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Enzymatic preparation of wheat bran xylooligosaccharides and their stability during pasteurization and autoclave sterilization at low pH

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ABSTRACT

Xylooligosaccharides (XOS) were prepared from wheat bran insoluble dietary fiber (WBIDF) by treatment with commercial xylanase preparation Sunzymes. XOS, with a purity of 95% (w/w) and degree of polymerization of 2-7 and the ratio of arabinose to xylose of 0.27, was obtained with a yield of approximately 31.2% of WBIDF. Their stability was evaluated by comparing with that of commercial fructooligosaccharides (FOS) during pasteurization (60–100 °C, 30 min) and autoclave sterilization (121 °C, 1 kg/cm^2, 10–50 min) at pH 2.0–4.0. XOS was characterized by a high thermal stability during pasteurization at pH 2.5–4.0 and sterilization at pH 3.0–4.0. Even at pH 2.0, the remaining XOS reached 97.2% (w/w) and 84.2% (w/w) during pasteurization (100 °C, 30 min) and sterilization (50 min), respectively. Compared with FOS, XOS was strongly resistant to lower acidic conditions. The results revealed that XOS was considered to be more suitable for use as functional food ingredients.

1. Introduction

Oligosaccharides are generally defined as saccharides containing between 2 and 10 sugar moieties and can be classified as digestible or non-digestible based on the physiological properties. Non-digestible oligosaccharides (NDOs) resist digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. The main categories of NDOs presently available or in development as food ingredients include carbohydrates in which the monosaccharide unit is fructose, galactose, glucose and/or xylose (Mussatto & Mancilha, 2007). NDOs, such as fructooligosaccharides (FOS), galactooligosaccharides and soybean oligosaccharides, are commercially produced by extraction from natural sources, by hydrolyzing polysaccharides, and by enzymatic and chemical synthesis from disaccharide substrates.

In the western world, the market leaders are the fructans FOS, oligofructose and inulin. FOS is amongst the best studied prebiotic NDOs. However, the remarkable potential of xylooligosaccharides (XOS) as sources of novel prebiotic oligosaccharides has started to receive some attention recently. XOS can be used as low-calorie sweeteners and soluble dietary fiber since they are not metabolized by the human digestive system. Further, they exhibit many excellent physiological properties, including improvement in bowel function, calcium absorption, lipid metabolism and reduction of the risk of colon cancer by forming short-chain fatty acids in the large intestine during fermentation and a prebiotic effect promoting the growth of beneficial intestinal bacteria, such as *Bifidobacterium* and *Lactobacillus* (Grootaert et al., 2007; Kabel, Kortenoeven, Schols, & Voragen, 2002; Vázquez, Alonso, Domínguez, & Parajó, 2000). In addition, XOS has acceptable organoleptic properties and do not exhibit toxicity or negative effects on human health (Montané, Nabarlatz, Martorell, Torné-Fernández, & Fierro, 2006). Like other prebiotic NDOs, such as FOS, soybean oligosaccharides, galactooligosaccharides, XOS as a valuable food sweetener or additive is added to beverages, yogurts and other fermented dairy products, as well as various types of health functional foods.

XOS can be attained by chemical and/or enzymatic methods from a variety of xylan-containing raw materials. Currently, more and more effort is directed towards the environmental friendly way. It has been reported that the steam or hydrolytic degradation of xylan-rich biomass, known as autohydrolysis, is a suitable process for the production of XOs (Montané et al., 2006). This method eliminates the use of corrosive chemicals needed in the extraction of xylan, such as alkali and acid. However, it requires special equipment that can be operated at high temperatures. The production of XOS with direct enzymatic treatment of xylan-containing materials is the only suitable method. Wheat bran, from the outer tissues of wheat kernel, is mainly composed of cell wall polysaccharides, among which xylans represent 40% of dry matter (Thiago & Kellaway, 1982). Xylans consist of a linear backbone of β-(1 → 4) linked D-xylopyranosyl residues containing individual α-L-arabinofuranosyl residues attached through O-2 and/or O-3 (Izydorczyk & Biliaderis, 1993). The xylan backbone can be hydrolyzed randomly by endoxylanases (endo-1,4-β-D-xylan xylanohydrolase, EC

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3.2.1.8). Endoxylanases attack the xylan main chain internally in a random manner to release a mixture of various XOs.

A number of prebiotic NDOs have been introduced as functional food ingredients during the last few decades, and their industrial applications are continuously increasing. Major uses focus in beverages (fruit drinks, coffee, cocoa, tea, soda, health drinks and alcoholic beverages), milk products (fermented milk, instant powders, powdered milk and ice cream), probiotic yogurts (based on live microorganisms that exert beneficial effects for the host via improvement of the microbiological balance in the intestine) and symbiotic products (containing a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host welfare) (Mussatto & Mancilha, 2007). During production the majority of food products are exposed to significant heat-flow density due to preservation processes. For prebiotic NDOs to serve as functional food ingredients, they must be chemically stable to food processing treatments, such as heat, low pH and Maillard reaction conditions. The prebiotic activity of FOS was stable for treatments of low pH and Maillard reaction conditions, whereas heating at low pH resulted in a reduction in prebiotic activity (Huebner, Wehling, Parkhurst, & Hutkins, 2008). To date, the physicochemical characteristics of the inulin and oligofructose have been studied most intensively. FOS is liable to hydrolysis in the conditions occurring during the pasteurization of fruit juices and drinks, and the amount of hydrolyzed saccharides is greater the lower the pH and the longer the heat effect (Klewicki, 2007). The continuously increasing applications of XOS as a functional component in food industry bring about the question of stability. The purpose of the present study is to prepare XOS from wheat bran insoluble dietary fiber (WBIDF) by treatment with commercial xylanase preparation Sunzymes and evaluate their stability during pasteurization (60–100 °C, 30 min) and autoclave sterilization (121 °C, 1 kg/cm^2, 10–50 min) at pH 2.0–4.0.

2. Materials and methods

2.1. Materials

Wheat bran was obtained from Beijing Gongdeli Flour Factory (Beijing, The People's Republic of China). The bran was milled and passed through a 0.5 mm sieve. Sunzymes, which contains the glycoside hydrolase 10 family endo-1,4-β-xylanase from *Bacillus subtilis*, was obtained from Sunhy Biology Co., Ltd., Wuhan, China. Heat-stable α-amylase Termamyl 120 L (EC 3.2.1.1 from *Bacillus licheniformis*, 120 KNU/g), protease Alcalase 2.4 L (EC 3.4.21.62, from *B. licheniformis*, 2.4 AU/g) and amyloglucosidase AMG 300 L (EC 3.2.1.3, from *Aspergillus niger*, 300 AGU/g) were from Novo Nordisk (Bagsvaerd, Denmark). Amberlite XAD-2 was obtained from Rohm and Haas Company (Philadelphia, USA). D(+)-Xylose (Sigma chemical Co.), 1,4-β-D-xylobiose, 1,4-β-D-xylotriose, 1,4-β-D-xylotetraose, 1,4-β-D-xylopentaose, 1,4-β-D-xylohexaose (Megazyme, Bray, Ireland) were used as carbohydrate standards. The chicory root inulin-derived FOS 'RAFTILOSE® P95' was obtained from Orafti Group (Tienen, Belgium) and consisted mainly of oligofructose (degree of polymerization 2–7 $\geqslant$ 95%) and some trace of other saccharides $\leqslant$5%. All other chemicals and solvents used were of analytical grade.

2.2. Preparation of WBIDF

Wheat bran (100 g) was autoclaved for 45 min at 121 °C in order to destroy endogenous enzymatic activities (Zilliox & Debeire, 1998) and subsequently swollen at 60 °C for 6 h in water (1 L) with continuous stirring. Then, α-amylase (7.5 mL) was added in the suspension. Beakers with 1 L wheat bran suspension were heated in a boiling water bath for 40 min and shaken gently every 5 min. The pH was adjusted to 7.5 with 275 mM NaOH, and the samples were incubated with protease (3.0 mL) at 60 °C for 30 min with continuous mild agitation. After the pH had been adjusted to 4.5 with 325 mM HCl, amyloglucosidase (3.5 mL) was added and the mixture was incubated at 60 °C for 30 min with continuous mild agitation. The suspension was centrifuged (10,000*g*, 10 min). The residue was stirred in hot distilled water, washed repeatedly by decantation with large volumes of hot water, and then washed with cold distilled water until no cloudiness was evident. Finally, the residue was washed twice with hot distilled water, 95% (v/v) ethanol and acetone successively and then dried at 40 °C overnight in a vacuum oven to get WBIDF (Bunzel, Ralph, Marita, Hatfield, & Steinhart, 2001). The process described was repeated several times to get sufficient WBIDF for the production of XOS.

2.3. Enzymatic preparation of XOS

Hydrolysis of WBIDF (40 g) was performed in a 2 L enzymatic reactor with a working volume of 1 L of 50 mM acetate buffer (pH 5.0) containing 0.4% (w/w) Sunzymes at 50 °C in the dark for 16 h with constant stirring. After heat inactivation of the enzyme (100 °C, 10 min), the hydrolysate of WBIDF was obtained by centrifugation (10,000*g*, 20 min). The supernatant solution was passed through 0.45 μm filter and concentrated to 100 mL by rotary evaporation. The concentrated solution was applied to an open column (80 × 2.5 cm i.d.) packed with Amberlite XAD-2 (previously washed with 95% (v/v) ethanol and then water). Elution was successively carried out with 4 column volumes of distilled water. The eluted fraction was concentrated and lyophilized with a freeze dry system (AlPHA1-4, Christ, Germany) to get XOS for further analysis.

2.4. Evaluation of the stability of XOS during pasteurization and autoclave sterilization

The evaluation of the stability of XOS was compared with that of FOS by employing two types of thermal processing, namely pasteurization and autoclave sterilization. A 10% (w/w) of XOS or FOS was added to 20 mM citrate–phosphate buffer at pH 2.0–7.0. Citrate–phosphate buffer was prepared using citric acid and disodium phosphate according to the procedure established by McIlvaine (1921) and diluted to a 20 mM of concentration. Each of the sample solutions was maintained 30 min in water bath with constant shaking at appropriate temperature (60–100 °C) during pasteurization, and sterilized for 10–50 min at 121 °C during autoclave sterilization (1 kg/cm^2 of pressure) at pH 2.0–4.0, respectively. Following the termination of thermal processing, the samples under investigation were cooled down to room temperature. The final samples were subjected to chromatographic analysis and the percentages of the remaining XOS or FOS were calculated. Each experiment was replicated three times.

2.5. Analytical methods

All values were calculated on a moisture-free basis. Ash content was determined gravimetrically by incineration at 525 °C for 8 h. Nitrogen content was determined by the Kjeldahl method. Protein was calculated as N × 6.25. Starch was determined enzymatically according to Karkalas (1985). The neutral sugar composition of wheat bran, WBIDF and XOS was determined on a Finnigan (GC–MS) chromatograph using a SP-2330 column (30 m × 0.25 mm)