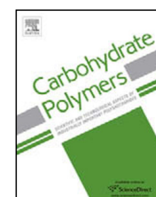
Carbohydrate Polymers 154 (2016) 305–312

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol



Birch pulp xylan works as a food hydrocolloid in acid milk gels and is fermented slowly *in vitro*

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ARTICLE INFO

Article history:
Received 9 February 2016
Received in revised form 18 May 2016
Accepted 6 June 2016
Available online 15 June 2016

Keywords:
Birch pulp xylan
Acid milk gel
In vitro colonic fermentation
Gas pressure evolution
Short-chain fatty acid

ABSTRACT

The objective was to evaluate the potential of birch xylan as a food hydrocolloid and dietary fibre. High-molecular weight xylan was isolated from birch kraft pulp by alkaline extraction, and enzymatically hydrolysed. Fermentability of xylans was evaluated using an *in vitro* colon model and performance as a hydrocolloid was studied in low-fat acid milk gels (1.5% and 3% w/w). Texture of the gels and water holding capacity of xylans were compared with inulin, fructooligosaccharide and xylooligosaccharide. Xylans showed slower fermentation rate by faecal microbiota than the references. Xylan-enriched acid milk gels (3% w/w) had improved water holding capacity (over 2-fold) and showed lower spontaneous syneresis, firmness and elasticity when compared to control (no hydrocolloids) or to references. In conclusion, birch xylan improved texture of low-fat acid milk gel applications, and the slow *in vitro* fermentation rate predicts lower incidence of intestinal discomfort in comparison to the commercial references.

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1. Introduction

Food industry is in pressure of creating healthy less energy-dense food structures and thus there is constant need for new structuring and bulking agents to build novel food matrices that contain water and/or air. Non-digestible polysaccharides can act as both performance ingredients (i.e. texturing and stabilising agents), but also as dietary fibres (DF) providing potential health effects (Collins et al., 2010). Hemicelluloses, comprising the non-cellulose cell-wall polysaccharides of plants, represent an immense renewable resource of attractive biopolymers, which can be utilized in their native or modified forms in various areas, including food applications (Ebringerová, Hromádková, & Heinze, 2005). Hardwood contains 15–30% xylan which consists of β(1–4) linked xylopyranose backbone substituted with acetyl groups at positions C2 or C3, and α(1–2) linked methylglucuronic acid (Willför, Sundberg, Pranovich, & Holmbom, 2005). Xylan from wood or agro-based materials can be isolated using a variety of extraction methods (Glasser, Kaar, Jain, & Sealey, 2000; Huang, Ramaswamy, Tschirner, & Ramarao, 2008; Sjöström, 1993). Up to 60% of the xylan present in bleached hardwood pulp can be isolated by alkaline extraction followed by precipitation and ultrafiltration to obtain a pure, high-molecular weight and little substituted xylan product (Laine et al., 2013; Teleman, Larsson, & Iversen, 2001).

The potential use of polysaccharides as food ingredient is often based on their properties of thickening and inducing gel-like characteristics, thus controlling the structure and texture of products (Banerjee & Bhattacharya, 2012). Furthermore, plant origin non-digestible polysaccharides represent an important source of DF that is digested by gut microbiota maintaining healthy gastrointestinal function. Wood xylans have shown to be substrates of xylanolytic microbial community from human faeces *Roseburia* Sp. and *Bacteroides* Sp. being the predominating ones (Chassard et al., 2007). Many health effects are believed to be related to DF and its fermentation products, short-chain fatty acids in the large intestine (Raninen, Lappi, Mykkänen, & Poutanen, 2011; Wong, Russell de Souza, Kendall, Emam, & Jenkins, 2006). The physiological effects of arabinoxylans as isolated polymers or present in diverse cereal matrices have been extensively studied *in vitro* and *in vivo* (Garcia et al., 2007; Lu, Walker, Muir, Mascara, & O'Dea, 2000; Nordlund et al., 2012; Rosa et al., 2013). The beneficial effects of colonic degradation of arabinoxylans as well as arabinoxylan-oligosaccharides and xylooligosaccharides (XOS) include production of short-chain fatty acids (SCFA) especially butyrate, increase of bifidobacterial levels, and shortening transit time (Broekaert et al., 2011). Concerning the physiological effects of xylans originating from wood or wood pulp, only few studies have been performed. Falck et al. (2013) showed that hardwood xylan hydrolysed by endoxy-

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http://dx.doi.org/10.1016/j.carbpol.2016.06.028
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lanases presented prebiotic properties (growth of *Lactobacillus brevis* and *Bifidobacterium adolescentis*), whereas non-treated polymeric xylans were not fermented by any of the strains.

Dairy products such as yogurts are a potential target for using non-digestible carbohydrates both as hydrocolloids for texture improvement and as a source for DF enrichment. Many different types of soluble or insoluble DF such as inulin (Meyer, Bayarri, Tárrega, & Costell, 2011; Paseephol, Small, & Sherkat, 2008), dextran (Mende et al., 2013), xylooligosaccharide (Mumtaz, Salim-Ur-Rehman, Huma, Jamil, & Nawaz, 2008), pectin (Jensen, Rolin, & Ipsen, 2010), β-glucan (Brennan & Tudorica, 2008; Lazaridou, Vaikousi, & Biliaderis, 2008), fibres from apple (Dello Staffolo, Bertola, Martino, & Bevilacqua, 2004), wheat bran (Aportela-Palacios, Sosa-Morales, & Vélez-Ruiz, 2005) and fibre from asparagus shoots (Sanz, Salvador, Jiménez, & Fiszman, 2008) have been used for enrichment of acid milk gels/yogurts. Besides nutritional potential of yogurt enriched in DF, these studies have focused on texture and reduction of syneresis, decisive for consumer acceptance. Moreover, these polysaccharides can act as fat mimetics in low-fat products (Li & Nie, 2014), as the structure of a dairy matrix is affected by protein-polysaccharide interactions. These interactions are related with the nature and concentration of the biopolymers, molecular weight, charge, distribution of charges as well as environmental conditions, such as pH (Corredig, Sharafbafi, & Kristo, 2011).

Birch pulp xylans could have potential as food hydrocolloids for dairy applications due to their structural characteristics, such as backbone with little substitution degree, and the white colour of isolated xylan (Laine et al., 2013). This specific xylan is of high purity without significant amounts of lignin, wood extractives or inorganic compounds. The objective of this work was to evaluate the potential of wood xylan extracted from birch pulp to be used as a dietary fibre and hydrocolloid in dairy application. In this perspective, the availability of birch xylan from industrial sources in large quantities using relatively easy and inexpensive processes could be a feasible source of xylan. With this aim, xylan was isolated from bleached pulp and enzymatically hydrolysed to shorten it chain length. The fermentability of xylans as DF was evaluated in an *in vitro* colon model and compared to inulin, fructooligosaccharide and xylooligosaccharide. The ability of both isolated and hydrolysed xylan to act as hydrocolloid was evaluated by analysing the textural properties and water holding capacity of low fat acid milk gels.

2. Materials and methods

2.1. Extraction and hydrolysis of xylan from birch pulp

Birchwood cellulose pulp obtained from a pulp mill in Finland was used as raw materials for xylan extraction. A total of 125 kg bleached birch kraft pulp was extracted in approximately 40 kg batches at 4.2% consistency in 1 M NaOH for 1 h in 1000 m^3 container. The fibres were separated using a decanter centrifuge and bag filter. Xylan was isolated from the alkaline filtrate by ultra- and diafiltration using UFX10 pHt membranes with 15 m^2 area yielding a dispersion containing about 7.7% xylan (dw). This isolated xylan dispersion is called as “Xyl” in this manuscript. Part of the xylan was hydrolysed using a food grade xylanase enzyme product Depol 740L from Biocatalysts Ltd (Cardiff, United Kingdom). Prior to hydrolysis the pH was adjusted to 5 with 1 M HCl. The 2-h hydrolysis was carried for at 7.4% dry matter content, 50 °C, using 1000 nkat xylanase/g of substrate. The xylanase activity of the preparate was assayed as described in Bailey, Biely, and Poutanen (1992). This hydrolysed xylan is called as “Hyd-Xyl”.

2.2. Characterization of the xylan samples

To determine the carbohydrate composition, xylan samples were hydrolyzed with sulphuric acid and analysed according to Willför et al. (2009). The resulting monosaccharides were determined by HPAEC with pulse amperometric detection (Dionex ICS 3000A) equipped with CarboPac PA1 column. Molar mass measurements of xylan were performed by size exclusion chromatography (SEC) in 0.1 M NaOH using PSS’s MCX 1000 and 100 000 columns with a pre-column (0.5 ml/min, T = 30 °C). Refractive index (RI) detector was used and the molar mass distributions of xylan were calculated in relation to pullulan standards using Waters Empower 2 software.

Viscosity of xylan samples was measured at 4 °C with a stress-controlled rotational rheometer (AR-G2; TA Instruments, Crawley, West Sussex, UK) equipped with a cylindrical sample cup (30 mm) and a four-bladed vane geometry (28 mm). About 50 ml of the sample was poured into the measuring cup and the vane was lowered into it so that the blades were just immersed. The steady-state viscosity was measured with a gradually increasing shear stress with values resulting in shear rates in the range 0.01–500/s. The viscosity was analysed in triplicate. For the measurement of water holding capacity (WHC), the xylans extracts were weighed in an Eppendorf tube and centrifuged at 12,100*g* for 15 min, 20 °C. After centrifugation, the supernatant was carefully removed and the wet pellet was weighed. The WHC was calculated as gram of water retained per gram of sample dry matter.

2.3. *Metabolical* in vitro *colon model*

The xylan samples were concentrated using a rotary evaporator (Heidolph Instrument GMbH & Co. KG, Schwabach, Germany) (40 °C, 80 rpm) before the *in vitro* colonic fermentation. After drying, the samples were homogenised and their moisture content was determined by oven drying (105 °C, overnight). The dry matter reached was 53.5 and 59.7 (g dry matter/100 g sample) for native and hydrolysed xylans, respectively. Long-chain inulin (INU) (Orafti® HP, Beneo, average DP 23), fructo-oligosaccharides (FOS) (Orafti® P95, Beneo; DP 2 – 8), *xylo*-oligosaccharides (XOS) (Shandong Longlive Bio-tech Co. Ltd, Japan, DP 1–6) were used as reference compounds.

In vitro colon model was performed according to Barry et al. (1995) with the following modifications: 100 mg (on dry weight basis) of supplements were weighed in the bottles (50 ml), and hydrated with 2 ml of medium one day before inoculation. Human faeces were collected from 5 healthy volunteers, who had not received antibiotics for at least 6 months and had given a written consent. The collection of faecal samples was performed with an approval of and according to the guidelines given by the Ethical Committee of Technical Research Centre of Finland. Freshly passed faeces were immediately taken in an anerobic chamber or closed in a container with an oxygen consuming pillow (Anaerocult Mini; Merck, Darmstadt, Germany) and a strip testing the anaerobiosis (Anaerotest; Merck, Darmstadt, Germany). Faecal suspension was prepared under strictly anaerobic condititions. Equal amounts of faecal samples were pooled and diluted to a 12.5% (w/v) suspension, 8 ml of which was dosed to the fermentation bottles to obtain a 10% (w/v) final faecal concentrations described previously Aura et al. (2006). Incubation was performed at 37 °C in tightly closed bottles and in magnetic stirring (250 rpm). Faecal control was incubated without addition of the supplements. A time course of 0, 2, 4, 6, 8 and 24 h was followed using the same inoculum for all the supplements by taking 1.5 ml aliquots from the incubated suspensions. The fermentation experiments were performed in triplicate.

For gas pressure (bar) evolution rate and extent measurement in the *in vitro* colon model as described in Nordlund et al. (2012),

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