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Assessment of the bifidogenic effect of substituted xylo-oligosaccharides obtained from corn straw

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ABSTRACT

This work evaluates the bifidogenic potential of substituted xylo-oligosaccharides (XOS) obtained from a lignocellulosic feedstock (corn straw). Autohydrolysis was used to selectively hydrolyse the xylan-rich hemicellulosic fraction and the soluble oligosaccharides were purified by gel filtration chromatography. Selected oligosaccharides fractions within the target ranges of polymerization degree (4–6 and 9–21, samples S1 and S2, respectively) were characterized and their bifidogenic potential was investigated by *in vitro* fermentations using human fecal inocula. Bacterial growth was assessed by fluorescent *in situ* hybridization (FISH). XOS consumption and short-chain fatty acids (SCFA) production were evaluated and compared with commercial oligosaccharides. Under the tested conditions, all the substrates were utilized by the microbiota, and fermentation resulted in increased bifidobacteria populations. Samples S1 and S2 increased bifidobacteria populations and the production profile of SCFA was similar for XOS samples and commercial oligosaccharides although XOS samples displayed the highest concentration of SCFA on longer fermentation times.

1. Introduction

The scientific and commercial interest of oligosaccharides has increased significantly in the last two decades. This is mainly due to the identification of several health and technological properties benefited from these compounds. On the health aspect, the most recognized trait is their ability to stimulate the growth of beneficial microflora in the gut which has been associated to a prebiotic effect (Rastall, 2010). Certain classes of oligosaccharides have also demonstrated bioactive properties such as antioxidant activity, antimicrobial activity, immunostimulatory activity and anti-allergen activity, among others (Rastall, 2010). Furthermore, these compounds also have some advantageous technological properties, in particular their high pH and thermal stability which makes their use as food additives very interesting but also with potential to be used in the feed, food, pharmaceutical, chemical and biotechnological industries.

The commercially available oligosaccharides are mainly obtained by enzymatic reactions of lactose, sucrose or inulin (Moreno, Montilla, Villamiel, Corzo, & Olano, 2014). The renewable lignocellulosic biomass is an emerging resource that presents high potential to be used as feedstock for the production of novel oligosaccharides which is still almost unexplored. In fact, hemicellulosic oligosaccharides such as xylo-oligosaccharides (XOS) for example, are the only oligosaccharides in the market obtained from lignocellulosic materials and their market is still very small, particularly in the EU and US. The current production of commercial XOS (Suntory Ltd., Japan) is carried out using an alkaline extraction of hemicelluloses from biomass followed by enzymatic hydrolysis. These processes lead to obtain non-substituted xylo-oligosaccharides (nXOS) with low DP, which may display a different fermentation behavior and bioactivity potential (Kabel, Kortenoeven, Schols, & Voragen, 2002; Van Laere, Hartemink, Bosveld, Schols, & Voragen, 2000).

Xylose-based oligomers such as XOS have shown potential prebiotic effect by stimulating the growth of microflora in the gastrointestinal tract, *i.e.*, *Bifidobacterium* spp. and *Lactobacillus* spp., that are related to the prevention and treatment of several gut

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health disorders and thus, can enhance health (Moure, Gullon, Dominguez, & Parajó, 2006).

Amongst the biomass sources available, agricultural residues are interesting and potentially low-cost biomass. Corn (*Zea mays* L.) is one of the most produced crops worldwide and its residues, namely straws, contribute to large quantities of renewable lignocellulosic biomass. Currently, this biomass is mainly used for low value applications but due to their chemical composition, availability and low-cost, they are an attractive feedstock.

As alternative to the processes already in use for the production of commercial (n)XOS, the selective fractionation of hemicelluloses using mild hydrothermal pretreatments, such as autohydrolysis enables the selective hydrolysis of hemicelluloses, and a high recovery of soluble XOS (Kabel, Carvalheiro, et al., 2002; Moniz, Pereira, Duarte, & Carvalheiro, 2014). Besides hemicellulose-derived oligosaccharides, the hydrolysates from hydrothermal processing also contain monosaccharides and other compounds, which should be separated from target compounds in order to improve the purity. This can be achieved by membrane processing but sequential membrane-based steps are needed for concentration and fractionation to achieve high-purity XOS. This method has already been employed in a multistage purification process (dos Santos et al., 2011; Gonzalez-Munoz, Rivas, Santos, & Parajó, 2013). In contrast to membrane processes, chromatographic separation has the advantage to yield oligosaccharides with high purity and separated by molecular weight and/or chemical structure (Gírio et al., 2003). Gel filtration chromatography (GFC) was used for the purification of XOS-rich hydrolysates obtained by autohydrolysis of olive tree prunings, corn cobs and rice straw enabling their fractioning by degree of polymerization (DP), and excluding low molecular weight components, such as monosaccharides, and by-products such as furfural and hydroxymethylfurfural (HMF) (Cara et al., 2012; Ho et al., 2014; Moura et al., 2007; Moniz et al., 2014).

Different types of oligosaccharides have been studied by various *in vitro* methods, animal models and human clinical trials. Xylo-oligosaccharides for example, have shown intestinal improvement, hypolipidemic activities and antimicrobial activity against some bacteria (Christakopoulos et al., 2003). Furthermore, XOS also improved *in vitro* growth of *Bifidobacterium* spp. (Moura et al., 2007); which was as effective as raffinose and higher than fructo-oligosaccharides (FOS) (Vazquez, Alonso, Dominguez, & Parajo, 2000). The majority of the studies on prebiotics have focused on inulin, FOS, galacto-oligosaccharides (GOS), and lactulose. These groups of carbohydrates are the leading commercial prebiotics due to their efficacy in humans (Moreno et al., 2014), and history of safe commercial use (Bouhnik et al., 2004; Macfarlane, Steed, & Macfarlane, 2008). Nevertheless, there is an increase in the interest to develop potentially new prebiotic ingredients, with different structures from both conventional and non-conventional sources, including wood or straws.

In this work, corn straw was subjected to non-isothermal autohydrolysis in order to obtain substituted xylo-oligosaccharides (XOS). The oligosaccharides in the hydrolysates were purified and separated according to their molecular mass using gel filtration chromatography (GFC). The purification effects attained by GFC were measured, and two fractions of refined XOS from corn straw were assayed for bifidogenic properties (generation of SCFA and bifidogenic potential) by *in vitro* fermentations using fecal inocula.

2. Material and methods

2.1. Raw material

Corn straw was supplied by Estação Nacional de Melhoramento de Plantas (Elvas, Portugal) as a heterogeneous sample containing stalks and leaves. Upon arrival, the raw material was ground with a knife mill (Fritsh Industriestr, Germany) to particle size < 6 mm and set in a homogenized lot as described before (Moniz, Pereira, Quilhó, & Carvalheiro, 2013).

2.2. Hydrothermal processing of corn straw

Autohydrolysis treatments of the corn straw lot were performed in a stainless steel reactor (Parr Instruments Company, USA) with a total volume of 600 mL, under previously optimized conditions (Moniz et al., 2013). Briefly, the raw material was mixed with water in the reactor in order to obtain a liquid-to-solid ratio (LSR) of 10 (g water/g dry raw material). The agitation speed was set at 150 rpm and the reactor heated to reach a final temperature of 215 °C, after which, the reactor was rapidly cooled down and the liquid and solid phases were recovered by pressing (up to 190 bar) using a hydraulic press (Sotel, Portugal). The liquid phase was filtered using Whatman filter paper no. 1 and the hydrolysates from several runs were combined in a single lot and used for further purification.

2.3. Purification of corn straw hydrolysates

Oligosaccharides purification was carried out using preparative gel filtration chromatography in an Amersham Pharmacia Biotech system (Sweden) equipped with a refractive index detector (K-2401 Knauer, Germany). A 400 mL XOS-rich hydrolysate was eluted with deionized water at a flow rate of 25 mL min^{-1} through a BPG 100/950 column (Amersham Pharmacia Biotech, Sweden) with a Superdex 30^{TM} gel bed volume of 4.2 L, using a similar strategy reported for the separation of the rice straw oligosaccharides (Moniz et al., 2014). The sample was fractionated into 28 fractions of 125 mL, and collected every 5 min using a Super-frac^{TM} collector (Amersham Pharmacia Biotech, Sweden). All fractions were freeze-dried (Labconco, MO, USA), weighted for mass quantification, and analyzed for molecular weight and chemical composition.

2.4. Fermentation

2.4.1. Fecal inocula

Fecal samples were obtained from three healthy human volunteers who were free of known metabolic and gastrointestinal diseases (*e.g.*, diabetes, ulcerative colitis, Crohn's disease, irritable bowel syndrome, peptic ulcers and cancer). The samples were collected on site, kept in an anaerobic cabinet (10% H_2, 10% CO_2, and 80% N_2), and used within a maximum of 15 min after collection. Samples were diluted 1/10 (w/w) in anaerobic phosphate-buffered saline (PBS; 0.1 mol L^{-1}, pH 7.4) and homogenized in a stomacher (Stomacher 400, UK) for 2 min at normal speed.

2.4.2. In vitro *fermentations*

Sterile stirred batch culture fermentation systems (50 mL working volume) were set up and aseptically filled with a 45 mL volume of sterile, basal medium: peptone water (Oxoid, UK), 2 g L^{-1} yeast extract (Oxoid, UK), 0.1 g L^{-1} NaCl, 0.04 g L^{-1} K_2HPO_4, 0.04 g L^{-1} KH_2PO_4, 0.01 g L^{-1} $MgSO_4{\cdot}7H_2O$, 0.01 g L^{-1} $CaCl_2{\cdot}6H_2O$, 2 g L^{-1} $NaHCO_3$, 2 mL Tween 80 (BDH, UK), 0.05 g L^{-1} haemin, 10 μL vitamin K1, 0.5 g L^{-1} cysteine HCl, 0.5 g L^{-1} bile salts, pH 7.0, and gassed overnight with oxygen-free nitrogen (15 mL min^{-1}). The samples of substituted XOS mixtures (S1 and S2), commercial XOS (avDP2, Shandong Longlive Biotechnology Co. Ltd., China) and FOS (avDP4, Orafti® P95, Beneo, Tienen, Belgium), in the concentration of 10 g L^{-1}, were added to the respective fermentation just prior to the addition of the fecal inoculum. The temperature was kept at 37 °C, and the pH was kept between 6.7 and 6.9 using an automated pH controller (Fermac 260; Electrolab, UK). Each vessel was inoculated with 5 mL of fresh fecal slurry (1/10, w/w). The batch cultures