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Preparation of xylooligosaccharides from xylan by controlled acid hydrolysis and fast protein liquid chromatography coupled with refractive index detection

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ABSTRACT

Xylooligosaccharides (XOSs) show many remarkable biological activities and have been widely used in pharmaceuticals, agricultural and functional food-related areas. However, the preparation of pure XOSs standards has not been satisfactory, despite that such standards were crucial for the quality control of XOSs and their products. It is necessary to develop an efficient method for preparation of XOSs. In this study, crude XOSs were obtained by controlled acid hydrolysis of xylan. Parameters of acid hydrolysis such as sulfuric acid concentration, hydrolysis time, and temperature were investigated, allowing optimum conditions to be determined (0.1 M H_2SO_4, 90 °C, 120 min). Then the hydrolysates were isolated and separated by FPLC-RID based on anion exchange chromatography (AEC) using DEAE cellulose and size exclusion chromatography (SEC) using Bio-Gel P-2, respectively. Five purified fractions were obtained, and their purities were above 95% determined by using HPTLC and HPLC-CAD. The fractions were also identified as xylooligosaccharides with degrees of polymerization of 2–6 by using MALDI-TOF-MS and GC–MS analysis. The results indicated that the developed FPLC-RID was an efficient and automatic approach for the separation and purification of XOSs with high purity from controlled acid hydrolysis of xylan, which is helpful to quality control of XOSs and their products.

1. Introduction

Xylooligosaccharides (XOSs) are sugar oligomers made up of xylose units, which could be found in the major component of plant hemicelluloses [1,2]. The most common XOSs are white powders containing a main backbone of 2–9 xylose units linked together by β-1,4 bonds [3]. Pharmacological studies have shown that XOSs exhibit prebiotic properties by selectively promoting the proliferation of *lactobacilli* and *bifidobacterium*, which are beneficial for microorganisms in colons and intestines [4–7]. Furthermore, XOSs also demonstrated beneficial effects on reinforce immune and cardiovascular system [8,9]. Due to their beneficial bioactivities, XOSs have been widely used as ingredients of functional food, and thus the demand of XOSs has been greatly increased in recent years [8–12]. However, qualitative and quantitative analysis of XOSs in food and health products, which is crucial to control their efficacy and safety, is challenge due to the absence of XOS reference compounds. Therefore, it is important to prepare XOSs with high purity.

Acidic hydrolysis of xylan is an effective and practical method to produce XOSs [1,13–15]. The controlled hydrolysis condition was optimizing for the production of XOSs with desired degree of polymerization [1,13,16–18]. Many materials include cereal straws, sugarcane bagasses, rice hulls, malt cakes, tobacco stalks had been used for XOS production [19,20]. However, most of them contain a variety of components that are not xylan, such as arabinose, glucuronic acid, acetyl or methyl groups [5,21], which interfere to the process of getting XOSs with high purity. In this study, beechwood was chosen as material to prepare XOS because it is consists of 90% xylose residues [22,23]. However, separation and purification of XOSs with high purity, especially XOSs with high

Abbreviations: AEC, anion exchange chromatography; CAD, charged aerosol detector; FPLC-RID, fast protein liquid chromatography-refractive index detection; HPTLC, high performance thin layer chromatography; XOSs, xylooligosaccharides.

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degrees of polymerization (DP), remains a challenge due to the high polarity. The current methods for separating XOSs include charcoal chromatography [24], anion-exchange chromatography (AEC) [25] and size-exclusion chromatography (SEC) [26–29], but each of them has its limitations when used alone. For instance, it is difficult to separate XOSs with high DP using charcoal chromatography; XOSs with high purity are hard to be separated only using AEC due to its low resolution; and SEC can hardly separate xylan-hydrolyzed constituents, which are usually mixed with acidic XOSs (the XOSs with a major side chain of 4-O-methyl-glucuronic acid and other side chains of glucuronic acid [30]). Acidic XOSs may interfere with the separation by overlapping with XOSs during the chromatography because of their similar molecular weight. The combination of AEC and SEC methods could not only eliminate the acidic sugars from XOSs, but also improve the efficiency of separation XOSs with high purity. Therefore, we attempted to combine the current methods for optimal results in preparation and purification of XOSs with high purity.

Fast protein liquid chromatography (FPLC), a form of liquid chromatography, is widely applied in purification of protein, RNA, and polysaccharides. More recently, FPLC coupled with refractive index detection (RID) method has also been successfully applied for separation and purification of oligosaccharides [31,32], which has been proven to be of high automation and high resolution. Therefore, in this study, XOSs were fractionized and purified by FPLC-RID coupled with AEC and SEC method. Subsequently, the purity of fractioned XOSs was determined using HPTLC and high performance liquid chromatography coupled with charged aerosol detector (HPLC-CAD), the XOSs were characterized by using MALDI-TOF-MS, and methylation analysis using GC–MS, respectively.

2. Materials and methods

2.1. Materials and chemicals

Beechwood xylan (⩾90% xylose residues), DEAE Cellulose D0909 and Super-DHB (2, 5-dihydroxybenzoic acid + 10% 5-methoxysalicylic acid) were purchased from Sigma Aldrich (St. Louis, Mo, USA). Acetonitrile for HPLC and Silica gel 60 TLC plates were purchased from Merck (Darmstadt, Germany). Bio-Gel P-2 extra fine was purchased from the Bio-Rad company (Hercules, CA, USA). Deionized water was prepared using a Millipore Milli-Q Plus system (Millipore, Bedford, MA, USA). All other chemicals and reagents were of analytical grade.

2.2. Controlled acid hydrolysis of xylan

The acid hydrolysis conditions (concentration of sulfuric acid, hydrolysis time and reaction temperature) of beechwood xylan have been investigated and optimized using univariate design. Oligosaccharides was determined using high performance thin layer chromatography (HPTLC) coupled with densitometry analysis. The relative peak area (RPA) was used to compare the content of XOS under each hydrolysis condition. Briefly, the RPA of the optimum condition for each parameter was set to be 100 and that of the others were calculated as: RPA (B) = (B/A) ∗ 100; (A was the peak area of the optimum condition for each factor; B was the peak area of other conditions). Furthermore, based on the univariate approach experiment results, an orthogonal design Taguchi L9 (3^4) was used to evaluate the combination effects of three parameters on the yield of XOSs derived from xylan. Finally, beechwood xylan (2.0 g) was treated with H_2SO_4 at a final concentration of 0.1 M in a total volume of 500 mL. Then the solution was incubated at the temperature of 90 °C for 120 min under magnetic stirring at 150 rpm. After cooling down, the hydrolysates were neutralized with barium carbonate, and the precipitated salt was removed by centrifugation (3720 × g, 10 min, 25 °C). The supernatant was concentrated and stored at 4 °C before further purification.

2.3. Purification of XOSs

2.3.1. FPLC-RID coupled with AEC

The separation was performed on FPLC-RID system. FPLC-RID consisted of an Akta FPLC system (AKTA Explorer 10, GE Healthcare, Uppsala, Sweden) and a refractive index detector (Agilent Technologies, Palo Alto, CA, USA). The mixed XOSs (0.5 g) were dissolved in 1.0 mL of deionized water. The sample was then filtered through a 0.45 μm Millipore membrane and injected into the anion-exchange chromatography (AEC) column (2.6 × 70 cm) with DEAE Cellulose resin for FPLC separation. The neutral xylooligosaccharides fractions were eluted with water as the mobile phase at a flow rate of 1.0 mL/min. Then acidic xylooligosaccharides were eluted with 0.1 M NaCl in the isocratic mode at the same flow rate of 1.0 mL/min. Then, the neutral xylooligosaccharides fractions were concentrated and further purified using SEC.

2.3.2. FPLC-RID coupled with SEC

The Bio-Gel P-2 packed column (1.6 × 100 cm) was used. The 1 mL of neutral XOSs (0.1 g/mL) was filtered through a 0.45 μm Millipore membrane, and subjected to the gel filtration chromatography with deionized water at a flow rate of 0.3 mL/min for FPLC separation [32]. The fractions were collected in 3 mL per tube.

2.3.3. Purity analysis of XOSs using HPTLC and HPLC-CAD

The purity of fractioned XOSs was determined using HPTLC and HPLC-CAD, respectively. The fractioned XOSs were applied on HPTLC silica gel G60 plates (Merck, Darmstadt, Germany), and followed by scanning with an AS30 HPTLC Applicator (Desaga GmbH, Germany). The quaternary solvent system, 1-butanol/ isopropanol/ acetic acid/ water, (7:5:1:2, v/v/v/v) was used as mobile phase [33]. The plates were dried and colorized with aniline–diphenylamine–phosphoric acid solution, then heated at 105 °C for 5 min on a YOKO-XR plate heater (Wuhan YOKO Technology Ltd., China). In addition, the purity of isolated XOSs were also determined using HPLC-CAD [34]. In brief, the column was a Prevail Carbohydrate ES column (4.6 × 250 mm, 5 μm, i.d) at 30 °C. The mobile phase consisted of water (A) and acetonitrile (B), and the gradient elution mode was as follows: 80–45% B at 0–25 min, 45–30% B at 25–26 min, and 30% B maintained at 26–31 min. The flow-rate was 1.0 mL/min and the injection volume was 10 μL. The nitrogen inlet pressure was set to 35 psi, the response range was 100 pA, and the digital filter was set to 3.

2.4. Characterization of XOSs

2.4.1. MALDI-TOF-MS analysis of XOSs

The MALDI-TOF-MS was performed on a New Ultraflextreme mass spectrometer (Bruker, Bremen, Germany) system, in order to provide the structural elucidation data of the purified XOSs. The lyophilized XOSs powder (3.0 mg) was dissolved in 1.0 mL of deionized water to make up the stock solutions. The Super-DHB was dissolved in 30% (v/v) acetonitrile containing 0.1% TFA to prepare the matrix solution at a final concentration of 5 mg/mL. Then, 10 μL of matrix was mixed with the equivalent volume of XOSs stock solutions. Finally, the obtained solutions were mixed with sodium chloride aqueous solution at a final concentration of 20 μmol/mL. Then, 0.7 μL of the mixed solutions were spotted directly on an MTP AnchorChip target and dried at room temperature. The instrument was equipped with a N_2 laser (337 nm). MS spectra were recorded in the reflector positive mode with two ion source voltages of 25 kV and 23.7 kV, and a mass scan over