Development, validation and application of a hydrophilic interaction liquid chromatography-evaporative light scattering detection based method for process control of hydrolysis of xylans obtained from different agricultural wastes

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ABSTRACT

Purified standards of xylooligosaccharides (XOSs) (DP2-6) were first prepared from a mixture of XOSs using solid phase extraction (SPE), followed by semi-preparative liquid chromatography both under hydrophilic interaction liquid chromatography (HILIC) modes. Then, an accurate quantitative analysis method based on hydrophilic interaction liquid chromatography-evaporative light scattering detection (HILIC-ELSD) was developed and validated for simultaneous determination of xylose (X1), xylobiose (X2), xylotriose (X3), xylotetraose (X4), xylopentaose (X5), and xylohexaose (X6). This developed HILIC-ELSD method was applied to the comparison of different hydrolysis methods for xylans and assessment of XOSs contents from different agricultural wastes. The result indicated that enzymatic hydrolysis was preferable with fewer by-products and high XOSs yield. The XOSs yield (48.40%) from sugarcane bagasse xylan was the highest, showing conversions of 11.21 g X2, 12.75 g X3, 4.54 g X4, 13.31 g X5, and 6.78 g X6 from 100 g xylan.

1. Introduction

Currently, there is worldwide interest in the use of agricultural residues as renewable raw materials for functional food production. Lignocellulosic materials represent the most abundant resources in agricultural residues (Carvalho, Neto, da Silva, & Pastore, 2013). The main chemical components constituting lignocellulosic materials are cellulose (40–55%), hemicellulose (25–35%), lignin (20–30%), and extractants (1–4%) (Deutschmann & Dekker, 2012). In hardwoods and cereals, the most common hemicelluloses are glucuronoxylans and arabinoxylans, respectively. Both of these are classed as xylans. Xylans are heteropolysaccharides built with a linear β-1,4-linked xylose backbone, decorated with various substituents, such as acetyl groups or α-L-arabinofuranoside at the O-2 or O-3 positions or both, α-D-glucuronic acid at O-2 (Ebringerová, Hromádková, & Heinze, 2005; Stolarski et al., 2015). The type of substituents, pattern of substitution along the xylans backbone, and the molecular weight of xylans differ depending on origin, part, and age of the plants, as well as the processing method for their purification (Akpinar, Erdogan, & Bostanci, 2009; Cheng et al., 2012; Van Dongen, Van Eylen, & Kabel, 2011). Xylooligosaccharides (XOSs) are sugar oligomers containing two-seven xylose units, mainly produced during the hydrolysis of xylan. XOSs are not digested by humans, but can be utilized by beneficial microorganisms (Moure, Guillón, Domínguez, & Parajó, 2006). As functional food ingredients in the pharmaceutical industry, feed formulation and agriculture, XOSs exhibit a variety of health benefits for the body, including improvement of gut health, immunomodulatory, anti-obesity and anti-infection benefits (Mudgil & Barak, 2013; Samanta et al., 2015; Singh, Banerjee, & Arora, 2015).

Production of XOSs from agricultural residues is mainly carried out in two processes: (1) alkaline extraction of xylan from biomass materials; (2) enzymatic hydrolysis or partial acid hydrolysis of xylan (Yang, Xu, Wang, & Yang, 2005). Enzymatic hydrolysis is preferable because it yields fewer undesirable by-products or monosaccharides (Akpinar, Erdogan, Bakir, & Yılmaz, 2010). The xylanolytic enzyme system contains a wide range of xylanases. Among them, endoxylanase and β-xylanosidase, are the two critically

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important types of enzyme for hydrolysis of the xylan backbone (Chavez, Bull, & Eyzaguirre, 2006). Endoxylanase can cleave the β-1,4 glycoside linkage between xylose units in the backbone, while β-xylodidade hydrolyzes short oligosaccharides from the non-reducing end to liberate xylose (Zeng, Xue, Peng, & Shao, 2007). Generally, commercial xylanase preparation is combinatorial; enzymes with a high endoxylanase activity and a low β-xylodidade activity.

The fast growth of the functional food market and sustainable production forces researchers to explore different methods and sources for converting XOSs from agricultural residues with high yields. Researchers are studying extraction methods of xylan, comparison of XOSs production by acid and enzymatic hydrolysis, characterization and application of novel xylanases, and conjecture of enzymatic mechanisms (Akpinar et al., 2010; Goncalves et al., 2012; McCleary et al., 2015; Nabarat, Ebringerová, & Montané, 2007; Peng et al., 2009). What is more, degree of polymerization (DP) is a significant factor for the industrial application of XOSs, because XOSs with different DP have varying degrees of beneficial health effects. The preferred DP range for food application is 2–4 (Loo et al., 1990).

Consequently, development of a comprehensive quantitative analysis method is urgently needed because the content of XOSs is of considerable interest in the control of the hydrolysis process for research or for industrial application.

In view of the techniques described for the analysis of XOSs over the past 10 years, high performance anion-exchange chromatography in combination with pulsed amperometric detection (HPAEC-PAD) and capillary electrophoresis (CE) with UV/VIS spectrophotometric detection have commonly been applied in the analysis of XOSs products (Hiltunen & Siren, 2013; Metsämäki, 2013; Sriensri, 2013; Riviere et al., 2013). With CE an extremely high pH (pH > 12) used for dissociation of the hydroxyl groups on oligosaccharide molecules brings in low frequency noise. On the other hand, non-volatile buffer salts with high concentration in the mobile phase of HPAEC cause the problem of baseline drift. Hydrophilic interaction liquid chromatography (HILIC) was defined by Alpert in 1990 (Alpert, 1990). It is a convenient method for separating highly polar samples which have poor retention on reverse-phase column by passing a highly polar organic-aqueous mobile phase across a polar stationary phase. The ability of HILIC to retain polar compounds with high selectivity makes it one of the most suitable chromatographic modes for the oligosaccharides separation (Buszewski & Noga, 2012; Karlsson, Winge, & Sandberg, 2005). Derivatization of carbohydrates for the improvement of extinction coefficients in UV/VIS spectrophotometry is currently a major hindrance for carbohydrates analysis. ELSD is a universal detector, which has been applied in detecting analytes without UV absorption (Condezo-Hoyos, Perez-Lopez, & Ruperez, 2015). From what has been discussed, HILIC-ELSD seems to be the most promising method for the analysis of oligosaccharides. Moreover, this approach was reported to be useful for the separation, purification and quantification of raffinose family oligosaccharides from Lycopus lucidus Turcz (Li et al., 2015).

In this study, standards of X2–X6 were purified from a mixture of XOSs sample through HILIC-solid phase extraction (SPE) and semi-preparative HILIC methods. A quantitative analysis method based on HILIC-ELSD was developed for simultaneous determination of X1–X6. A quantitative comparison between enzymatic and acid hydrolysis of beech xylan was also studied. Finally, the HILIC-ELSD was applied in the quantification of XOSs obtained from enzymatic hydrolysis of xylans from agricultural wastes. The present work aims to provide a demonstration for process control of XOSs production by a validated quantitative method.

2. Materials and methods

2.1. Chemicals

The sample containing a mixture of XOSs was obtained from Shandong Longlive Bio-technology Co., Ltd (Shandong, China). Beech xylan and xylose were obtained from Sigma-Aldrich (St. Louis, MO). Wheat bran, sugarcane bagasse and corncob were collected from a farmers’ market. Xylanase from Pichia pastoris was obtained from SunHY Bio Co., Ltd. (Hubei, China). Water was from a Milli-Q water purification system (Billerica, MA, USA). Acetonitrile (ACN) of HPLC grade and ethyl alcohol (EtOH) of HPLC grade were purchased from J&K (Beijing, China). The zwiterionic HILIC stationary phase (named Click TE-Cys) was synthesized according to a previous report (Shen, Guo, Yu, Cao, & Liang, 2011). The Click TE-Cys (20–30 μm) was used as a solid phase extraction sorbent. All of the other chemicals and reagents were of analytical grade.

2.2. Instrumentation and HILIC-ELSD analysis conditions

Experiments were performed on an Alliance HPLC system equipped with a Waters 2695 HPLC pump and a Waters 2424 evaporative light scattering detection (ELSD) system (Waters, Milford, MA, USA). Chromatograms were recorded using Waters Empower 3 software. MS analysis was carried out on an Agilent Technologies 6450 UHD Accurate-Mass Q-TOF (Agilent, USA) operating on ESI+ mode. NMR spectra were recorded at 500 MHz for 1H and 13C on a Bruker DRX-500 spectrometer.

Chromatographic analysis of each eluate from SPE was carried out on a Click TE-Cys column (4.6 × 150 μm i.d., 5 μm). The column temperature was 40 °C. The mobile phase consisted of H2O (A) and ACN (B) with gradient elution: 15% A-40% A at 0–40 min. The flow rate was 1.0 ml/min. The purity analysis and quantitative analysis were performed on a diol column (4.6 × 150 μm i.d., 5 μm, Tokyo, Japan). The column temperature was 60 °C. The mobile phase consisted of H2O (A) and ACN (B) with gradient elution: 5% A-20% A at 0–25 min; 20% A-90% A at 25–40 min. The flow rate was 1.0 ml/min. ELSD parameters: gas pressure was 20 psi, drift tube temperature was 50 °C, nebulizer temperature was 30 °C, and the value of the gain was 10.

2.3. Preparation of pure X2–X6

The mixture of XOSs (20 g) was dissolved in 40 ml H2O as the sample for SPE. A home-made cartridge (20 g, 60 ml) was activated with 70 ml of H2O and equilibrated with 70 ml of ACN. After that, the cartridge was loaded with 2 ml of sample and eluted with 300 ml of 80% ACN (eluate a), 150 ml of 75% ACN (eluate b), 90 ml of 70% ACN (eluate c), 50 ml of 65% ACN (eluate d), 40 ml of 60% ACN (eluate e) successively. The SPE process was repeated 20 times and each eluate was concentrated under vacuum and freeze-dried.

A semi-preparative Click TE-Cys column (7.8 × 150 mm i.d., 10 μm) was used to further prepare pure X2–X6. The flow rate was 3.0 ml/min. The split ratio of eluent between detection and collection was set at 1:12. The chromatographic conditions for each eluate from SPE step were as follows: mobile phase A was H2O, mobile phase B was EtOH, mobile phase C was ACN. Eluate a: 0–30 min, A/B: 5/95(v/v), column temperature was set at 40 °C, sample concentration was 200 mg/ml (dissolved in 50% EtOH), injection volume was 80 μl. Eluate b: 0–40 min, A/B: 12/88 (v/v), column temperature was set at 40 °C, sample concentration was 200 mg/ml (dissolved in 50% EtOH), injection volume was 100 μl. Eluate c: 0–40 min, A/C: 27/73 (v/v), column...
temperature was set at 40 °C, sample concentration was 200 mg/ml (dissolved in H2O), injection volume was 80 µl. Eluate d: 0–40 min, A/C: 29/71 (v/v), column temperature was set at 60 °C, sample concentration was 200 mg/ml (dissolved in H2O), injection volume was 50 µl. Eluate e: 0–45 min, A/C: 30/70 (v/v), column temperature was set at 60 °C, sample concentration was 200 mg/ml (dissolved in H2O), injection volume was 50 µl. Fractions collected by time mode from semi-preparative HILIC were concentrated under vacuum and freeze-dried.

2.4. Standard stock solutions and sample preparation for method validation

Standard stock solutions of X1–X6 were prepared by dissolving accurately weighed portions of standards in H2O. The exact concentrations of X1–X6 were 40.12 mg/ml, 38.98 mg/ml, 40.03 mg/ml, 20.39 mg/ml, 20.3 mg/ml and 10.22 mg/ml, respectively. A series of working standard solutions were obtained by preparing appropriate volumes of standard stock solutions and diluting with H2O. Sample solution was prepared by enzymatic hydrolysis of beech xylan at 37 °C for 4 h. The enzyme concentration was 50 U/ml. The detailed process of enzymatic hydrolysis can be found in Section 2.5. All of the standard stock solutions were stored in a refrigerator at 4 °C.

The HILIC-ELSD method for quantitative analysis was validated to determine the linearity, repeatability, precision, stability, limit of quantification (LOQ) and limit of determination (LOD), and accuracy for each analyte. Calibration curves were constructed using a range of concentrations of working standard solutions. Each line was based on five concentrations of working standard solutions and each concentration was determined for triplicate injections. LOD, LOQ were determined at a signal-to-noise ratio (S/N) of 10 and 3. Intra-day precision was evaluated by six replicates in one day and inter-day precision was evaluated by five replicates in three consecutive days. Repeatability was determined by preparation and analysis of six parallel samples. Stability was tested with a mixed standard solution at 0, 12, 24, 48, 72 h. Recovery was determined by spiking three concentration levels (50%, 100%, 150%) of the standard to the samples.

2.5. Comparison of enzymatic hydrolysis and acid hydrolysis

Beech xylan was used as a standard substrate for comparison of two hydrolysis methods. Commercial enzyme preparations were assayed for desired enzyme activities according to endoxylanase activity as defined by Bailey (Bailey, Biely, & Poutanen, 1992). One unit is defined as the quality of enzyme which liberates 1.0 µmol of xylose equivalent per minute under described conditions. The recommended enzymatic conditions of Xylanase from Pichia pastoris were 37 °C and pH 7.0 according to the merchandise description. Substrate concentration was 10 mg/ml. In order to study the effects of enzyme concentrations and reaction periods, the reactions were performed at different enzyme concentrations (2, 5, 8, 10 U/ml) and different hydrolysis periods (4, 8, 12, 24 h). The hydrolysis was started by adding xylanase preparation. After incubation for the desired time, hydrolysis was stopped by heating to 100 °C for 5 min to inactivate the enzyme followed by centrifugation at 2000 r/min for 1 min. An appropriate volume of suspension was taken and dried with nitrogen, dissolved in 1 ml H2O, and filtered through a 0.22 µm filter for further analysis.

To evaluate the consequence of acid hydrolysis, trifluoroacetic acid (TFA) was used as the acid reagent to hydrolyze beech xylan. 10 mg of beech xylan was added in 2 ml suspension which was then incubated at different acid concentrations (0.25, 0.5, 1.0, 1.5 M); temperature (80, 100, 120, 140 °C) and hydrolysis periods (0.5, 1.0, 1.5, 2.0 h). After the reaction, the suspension was dried with nitrogen, dissolved in 1 ml H2O, and filtered through a 0.22 µm filter for further analysis.

2.6. Extraction of xylans from sugarcane bagasse, corncob, and wheat bran

Xylans extracted from different agricultural wastes were extracted according to Reza Farjah with slight modification (Faryar et al., 2015). The collected sugarcane bagasse, corncob and wheat bran were washed with water to remove dirt. Then they were dried overnight at 50 °C and ground to particle size of 5–10 mm. A 2 g sample of each was then soaked in 2% (w/v) NaOH at 80 °C in a water bath for 2 h. The mixtures were cooled and filtered. The supernatants of alkaline solubilized xylans were acidified with a 0.01-fold volume of glacial acid and precipitated by adding 2-fold volumes of 95% ethanol. The xylans extracted from different origins were dried in hot air at 50 °C until constant weight was reached.

2.7. Enzymatic hydrolysis of sugarcane bagasse xylan, corncob xylan, and wheat bran xylan

Xylans extracted from sugarcane bagasse, corncob and wheat bran were used as substrates of enzymatic hydrolysis. Hydrolysis was conducted at 37 °C, pH 7.0. Substrate concentration was 10 mg/ml. Enzyme concentrations and hydrolysis periods for xylans from different origins were investigated and the optimized concentrations and hydrolysis periods were as follows: enzyme concentration for sugarcane bagasse xylan and corncob xylan was 10 U/ml, and for wheat bran xylan was 100 U/ml; hydrolysis period for these three sources of xylans was 8 h. Hydrolysis was stopped by heating to 100 °C for 5 min to inactivate the enzyme followed by centrifugation at 2000 r/min for 1 min. An appropriate volume of suspension was taken and dried with nitrogen, dissolved in 1 ml H2O, and filtered through a 0.22 µm filter for further analysis.

3. Results and discussion

3.1. Purification and characterization of X2–X6 from the mixture sample of XOSs

In order to obtain the standards of pure X2–X6 from the sample containing a mixture of XOSs, a systematic method of solid phase extraction (SPE) followed hydrophilic interaction liquid chromatography at semi-preparative scale was developed. The specific procedure of SPE is depicted in Section 2.3. As shown in the Fig. 1 (A–F), peaks 1–6 in the chromatograms were X2–X6. In the sample containing a mixture of XOSs, xylooligosaccharides (XOSs) were the main substances, but other impurities (saccharides and nonsaccharide compounds) were also present. After pretreatment by SPE, the sample containing a mixture of XOSs was fractionated into five eluates. Besides some impurities, the dominant components in eluates a–e were X2, X3, X4, X5 and X6, respectively. The sample complexity was reduced and XOSs of low concentration in the mixture sample were enriched, which enabled increased loading amounts and the removal of impurities prior to further purification by semi-preparative HILIC. The separation conditions for each eluate were optimized, including mobile phase, solvent for the eluate, injection volume, and column temperature. The chromatograms for eluates a–e separated on the semi-preparative Click TE-Cys column with isocratic elution are shown in Fig. 1(G–K). Milligram quantities of pure X2–X6 were obtained after semi-preparative HILIC.
In the HILIC mode, the simple mobile phase consisting of H₂O and organic phase made the methodology of SPE followed by semi-preparative HILIC highly reproducible for the purification of XOSs. After semi-preparative HILIC, fractions of X₂–X₆ collected by time mode were concentrated under reduced pressure, and the high percentage of organic phase in the mobile phase allowed this post-treatment to be completed in a short time. The chromatographic purities of obtained standards were determined by HILIC-ELSD, their purities were more than 99%. The molecular weights and structures of these compounds were determined by ESI-Q-TOF-MS/MS in negative mode and further confirmed by NMR spectroscopy (data not shown). In negative mode, oligosaccharides showed generally good response as singly charged \([M-H]^-\) or \([M+COOH-H]^+\). The deprotonated molecules of X₂–X₆ were \(m/z\) 327.0925, 459.1305, 591.1780, 677.2133, and 809.2700, respectively. These standards could be applied in the development of the method for process control of hydrolysis of xylans.

3.2. Development and validation of quantitative method

Although relative quantification (using average peak areas of samples compared with standards) is currently the popular way for estimating the contents of XOSs during the production process, a simple, rapid and systematically validated quantitative method is still lacking. HILIC is a feasible way for separating polar compounds, which has been proven to be effective for the purification of XOSs in the above experiment, but was never applied to quantification of XOSs according to previous reports. In this study, a reliable quantitative method based on HILIC-ELSD was developed by the standards of xylose and purified X₂–X₆. The HILIC-ELSD chromatograms of the standard solution and the sample are shown in Fig. 2. In our previous study (Fu et al., 2010), column temperature was considered as an important factor that influenced the separation of oligosaccharides. A higher column temperature would provide an increased rate of anomer mutarotation, eliminating the formation of doublet peaks of saccharide diastereomers. A column temperature 60 °C was used and hence better peak shape in the separation of oligosaccharides was obtained. Without non-volatile salt buffer, a simple mobile phase consisting of H₂O and ACN was applied in this study, which also provided a smooth baseline. By using an analytical diol column with weaker retention capacity, separation of X₁–X₆ in 20 min was observed.
The results of calibration curve, calibration range, correlation coefficient and LOQ are summarized in Table 1. Calibration curves were plotted using the logarithm of the peak areas with the logarithm of concentration values. The data indicated the calibration curves of the analytes had good linearity within test ranges, with values of regression coefficient all above 0.9991. The LOQ and LOD ranges of X1–X6 were from 0.1585–0.5259 µg/µl and 0.0916–0.3150 µg/µl, respectively. In order to test the repeatability, six parallel sample preparations were determined and peak area variation (RSD) of analytes was below 4.8%. The intra-day and inter-day precision were within 4.1% and 4.5%, respectively. For determination of stability, sample solution stored at room temperature was investigated by multiple analyses at 0, 12, 24, 48, and 72 h. The peak area variations of X1–X6 were 3.1%, 6.4%, 5.9%, 4.7%, 2.3%, and 0.9%, respectively. The analytes were stable during the tested period. Recoveries ranged from 95.0% to 110.8%.

The developed HILIC-ELSD method was proven to be repeatable, precise, and accurate by validation tests. As an accurate quantification method, it would be applied in the determination of XOSs products.

### 3.3. Comparison of enzymatic and acid hydrolysis of beech xylan for XOSs production

Utilizations of enzymatic hydrolysis and acid hydrolysis for production of XOSs have been extensively reported. In order to select the best hydrolysis mode, both were applied to hydrolysis of beech xylan purchased from Sigma-Aldrich. Effects of enzyme concentrations, hydrolysis periods, acid concentrations, and temperatures were studied.

Akpinar et al. (Akpinar et al., 2010) found that a high concentration of substrate led to poor yield of XOSs, most likely due to inhibition of the enzyme with the increase in viscosity and density of the reaction mixture. Hence, the concentration of substrate was 10 mg/ml in this study. Enzymatic hydrolysis was carried out at 37 °C and pH 7.0 according to the manuscript description. Fig. 3 (a) illustrates the effect of enzyme concentration on XOSs production from beech xylan. The major constituents were X2, X3, X5, and X6 with small amounts of X1 and X4. Increase in enzyme concentration from 2 to 5 U/ml resulted in increased yield of XOSs. When the enzyme concentrations were over 5 U/ml, the observed yields of XOSs stopped increasing. Therefore, an enzyme concentration of 5 U/ml was used in further enzymatic hydrolysis of beech xylan. The effect of hydrolysis period on XOSs production is given in Fig. 3 (b). A hydrolysis period of 8 h seems reasonable, because the amount of XOSs incubated for 8 h was higher than that incubated for 4 h, and similar to amounts obtained after incubation for 12 h or 24 h.

To investigate the probability of more efficient release by acid hydrolysis, this was also used for production of XOSs. Fig. 3 (c) illustrates the effect of acid concentration on XOSs production. Reactions were carried out with 0.2 M, 0.5 M, 1.0 M, and 1.5 M TFA at 100 °C for 1.0 h, respectively. The result showed that the monosaccharide was the major component when acid concentration was higher than 0.5 M. However, a poor conversion was observed when acid concentration was 0.2 M. Therefore, an acid concentration of 0.5 M was used for the further experiments. Fig. 3(d) and (e) show the effects of temperature and hydrolysis period on XOSs production, which were similar to the effect of acid concentration. Increasing the temperature or time course resulted in an increase of the monosaccharide concentration. On the other hand, yields of XOSs were poor at the lowest temperature or for the shortest hydrolysis time. As observed from Fig. 3(d) and (e), a better result could be obtained when xylan are hydrolyzed at 100 °C for 1.0 h.

Comparison between enzymatic hydrolysis and acid hydrolysis was carried out under their optimized conditions and is depicted in Table 2. Both enzymatic hydrolysis and acid hydrolysis can be used for the production of XOSs from beech xylan. Acid hydrolysis was faster (1.0 h), but needed to be operated at a higher temperature (100 °C). Another disadvantage of acid hydrolysis is the high amount of xylose, which accounted for its lower XOSs conversion compared with the conversion of enzymatic hydrolysis. Period of enzymatic hydrolysis was longer than that of acid hydrolysis, but enzymatic hydrolysis was still preferable with fewer xylose, a high XOSs yield, and the mild condition. To enhance the production of XOSs and minimize the xylose, enzymatic hydrolysis was chosen for use in the production of XOSs from agricultural wastes.

### 3.4. Determination of XOSs from sugarcane bagasse xylan, corn cob xylan, and wheat bran xylan by enzymatic hydrolysis

In lignocellulosic materials, xylan links with lignin via arabinose feruloyl ester bridges (Browne, Dian, Hector, Sarah, & Cotta, 2012). Alkaline extraction saponifies these ester bonds, separating xylan and lignin, removing acetyl groups and dissolving xylan. Alkaline extracted xylans from sugarcane bagasse, corn cobs and wheat bran amounted to 7.9%, 4.1% and 7.1%, respectively of the total dry weight.

After alkali extraction of xylans, enzymatic hydrolysis was performed. Since xylan is a heterogenous polymer, the composition and structure of these xylans are different from each other. The structural differences might affect not only the XOSs concentrations but also the level of enzymatic hydrolysis. To obtain desired XOSs products with fewer xylose and high conversions of XOSs, enzyme concentrations and hydrolysis periods for xylans from different origins were investigated (data of optimization process not shown) and the optimized conditions are given in Section 2.7.

The quantitative method based on HILIC-ELSD described above was applied for determination of enzymatic hydrolysis products.

### Table 1

<table>
<thead>
<tr>
<th>Samples</th>
<th>Calibration curve</th>
<th>Range (µg/µl)</th>
<th>R²</th>
<th>LOQ (µg/µl)</th>
<th>Repeatability RSD (%) n = 6</th>
<th>Intra-day RSD (%) n = 6</th>
<th>Inter-day RSD (%) n = 3</th>
<th>Recovery (%)</th>
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</thead>
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<tr>
<td>X1</td>
<td>y = 2.1983x + 4.7221</td>
<td>0.2166–2.0061</td>
<td>0.9999</td>
<td>0.1585</td>
<td>4.3</td>
<td>2.5</td>
<td>3.9</td>
<td>99.5</td>
</tr>
<tr>
<td>X2</td>
<td>y = 2.1371x + 4.3008</td>
<td>0.4210–3.8982</td>
<td>0.9999</td>
<td>0.2460</td>
<td>2.5</td>
<td>2.3</td>
<td>3.8</td>
<td>99.6</td>
</tr>
<tr>
<td>X3</td>
<td>y = 2.1834x + 4.2778</td>
<td>0.4324–4.0036</td>
<td>1.0000</td>
<td>0.2968</td>
<td>4.2</td>
<td>2.3</td>
<td>2.2</td>
<td>108.6</td>
</tr>
<tr>
<td>X4</td>
<td>y = 2.3404x + 4.2830</td>
<td>0.4404–2.0390</td>
<td>0.9998</td>
<td>0.3342</td>
<td>2.3</td>
<td>2.9</td>
<td>3.6</td>
<td>100.0</td>
</tr>
<tr>
<td>X5</td>
<td>y = 2.3049x + 4.0859</td>
<td>0.6093–4.0624</td>
<td>0.9999</td>
<td>0.3865</td>
<td>4.8</td>
<td>4.1</td>
<td>4.4</td>
<td>102.0</td>
</tr>
<tr>
<td>X6</td>
<td>y = 2.3763x + 3.9015</td>
<td>0.6133–4.0888</td>
<td>0.9991</td>
<td>0.5259</td>
<td>2.9</td>
<td>3.0</td>
<td>4.5</td>
<td>107.0</td>
</tr>
</tbody>
</table>

[a] LOQ refers to the limit of quantity, S/N = 10.
[b] Recovery % = (found – original) × 100/added.
[c] Average of three determinations.
[d] Average of nine determinations.
from sugarcane bagasse xylan, corncob xylan and wheat bran xylan. The contents and yields of XOSs in hydrolysis products from different xylan sources are shown in Table 3. For the XOSs contents of varying degrees of polymerization, XOSs contents from sugarcane bagasse were X5 > X3 > X2 > X6 > X4, while XOSs contents from corncob were X2 > X3 > X5 and XOSs contents from wheat bran were X5 > X6 > X3 > X2. The yield of XOSs from sugarcane bagasse xylan was 48.40%, higher than that from corncob xylan (22.47%), and from wheat bran xylan (16.26%). Xylans from different sources were hydrolyzed under their optimized conditions. Although the wheat bran xylan was hydrolyzed at the highest enzyme concentration (100 U/ml), the XOSs yield was the lowest. Generally, endoxylanase has different specificities for xylans from different sources. Most of the endoxylanases are sterically hindered by the substituents in the xylan. The XOSs extracted from sugarcane bagasse, corncob and wheat bran are arabinoxylan compounds which have a backbone of (1 → 4)-b-xylan and are mainly substituted with arabinofuranosyl residues (Bian et al., 2013; 2014).
Mandalarla et al., 2005; Uçkıran Kiran, Akpinar, & Bakır, 2013). The commercial xylanase used in this study has a high activity of endoxylanase. The lowest XOSs yield of wheat bran arabinoxylan indicated that the wheat bran arabinoxylan had more arabinofuranosyl substituents than the other two arabinoxylans.

4. Conclusion
To the authors’ knowledge, this is the first time that a validated accurate quantification method for XOSs separation has been developed and applied. The HILIC-ELSD quantitative method developed in this study allowed simultaneous quantification of X1–X6, xylooligosaccharides from different hydrolysis methods or from different agricultural wastes. Compared with HPAEC and CE, the HILIC mode used in this study could provide a simple baseline because of a simple mobile phase which consisted of H2O and ACN. The HILIC-ELSD can not only be used for better process control of XOSs production, but also it can be applied to the analysis of other functional oligosaccharides. Therefore, this study has important significance for improvement of the quality of functional oligosaccharides.

Conflict of interest
Fangbing Li, Hui Wang, Huaxia Xin, Jianfeng Cai, Qing Fu, and Yu Jin declare that they have no conflict of interest.

Ethical approval
This article does not contain any studies with human or animal subjects.

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Conflict of interest
Fangbing Li, Hui Wang, Huaxia Xin, Jianfeng Cai, Qing Fu, and Yu Jin declare that they have no conflict of interest.

Ethical approval
This article does not contain any studies with human or animal subjects.

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References

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