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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 microorganism (Moure, Gullón, 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, & Yilmaz, 2010). The xylanolytic enzyme system contains a wide range of xylanases. Among them, endoxylanase and β-xylosidase, are the two critically

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important types of enzyme for hydrolysis of the xylan backbond (Chavez, Bull, & Eyzaguirre, 2006). Endoxylanase can cleave the β-1,4 glycoside linkage between xylose units in the backbone, while β-xylosidase 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-xylosidase 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; Nabarlatz, 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; Metsämuuronen, Lyytikäinen, Backfolk, & Sirén, 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 (Liang 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 zwitterionic 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 H_2O (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 H_2O (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 H_2O as the sample for SPE. A home-made cartridge (20 g, 60 ml) was activated with 70 ml of H_2O 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 H_2O, 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