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Centrifugal partition chromatography in a biorefinery context: Optimisation and scale-up of monosaccharide fractionation from hydrolysed sugar beet pulp☆

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ABSTRACT

The isolation of component sugars from biomass represents an important step in the bioprocessing of sustainable feedstocks such as sugar beet pulp. Centrifugal partition chromatography (CPC) is used here, as an alternative to multiple resin chromatography steps, to fractionate component monosaccharides from crude hydrolysed sugar beet pulp pectin. CPC separation of samples, prepared in the stationary phase, was carried out using an ethanol: ammonium sulphate (300 g L^{-1}) phase system (0.8:1.8 v:v) in ascending mode. This enabled removal of crude feedstream impurities and separation of monosaccharides into three fractions (L-rhamnose, L-arabinose and D-galactose, and D-galacturonic acid) in a single step. Throughput was improved three-fold by increasing sample injection volume, from 4 to 16% of column volume, with similar separation performance maintained in all cases. Extrusion of the final galacturonic acid fraction increased the eluted solute concentration, reduced the total separation time by 24% and removed the need for further column regeneration. Reproducibility of the separation after extrusion was validated by using multiple stacked injections. Scale-up was performed linearly from a semi-preparative 250 mL column to a preparative 950 mL column with a scale-up ratio of 3.8 applied to mobile phase flow rate and sample injection volume. Throughputs of 9.4 g L^{-1} h^{-1} of total dissolved solids were achieved at the preparative scale with a throughput of 1.9 g L^{-1} h^{-1} of component monosaccharides. These results demonstrate the potential of CPC for both impurity removal and target fractionation within biorefinery separations.

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1. Introduction

Sugar beet pulp (SBP) is an abundant low value by-product from the sugar beet processing industry, which, in the UK, utilises around 8 million tonnes of sugar beet per year. SBP is a rich source of carbohydrates, primarily consisting of cellulose and pectin with a low lignin content (1–2%) [1]. Sugar beet pectin has poor gelling properties due to its high degree of esterification [2], limiting its use as a gelling agent to that of a thickener [3]. As a result SBP has generally been dried and pelleted for sale as low value animal feed [4]. However, its abundance, low cost and high carbohydrate content indicates that SBP could be a significant sustainable feedstock for the production of chemical and pharmaceutical intermediates, while simultaneously undergoing waste valorisation within an integrated sugar beet biorefinery.

Previous work, as part of a wider project on sugar beet pulp fractionation and utilisation, has demonstrated that steam explosion, a high pressure steam treatment, can effectively and selectively convert SBP into two fractions [5]: solubilised pectin and enriched insoluble cellulose. We have demonstrated that the latter provides an effective glucose-based feedstock for fermentation into bioethanol after complete enzymatic hydrolysis [5]. This work focuses on the solubilised pectin fraction, which can be sub-

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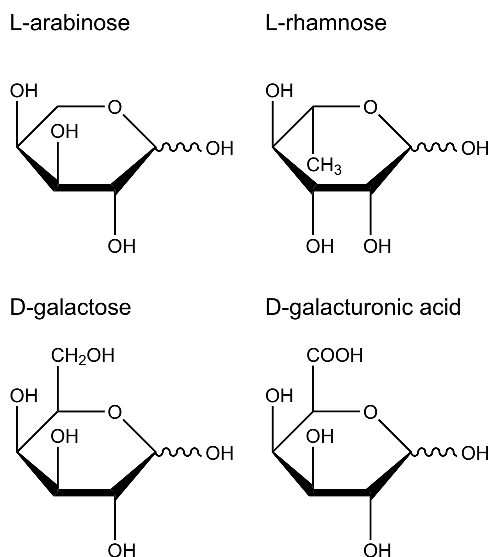

Fig. 1. Structures of the main monosaccharides present in SBP pectin.

jected to complete acid hydrolysis. This is an important first step in the conversion of complex carbohydrate heteropolymers like pectin [6], breaking them down into monosaccharides. In this case, the monosaccharides are primarily L-arabinose (Ara) and D-galacturonic acid (GA), as well as lesser amounts of L-rhamnose (Rha), D-galactose (Gal) and D-glucose (Glu). The structures of these four monosaccharides are shown in Fig. 1.

The pectic monosaccharides have a number of potential applications. GA, as an oxidised sugar, can be used to produce hyperbranched polyesters and plasticisers [7]. Ara can be used to produce biopolymers [8] or reduced to arabinitol, listed as an important value added chemical from biomass for the production of unsaturated polyester resins [7]. Ara can also be upgraded via biocatalysis using transketolase to produce L-gluco-heptulose, which has potential therapeutic applications [9].

Large scale monosaccharide separations are generally performed using simulated moving bed chromatography (SMB) on strong cation exchange resins [10]. SMB can be used to separate fructose and glucose for the production of high fructose corn syrup [11,12], however, SMB is generally limited to binary separations and requires clean input streams. In reality, process streams from biomass tend to contain multiple monosaccharides and a large number of contaminants. Consequently, a series of chromatography steps are required prior to SMB such as deashing and decolourisation [13]. In order to reduce the number of operating steps, a method capable of processing highly complex and contaminated samples is needed.

Centrifugal partition chromatograph (CPC) is a liquid–liquid chromatography technique that partitions solutes between two immiscible liquid phases. One liquid phase is held stationary and retained in the column via centrifugal forces, while the mobile phase is pumped past it in a series of interconnected chambers where the two phases mix and solute partitioning occurs. It has widely been used for the isolation of natural products [14], with only a small number of articles examining the separation of oligosaccharides [15] and glycosides [16], while the separation of monosaccharides has been restricted to the use of counter-current chromatography [17,18]. The lack of a solid stationary phase, simplicity of complete column regeneration and no irreversible adsorption, makes CPC an interesting technology for the separation of crude process streams with little pretreatment, potentially capable of removing impurities and isolating multiple target compounds in a single step.

In our previous CPC work, a synthetic mixture of Ara, GA, Rha and Gal was separated into three fractions (Rha, Ara and Gal, and GA) with an ethanol: ammonium sulphate ($300\,g\,L^{-1}$): DMSO (0.8:1.8:0.1 v:v:v) phase system [19]. This highly hydrophilic two-phase system is capable of separating these structurally similar hydrophilic monosaccharides. In this study, the previously developed methodology was further optimised and applied for the first time for CPC separation of a fully hydrolysed crude sugar beet pectin fraction in order to remove impurities and fractionate Rha, Ara and Gal, and GA. Sample preparation was optimised for the crude material while throughput was improved by increasing sample volume and operating in elution-extrusion mode. Reproducibility of the elution-extrusion method was demonstrated and successful scale-up was performed linearly from a semi-preparative to preparative column.

2. Materials and methods

2.1. Reagents

L-Arabinose (99%), L-rhamnose (99%), D-galacturonic acid sodium salt (98%), D-galactose (99%), analytical grade ammonium sulphate and ethanol were purchased from Sigma-Aldrich (Gillingham, UK). Water was purified using a Millipore Synergy UV Water Purification System (Watford, UK). Sodium acetate trihydrate (ED grade) was purchased from Fisher Scientific (Loughborough, UK). Sugar beet pulp was provided by AB sugar (Wissington, UK).

2.2. Phase system

The two-phase system used throughout this work was ethanol: aqueous ammonium sulphate ($300\,g\,L^{-1}$) (0.8:1.8 v:v). The phase diagram and the exact composition of each phase was determined as described by Ward et al. [19].

2.3. Sample preparation

Acid hydrolysed sugar beet pectin, referred to here as the ‘crude’ material, was prepared using steam explosion to fractionate the sugar beet pulp into insoluble cellulose and soluble pectin phases, as described in Hamley-Bennett et al. [5]. Then, the soluble sugar beet pectin was fully hydrolysed with 2.5% (v/v) sulphuric acid, heated to 121 °C for 1 h in an autoclave and then adjusted to pH 6 with NaOH. The crude material contained a total dissolved solids content of $\sim 100\,g\,L^{-1}$ with a total sugars concentration of $\sim 20\,g\,L^{-1}$.

Crude samples were prepared for CPC by using the crude hydrolysate as the water proportion of the lower phase and adding appropriate amounts of ammonium sulphate ($332\,g\,L^{-1}$) and ethanol (13% v/v). Samples were filtered through a 0.45 μm filter prior to injection.

‘Synthetic crude’ samples were prepared by dissolving the required masses of pure monosaccharides in water and adding the appropriate amounts of ammonium sulphate ($332\,g\,L^{-1}$) and ethanol (13% v/v). The prepared monosaccharide composition contained Ara, GA, Gal and Rha at 43, 41, 11 and $5\,g\,L^{-1}$ respectively, as in our previous work to give a total sugars concentration of $100\,g\,L^{-1}$ [19]. Samples were filtered through a 0.45 μm filter prior to injection.

2.4. CPC equipment and operating conditions

CPC separations were performed on a Kromaton FCPC-A (fast centrifugal partition chromatography – Rousselet Robatel Kromaton, Annonay, France) on a semi-preparative and a preparative column with experimentally determined total volumes of 250 mL and 950 mL respectively. Both columns feature a twin-cell design

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