855.
(37) Yadav, M. P.; Johnston, D. B.; Hotchkiss, A. T.; Hicks, K. B. Corn fiber gum: A potential gum arabic replacer for beverage flavor emulsification. *Food Hydrocolloids* **2007**, *21*, 1022–1030.
(38) Redgwell, R. J.; Fischer, M. Dietary fiber as a versatile food component: An industrial perspective. *Mol. Nutr. Food Res.* **2005**, *49*, 421–535.

Received for review August 20, 2009. Revised manuscript received November 16, 2009. Accepted November 23, 2009. The study was funded by the Academy of Finland (Contract No. 117765).