Simultaneous Separation and Quantification of Linear Xylo- and Cello-Oligosaccharides Mixtures in Lignocellulosics Processing Products on High-Performance Anion-Exchange Chromatography Coupled with Pulsed Amperometric Detection

Yong Xu,[a,b,c,*] Li Fan,[a,b,c] Xing Wang,[a,b,c] Qiang Yong,[a,b] and Shi-Yuan Yu [a,b]

A simple one-step method was developed for rapid separation and quantification of the linear xylo-oligosaccharides (XOS) and cello-oligosaccharides (COS) mixtures by using high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD). By exploiting small ion-exchange behavioral differences of various oligosaccharide components on the CarboPac PA200 column, a two-stage binary gradient elution program of NaOAc-NaOH solution was established. Subsequently, nine linear oligomers were separated simultaneously and readily within 30 min, in the order of: xylobiose, cellobiose, xylotriose, xylotetraose, cellotriose, xylopentaose, cellotetraose, xylohexaose, and cellopentaose. The method was applied successfully in the analysis and determination of different lignocellulosics processing products. The system provides a convenient and powerful analytical tool for technical research and development on polysaccharide components bioconversion in lignocellulosic biomass processing.

Keywords: Linear xylo- and cello- oligosaccharides mixtures; Simultaneous separation and quantification; Lignocellulosic processing products; HPAEC-PAD

*Contact information: a: College of Chemical Engineering, Nanjing Forestry University, Nanjing 210037 China; b: Key Laboratory of Forest Genetics & Biotechnology, Ministry of Education of China, Nanjing 210037 China; c: Jiangsu Key Lab of Biomass-based Green Fuels and Chemicals, Nanjing 210037 China; * Corresponding author: xuyong@njfu.edu.cn*

INTRODUCTION

Lignocellulosic materials have been identified as one of the most promising sources for biomass-based fuels and chemicals production because of their abundance, sustainable supply, and relatively low cost. Efforts in recent decades have mainly focused on its bioconversion (Ragauskas *et al.* 2006; Ohlrogge *et al.* 2009; Waltz 2010; Menon and Rao 2012). During feedstock processing with steam-explosion, hot-compressed water, alkaline pulping, and acidic and enzymatic hydrolysis, a variety of oligosaccharides containing xylo-oligosaccharides (XOS), cello-oligosaccharides (COS), or other oligosaccharides are released simultaneously or sequentially from hemicellulose and cellulose degradation (Sun *et al.* 2004; Wyman *et al.* 2005; Yang and Wyman 2008; Alvira *et al.* 2010; Otieno and Ahring 2012). Furthermore, some oligosaccharides deserve increasing attention as high added-value prebiotics. They are exhibiting more and more positive effects on human health and domestic animal productivity and have a promising future (Loo *et al.* 1999; Remaud *et al.* 2003; Moure *et al.* 2006; Xu *et al.* 2009; Gobinath *et al.* 2010). Therefore, more emphasis will be given to the quantitative analysis of these

oligosaccharides. It is useful not only for basic research and technical development of polysaccharides bioconversion, but also necessary for the oligosaccharide product quality testing.

Several methods have been proposed for the identification and determination of oligosaccharides. The method under study was developed from thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC), which were incorporated into a system of high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD). Various chromatographic devices combined with a mass spectrometry (MS) detector have been suggested. However, TLC and HPLC are suitable for mono-, bi-, and a few trisaccharides rather than for higher oligo- or polysaccharides (Gauch *et al.* 1979; Raessler 2011), whereas HPAEC-PAD has been applied just for gluco- or fruco-oligosaccharides from starch, alginate, and inulin (Van der Hoeven *et al.* 1992; White *et al.* 2003; Balance *et al.* 2005; Corradini *et al.* 2012). Despite the apparent priority of molecular structural analysis and qualitative identification of compounds, chromatography combined with MS detection has been somewhat inconvenient in terms of a permanent online application due to the extended experimental setup. Moreover, MS is usually labor-intensive with respect to sample preparation work for the necessary purification and derivatization; special knowledge and experience is also required to obtain usable mass spectra and data (Van der Hoeven *et al.* 1998; Kabel *et al.* 2001; Reis *et al.* 2003; Min *et al.* 2007; Westphal *et al.* 2010; Guadalupe *et al.* 2012). Thus, it is desirable to develop a new convenient assay for quantitative determination of XOS and COS in lignocellulosics processing products.

HPAEC-PAD is a widely used tool for the separation and determination of mono- and oligosaccharides because of its distinctive separation capacity on these sugars with gradient elution of alkaline solution. In nature, carbohydrates usually act as general acids having pK_a values ranging between 12 and 14 according to the Brønsted-Lowry definition; for example, glucose has a pK_a value of 12.28 and xylose has a value of 12.15 (Yu Ip *et al.* 1992). Under high pH conditions, the multiple hydroxyl functional groups of oligosaccharides will be either completely or partially ionized to form anions, depending on their pKa values. Thus the ionized carbohydrates could be separated by means of anion-exchange chromatography (AEC) under alkaline conditions (pH > 12) despite AEC not being a technique commonly associated with the analysis of neutral carbohydrates. Due to the use of high pH-resistant polymeric-based strong anion-exchange columns and extreme alkaline mobile phase and high-pressure elution in combination with a highly sensitive pulsed amperometer detector, the capability of HPAEC-PAD is improved so greatly in terms of separation efficiency and detection sensitivity that it allows direct separation and quantification of un-derivatized carbohydrates in a single run. For these reasons the HPAEC-PAD system was selected for the determination of XOS and COS.

The aim of this work was to develop a direct and simultaneous quantification method for the various XOS and COS mixtures on an HPAEC-PAD system. Nine linear oligomers were studied, *i.e.* xylobiose, xylotriose, xylotetraose, xylopentaose, xylohexaose, cellobiose, cellotriose, cellotetraose, and cellopentaose. The method for respective quantification of XOS or COS was investigated on the chosen column. On this basis, the gradient elution program and other chromatographic operating parameters were further optimized for simultaneous quantification of XOS and COS mixtures. Ultimately, a simple and rapid method was established and applied in crude lignocellulosics processing products. To the best of our knowledge, it is the first time that the various linear XOS and

COS mixtures have been determined simultaneously and readily by a one-step method performed with such an HPAEC-PAD system.

EXPERIMENTAL

Materials

1,4-β-D-(+)-xylobiose / xylotriose / xylotetraose / xylopentaose / xylohexaose (Megazyme), and D-(+)-xylose / glucose and 1,4-β-D-(+)-cellobiose / cellotriose / cellotetraose / cellopentaose (Sigma) were used as chemical reference standards for the HPAEC-PAD analysis. α-Cellulose powder, beech xylan powder, cellulase aqueous solution (C2730), and xylanase lyophilized powder (X3876) were purchased from Sigma. Anhydrous sodium acetate and aqueous sodium hydroxide (50%, w/w) were obtained from Fluka. All chemicals used were of analytical grade or higher. Air-dried corncob powder (40–80 mesh) was obtained from Northeast China. Deionized water was prepared from a Milli-Q purification system.

Preparation of Standard Solution

The individual chemical reference standard was mixed for different standard mixture solutions with deionized water in optimum concentration. All the prepared standard solutions were kept frozen in airtight vials. Upon thawing, the standards were mixed vigorously on a lab Vortex Mixer to ensure thorough mixing until used.

Preparation of Lignocellulosics Processing Products

The steam-exploded corncob was contained in a 2.2 L stainless steel steam-gun (190 °C, 10 min), and 200 g of corncob was fed into a batch run. By washing thoroughly with 1.0 L of deionized water divided into three equal portions, the washed solid and the solution were obtained from the steam-exploded corncob, respectively. Enzymatic hydrolysis was carried out in a 250-mL Erlenmeyer shaken flask sealed with a plastic tap (50 °C, 150 rpm, 6 h). The sample (1.0 g of α-Cellulose powder or beech xylan powder or the washed solid from the steam-exploded corncob) was suspended in 50 mL of citrate buffer (0.05 M, pH 4.80), and cellulase (5 FPIU cellulase/g cellulose) and xylanase (20 U xylanse/g xylan) were added in terms of substrates. The reaction was stopped by the addition of 0.1 mL of sulfuric acid (98%). All samples were prepared in duplicate with relative errors less than 10%.

Determination and Quantification on HPAEC-PAD

HPAEC-PAD analysis was performed on a Dionex ICS-3000 system equipped with an analysis anion-exchange column of CarboPac PA200 (3 mm × 250 mm) in combination with a guard column of CarboPac PA200 (3 mm × 50 mm) at 30 °C. The Dionex ED40 Electrochemical Detector was used for the detection of carbohydrates in pulsed amperometry mode through standard quadruple waveform (t= 0 – 0.40 s, p = 1.00 V; t = 0.41–0.42 s, p = −2.00 V; t = 0. 43 s, p = 6.00 V; t = 0.44 – 0.50 s, p = −1.00 V). Two eluents were prepared as the mobile phase in plastic bottles pressured with inert nitrogen gas at 6 to 9 psi pressure, which consisted of 0.1 M NaOH solution and 0.5 M NaOAc containing 0.1 M NaOH solution (NaOAc-NaOH). The gradient elution was performed at 0.3 mL/min using the programs given in Tables 1 and 2, respectively. The first program is for the respective determination of XOS or COS, and the second one is

for the simultaneous determination of the XOS and COS mixture. Data and graphs were collected and analyzed on computers equipped with Dionex Chromeleon 6.7 software.

All the samples needed centrifugation at 10,000 rpm for 5 min prior to filtration using a 0.22-μm nylon Acrodisc syringe filter, and the injection volume was typically 10 μL. The external standards were used in samples analysis. Samples were stored in an airtight container and refrigerated. Samples were mixed vigorously on a lab Vertex Mixer to ensure thorough mixing and dilution for measurement.

Table 1. Elution Program for Respective Determination of XOS or COS

Time (min)	NaOH (%)	NaOAc-NaOH (%)
0	100	0
25	80	20
25.1	100	0
35	100	0

Table 2. Elution Program for Simultaneous Determination of XOS and COS Mixtures

Time (min)	NaOH (%)	NaOAc-NaOH (%)
0	100	0
9	100	0
26	92	8
26.1	50	50
40	50	50
40.1	100	0
50	100	0

RESULTS AND DISCUSSION

Respective Determination of XOS or COS

On a Dionex ICS-3000 chromatography system, two main commercially available anion-exchange columns based on styrene-divinylbenzene copolymer resin were first compared; these columns were designed especially for oligosaccharides (Swennen *et al.* 2005; Fan *et al.* 2011). The CarboPac PA200 column was chosen for its prior separation performance to the CarboPac PA100 column (unpublished data). Based on earlier works on XOS (Fan *et al.* 2011), the elution program was further modified and generalized for XOS as well as COS. Compared with sodium hydroxide, sodium acetate is also particularly suitable as a stronger pusher ion for gradient elution because pulsed ampere-metric detectors are relatively insensitive to ionic strength changes of sodium acetate gradient (Pérez and Frey 2005; Corradini *et al.* 2012). So a higher slope of sodium acetate gradient elution was used to increase the ionic strength of the mobile phase and decrease the elution time of higher polymeric XOS or COS components, allowing faster analysis (Table 1).

Linear XOS (from X1 to X6), as well as linear COS (from G1 to G5) were separated completely for determination and quantification within 20 min by using the binary gradient elution program with NaOAc-NaOH solution (Fig. 1). The following prolonged elution procedure with NaOAc-NaOH solution for 5 minutes and NaOH

solution for 15 min were needed for impurity cleaning in crude sampling and for renewal of the chromatographic system. With the elution ionic strength increasing, all components of XOS or COS were eluted out in order of the ascending degree of polymerization (DP) due to their ion-exchange behavior differences on the column. It was found to be relatively easy to create calibration curves for quantification of every oligosaccharide component in XOS or COS solution.

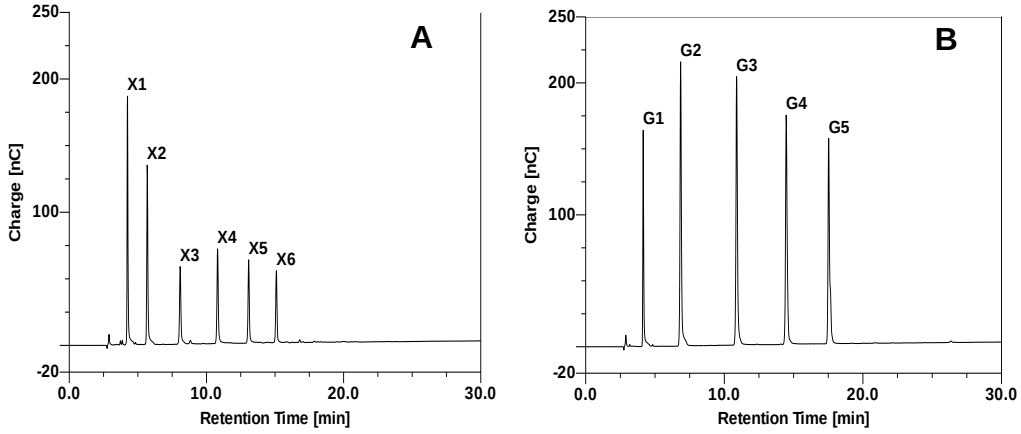

Fig. 1. Chromatograms of XOS (A) or COS (B) standards.
Peak identifications: X1, xylose; X2, xylobiose; X3, xylotriose; X4, xylotetraose; X5, xylopentaose; X6, xylohexaose; G1, glucose; G2, cellobiose; G3, cellotriose; G4, cellotetraose; G5, cellopentaose.

Furthermore, it was interesting to see the good-fit linear relationship between DP and retention time (RT) of XOS or COS components on the CarboPac PA200 column, respectively. For instance, xylose (RT=4.25 min), xylobiose (RT=5.68 min), xylotriose (RT=8.08 min), xylotetaose (RT=10.82 min), xylopentaose (RT=13.08 min), and xylohexaose (RT=15.10 min) were fitted to the equation: y (min) = 2.263 x + 1.583 (y, RT of XOS; x, DP of XOS; the correlation coefficient squared, R^2 = 0.994). And glucose (RT=4.15 min), cellobiose (6.85 min), cellotriose (10.88 min), cellotetaose (14.47 min), and cellopentaose (17.53 min) were fitted to the equation: y (min) = 3.44 x + 0.46 (y, RT of COS; x, DP of COS; the correlation coefficient squared, R^2 = 0.997). Therefore, the equation between RT and DP may be useful for prediction of some unknown higher linear oligosaccharides components of XOS or COS on the HPAEC-PAD system.

Simultaneous Separation and Determination of the XOS and COS Mixtures

Obviously, several components in the XOS and COS mixtures solution were indistinguishable on the elution program described in Table 1, *e.g.*, xylose and glucose, xylotetraose and cellotriose. Their small differences of ion-exchange behaviors on the CarboPac PA200 column should be utilized. Since sodium acetate is a stronger eluent (Pérez and Frey 2005; Corradini *et al.* 2012), a low gradient slope of sodium acetate solution was used as a possible means to separate the closely similar components of sugar. Taking into account the twist in between xylose and glucose, the NaOAc-NaOH solution was decreased somewhat or replaced completely by the NaOH solution in the beginning elution phase; as for the peaks overlap between xylotetraose and cellotriose, the slope of the sodium acetate gradient elution was decreased in the middle elution phase. These

endeavors succeeded with xylotetraose and cellotriose but failed with xylose and glucose. Based on these results, it appears that there is a natural characteristic limit of the CarboPac PA200 column, which is designed specifically for oligosaccharides rather than for monosaccharides.

The elution mode, flow rate, and other chromatographic conditions were further optimized according to column efficiency, chromatographic resolution, peak symmetry, and other factors. On this basis, the two-stage binary gradient elution program was chosen for separation of the mixtures solution in a one-step analysis (Table 2), and oligomers were eluted out in the order of: xylobiose, cellobiose, xylotriose, xylotetraose, cellotriose, xylopentaose, cellotetraose, xylohexaose, and cellopentaose (Fig. 2). At the same time, there are various columns routinely suitable for determination of xylose and glucose on the HPAEC-PAD system, *e.g.*, the columns of CarboPac PA1, PA10, and PA20 (Wang *et al*. 2012). In addition, the HPLC system is also commonly used for xylose and glucose detection on the column of Bio-Rad HPX-87P or 87H.

By using the two-stage binary gradient elution program, nine oligosaccharide components in addition to one monosaccharide (X1 + G1) in the XOS and COS mixtures standard solution were distinguished and detected simultaneously and readily within 30 min (Fig. 2). In consideration of the cleaning and renewal requirements of the column and chromatographic system, the elution step with NaOAc-NaOH solution and subsequently the NaOH solution were arranged so that the total operation time was extended to 50 min. All ten components were separated completely, and the value of the chromatographic resolution between cellotriose (RT=14.50 min) and xylotetraose (RT=15.07 min) was more than 1.50. As a result, quantitative calibration curves were listed by using linear regression for determination of every component. However, the RT of components did not exhibit a linear response to the DP of various oligosaccharides because of the non-linear gradient elution operation and other factors.

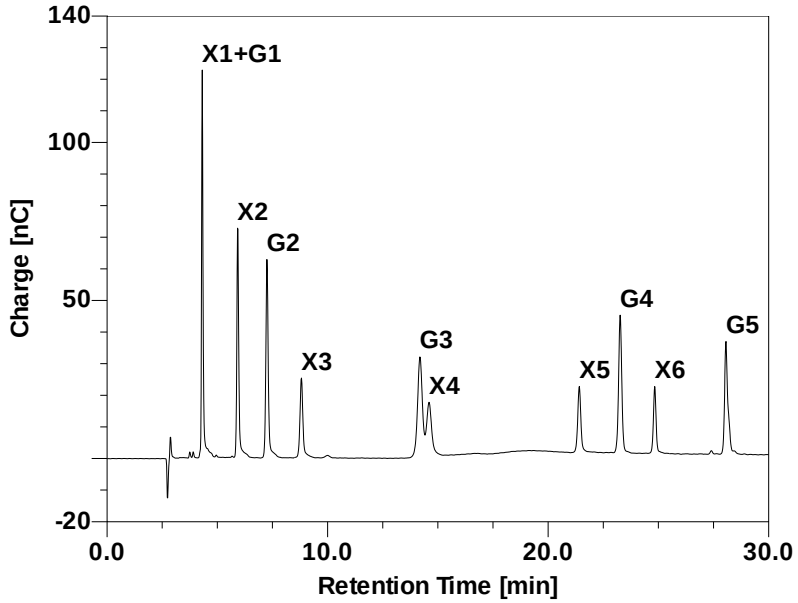

Fig. 2. Chromatograms of XOS and COS Mixture Standards; Peak identifications: X, xylose; X2, xylobiose; X3, xylotriose; X4, xylotetraose; X5, xylopentaose; X6, xylohexaose; G1, glucose; G2, cellobiose; G3, cellotriose; G4, cellotetraose; G5, cellopentaose

The established method was validated in terms of linearity range, limits of detection (LODs), limits of quantification (LOQs), repeatability, and precision. Seven samples were analyzed for method verification, and the results are listed in Table 3. The linear range of 0.50 to 5.00 mg/L of XOS and 0.40 to 4.00 mg/L of COS were defined with a good linear correlation coefficient ($R2 \geq 0.998$). Furthermore, LODs and LOQs of every oligosaccharide component were quantified from the threefold and tenfold signal-to-noise ratio, and gave the satisfactory ranges for LODs of 0.04 to 0.08 mg/L and for LOQs of 0.16 to 0.38 mg/L, respectively.

Data obtained by intermittent determination of six replicate runs of an unknown-amount standards mixtures solution were calculated for analysis of method repeatability. Relative standard deviations (RSD) of retention time and peak area were less than 0.48% and 7.43%, respectively.

Table 3. Quantitative Calibration Curves for Components of XOS and COS Mixtures

Component	Retention Time (min)	Linearity Range (mg/L)	Calibration Curve[a]	Correlation Coefficient Squared	LODs[b] (mg/L)	LOQs[b] (mg/L)	RSD[c] (%)	
							RT	PA
Xylobiose	6.02	0.50–5.00	y=2.92 x + 0.07	1.000	0.07	0.25	0.14	0.78
Cellobiose	7.37	0.40–4.00	y=3.93 x + 0.02	0.999	0.07	0.22	0.12	0.42
Xylotriose	9.02	0.50–5.00	y=1.52 x + 0.04	1.000	0.08	0.28	0.15	0.91
Cellotriose	14.50	0.40–4.00	y=3.62 x + 0.10	0.999	0.05	0.17	0.32	0.51
Xylotetraose	15.07	0.50–5.00	y=1.54 x + 0.05	0.998	0.07	0.25	0.48	3.18
Xylopentaose	21.35	0.50–5.00	y=1.29 x + 0.02	0.998	0.07	0.22	0.27	7.43
Cellotetraose	23.13	0.40–4.00	y=3.46 x + 0.09	1.000	0.06	0.18	0.19	1.57
Xylohexaose	26.53	0.50–5.00	y=0.98 x + 0.00	0.998	0.04	0.38	0.12	1.87
Cellopentaose	27.63	0.40–4.00	y=2.74 x + 0.05	0.999	0.05	0.16	0.20	1.07

[a] seven samples were analyzed for method verification. y, the concentration of oligosaccharide component (mg/L); x, the chromatographic peak area of the oligosaccharide component (nC· min).
[b] LODs, limits of detection. LODs = 3 sb/b; LOQs, limits of quantification. LOQs = 10 sb/b. sb was the standard deviation value calculated on 20 measurements, and b was the slope of the calibration curve for each oligosaccharide component.
[c] RSD, repeatability relative standard deviation, was calculated on 6 replicate runs. RT, retention time (min). PA, peak area (nC · min).

To perform the recovery test, known amounts of each oligosaccharide component solute were spiked to the identified mixture solutions of XOS and COS, and the resulting spiked samples were subjected to the entire analytical procedure. All analyses were carried out in triplicate.

On considering all nine oligosaccharides, components recoveries were found to range between 93.1% and 105.4%, and the RSD values fell within the range 0.32% to 10.0% (Table 4). The one-step method repeatability, sensitivity, precision, and accuracy were satisfactory and acceptable for simultaneous determination and quantification of linear XOS and COS mixtures.

Table 4. Recovery of Oligosaccharide Components in the Spiked Samples

Component	Background (mg/L)	Spiked (mg/L)			Found (mg/L)			Average Recovery (%)	RSD[a] (%)
		1	2	3	1	2	3		
Xylobiose	1.45	0.82	1.15	1.58	2.25	2.52	2.94	95.5	2.61
Cellobiose	2.60	0.66	0.96	1.25	3.24	3.56	3.88	99.8	1.01
Xylotriose	2.52	0.83	1.17	1.66	3.32	3.71	4.21	100.0	1.31
Cellotriose	0.91	0.66	1.00	1.33	1.55	1.88	2.28	99.0	3.94
Xylotetraose	1.81	0.83	1.16	1.66	2.53	2.87	3.49	93.1	4.28
Xylopentaose	1.24	0.84	1.18	1.72	2.00	2.35	3.07	97.0	8.83
Cellotetraose	0.42	0.65	0.91	1.32	1.07	1.33	1.74	100.0	0.32
Xylohexaose	0.57	0.83	1.18	1.69	1.40	1.77	2.31	104.0	5.19
Cellopentaose	0.30	0.66	1.00	1.45	0.99	1.39	1.79	105.4	10.0

[a] RSD, recoveries relative standard deviation.

Application of the Method in Crude Lignocellulosics Processing Products

Using the one-step method, four representative products from lignocellulosics processing were analyzed and determined directly on the HPAEC-PAD system. All samples were analyzed in several dilutions to make sure that every oligosaccharide component fell within its linearity range described in Table 3. Figure 3 shows that various XOS and COS components in crude samples were readily detected with complete resolution. Based on chromatograms and the established quantitative calibration curves, we figured out every oligosaccharide's contents in four samples (Table 5).

The data showed that α-cellulose produced absolutely only linear cello-oligosaccharides, and beech xylan released almost-linear XOS after enzymatic hydrolysis. In the same way, a series of XOS and COS components were found together in the solution of steam-exploded corncob, but there were trace amounts of XOS in the enzymatic hydrolysate of its washed solid. It is well known that cellulose seems inert, while xylan is labile when lignocellulosic feedstock is pretreated with furious steam explosion. So there are usually three possible outcomes: "One third of xylan" in the steam-exploded corncob, *i.e*. the first portion of soluble mono- and oligosaccharides degradations, the second portion of insoluble rigid xylan, and the third portion of loss and derived compounds (Sun *et al.* 2004; Wyman *et al.* 2005; Moure *et al.* 2006; Yang *et al.* 2008; Gobinath *et al.* 2010; Alvira *et al.* 2010; Fan *et al.* 2011; Otieno and Ahring 2012). The results of this study were reverse quantitative data between the solution and the enzymatic hydrolysate of the washed solid. More XOS than COS were obtained in the solution because the labile xylan is easier to degrade than the inert cellulose during steam explosion; at the same time, we obtained COS and a trace amount of XOS in the washed solid enzyme hydrolysate because the insoluble rigid xylan that remained in the solid is more resistant to cellulosic enzymes compared with cellulose. That is to say, there were no unreasonable oligosaccharide components in four products to be detected by the method. For that matter, this result further demonstrated the method accuracy and the precision in its application.

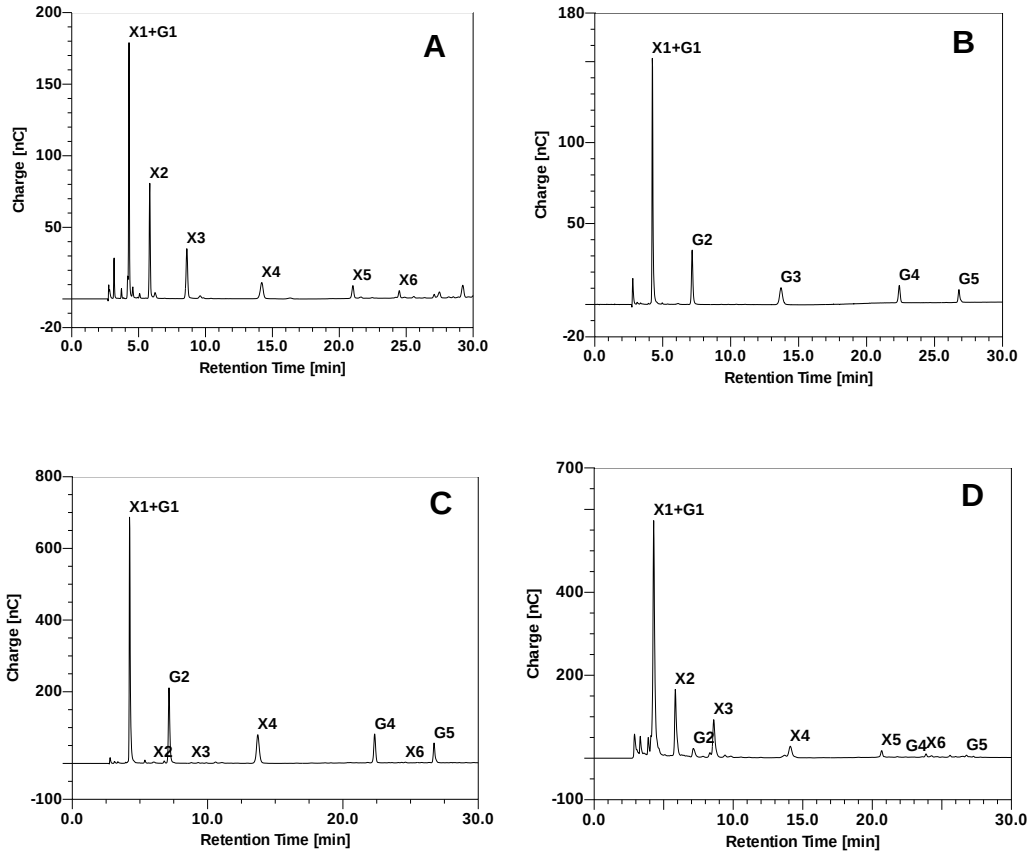

Fig. 3. Chromatograms of crude lignocellulosics processing products.
A, the enzymatic hydrolysate of xylan (×1/500); B, the enzymatic hydrolysate of cellulose (×1/500); C, the enzymatic hydrolysate of washed solid of steam-exploded corncob (×1/500); D, the solution of steam-exploded corncob (×1/1000).
Peak identifications: X1, xylose; X2, xylobiose; X3, xylotriose; X4, xylotetraose; X5, xylopentaose; X6, xylohexaose; G1, glucose; G2, cellobiose; G3, cellotriose; G4, cellotetraose; G5, cellopentaose.

Table 5. Quantification of Oligosaccharide Components in Crude Lignocellulosics Processing Products[a] (g/L)

Components	A	B	C	D
Xylobiose	1.39	0	0.03	5.93
Cellobiose	0	1.64	1.12	0.32
Xylotriose	1.84	0	ND	7.45
Cellotriose	0	0.25	1.04	ND
Xylotetraose	0.93	0	0	5.44
Xylopentaose	0.64	0	0	3.36
Cellotetraose	0	0.02	0.21	0.02
Xylohexaose	0.31	0	ND	ND
Cellopentaose	0	0.06	0.19	0.01

[a] A, the enzymatic hydrolysate of xylan (×1/500); B, the enzymatic hydrolysate of cellulose (×1/500); C, the enzymatic hydrolysate of washed solid of steam-exploded corncob (×1/500); D, the solution of steam-exploded corncob (×1/1000). ND, not detected with response signals less than LODs.

Furthermore, the method recorded more details about the distribution profiles of various degradation products from cellulose and xylan. Such results can be helpful toward the discovery of reaction kinetics and the mechanism of polysaccharides bioconversion with an exact and sensitive performance. Ultimately, the method was judged to be a convenient and powerful tool for research and development of lignocellulosic biomass processing. It is certainly necessary and desirable to keep making progress with respect to the development of analytical techniques capable of separating, identifying, and quantifying more complex branched and derived XOS and COS components (Broberg *et al.* 2000; Westphal *et al.* 2010; Radva *et al.* 2012).

CONCLUSIONS

1. A simple and rapid method was employed for separation and quantification of linear oligosaccharide components in XOS and COS mixtures on the HPAEC-PAD system.
2. For identification of undistinguished components in the mixtures, small anion-exchange behavioral differences among the oligosaccharides on the CarboPac PA200 column were exploited effectively by use of a two-stage binary gradient elution program.
3. Nine oligosaccharides were quantified directly within 30 min in one step.
4. Thereafter, the method was easily applied to characterize and quantify crude lignocellulosics-processing products with satisfactory accuracy.
5. Altogether, the method can provide a convenient and powerful analytical tool for the degradation of xylan and cellulose during lignocellulosic biomass processing.

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