Carbohydrate Polymers 131 (2015) 50–56

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

In vitro digestion and fermentation properties of linear sugar-beet arabinan and its oligosaccharides

Jin Seok Moon [a], So Yeon Shin [a], Hye Sun Choi [b], Wooha Joo [a], Seung Kee Cho [a], Ling Li [a], Jung-Hyun Kang [a], Tae-Jip Kim [a], Nam Soo Han [a,*]

[a] *Brain Korea 21 Center for Bio-Resource Development, Division of Animal, Horticultural, and Food Sciences, Chungbuk National University, Cheongju, Republic of Korea*

[b] *Department of Agro-food Resource, National Academy of Agricultural Science, RDA, Jeonju, Republic of Korea*

ARTICLE INFO

Article history:
Received 5 March 2015
Received in revised form 1 May 2015
Accepted 5 May 2015
Available online 19 May 2015

Keywords:
Digestibility
Sugar-beet arabinan
Linear arabino-oligosaccharides (LAOS)
Prebiotics
In vitro fermentation

ABSTRACT

This study was conducted to investigate the prebiotic effects of linear arabino-oligosaccharides (LAOS) and debranched (linear) sugar beet arabinan (LAR) for the development of new prebiotics. LAOS were prepared from LAR by enzymatic hydrolysis with endo-arabinanase from *Bacillus licheniformis*, followed by removal of the arabinose fraction by incubation with resting cells of *Leuconostoc mesenteroides*. The resulting LAOS contained DP2 (28.7%), DP3 (49.9%), DP4 (20.1%), and DP5 (1.16%). A standardized digestibility test showed that LAOS and LAR were not digestible. Individual cultures of 24 strains of gastrointestinal bacteria showed that LAOS and LAR stimulated growth of *Lactobacillus brevis*, *Bifidobacterium longum*, and *Bacteroides fragilis*. *In vitro* batch fermentation using human fecal samples showed that LAOS had higher bifidogenic properties than LAR; LAOS increased the population of bifidobacteria which produced short-chain fatty acids (SCFAs). LAOS was fermented slowly compared to fructo-oligosaccharides and this may permit SCFA production in the distal colon. This study demonstrates that LAOS prepared from LAR are promising dietary substrates for improvement of human intestinal health.

1. Introduction

Recently, the use of indigestible carbohydrates as functional food components targeted at improving gut health has attracted much attention (Mussatto & Mancilha, 2007; Roberfroid & Slavin, 2000). Some polymers or oligosaccharides are selectively fermented in the human colon, and can be described as prebiotics. The currently accepted criterion for prebiotic activity is an increase in populations of *Bifidobacterium* spp. and *Lactobacillus* spp., with a decrease in less desirable bacterial groups such as species of *Clostridium* and *Bacteroides* (Gibson & Roberfroid, 1995). Although the prebiotic effects of various carbohydrates have been investigated (Rycroft, Jones, Gibson, & Rastall, 2001), only a small number have been commercialized to date (Crittenden & Playne, 1996). In view of the growing interest in prebiotic carbohydrates, it is of interest to investigate the potential for generation of novel forms from readily available and renewable carbohydrate sources. Arabinan is a pectic polysaccharide consisting of highly branched α-1,5-linked chains of arabinofuranoside residues (Oosterveld, Beldman, & Voragen, 2002). Arabinan has a molecular weight ranging from range 5700 to 10,000 kDa. Substantial potential exists for converting such by-product from sugar industry into new value-added products. Several types of indigestible fibers and oligosaccharides from plant cell walls have shown the capability to modulate the microbial composition in the human gut (van Laere, Hartemink, Bosveld, Schols, & Voragen, 2000). By *in vitro* fermentation in human fecal slurry, it was shown that various types of arabino-oligosaccharides (AOS) derived from sugar beet pulp had a bifidogenic effect (Holck et al., 2011; Sulek et al., 2014; Vigsnæs, Holck, Meyer, & Licht, 2011). Previous studies have used branched AOS or mixed AOS (containing linear and branched), but did not use the purified linear form of AOS although the linear structure might be easily fermentable by diverse intestinal bacteria. In addition, the AOS used in the previous studies contained arabinose monomers which might give effect to the results. Furthermore, the digestibility of the AOS was not tested before investigating its fermentation properties, although arabinan is susceptible to breakdown in mildly acidic conditions (Kusema et al., 2010). For evaluation of the prebiotic effects of AOS, hydrolysis of AOS in a digestive system (in gastric and intestinal fluids) should be investigated before testing their bifidogenic effects by following a standardized procedure.

* Corresponding author. Tel.: +82 43 261 2567; fax: +82 43 271 4412.
E-mail address: namsoo@chungbuk.ac.kr (N.S. Han).

http://dx.doi.org/10.1016/j.carbpol.2015.05.022
0144-8617/

In this study, linear arabino-oligosaccharides (LAOS) and debranched (linear) sugar beet arabinan (LAR) were tested for their digestibility in the human gastrointestinal tract and their prebiotic effects in the large intestine. LAOS were prepared from LAR by enzymatic hydrolysis with endo-arabinanase from *Bacillus licheniformis*, and subsequent removal of the arabinose fraction by incubation with resting cells of *Leuconostoc mesenteroides*. For the digestibility test, a recent standard procedure was employed, using artificial salivary, gastric, and small intestinal fluids. For the prebiotic tests, *in vitro* batch fermentations were conducted in test tubes and fermenters using intestinal type cultures (24 strains) and human fecal samples. In addition, the production of short-chain fatty acids (SCFAs) and gas during the batch fermentation using LAOS and LAR was analyzed. For comparative purposes, a commercial fructo-oligosaccharides (FOS) were also used.

2. Materials and methods

2.1. Materials

A debranched sugar beet arabinan (LAR) with average molecular weight of 18 kDa was purchased from Megazyme Co. (Lot 100401b, Wicklow, Ireland), which was prepared by treatment of sugar-beet arabinan with α-L-arabinofuranosidase to remove all 1,2- and 1,3-α-L-arabinofuranosyl branch units. Fructo-oligosaccharides (FOS) were composed of β-1,2-linked fructose oligomers and a mixture of 1-kestose, nestose, and 1-fructofuranosyl-D-nystose (Wako, Osaka, Japan). The de Man, Rogosa and Sharpe (MRS) medium and yeast extract were obtained from Difco (Detroit, USA) and the peptone water was obtained from Oxoid (Basingstoke, UK). All materials were of standard analytical grade.

2.2. Preparation of LAOS from sugar beet arabinan

Recombinant arabinanase (BLABNase) (Park et al., 2012) from *B. licheniformis* was expressed in *Escherichia coli* MC 1061, and the enzyme linked with the C-terminal 6-histidine tag was purified using Ni-NTA column chromatography. Using this recombinant BLABNase, debranched sugar beet arabinan (LAR) was partially hydrolyzed in 50 mM sodium acetate buffer (pH 6.0). Residual monosaccharides were removed by fermentation with *L. mesenteroides* subsp. *mesenteroides* ATCC 8293 at 30 °C for 24 h. Arabinose residue was successfully removed from the LAOS (Supplementary data, Fig. S1) and the concentrations of the resulting oligomers were DP2 (4.44 mg/ml), DP3 (7.73 mg/ml), DP4 (3.12 mg/ml), and DP5 (0.18 mg/ml).

2.3. In vitro *digestibility of LAOS*

The *in vitro* digestibility of LAOS and LAR were investigated according to the method published by Minekus et al. (2014). Experimental protocol (oral): LAOS and LAR (250 μl) were thoroughly mixed with 175 μl of simulated salivary fluid (SSF) electrolyte stock solution (KCl, 15.1 mmol/l, KH_2PO_4 3.7 mmol/l, $NaHCO_3$ 13.6 mmol/l, $MgCl_2(H_2O)_6$ 0.15 mmol/l, $(NH_4)_2CO_3$ 0.06 mmol/l, $CaCl_2(H_2O)_2$ 1.5 mmol/l, pH 7.0), 25 μl salivary α-amylase (from human saliva Type IX-A, Sigma) solution of 1500 U/ml made up in SSF electrolyte stock solution, 1.25 μl of 0.3 M $CaCl_2$ and 48.75 μl of water. Enzyme reaction was ceased after 5 min heating at 100 °C. Experimental protocol (gastric): LAOS and LAR (250 μl) was mixed with 187.5 μl of simulated gastric fluid (SGF) electrolyte stock solution (KCl 6.9 mmol/l, KH_2PO_4 0.9 mmol/l, $NaHCO_3$ 25 mmol/l, NaCl 47.2 mmol/l, $MgCl_2(H_2O)_6$ 0.1 mmol/l, $(NH_4)_2CO_3$ 0.5 mmol/l, $CaCl_2(H_2O)_2$ 0.15 mmol/l, pH 3.0), 40 μl of porcine pepsin stock solution (25,000 U/ml) made up in SGF electrolyte stock solution (pepsin from porcine gastric mucosa 3200–4500 U/mg protein, Sigma), 0.125 μl of 0.3 M $CaCl_2$, 1 M HCl to adjust pH to 3.0, and rest of volume with water. The sample was neutralized to pH 7.0 by adding $NaHCO_3$ (0.5 M) that was filtered through a 0.22 μm filer. Experimental protocol (intestinal): 250 μl of LAOS, LAR is mixed with 137.5 μl of simulated intestinal fluid (SIF) electrolyte stock solution (KCl 6.8 mmol/l, KH_2PO_4 0.8 mmol/l, $NaHCO_3$ 85 mmol/l, NaCl 38.4 mmol/l, $MgCl_2(H_2O)_6$ 0.33 mmol/l, $CaCl_2(H_2O)_2$ 0.6 mmol/l, pH 7.0), 62.5 μl of a pancreatin solution 800 U/ml based on pancreatin α-amylase activity made up in SIF electrolyte stock solution (pancreatin from porcine pancreas, Sigma), 31.25 μl of SIF electrolyte stock replacing bile, 5 μl of 0.3 M $CaCl_2$, NaOH to adjust pH to 7.0, and water filling rest of the volume. The amount of NaOH-HCl to be added for pH adjustment is pre-determined through pilot experiment. Enzyme reaction is ceased after 5 min heating at 100 °C.

2.4. Utilization of LAOS by intestinal bacterial species

Bacterial strains (Supplementary, Table S1) were obtained from the American Type Culture Collection (Manassas, USA) and the Korean Collection for Type Cultures (Daejeon, Korea). Seed cultures were incubated overnight until the optical density at 580 nm reached 0.5. To study the growth of individual bacterial strains in the presence of LAOS and LAR (0.2% w/v), sugar-free basal medium (BHI) was prepared according to Zhang, Mills, and Block (2009). LAOS and LAR were dissolved in BHI medium for 1 h before inoculation with 1% w/v bacterial slurry in phosphate-buffered saline (PBS). Fermentation was conducted under anaerobic conditions at 37 °C, and culture broth collected at 48 h was used to measure microbial growth in a microtiter plate at 580 nm. Each data point for an experiment was the average of 3 wells and all experiments were carried out in triplicate.

2.5. In-vitro *fecal batch fermentation*

In vitro fecal batch fermentation of LAOS and LAR was conducted according to Mandalari, Nueno-Palop, Bisignano, Wickham, and Narbad (2008). Water-jacketed fermenter vessels (300 ml) were filled with 135 ml of pre-sterilized basal growth medium (2 g/l peptone water, 2 g/l yeast extract, 0.1 g/l NaCl, 0.04 g/l K_2HPO_4, 0.04 g/l KH_2PO_4, 0.01 g/l $MgSO_4{\cdot}7H_2O$, 0.01 g/l $CaCl_2{\cdot}6H_2O$, 2 g/l $NaHCO_3$, 2 ml Tween 80, 0.02 g/l hemin, 10 ml vitamin K_1, 0.5 g/l cysteine HCl, 0.5 g/l bile salts, pH 7.0). They were inoculated with 15 ml of fecal slurry, prepared by homogenizing 10% (w/v) freshly voided fecal material from three healthy adult donors in 0.1 M PBS at pH 7.0. LAOS, LAR, and FOS were added to a final concentration of 1% (w/v). Each vessel was magnetically stirred, the pH was automatically maintained at pH 6.8, and the temperature was set at 37 °C. Anaerobic conditions were maintained by sparging the vessels with oxygen-free nitrogen gas at 15 ml/min. Samples (5 ml) were removed at 12 h and 24 h for the enumeration of bacteria and short-chain fatty acid analysis. Fermentation was conducted three separate times. For enumeration of intestinal bacteria, a quantitative real-time polymerase chain reaction (PCR) was performed using genus-specific primer sets (Supplementary, Table S2) according to Vigsnæs et al. (2011).

2.6. Experimental analyses

All hydrolytic products were analyzed and compared *via* high-performance anion exchange chromatography (HPAEC) with a Carbopac PA-1 column (Bio-LC 3000, Dionex, CA, USA). SCFAs in culture supernatants were quantified using a normal phase HPLC system consisting of a Young-Lin M930 solvent delivery pump