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Capillary electrophoretic profiling of wood-based oligosaccharides

Salla Hiltunen · Heli Sirén · Isto Heiskanen · Kaj Backfolk

Abstract A method based on direct UV-detection was developed for the qualitative and quantitative determination of oligosaccharides simultaneously separated by capillary electrophoresis (CE). Reference compounds consisting of five xylo- three manno- and five cello-oligosaccharides were concurrently measured in a highly alkaline solution without derivatization. Sodium and potassium cations were used in the electrolyte solution to adjust the electrokinetic properties of the oligomers by controlling the mobility of charged species and to improve the resolution in baseline separation. The quantification range extended from 25 to 125 mg/L with linear correlation $R^2 = 0.986-0.997$ for all analytes other than xylobiose and cellobiose, for which the range was 50–200 mg/L ($R^2 = 0.995$). The CE method developed was further applied to determine oligosaccharides from hot-water extracts of a bleached birch and pine kraft pulp, in which the oligosaccharides typically exist as complex mixtures.

Introduction

Oligosaccharides are typical compounds formed in biomass processing. The enzymatic treatment of fibers and the hydrolysis of lignocellulose to produce bioethanol are examples of processes that produce oligosaccharides (Rydlund and Dahlman 1997; Liang et al. 2013). Xylo-oligosaccharides and manno-oligosaccharides are subjects of current research due to their biochemical and prebiotic activities (Mussatto and Mancilha 2007; Qiang et al. 2009; Moure et al. 2006; Carvalho et al. 2013; Otieno and Ahring 2012). In sample matrices, oligosaccharides typically exist in linear and branched forms in complex mixtures whose composition is highly dependent on the processing conditions and on the raw material used. Lignocellulose-based oligosaccharides can exist both in neutral and acidic forms, and the heterogenity of the samples poses great challenges for the analysis procedure. On the other hand, carbohydrates can form multiple stereoisomers that differ only in their three-dimensional spatial structure. This structural similarity of oligosaccharides

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requires high resolution and precise optimization of the separation conditions in order to avoid peak overlapping (Ristolainen 1999; Sjöberg et al. 2004).

Due to its high separation efficiency, capillary electrophoresis has been shown to be applicable for the characterization of oligosaccharides (El Rassi 1999; Kabel et al. 2006). However, certain problems related to ionization and detection have to be resolved before the analysis. Since only some of the oligosaccharides have the charge required for electrophoretic separation (such as uronic acid group), several approaches have been used to transform neutral oligosaccharides to charged species. The most common is to apply borate-based electrolyte media (400–500 mM H_3BO_3) (Rydlund and Dahlman 1997; Ristolainen 1999; Doliska et al. 2009). Borates form negatively charged carbohydrate-borate complexes under alkaline conditions (pH = 8–10). The charge of the complexes depend on the stereochemistry of carbohydrates and is a basic prerequisite for separation (Hoffstetter-Kuhn et al. 1991; El Rassi 1999). Another approach is to derivatize the oligosaccharides with with a suitable charged tag before the analysis. Sulfonated aromatic amines, which introduce a negative charge, have been used in the separation of wood-based oligosaccharides (Kabel et al. 2006; Jáĉ et al. 2014). A third option is to apply highly alkaline (pH = 12–14) conditions, in which the hydroxyl groups of oligosaccharides are partly or completely ionized (El Rassi 1999; Carvalho et al. 2003).

UV absorption is the most common detection method in commercial CE instruments and, since the oligosaccharides lack UV-absorbing chromophores, they are typically derivatized using reductive amination with aminobenzoic acid ethyl ester (ABEE), 4-aminobenzonitrile (4-ABN) or 6-aminoquinoline (6-AQ) (Ristolainen 1999; Rydlund and Dahlman 1997; Sartori et al. 2003). Rydlund and Dahlman (1996) demonstrated the separation of neutral and acidic xylo-oligosaccharides after chemical and enzymatic hydrolysis of spruce wood xylan. These oligosaccharides were separated as their borate complexes and were derivatized with 6-AQ before the analysis. Electrophoretic separation can also be coupled with laser-induced fluorescence detection (CE-LIF) (Chen and Evangelista 1995; Kabel et al. 2006; Jáĉ et al. 2014). In this case, the oligosaccharides are labeled with a fluorescent substance such as 9-aminopyrene-1,4,6-trisulfonate (APTS) or 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS). Kabel et al.

(2006) analyzed hydrothermally treated Eucalyptus wood with CE-LIF and recently Jáĉ et al. (2014) applied CE-LIF in the analysis of hydrothermally isolated xylan from beech wood.

Relatively recently it has been shown that saccharides can be separated and analyzed directly with UV under highly alkaline conditions (Rovio et al. 2007, 2008; Metsämuuronen et al. 2013). The precise nature of this direct detection method for saccharides is the subject of some debate. It has been suggested that the UV absorbance observed at a wavelength of 270 nm is based on a photo-oxidation reaction of saccharides in the detection window (Sarazin et al. 2011). The UV-absorbing compound has been claimed to be malondialdehyde (Sarazin et al. 2011; Schmid et al. 2015) or carboxylate (Oliver et al. 2014). The advantage of direct detection is that it simplifies the analysis procedure by reducing the sample pretreatment necessary and facilitating a greater automatization during the analysis procedure. In addition, the use of expensive and hazardous derivatization chemicals can be avoided. The drawbacks are that acetylated oligosaccharides cannot be quantified, since acetyl groups are easily cleaved under alkaline conditions. Two studies (Metsämuuronen et al. 2013; Alinat et al. 2015) discuss the separation of oligosaccharides in highly alkaline conditions using direct UV detection, and in both cases the application was related to homologous series of oligosaccharides.

The aim of the present study was to extend the separation and quantification of underivatized oligosaccharides in mixtures. In this study, 13 neutral and unsubstituted oligosaccharides were chosen for analysis: xylo-oligosaccharides having a degree of polymerization (DP) of 2–6, manno-oligosaccharides (DP = 2–4) and cello-oligosaccharides (DP = 2–6). These oligosaccharides can all be released from lignocellulosic matrices as a result of thermal treament and then are also compounds that may interfere with each other during the analysis. Arabinose- and galactose-oligomers were excluded from the study, because the amounts of arabinogalactan in normal hardwood and softwood species are insignificantly low (Ristolainen 1999). Highly alkaline conditions and direct UV detection were chosen in order to reduce sample pre-treatment by avoiding the derivatization procedure. An earlier established method for monosaccharide separation (Rovio et al. 2007) was first used to monitor the compounds. Since the resolution was not satisfactory,

the composition of the electrolyte solution and the parameters of the CE instrument were carefully optimized. The method developed was subsequently applied to the characterization of the neutral oligosaccharides liberated from a hot-water-treated bleached kraft pulp.

Materials and methods

Chemicals

Linear unsubstituted oligosaccharides 1,4-β-D-Xylobiose (x2, purity 90 %), 1,4-β-D-Xylotriose (x3, purity 95 %), 1,4-β-D-Xylotetraose (x4, purity 95 %), 1,4-β-D-Xylopentaose (x5, purity 95 %), 1,4-β-D-Xylohexaose (x6, purity 95 %), 1,4-β-D-Cellotriose (c3, purity 95 %), 1,4-β-D-Cellotetraose (c4, purity 95 %), 1,4-β-D-Cellopentaose (c5, purity 95 %), 1,4-β-D-Cellohexaose (c6, purity 90 %), 1,4-β-D-Mannobiose (m2, purity 95 %), 1,4-β-D-Mannotriose (m3, purity 95 %), 1,4-β-D-Mannotetraose (m4, purity 95 %) were purchased from Megazyme International Ireland. 1,4-β-D-Cellobiose (c2, purity 98 %) was purchased from Alfa Aesar, Karlsruhe, Germany.

Sodium phosphate tribasic (Na_3PO_4, 96 %) was purchased from Sigma-Aldrich, Gillingham, Dorset, United Kingdom, Tri-potassium phosphate-7-hydrate ($K_3PO_4·7H_2O$, 98 %), sodium hydroxide (NaOH, 99 %) and potassium hydroxide (KOH, pro analysis $\geq$ 85 %) were purchased from Merck, Darmstadt, Germany. Ultra pure water (18 mΩ cm) was prepared with an Elga Centra-R 60/120 purification system.

Instruments

The oligosaccharides were analysed using a Beckman-Coulter P/ACE MDQ (Fullerton CA, USA) capillary electrophoresis instrument, with a photodiode array detector (PDA) at a wavelength of 270 nm and a bandwidth of 10 nm. The capillary (Polymicro Technologies) was made of fused silica (total length 96 cm, separation distance to the detector 86 cm and inner diameter 50 μm). The temperature of the capillary was thermostatted at 15 °C and controlled with a liquid coolant during the separations. The capillary was conditioned by rinsing with 0.1 M sodium hydroxide, ultra high purity water, and the electrolyte solution for 30 min each. Between analyses, the capillary was rinsed with electrolyte solution for 5 min. The samples

were introduced by hydrodynamic injection with a pressure of 0.7 psi for 19 s.

Electrolyte and standard solutions

The background electrolyte solution consisted of 35 mM Na_3PO_4, 35 mM K_3PO_4, 90 mM NaOH, and 65 mM KOH in aqueous solution. The working solution of the buffer was prepared volumetrically from stock solutions by mixing 1 M NaOH, 1 M KOH, 100 mM K_3PO_4, 80 mM Na_3PO_4 and purified water. The electrolyte solutions used for the separations were degassed in an ultra-sonic bath for 20 min before use.

Stock solutions of 10,000 mg/L of xylobiose, xylotriose, xylotetraose, mannobiose, mannotriose, cellobiose, cellotriose and cellotetraose were prepared together with stock solutions of 5000 mg/L for mannotetraose, cellopentaose, cellohexaose, xylopentaose and xylohexaose. The working solution of the 13 oligosaccharides was 500 mg/L for each individual oligosaccharide.

Hot water extraction of pulp

The hot water extractions were carried out for different times (15–90 min) at different temperatures (100−160 °C). Four grams of dried industrial elemental chlorine-free bleached birch kraft pulp or dried pine kraft pulp was mixed with 30 mL of ultra high purity water. The mixture was placed in a smalll stainless steel container having a volume of 36 mL. The samples were heated to the reaction temperature in an oven and the time was measured for the time when the temperature reached the desired value. After heat treatment, the samples were cooled in cold water for 15 min and the solids were separated by centrifugation (500g, ∼2000 rpm) for 30 min. The concentrations and types of oligosaccharides remaining in the aqueous phase were determined using the developed CE method.

Results and discussion

Development of method for oligosaccharide separation

The composition of the electrolyte originally proposed by Rovio et al. (2007), was optimized by modification
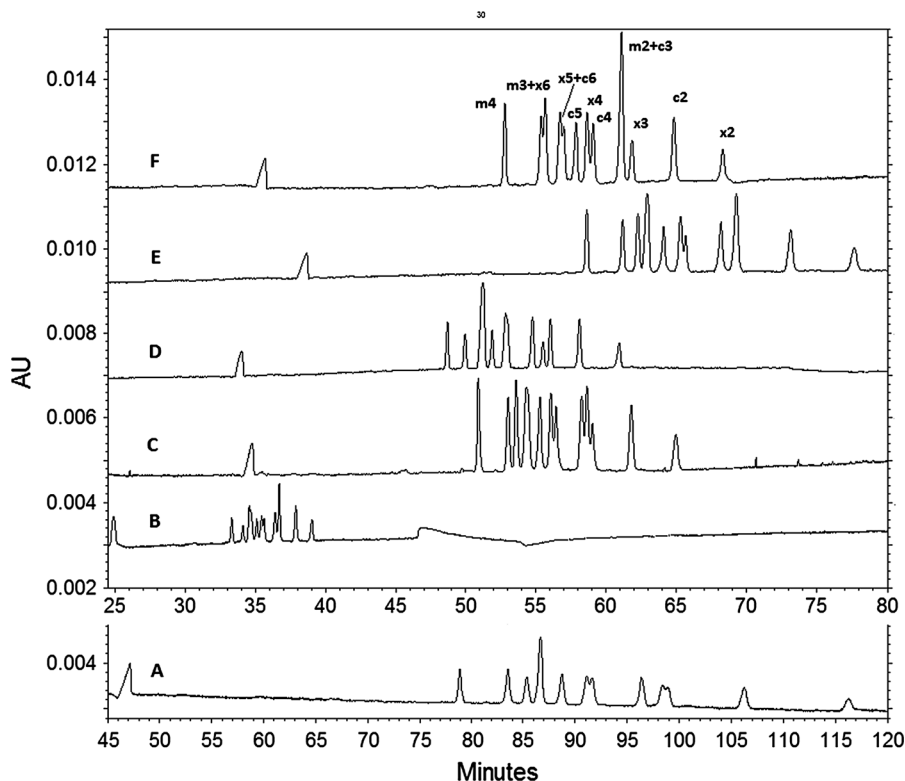
Fig. 1 Electropherogram showing the effect of electrolyte composition on the separation of oligosaccharides. (A) 130 mM NaOH + 36 mM Na$_3$PO$_4$, (B) 130 mM KOH + 36 mM K$_3$PO$_4$, (C) 65 NaOH + 65 KOH + 18 mM K$_3$PO$_4$ + 18 mM Na$_3$PO$_4$, (D) 130 mM KOH + 36 mM Na$_3$PO$_4$, (E) 86,667 mM NaOH + 24 mM Na$_3$PO$_4$ + 43,333 mM KOH + 12 mM K$_3$PO$_4$, (F) 130 mM NaOH + 36 mM K$_3$PO$_4$. Instrumental parameters: capillary 116/126 cm (L$_{det}$/L$_{tot}$), applied voltage +20 kV (normal polarity), injection 19 s 0.7 psi, UV-detection at 270 nm, temperature 20 °C. C = cello-oligosaccharide, m = manno-oligosaccharide and x = xylo-oligosaccharide. Degree of polymerisation is marked with a number

of the sodium or potassium hydroxide solutions with trisodium or tripotassium phophate salts. The phosphate and hydroxide ion concentrations in the the electrolyte solutions were maintained constant at 36 and 130 mM respectively (as in the work of Rovio et al. 2007), but the ratio of Na$^+$ to K$^+$ in the system was varied. Figure 1 presents results with different combinations of NaOH, KOH and K$_3$PO$_4$ and Na$_3$PO$_4$. Electrolyte solution consisting only of sodium salts NaOH-Na$_3$PO$_4$ (Fig. 1a) resulted notably lower electrophoretic mobilities and a better resolution of oligosaccharides than the potassium-based electrolyte KOH-K$_3$PO$_4$ (Fig. 1b), but the oligosaccharides were still not resolved. On the other hand, potassium salts (KOH-K$_3$PO$_4$), resulted fast migration times but insufficient resolution between oligosaccharides (Fig. 1b). A compromise between analysis time

and resolution was achieved with mixed combinations of NaOH, KOH, Na$_3$PO$_4$ and K$_3$PO$_4$ (Fig. 1c–f).

It was found that a higher phosphate and higher hydroxide ion concentration improved the resolution, but that analysis time increased (Fig. 2a–c). A more concentrated electrolyte was beneficial, especially for the separation of x3, c3, m2 and m3, x6, x5. The analysis time could be somewhat shortened by reducing the capillary length: lengths of 86 cm (L$_{det}$) and 96 cm (L$_{tot}$) gave the best combination of analysis time, acceptable resolution and low current during separation.

Figure 2d show the separation of a standard mixture containing 13 different oligosaccharides under optimized conditions. The primary goal in the use of alkali metal hydroxides was to ionize the hydroxyl groups of the oligosaccharides, and this emphasized
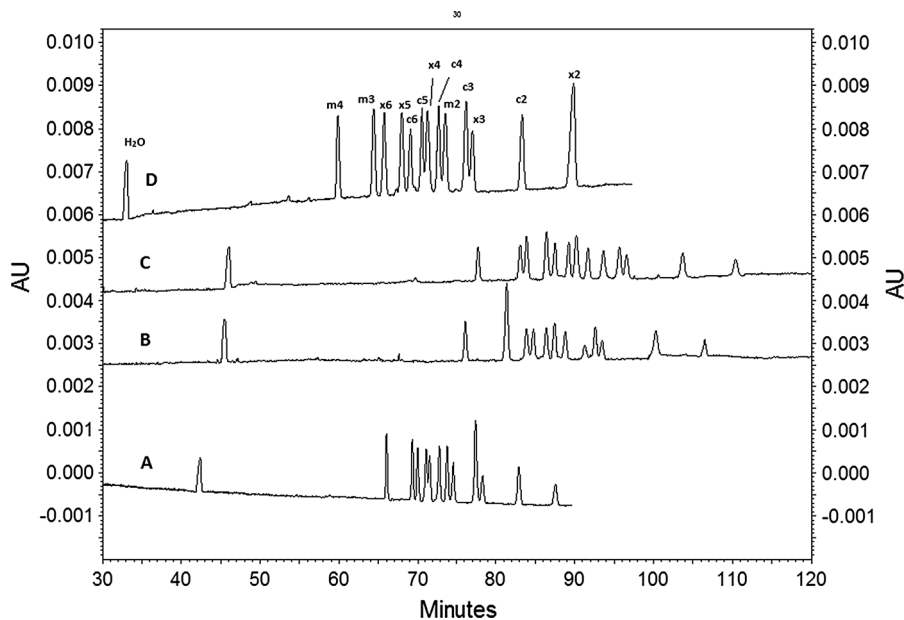
Fig. 2 Electropherograms showing the effect of buffer composition on separation of oligosaccharides. (*A*) 65 NaOH + 55 KOH + 25 mM K_3PO_4 + 25 Na_3PO_4, (*B*) 75 NaOH + 65 KOH + 35 mM K_3PO_4 + 35Na_3PO_4, (*C*) 80 mM NaOH + 65 mM KOH + 35 mM Na_3PO_4 + 35 mM K_3PO_4 instrumental parameters: capillary 116/126 cm (L_{det}/L_{tot}), applied voltage +20 kV (normal polarity), injection 19 s 0.7 psi, UV-detection at 270 nm. Temperature 15 °C. (*D*) 90 mM NaOH, and 65 mM KOH, 35 mM Na_3PO_4, 35 mM K_3PO_4, instrumental parameters: capillary 86/96 cm (L_{det}/L_{tot}) applied voltage +20 kV (normal polarity), injection 19 s 0.7 psi, UV-detection at 270 nm. Temperature 15 °C. C = cello-oligosaccharide, m = manno-oligosaccharide and x = xylo-oligosaccharide. Degree of polymerisation is marked with a number

the differences in the pKa-values of the individual OH-groups of the oligosaccharides. Phosphate provided some buffering capacity and because the mobility of phosphate anions is less than that of hydroxide ions (Carvalho et al. 2003) oligosaccharides migrated more slowly and the resolution was improved. Sodium and potassium were used in electrolyte solution to control the electro-osmotic flow and the mobility of the oligosaccharides. Colon et al. (1993) suggested that sodium and potassium affect the electro-osmotic flow differently because they have different ratios of ionic charge to ionic radius and this leads to differences in the sphere of solvation of the alkali metals. It is also possible that sodium and potassium participate in forming weak ion-pairs between the alkali metals and the ionized hydroxyl groups. Several studies (Rendleman 1967; Moulik and Khan 1975; Angyal 1980; Ortiz et al. 2005) have discussed the interaction between sugars and alkali metals. The interaction depends on several factors, such as the orientation of the hydroxyl groups, the charge of the cation and its atomic radius. In the present case both the alkali metal cations are univalent

and thus neither is favoured over the other. An optimal ionic radius of the cation is 100–110 picometers (pm) (Angyal 1980; Brown 1994) and this slightly favours sodium (102 pm) over potassium (138 pm). With regard to the orientation of the hydroxyl groups, manno-oligosaccharides differ from cello and xylo-oligosaccharides in that manno-oligosaccharides have cis diol on a six-membered ring (at the C2 and C3 positions) which is more favorable to interaction with the alkali metal cations than the trans diol of the cello-oligosaccharides. This may give manno-oligosaccharides a fast mobility in the applied electrolyte solution; as is seen in Fig. 2 where manno-oligosaccharides migrate to the detector before the cello-oligosaccharides despite the fact that cello-oligosaccharides are diastereomers with manno-oligosaccharides and thus have the same molar masses and an equal number of hydroxyl groups.

Calibration and detection limits

The structural similarity of the oligosaccharides posed great challenges for the separation process. A

satisfactory separation and resolution were achieved only with a highly concentrated electrolyte solution and with a long capillary. This led to small electrophoretic mobility values of the oligosaccharides, as can be seen in Table 1. The analysis time with this method (approximately 90 min) is much longer than those reported for homological series of oligosaccharides under highly alkaline conditions (20–30 min) (Metsämuuronen et al. 2013; Alinat et al. 2015) or with borate-based electrolytes (<20 min) (Ristolainen 1999; Sartori et al. 2003; Jáĉ et al. 2014). The long analysis time is a notable drawback when considering multiple samples despite the fact that derivatization is not needed and the analyses are carried out automatically. The resolution values between the compounds separeted in the current study varied from 0.7 to 4.2. Generally, it can be stated that separation has been achieved if the resolution value is >1. With the current method, baseline-to-baseline separation was not achieved for cellohexaose, xylotetraose and xylotriose. The difficulty in separating oligosaccharides as their mixtures has been reported for derivatized oligosaccharides (Ristolainen 1999; Sartori et al. 2003; Jáĉ et al. 2014)

The CE method developed in the present work was calibrated with aqueous standard solutions. Cellobiose and xylobiose were calibrated using a five-point calibration at concentration levels of 50–200 mg/L. Other oligosaccharides were calibrated using a five-

point calibration in the 25–125 mg/L range. The calibration results are shown in Table 2. The correlation coefficients (R^2) of the resulting calibration curves varied from 0.986 to 0.997. The detection limits (LOD) for the compounds calculated from the signal area using using signal-to-noise ratio S/N = 3 and limit of quantification (LOQ) S/N = 10 are presented in Table 2. The LOD and LOQ values of the xylo-and cello-oligosaccharides are in the same range as those reported in a previous study (Sartori et al. 2003) where LOQ varied from 9.2 to 45 mg/L.

Analysis of hot-water-treated bleached pulp samples

The method was applied to hot-water-extracted bleached pulp samples, where the existence of the oligosaccharides in the samples was determined by comparing the electrophoretic mobilites and by spiking the samples with pure standards. The results are shown in Table 3. Two different raw materials were used in the hot-water treatment, birch (B) and pine (P). The hot-water treatment was carried out for different temperatures and different times between 100 and 160 °C and between 15 and 90 min.

Figure 3 presents electropherograms of undiluted hot-water-treated birch and pine samples prepared at temperatures from 130 to 160 °C. In the case of the birch pulp, at the lowest temperature, 100 °C, and,

Table 1 Electrophoretic mobility and resolution of oligosaccharides

Analyte	Migration time (min)	RSD (%)	Mobility ($m^2\ V^{-1}\ s^{-1}$)	RSD (%)	Resolution
EOF	33.7	3.8	2.04E−08	3.9	
m4	59.0	4.7	−8.76E−09	3.2	
m3	63.4	4.8	−9.58E−09	3.1	3.0
x6	64.7	5.0	−9.79E−09	3.0	0.9
x5	66.9	5.0	−1.01E−08	3.0	1.4
c6	67.8	5.1	−1.03E−08	2.9	0.7
c5	69.3	5.2	−1.05E−08	2.9	1.3
x4	70.1	5.1	−1.06E−08	3.0	0.7
c4	71.4	5.2	−1.08E−08	3.0	1.3
m2	72.3	5.1	−1.09E−08	3.1	0.7
c3	74.8	5.3	−1.12E−08	3.0	1.9
x3	75.6	5.3	−1.13E−08	3.0	0.7
c2	81.7	5.5	−1.20E−08	3.0	4.2
x2	87.9	5.8	−1.26E−08	3.0	2.8

Relative standard deviations (RSD) are calculated using seven five-point calibrations

Table 2 Limits of quantification (LOQ), limits of detection (LOD), regression equations (based on peak areas) and correlation coefficients (R^2) of the different oligosaccharides

Analyte	LOD (mg/L)	LOQ (mg/L)	Equation	R^2 (%)
m4	3.8	12.8	$y = 467x - 1155$	0.992
m3	3.1	10.3	$y = 682x - 1894$	0.995
x6	5.7	18.9	$y = 320x - 837$	0.989
x5	3.4	11.2	$y = 669x - 659$	0.991
c6	5.8	19.2	$y = 463x - 4495$	0.986
c5	3.4	11.3	$y = 679x - 2314$	0.995
x4	4.4	14.7	$y = 426x - 2983$	0.992
c4	4.9	16.2	$y = 587x - 3705$	0.995
m2	4.4	14.5	$y = 669x - 5788$	0.997
c3	4.3	14.3	$y = 797x - 5285$	0.994
x3	5.6	18.6	$y = 446x - 2220$	0.988
c2	4.5	14.9	$y = 850x - 11982$	0.995
x2	6.0	20.2	$y = 526x - 7145$	0.995

Table 3 Amounts of oligosaccharides found in hot-water-treated samples

Analyte	B 130 °C 53 min (mg/g$_{fiber}$)	RSD (%)	B 160 °C 15 min (mg/g$_{fiber}$)	RSD	B 160 °C 90 min (mg/g$_{fiber}$)	RSD (%)	P 160 °C 90 min (mg/g$_{fiber}$)	RSD (%)
m4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.37	1.37
m3	0.15	2.15	0.12	1.43	0.34	2.42	0.60	2.09
x6	0.23	2.63	0.15	0.68	0.70	2.05	0.60	1.85
x5	0.15	1.90	0.11	2.76	0.75	1.10	0.65	1.48
c6	n.d.	n.d.	n.d.	n.d.	<0.1	n.d.	<0.1	n.d.
c5	n.d.	n.d.	n.d.	n.d.	<0.1	n.d.	<0.1	n.d.
x4	0.21	0.77	0.18	0.17	1.80	4.16	0.70	4.13
cd c4	n.d.	n.d.	n.d.	n.d.	0.20	1.32	<0.1	n.d.
m2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.30	2.61
c3	n.d.	n.d.	n.d.	n.d.	0.18	2.61	<0.1	1.37
x3	0.33	1.41	0.25	3.09	5.76	0.36	1.30	1.11
c2	n.d.	n.d.	n.d.	n.d.	0.18	0.95	n.d.	n.d.
x2	0.30	2.99	0.25	2.43	11.60	3.37	2.75	3.36

RSD's are based on three repetitions

n.d. not detected

with reaction times of 15 and 90 min, oligosaccharides were not detected but oligosaccharides were detected at 130 °C and 53 min reaction time and at 160 °C and 15 and 90 min reaction times. The samples contained mainly xylo-oligosaccharides, xylobiose and xylotriose being the most abundant components. According to the analyses, the hot water treated birch samples contained also small amount of mannotriose. However, it is not likely that a single trimer without any corresponding oligomers occurs in the sample. For this reason, it is possible that an unknown component coeluates with mannotriose. The profiles were very similar at 130 °C for 53 min and at 160 °C for 15 min, i.e. the same results can be obtained by treating the birch kraft pulp for an hour at 130 °C or for 15 min at 160 °C. The highest temperature (160 °C) and longest reaction time (90 min) gave the largest amount of oligosaccharides. At this temperature, a small amount of cello-oligosaccharides was also detected which may indicate cellulose degradation. The sample at 160 °C and 90 min reaction time had to be diluted with ultra high purity water before the analysis in order to determine the amounts of the most abundant components (xylobiose, xylotriose, xylotetraose).
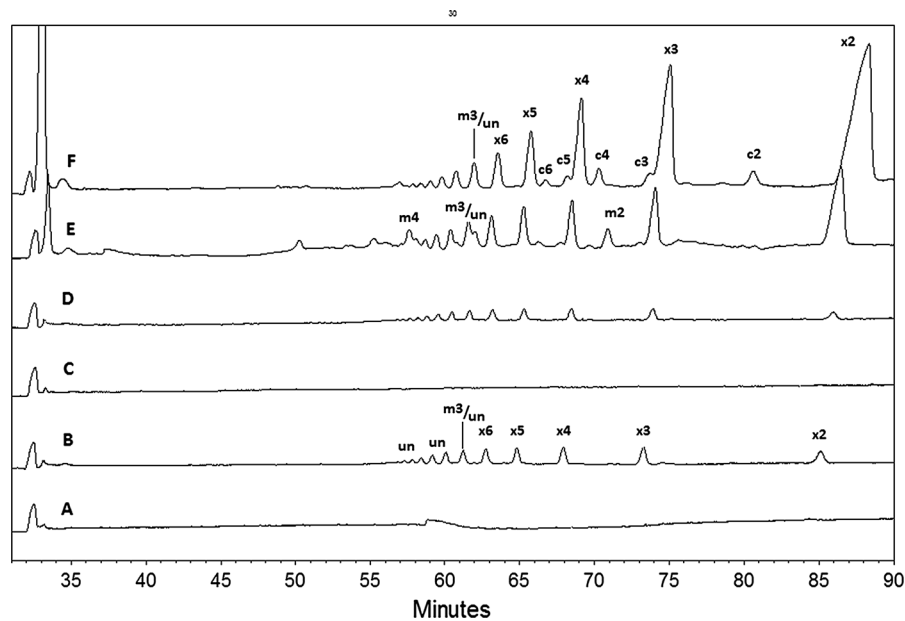
Fig. 3 Electropherograms of hot-water-treated bleached birch (B) and pine (P) pulp. (*A*) P 130 °C 53 min, (*B*) B 130 °C 53 min, (*C*) P 160 °C 15 min, (*D*) B 160 °C 15 min, (*E*) P160 °C 90 min, (*F*) B 160 °C 90 min B. Electrolyte 35 mM Na_3PO_4, 35 mM, K_3PO_4 65 mM KOH, 90 mM NaOH. Capillary 86/96 cm, injection 0.7 psi for 19 s, separation temperature 15 °C. C = cello-oligosaccharide, m = manno-oligosaccharide, x = xylo-oligosaccharide and un = unknown. Degree of polymerisation is marked with a number. The electropherograms have been stacked and are in the same scale to simplify the comparison

A similar set of experimets was carried out with bleached pine pulp. Only the highest temperature (160 °C) and longest reaction time (90 min) dissolved oligosaccharides from this sample, as can be seen in Fig. 3, electropherogram I. The amount of dissolved oligosaccharides was smaller than that from the birch sample treated with hot water under the same conditions. At lower temperatures or shorter reaction times, oligosaccharides were not detected. Interestingly, the amount of manno-oligosaccharides (DP = 2–4) in the pine samples was rather small, although (galacto)glucomannan is the most abundant hemicellulose in softwood. It is probable that (galacto)glucomannans were already dissolved from the fiber in the alkaline kraft cooking and bleaching stage and that the hydrolytic treatment did not hydrolyze the remaining galactoglucomman into oligosaccharides.

Concluding remarks

A method has been developed where three different linear oligosaccharide groups, 1,4-β-D-cello-, xylo, and manno-oligosaccharides have been separated from each other in a single analytical analysis. The studied cello- and mannooligomers (DP = 2-6) were separated from each other, although they are diastereomers with each other and have the same molar masses and the same number of hydroxide groups. Sodium and potassium were used in the electrolyte solution to adjust the electrophoretic mobility and to enhance resolution between the oligosaccharides. The oligosaccharides were detected using direct UV-detection, without any derivatization procedure, which simplifies the analysis and increases automatization. The CE method developed was further applied in determination of oligosaccharides from aqueous hot water treated pulp samples, in order to study the degradation of hemicelluloses into oligosaccharides as functions of time and temperature.

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