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Determination of uronic acids and neutral carbohydrates in pulp and biomass by hydrolysis, reductive amination and HPAEC-UV

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Abstract: The exact quantification of all carbohydrate constituents in wood and pulp is a challenge because of the various glycosidic linkages of the polysaccharides with different stabilities. The individual detector responses for the compounds in the hydrolysates additionally complicate the quantification as pure standards for 4-*O*-methyl-α-D-glucuronic acid (meGlcA) and related oligosaccharides are not commercially available for calibration. In the present paper, a new analytical procedure is presented, based on the reductive amination of the carbohydrates obtained via acidic and enzymatic hydrolysis of the polysaccharides before quantification by means of high performance anion exchange chromatography (HPAEC) and UV-detection. This approach was suitable for the analysis of neutral carbohydrates and uronic acids obtained via enzymatic hydrolysis from bleached pulps. In the case of unbleached pulps, the enzymatic hydrolysis was not complete and unhydrolyzed nano-scaled and micro-scaled particles remained in the hydrolysates as detected by dynamic light scattering (DLS) measurements. The new HPAEC-UV methodology was also applied to kraft pulps and a sulfite pulp; six different kinds of wood as well as wheat straw and bagasse. All relevant monosaccharides and the dimer of meGlcA and xylose could be detected in the hydrolysates. Accordingly, significantly higher yields of meGlcA were found compared to literature data.

Keywords: 2-aminobenzoic acid, anthranilic acid, biomass, dynamic light scattering, enzymatic hydrolysis, HPAEC-UV/VIS, labeling, 4-O-methyl-α-D-glucuronic acid, particle size, reductive amination, xylan

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Introduction

The traditional production of pulp and paper became a new impetus by the new biorefinery technologies, with many of them aiming at hemicelluloses prehydrolysis and a value added hemicelluloses utilization (Fernando et al. 2006; Park and Um Byung 2015; Reyes et al. 2015; Kleen et al. 2016; Lloyd and Murton 2016; Nebreda et al. 2016; Rivas et al. 2016; Roselli et al. 2016; Zhou et al. 2016). For an optimal process design in these new approaches, a detailed knowledge about the chemical composition of the feedstocks is necessary, which is also relevant for new products such as films and aerogels (Liebner et al. 2012; Alekhina et al. 2014; Zhao et al. 2016). Lignocellulosic materials consist of polysaccharides and lignin, while cellulose is the most abundant polymer followed by several hemicelluloses (xylan, mannan, and galactan) and pectin (Kačuráková et al. 2000; Ebringerová et al. 2005).

The analysis of the polysaccharide moiety of lignocellulosics and pulps requires a hydrolysis step, which is usually achieved via hydrolysis with sulfuric acid (Saeman et al. 1954; Willför et al. 2009). It is well known that a complete hydrolysis into monosaccharides is difficult to realize due to the side-reactions leading to furfural, 5-hydroxymethyl furfural, formic acid and levulinic acid (Dahlman et al. 2000; Palmqvist and Hahn-Hägerdal 2000; Pilath et al. 2010; Lorenz et al. 2016). On the other hand, too gentle conditions result in a partial hydrolysis, mainly with residual oligosaccharides of 4-*O*-methyl-α-D-glucuronic acid (meGlcA) and xylose. Moreover, xylose and meGlcA related oligosaccharides in the hydrolysates are difficult to identify and quantify as such compounds are not commercially available (Lorenz et al. 2016).

Delignified or steam treated biomass as well as the application of co-solvent systems enable the hydrolysis by mild enzymatic procedures, which minimize the structural modification (Dahlman et al. 2000; Schütt et al. 2013; Han et al. 2017). According to Dahlman et al. (2000), the total determined carbohydrate amounts are between 4.4% and 12.1% higher in case of enzymatic hydrolysis (EH) compared to acid hydrolysis (AH) of pulps. Though EH in combination with fermentation processes for ethanol or biogas

production is well developed in the meanwhile, there are still limiting factors detracting from this approach for quantitative analysis. For example, modification of the biomass surface after drying (crustification) may prevent close surface contact with the enzymes. Chemical interactions between lignin, cellulose and xylan may form complexes not accessible for the enzymes and an incomplete hydrolysis is the result (Hendriks and Zeeman 2009; Penttilä et al. 2013).

For the chromatographic separation and quantification of the products in the hydrolysates, high performance anion exchange chromatography (HPAEC), frequently used in combination with pulsed amperometric detection (PAD), is an established method (Gullón et al. 2011; Manns et al. 2014; Anders et al. 2015). PAD is known for high sensitivity and selectivity for oxidizable molecules, but its response to various compounds is very different. Therefore, PAD detection must be calibrated by pure standards. This procedure is suitable for most of the monosaccharides, but its application for meGlcA (or related oligosaccharides) quantification is aggravated, because pure standards are not commercially available. Therefore, these and similar compounds are significantly underestimated in the course of polysaccharide analysis.

Another analytical method is the depolymerization of polysaccharides combined with a simultaneous derivatization of the produced monomers by acid methanolysis followed by GC analysis (Bertaud et al. 2002). This approach is suitable for pectins and hemicelluloses leading to the exact quantification of uronic acids, including meGlcA. However, the method is not appropriate for the hydrolysis of cellulose in biomass and pulp feedstocks. According to Willför et al. (2009), the determined carbohydrate yields are around 23% for birch kraft pulp and between 33% and 49% for several other biomass samples, showing the incomplete quantification rate. Altaner and Saake (2016) published data regarding the characterization of lignocellulosics by ^{1}H-NMR spectroscopy. Though less sensitive than chromatographic procedures, this approach enables the fast and simultaneous analysis of carbohydrates and their degradation products such as furfural, 5-hydroxymethyl furfural and levulinic acid.

Lorenz et al. (2016) published a new approach for the analysis of isolated 4-*O*-methyl-α-glucurono-D-xylans, where the quantification of acidic oligosaccharides after partial acid hydrolysis has been described. The essence of the method is the reductive amination of all carbohydrates in the hydrolysates, which allows the quantification of the monomer meGlcA and oligosaccharides of meGlcA linked to xylose as well as of the other relevant monosaccharides. One of the appropriate derivatization agents for reductive amination is 2-aminobenzoic acid (2-AA) (Anumula 2014). The reduction of the unstable intermediate, which is produced during the reaction, is usually realized by sodium cyanoborohydride, as introduced by Borch et al. (1971). However, this compound is highly toxic. The substitution of this reducing agent with the non-toxic 2-picoline-borane complex (Figure 1) is a good alternative (Unterieser and Mischnick 2011).

The aim of the present study is the adaptation of the HPAEC-UV analysis to more complex pulp and biomass samples for a more precise determination of the total carbohydrate composition via hydrolysis in combination with the reductive amination. The recovery rates of carbohydrates by this approach were determined previously and the results are summarized in Table 1. The hydrolysis of pulps should be realized by means of AH and EH and the cleavage efficiencies should be compared. For comparison, pulps will also be analyzed by the established standard borate-HPAEC.

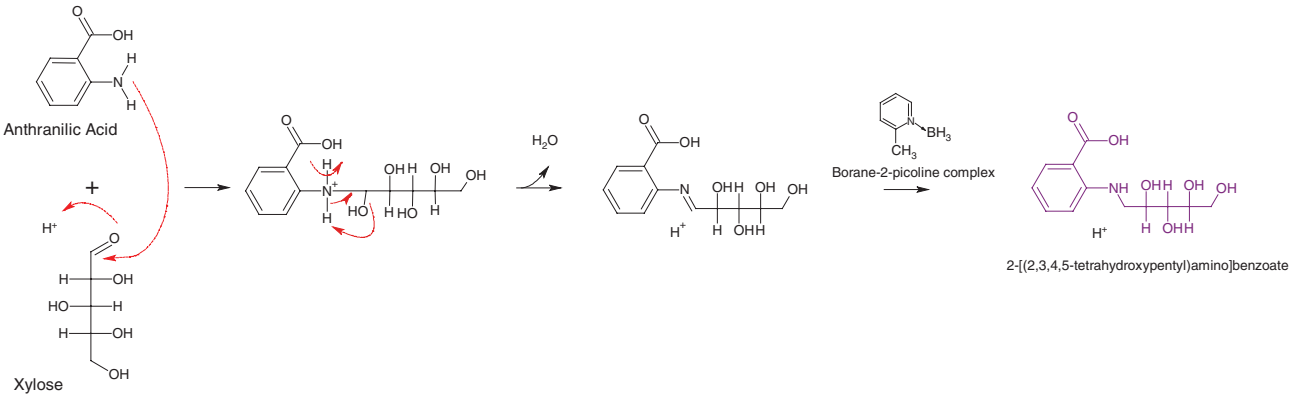

Figure 1: Reductive amination of carbohydrates, using the example of xylose.
The amine compound is anthranilic acid (2-AA), the reducing agent is borane-2-picoline complex.

Table 1: Recovery rates of carbohydrates after reductive amination and analysis by HPAEC-UV/VIS, adapted from Lorenz et al. (2016).

Carbohydrate	Recovery rate (%)
Xylose	95.9 ± 1.7
Galactose	100.7 ± 2.6
Glucose	95.9 ± 1.7
Arabinose	102.6 ± 3.1
meGlcA-Xyl	109.9 ± 3.6
meGlcA	94.3 ± 5.0
GalA	98.1 ± 3.4
GlcA	103.0 ± 3.2

Materials and methods

Preparation of pulps: The following kraft pulps (KPs) were analyzed: spruce unbleached KP (Zellstoff Rosenthal, Germany), spruce bleached (BKP) (Zellstoff Rosenthal, Germany), and beech unbleached KP (technical plant at Hamburg University, Germany; T_{max} 165°C, t 90 min, L:W ratio 4:1, NaOH 20%, Na_2S 30%). A bleached sulfite pulp (BSP) from beech (SAPPI, Stockstadt am Main, Germany) was also analyzed. The feedstocks were fluffed with a lab mill (A10, Janke und Kunkel, Staufen im Breisgau, Germany) for 90 s. Remaining alkali from the pulping or bleaching process was removed completely with a 0.1 M HCl solution and the purified pulps were dried before further treatment.

Preparation of wood and annual plants: Wood from *Picea abies* (L.) H. KARST, *Eucalyptus globulus* LABILL., *Fagus sylvatica* L., *Quercus alba* L., *Alnus glutinosa* (L.) GAERTN., and *Populus alba* L. was chopped into chips. The feedstocks wheat straw and bagasse were shred into 2–3 cm long pieces. The raw materials were dried for 72 h at 45°C. Afterwards, homogeneous matrices were produced by milling with a swing mill HP-M 100P (Herzog, Osnabrück, Germany) for 60 s.

Enzymatic hydrolysis (EH): The prepared pulps were submitted to EH (72 h, 45°C) by addition of 300 µl Cellic-C Tec 2 (cellulase complex with a filter paper activity of 101 FPU ml^{-1}; Novozymes A/S, Bagsvaerd, Denmark) and 50 µl Novozymes 188 (β-glucosidase with an activity of 227 CBU ml^{-1}; Novozymes A/S, Bagsvaerd, Denmark) per gram of sample material. The experiments were performed in ammonium acetat (Merck, Darmstadt, Germany) buffer (pH 4.8).

Two-stage acid hydrolysis (AH): The milled and dried biomass was hydrolyzed according to Lorenz et al. (2016). In short, 100 mg of each matter were treated with 1 ml of 72% H_2SO_4 (Honeywell, Seelze, Germany). The suspensions were conditioned to 30°C and pre-hydrolyzed for exactly 60 min. This first hydrolysis was stopped by adding 3 ml of water. After dilution with 25 ml water (leading to H_2SO_4 conc. of 2.5%), the samples were treated in an autoclave (Systex VX-75 from Systec GmbH, Linden, Germany) for 30 min at 120°C. The hydrolysis residues were filtrated, washed, dried and weighed (lignin content); the filtrated solutions were filled up to 50 ml.

Analysis of carbohydrates by borate-HPAEC: Borate-HPAEC was applied (Lorenz et al. 2016) as the standard procedure.

Analysis of reductively aminated carbohydrates by HPAEC-UV: Sulfate ions are detrimental for HPAEC-UV analysis and the sample preparation, thus, 5 ml of each hydrolyzed sample was mixed with 10 ml $Ba(OH)_2$ (40 gl^{-1}) (Merck Suchardt, Hohenbrunn, Germany) for sulfate removal. The precipitated $BaSO_4$ was removed after centrifugation and the residue was rinsed with 2 ml of water three times. The solutions were pooled and water was added up to a total volume of 25 ml. For UV-detection of carbohydrates, the analytes were reductively aminated according to Lorenz et al. (2016). Derivatization with 2-aminobenzoic acid (Merck Suchardt, Hohenbrunn, Germany) was catalyzed with acetic acid (Fisher Chemical, Loughborough, UK) and reduction was carried out with 2-picoline borane complex (Aldrich Chemical, Steinheim, Germany).

Subsequently, the solutions were dried with a N_2 stream to remove all volatile compounds, 50 µl of the internal standard 4-aminobenzoic acid (2 gl^{-1}) (Aldrich Chemistry, Steinheim, Germany) was added and the residue was dissolved in 1 ml of water. For chromatographic analysis, 10 µl of each sample was injected in a HPAEC-system ICS 3000 (Dionex). The system was operated with a guard column CarboPac™ PA200 3 × 50 mm (Dionex) and an analytical column CarboPac™ PA200 3 × 250 mm (Dionex) and two eluents, namely deionized water (A) and 1 M sodium acetate (ChemSolute, Renningen, Germany) in 200 mM NaOH (B) (Fluka Analytical, Steinheim, Germany). The gradient development was as follows: 7.5% of B for 30 min→increasing B within 30 min to 60%→keeping constant for 25 min. To clean the system, it was flushed with 200 mM NaOH for 20 min. The subsequent equilibration was done with 7.5% B for 10 min. The flow rate in the system was 0.4 ml min^{-1} at 30°C. UV detection occurred at 328 nm and quantified by means of calibration with 2-AA as an internal standard (IS), detected at 309 nm. All integrated peak areas are referred to that of 2-AA as IS. For quantification, the molar masses of the respective compounds were reduced by the mass of water and used for the calculations. In this way, the condensed forms of the saccharides in the polymer were taken into account. One end-group of each polysaccharide is not included in these calculations. Referring to the total mass of the polysaccharides, the influence of one water molecule can be neglected. Based on the general quantification limits determined previously (Lorenz et al. 2016), the limits of quantification for the applied conditions were around 0.15% referred to sample dry weight. Coefficients of variation were between 3% and 10%.

Kappa number (KN): The determination was done according to TAPPI (1999). If the KN is multiplicated by a factor of 0.13, the Klason lignin content can be approximated.

Particle size analysis: The distribution of the particle size in the hydrolysates of EH and AH was determined with a dynamic light scattering particle size analyzer Horiba LB-550 (Retsch Technology, Haan, Germany). For this purpose, 1.5 ml of each hydrolysate was placed in a disposable cuvette (Plastibrand, Brand GmbH, Wertheim, Germany) and the light-scattering intensity was detected 3 times for each sample. For graphical reports, the respective means were calculated.

Results and discussion

Determination of lignin

The data of acid hydrolysis (AH) with the weight of the unhydrolysable residues (equivalent to Klason lignin) are presented in the Tables 1–3. The kappa number (KN) is a parameter for the oxidizable unsaturated bonds and thus also for the remaining lignin content in a pulp by multiplication with the factor 0.13 (TAPPI 1999). In all the samples, the KN derived lignin contents were less than those determined based on the hydrolysis residues. This means that the residues might include further constituents as acid insoluble inorganic matter and extractives. The KN and AH based lignin contents are listed in Table 2.

Table 2: Kappa numbers (KN), calculated lignin amounts and hydrolysis residues of kraft pulps (KP) and a sulfite pulp (SP) obtained after two-stage sulfuric acid hydrolysis.

Pulp	Kappa number	Calculated lignin (%)[a]	Hydr. res. (%)
Spruce KP	23.2	3.0	6.4
Spruce BKP	1.7	0.2	0.7
Beech KP	17.2	2.2	2.4
Beech BSP	5.1	0.7	0.7

[a]KN × 0.13; B refers to bleaching, i.e. to bleached pulps.

Determination of carbohydrates in pulps

Bleached and unbleached pulps were also hydrolyzed by EH and AH. The subsequent analysis with the conventional borate-HPAEC and the new HPAEC-UV method resulted in the data presented in Table 3. The commercial enzyme mixtures contained significant amounts of fructose and glucose as well as traces of rhamnose, mannose and galactose. These carbohydrates were quantified by borate-HPAEC (data not shown) and offset against the carbohydrate content analyzed in pulp samples. In all hydrolysates, glucose, xylose and mannose were detected. Arabinose was found in some samples as well as galacturonic acid and meGlcA. Their yield depends on the natural source, sample preparation and the kind of analytical measurement. MeGlcA was detected in all EH samples analyzed by the standard and novel HPAEC approaches. MeGlcA was only absent in BKP_{spruce} (borate-HPAEC). As the dimer of meGlcA and xylose was not detected in any EH samples, it can be assumed that the applied enzyme mixtures also contained glucuronidase. On the other hand, meGlcA could be detected in two samples after AH in combination with the HPAEC-UV approach, indicating a structural degradation of this compound. KP_{spruce} contained 0.3% and KP_{beech} contained 4% meGlcA. Compared to the results of EH, the degradation rate of meGlcA during AH was between 43% and 44%. The behavior of meGlcA is in contrast to that of all neutral carbohydrates, as the amounts determined in EH treated pulps are generally lower than those in AH

Table 3: Analysis of enzymatic and acidic pulp hydrolysates.

Pulp		Yields of hydrolysis products (wt%)							
Method	Hydrolysis[a]	Glc	Xyl	Ara	Man	GalA	meGlcA	Resid.	Sum
Spruce KP	EH	63.8	5.6	n.d.	3.8	0.3	0.7	5.4	79.6
HPAEC-UV	AH	74.5	6.7	0.3	5.6	0.3	0.3	6.4	94.1
Spruce KP	EH	63.8	5.0	0.3	4.6	n.d.	0.4	5.4	79.5
Borate-HPAEC	AH	77.3	6.3	n.d.	6.1	n.d.	n.d.	6.4	96.1
Spruce BKP	EH	73.6	6.0	n.d.	4.5	n.d.	0.6	5.8	90.5
HPAEC-UV	AH	78.0	8.0	0.1	5.0	0.2	n.d.	0.7	92.0
Spruce BKP	EH	74.8	6.7	0.3	5.3	n.d.	n.d.	5.8	92.9
Borate-HPAEC	AH	80.7	7.2	0.5	5.7	n.d.	n.d.	0.7	94.8
Beech KP	EH	60.1	20.7	n.d.	0.5	1.0	0.9	3.0	86.2
HPAEC-UV	AH	65.6	20.2	n.d.	0.4	1.1	0.4	2.4	90.1
Beech KP	EH	62.6	18.4	n.d.	0.3	n.d.	0.8	3.0	85.1
Borate-HPAEC	AH	65.8	20.8	0.1	0.4	n.d.	n.d.	2.4	89.5
Beech BSP	EH	73.8	8.7	n.d.	1.0	n.d	0.6	7.2	91.3
HPAEC-UV	AH	80.4	8.4	0.2	1.3	0.3	n.d.	0.7	91.3
Beech BSP	EH	74.9	9.1	n.d.	1.4	n.d.	0.6	7.2	93.2
Borate-HPAEC	AH	82.3	9.5	n.d.	1.6	n.d.	n.d.	0.7	94.1

[a]EH, Enzymatic hydrolysis; AH, acid hydrolysis; KP, unbleached kraft pulp; BKP, bleached kraft pulp; BSP, bleached sulfite pulp; n.d., not determined. Resid. is the hydrolysis residue. The yields are based on dry material.

pulps. This observation is different from that of Dahlman et al. (2000), probably because of differences between the enzyme mixtures. Both bleached pulps show significantly more AH residues after enzymatic pretreatment, but this observation does not apply to unbleached pulps. This is partly due to the fragmentary enzymatic breakdown of the polysaccharides. The presence of xylan can additionally hinder the enzymatic hydrolysis of pulps by forming crystallite structures with cellulose (Dammstrom et al. 2009; Penttilä et al. 2013), which is not only affected by the amount of hemicelluloses but also by the steric orientation of their polymer chains to each other.

The monosaccharide yields are in the same range, but altogether the results obtained by borate-HPAEC are slightly higher. The sum of all total carbohydrate contents and the hydrolysis residues are in the range of 80%–95%. With the exception of KP_{spruce}, the cumulative amounts of AH yields are maximal about 5% higher than those of EH. This observation is surprising, because in case of an incomplete cleavage, the missing carbohydrates should be among the hydrolysis residues. The interpretation is that a large amount of carbohydrates within the dissolved polymeric structures are small enough to pass the sinter glass frits (pore size: 10–16 µm) used for filtration of the hydrolysis residue.

Therefore, dynamic light scattering (DLS) experiments were performed for the characterization of oligomeric and polymeric fragments in the hydrolysates (Figure 2). In the AH hydrolysates, no particles could be observed in the diameter range of 0.001 µm–6.000 µm. But in case of EH, particles with varying sizes are seen in three of the enzymatically treated samples. These particles might be due to unhydrolyzed xylan-lignin and xylan-cellulose complexes (Dammstrom et al. 2009; Hendriks and Zeeman 2009). KP hydrolysates contained particles with average sizes between 0.1 µm and 3 µm. These pulps also have significantly lower overall recovery rates compared to the BKP samples (Table 3). This might be explained by the circumstance that certain carbohydrate containing particles could not be analyzed by the HPAEC techniques applied. Particles with an average size >1 µm were detected in SP_{beech}, while the hydrolysate BKP_{spruce} does not contain any particles. Accordingly, DLS revealed that EH of pulps is not quantitative and unhydrolyzed particles may elude HPAEC quantification.

It can be safely concluded that EH is not appropriate for more complex matrices such as biomass, though it conserves the labile compound meGlcA in pulps. Thus, the wood and other lignocellulosics in the following section were analyzed only by AH with subsequent HPAEC-UV detection.

Determination of carbohydrates in biomass

Figure 3 shows exemplarily the AH analysis by HPAEC-UV. As visible, a big variety of monosaccharides and one short oligosaccharide are present. With retention

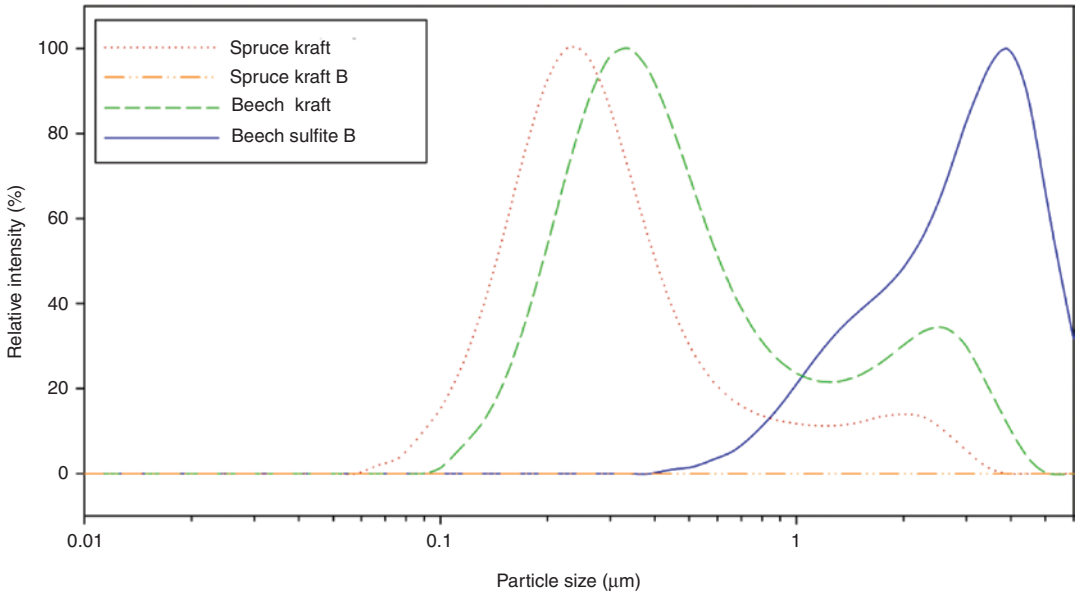

Figure 2: Normalized distribution of the particle size, as determined in the filtrates of enzymatic hydrolysis of pulps via dynamic light scattering (DLS).
The suffix B indicates bleached pulps. If not labeled, the pulps are unbleached.

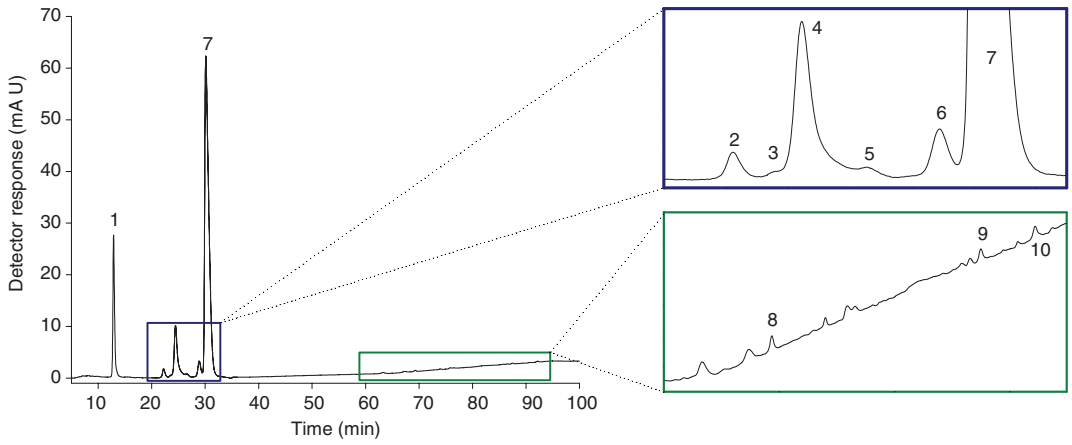

Figure 3: Chromatographic separation of reductively aminated carbohydrates in hydrolyzed *Picea abies* (spruce).
The following peaks have been assigned: (1) 4-aminobenzoic acid (internal standard), (2) xylose, (3) galactose, (4) glucose, (5) arabinose, (6) mannose, (7) 2-aminobenzoic acid (derivatization agent), (8) meGlcA-xylose, (9) meGlcA, (10) galacturonic acid.

times (RTs) between 20 min and 30 min, the neutral monosaccharides xylose, galactose, glucose, arabinose and mannose are detectable. Galactose and glucose are not baseline separated, which could detract from the accuracy of the galactose quantification. Acidic carbohydrates eluted with significantly longer RTs (60 min to 95 min). In this range, the dimer of meGlcA and one xylose unit (meGlcA-X) and the uronic acids meGlcA and galacturonic acid are detected. Neutral oligosaccharides as well as acidic oligosaccharides with higher degrees of polymerization (DPs) and non-methylated GlcA were absent in all the samples. The acidic oligosaccharide was detected at lower RTs than the acidic monosaccharides. The affinity of anionic compounds for the resin in the column could explain this finding. With increasing chain lengths, the overall ionic character, introduced by the 2-AA tag, decreased and accordingly, the corresponding compound eluted earlier in the chromatogram. Furthermore, several peaks were detected between 60 min and 70 min, which could not be assigned to particular structures. These signals may be observable due to further acidic oligosaccharides from pectins or from uronic acid containing polysaccharides. The HPAEC-UV detectable polysaccharides are compiled together with literature data in Table 4.

With the exception of poplar wood, the amounts of monomeric and dimeric meGlcA are in a ratio around 1:1. The monomeric glucose is the most abundant hydrolysis product followed by xylose. Only spruce wood hydrolysate contains more mannose than xylose. Mannose is originated from glucomannans, which is a typical marker for softwoods. While bagasse contains only traces of mannose (0.4%), this compound was not detected in wheat straw. The determined amounts of mannose in the remaining samples were between 1% and 3%. Bagasse and wheat straw contain the highest amounts of arabinose due to the high amounts of arabinoxylan in these feedstocks. In all wood samples, the amounts of arabinose were around 2%; only *Populus alba* contained more of this pentose sugar (3.2%). Low contents of galactose were quantified in alder as well as in poplar wood; in bagasse, the galactose content was below the detection limit. Relatively constant amounts of galactose between 1.2% and 1.6% are seen in the other lignocellulosic materials. The pectin typical galacturonic acid is detectable in all samples with yields below 1%. In spruce, this compound amounted to 0.8%. The cumulative analysis resulted to total yields around 90%, accordingly, a representative moiety the biomass could be characterized by.

The comparison with literature data, as presented in Table 3, is difficult because of the differences in raw materials and the analytical methods. The habitat of the original plant and its development stage and tissue type also influence the results (Ebringerova 2006). The comparison in Table 3 shows that the sum of meGlcA is higher compared to all reference values. The monomeric yields from oak and poplar are lower than those from the literature, probably because of the quantification of the uronic acids meGlcA, GalA and GlcA and due to different species compared. The galacturonic acid data are not in a systematic relation to each other. The wheat straw and spruce data are in good accordance with the literature, but bagasse, eucalyptus and beech have lower yields than those of the literature. The literature also reported on the presence of non-methylated glucuronic acid, which was not detectable in any of the analyzed materials.

Table 4: Monosaccharide compositions as detected by HPAEC-UV in comparison with literature data designated in the upper case indices d–h.

	Quantitative yields of the products in the hydrolysates (wt%)										
Biomass	**Glc**	**Xyl[a]**	**Ara**	**Man**	**Gal**	**meGlcA monom.**	**meGlcA-X dimer[b]**	**GalA**	**Σ Uronic acids**	**Residue**	**Sum total**
Bagasse	40.3	28.8	3.9	0.4	n.d.	0.5	0.5	0.3	1.3	17.9	92.3
Bagasse[d]	43.1	23.8	1.5	0.3	0.4		0.8	1.2	2.0	23.2	94.3
Wheat straw	35.6	25.1	4.6	n.d.	1.2	0.6	0.6	0.6	1.8	18.9	87.3
Wheat straw[e]	6.2[c]	24.4	3.4	0.3	1.1		0.9	0.6	1.5	n.d.	36.9[c]
Picea abies	41.3	9.4	2.3	10.7	1.2	0.7	0.9	0.8	2.4	25.2	92.2
Picea abies[f]	39.8	4.9	1.1	10.1	2.8		n.d.	0.6	0.6	29.8	89.1
Eucalyptus globulus	42.6	19.4	1.6	0.8	1.3	1.1	1.2	0.5	2.8	18.0	87.6
Eucalyptus grandis[d]	46.7	11.5	0.5	1.0	1.2		1.6	1.2	2.8	29.2	92.9
Fagus sylvatica	38.8	20.1	1.8	0.9	1.6	1.0	0.9	0.6	2.5	19.1	86.4
Fagus sylvatica[f]	37.3	16.5	0.8	1.5	2.2		n.d.	1.1	1.1	24.2	83.6
Quercus alba	31.9	20.0	1.8	1.4	1.2	0.8	0.9	0.4	2.1	28.3	86.7
Quercus falcata[g]	41.0	19.0	0.4	2.0	0.9				4.5	24.0	91.8
Alnus glutinosa[h]	36.9	19.7	1.8	1.3	0.5	0.7	0.9	0.4	2.0	27.1	89.3
Populus alba	46.2	19.4	3.2	2.8	0.6	0.6	1.0	0.4	2.0	19.3	93.4
Populus deltoides[g]	47.0	15.0	0.6	2.9	1.4				4.8	24.0	95.7

[a]Total xylose concentration, $c = c_{monomer} + c_{xylose\ in\ meGlcA\text{-}X}$; [b]concentration of meGlcA linked in the meGlcA-X dimer; [c]Glc concentration does not represent the total amount of cellulose; n.d., not determined. Comments to literature data d to h:

[d]Alves et al. (2010), HPLC-RID. Concentrations of uronic acids were quantified with methanolysis and GC. The amount of GalA represents the total concentration of GalA and GlcA.

[e]Willför et al. (2009), by methanolysis and GC.

[f]Altaner and Saake (2016), by ^{1}H-NMR spectroscopy. Concentrations were quantified as monomers, thus the results indicated in this table are adapted and calculated as homopolymers.

[g]Pettersen (1984), uronic acids were not specified in detail.

[h]No data available in the literature.

The determined glucose yields are very similar to literature values. The only significant discrepancy was seen in case of wheat straw with 35.6 mg Glc compared to 6.2 mg per 100 mg reported in the literature. Literature data are frequently determined via methanolysis and GC, a method, better suited for hemicelluloses analysis and less appropriate for cellulose characterization. The amounts of xylose in the present work are higher than those from the literature. All concentrations of arabinose were higher compared to literature data and no systematic differences concerning mannose and galactose yields are visible.

Conclusions

Reductive amination after two-stage acid hydrolysis and analysis by HPAEC-UV allows the detection of monomeric and dimeric meGlcA. To test the analytical power of this new approach, softwood and hardwood kraft pulps, which were also bleached and a sulphite pulp were analyzed via enzymatic and acid hydrolysis, while the traditional borate-HPAEC technique served for comparison. After acid hydrolysis, lower amounts of monomeric meGlcA were monitored compared to enzymatic cleavage, due to secondary degradation reactions in the acidic medium. But the yields of the other carbohydrates are higher after acid hydrolysis because of the incomplete enzymatic hydrolysis. As proved by dynamic-light scattering measurements, enzymatic hydrolysis releases small particles into the hydrolysate with sizes between 0.2 µm and 3 µm. Enzymatic hydrolysis is well suited for the determination of uronic acids. However, further improvements of the enzymatic cleavage protocols are needed for pulp analysis. Definitively, the performance of enzymatic hydrolysis in case of more complex biomasses than pulps is not satisfactory. This is the reason why six softwoods and hardwoods and wheat straw and bagasse were analyzed by acid hydrolysis followed by reductive amination of the hydrolysis products with subsequent HPAEC-UV analysis. The determined amounts of all neutral carbohydrates are in good accordance with literature data. In contrast to pulps, a disaccharide of meGlcA and xylose was detected in hydrolyzed biomass. This resulted in significantly higher amounts of meGlcA, referred to the literature data.

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