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Assessment of the prebiotic potential of oligosaccharide mixtures from rice bran and cassava pulp

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ABSTRACT

Hydrothermal treatments (135 °C, 0.22 MPa, 0.5–3 h) of two kinds of agricultural wastes, rice bran and cassava pulp, resulted in hemicellulosic oligosaccharide mixtures. Acidic hydrolyses of these oligosaccharide mixtures revealed that they were mostly composed of glucose, galactose and mannose. Microbial utilizations of the obtained oligosaccharide mixtures showed that they were able to promote the growth of two from three *Lactobacillus*, as well as three from five *Bifidobacterium* species tested. Neither of rice bran nor cassava pulp derived oligosaccharide mixtures promoted the growth of *Eubacterium cylindroides* and *Clostridium scindens*. From the three tested *Bacteriodes* strains one utilized the cassava pulp oligosaccharide mixture better than inulin, while two grew better on rice bran oligosaccharide mixture than on inulin. Furthermore, the two oligosaccharide mixtures were found to be stable at 85 °C for 30 min. Similarly, these mixtures were able to withstand their exposure to simulated human gastric juice (pH 1–5) and to pancreatin treatments for up to two hours. To the best of our knowledge this is the first report that describes bifidogenic effects of rice bran and cassava pulp oligosaccharides extracted by hydrothermal treatment.

1. Introduction

Countries with big agricultural output have an increasingly challenging task of making a good use of their agricultural wastes. Technological processing of crops like rice and cassava especially in tropical-subtropical countries generates substantial amounts of wastes which can be exploited to obtain novel substances with considerable nutritional and economical values. Such agricultural wastes are inherently rich in plant cell wall materials like hemicelluloses, which are complex heteropolymers consisting of several kinds of monosaccharides connected by various glycosidic linkages. Due to this variety of glycosidic bonds between the monomers, hemicelluloses are usually not digestible by human intestinal digestive enzymes, but could be utilized by members of the intestinal microbiota. Indeed, this very idea, to fuel certain members of the intestinal microbial community by carbohydrates indigestible for the human host is the prime focus of the prebiotic concept. According to a recent definition a prebiotic is “a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health” (Roberfroid, 2007). The prebiotic properties of hemicellulose derived carbohydrates such as xylo-oligosaccharides (Hespell & Whitehead, 1990; Hsu, Liao, Chung, Hsieh, & Chan, 2004; Kontula, von Wright, & Mattila-Sandholm, 1998; Okazaki, Fujikawa, & Matsumomo, 1990), manno-oligosaccharides (Chee, Iji, Choct, Mikkelsen, & Kocher, 2010; Walton et al., 2010) and arabino-oligosaccharides (Hopkins et al., 2003; Kontula et al., 2000; Van Laere, Hartemink, Bosveld, Schols, & Voragen, 2000) have been investigated yielding promising results. Based on those findings, this work aimed to isolate oligosaccharides from hemicellulose rich agricultural wastes rice bran and cassava pulp. The effects of obtained oligosaccharide mixtures on the *in vitro* growth of selected intestinal bacteria were investigated. In addition, the digestibility of the obtained oligosaccharide mixtures under simulated human intestinal conditions were also examined.

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2. Materials and methods

2.1. Materials

Oil-free rice bran, a waste produced during the rice bran oil manufacturing process, was received from Asia Star Animal Health Co. Ltd, Thailand, as a gift. Cassava pulp, which is a by-product of cassava starch production, was donated by Eiamrungruang Industry Co. Ltd., Thailand.

2.2. Hydrothermal treatments

Hydrothermal treatments are established methods to extract hemicellulosic materials where polysaccharides undergo hydrolysis at elevated temperature and pressure in the presence of hydronium ions generated by water autoionization, which act as catalysts (Gírio et al., 2010). Hydrothermal treatment of rice bran and cassava pulp was preceded by defatting the samples with Soxhlet extractor. For the investigation of the effect of pH on the hydrothermal treatment samples were suspended in deionized water (1%, w/v), then the pH was adjusted to 4.0, 5.0, 6.0 and 7.0 with 1 M HCl. Hydrothermal treatments were performed in autoclave at 135 °C (0.22 MPa) for 0.5, 1.5 and 3 h. Afterwards the resulting supernatants were collected by centrifugation at 5000 *g* for 5 min at room temperature, and then were freeze dried. The freeze-dried rice bran and cassava pulp extracts were analyzed on TLC plates as well as applied in microbial testing.

2.3. Analysis of oligosaccharide mixtures

2.3.1. Proximate analysis

The hydrothermal treatment extracted and freeze-dried materials obtained from rice bran and cassava pulp were subjected to proximate analysis to quantify the content of crude protein, ash, fat, and fiber (AOAC, 2000). Total carbohydrate was determined by phenol-sulfuric acid assay. (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956; Xie et al., 2010).

2.3.2. Thin layer chromatography

Samples were analyzed by TLC on Silica gel 60 F254 plates (Merck) using n-butanol: formic acid: H_2O = 3:4:1 as solvent (Nimptsch et al., 2010). The TLC plate was developed with thymol reagent (0.5% thymol in 5% H_2SO_4 in EtOH) at 100 °C for 10 min. Standard sugars of glucose, stachyose, raffinose, mannotetraose, and mannohexaose were used to estimate the length of liberated oligosaccharides (Xie et al., 2010; Yang, Prasad, Xie, Lin, & Jiang, 2011).

2.3.3. High performance liquid chromatography

The sugar composition of oligosaccharide mixtures was analyzed using HPLC after complete acid hydrolysis using 2 M trifluoroacetic acid (100 mg, dry wt./5 ml TFA). The hydrolysis was done at 100 °C for 4 h, then the reaction mixtures were cooled down and TFA was removed by vacuum rotary evaporation. The hydrolyzed sugars were redissolved with 1.5 ml deionized water (Dubois et al., 1956; Xie et al., 2010). The solution was filtered through 0.45 μm membranes before analysis by HPLC. The HPLC system (Waters) was equipped with a refractive index detector (Waters). The column (Vertisep™ SUGAR LMP; 7.8 × 300 mm) was operated at 75 °C using ultrapure water as mobile phase with a flow rate of 0.6 ml/min. Standard monosaccharides were used to identify the monomer components in the samples by comparing the retention times of the constituents detected in the samples to those of the standards.

2.4. Assessing the utilization of the extracted oligosaccharides

2.4.1. Bacterial strains

All the bacteria used in this study are type strains and were obtained from the Japan Collection of Microorganisms (JCM, Wako, Japan). Bifidobacteria and lactobacilli were routinely cultured in MRS (Beckton, Dickinson and Co., USA) broth under anaerobic conditions using a mixed gas (N_2:CO_2:H_2 = 8:1:1). Other intestinal bacteria were maintained in Wilkins-Chalgren broth under anaerobic atmosphere (mixed gas as above).

2.4.2. Glucose removal

Glucose removal was carried out before microbial testing for rice bran and cassava pulp oligosaccharide mixtures after hydrothermal treatment and centrifugation. Overnight culture of *Saccharomyces cerevisiae* BCC 12652, cultivated in YM broth at 37 °C, was centrifuged at 15,000 *g* at 4 °C for 5 min, then washed once with sterile saline. Freeze-dried hydrothermal extracts of rice bran and cassava pulp were dissolved in sterile distilled water at 10% (w/v) concentration and mixed with the washed *S. cerevisiae* cells then incubated for 24 h at 37 °C. Afterwards the mixtures were centrifuged (as above) to remove the yeast cells and freeze dried.

2.4.3. Microbial testing

Oligosaccharide mixtures were dissolved in distilled water and passed through 0.2 μm pore size sterile filters then the mixtures were added to MRS medium that lacked glucose to a final concentration of 0.5% (w/v) then inoculated with overnight cultures of bifidobacteria or lactobacilli (inoculum size: 1% (v/v)). The microbes were cultured in anaerobic atmosphere (80% N_2, 10% CO_2, 10% H_2) at 37 °C for 24 h then the absorbance of the cultures were measured at 660 nm.

The utilization of oligosaccharide mixtures by *Bacteroides* strains were carried out using a minimal medium (MM) described by Martens, Chiang, and Gordon (2008). While the growth of *Eubacterium* and *Clostridium* strains on oligosaccharide mixtures were tested in Wilkins-Chalgren broth lacking yeast extract and glucose. These media were supplemented by sterile filtered rice bran and cassava derived oligosaccharide mixtures to a final concentration of 5 g/l as described above. The above media were inoculated with 24 h seed cultures grown in Wilkins-Chalgren broths (inoculum size: 1% (v/v)), then incubated in anaerobic atmosphere (80% N_2, 10% CO_2, 10% H_2) at 37 °C for 24 h. Subsequently, the absorbances of the cultures were measured at 660 nm. For comparative purposes the growth on glucose and on inulin (each added to a final concentration of 5 g/l) in the appropriate medium was also tested for all test strains.

Microbial growth on different oligosaccharide mixtures was compared to that on glucose using the following growth index (GI): $GI = (A_{S24} - A_{S0}) - (A_{C24} - A_{C0})/(A_{G24} - A_{G0}) - (A_{C24} - A_{C0})$. Here the A_{S24}, A_{G24} and A_{C24} represent the absorbance of an appropriate medium either containing the sample oligosaccharide mixture (S) or glucose (G), or lacking any carbohydrate (control, C) after incubation for 24 h, respectively. While A_{S0}, A_{G0} and A_{C0} stand for the absorbance readings at 0 h.

2.5. Effect of heating and acidity on the oligosaccharide mixtures

The effect of elevated temperature commonly used for pasteurization process as well as acidity on the stability of our oligosaccharide mixtures was investigated following the method of Huebner, Wehling, Parkhurst, and Hutkins (2008). Freeze dried oligosaccharide mixtures or inulin were added into 20 mM citrate buffer (10%, w/v) at pH 3, 4, 5, 6 and 7. The solutions were incubated at 37 °C for 24 h and at 85 °C for 30 min. Afterwards the samples