



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

Jing Wang^{*}, Baoguo Sun, Yanping Cao, Yuan Tian, Chengtao Wang

College of Chemistry and Environment Engineering, Beijing Technology and Business University, 11 Fucheng Road, Beijing 100048, PR China

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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/cm², 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.

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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 pro-

moting 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 \rightarrow 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

^{*} Corresponding author. Tel.: +86 10 689 853 78; fax: +86 10 689 854 56.
E-mail address: jingw810@yahoo.com (J. Wang).

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/cm², 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-xylo-triose, 1,4- β -D-xylo-tetraose, 1,4- β -D-xylo-pentaose, 1,4- β -D-xylo-hexaose (Megazyme, Bray, Ireland) were used as carbohydrate standards. The chicory root inulin-derived FOS 'RAFTILOSE® P95' was obtained from Orafit Group (Tienen, Belgium) and consisted mainly of oligofructose (degree of polymerization 2–7 \geq 95%) and some trace of other saccharides \leq 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 (10,000g, 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 (10,000g, 20 min). The supernatant solution was passed through 0.45 μ m filter and concentrated to 100 mL by rotary evaporation. The concentrated solution was applied to an open column (80 \times 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/cm² 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 \times 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 \times 0.25 mm)

with *myo*-inositol as an internal standard described by Blakeney, Harris, Henry, & Stone (1983). The temperature of injection and detection (flame ionization detector) were 260 and 280 °C, respectively. The samples were treated with 72% H₂SO₄ (1 h, 30 °C) followed by hydrolysis with 1 M H₂SO₄ for 3 h at 100 °C and the constituent sugars released were analyzed as their alditol acetates. Oligosaccharides were determined on a Water 600 system high performance liquid chromatography (HPLC) using a Sugar-Pak™1 column (300 mm × 6.5 mm i.d.). The column was maintained at 85 °C. The detector signal was electronically monitored with a Waters 2401 refractive index detector. A sample volume of 10 µL was run at a flow rate of 0.5 mL/min with water as mobile phase. Xylose, xylobiose, xylotriose, xylotetraose, xylopentaose and xylohexaose were used as standards for XOS analysis.

2.6. Statistical analysis

Statistical analysis was performed using the software Statistica 6.0. Data were analyzed by an analysis of variance ($P < 0.05$) and the means separated by Duncan's multiple range test.

3. Results and discussion

3.1. Chemical composition of wheat bran and WBIDF

The chemical composition of wheat bran and WBIDF is given in Table 1. The extent of protein and starch removal was close 73% (w/w) and 98% (w/w), respectively, indicating that enzyme treatment effectively lowered the protein and starch content of wheat bran. WBIDF, the destarched and deproteinised wheat bran, was isolated from wheat bran with a yield of approximately 43%. The untreated wheat bran and WBIDF showed marked differences among the monosaccharides except for mannose and galactose. The relative neutral sugar composition of WBIDF polysaccharides was 42.78% xylose, 19.45% arabinose, 0.49% mannose, 1.48% galactose and 1.53% glucose. The isolated WBIDF are rich in arabinoxylan (approximately 55% of dry matter and A/X 0.45). Apart from removing physiologically active proteins such as endogenous enzyme and xylanase inhibitors, the combined enzyme and thermal treatments may have changed the three-dimensional architecture of water-unextractable arabinoxylan in WBIDF (Beaugrand, Crônier, Debeire, & Chabbert, 2004). Based on the literature data, wheat bran and WBIDF might also contain cellulose, lignin and fat (Bataillon, Mathaly, Nunes Cardinali, & Duchiron, 1998; Bergmans, Beldman, Gruppen, & Voragen, 1996).

3.2. Preparation of XOS

The potential of plant cell wall polysaccharides as sources of novel prebiotic oligosaccharides has started to receive some attention recently. These oligosaccharides can be released from plant cell wall polysaccharides by treatment with specific glycanases (Van Laere, Hartemink, Bosveld, Schols, & Voragen, 2000). Cereal biopro-

cessing through enzymatic reactions or through fermentation can also produce a large range of oligosaccharides with potential prebiotic properties. Arabinoxyloligosaccharides can be made from wheat by xylanases, which were fermented by bifidobacteria and shown prebiotic potential (Rastall & Maitin, 2002). β-Gluco-oligosaccharides can be produced from oat bran-β-D-glucan by enzymatic hydrolysis with endo-β-glucanase II enzyme, which have been shown to enhance the growth of lactic acid bacteria (Kontula, von Wright, & Mattila-Sandholm, 1998). The enzymatic degradation of WBIDF was performed at 50 °C and pH 5.0. The hydrolysate was centrifuged and passed through 0.45 µm filter to remove non-hydrolyzed WBIDF and other insoluble contaminants. It is well known that wheat bran arabinoxylan is associated with hydroxycinnamic acids, such ferulic and coumaric acids, via an ester bond. Feruloyl or cinnamoyl oligosaccharides were released from wheat bran by treatment with endoxylanase (Lequart, Nuzillard, Kurek, & Debeire, 1999; Rhodes, Sadek, & Stone, 2002). Therefore, in order to get pure XOS, the hydrolytic products of WBIDF incubated with Sunzymes were applied to Amberlite XAD-2, which is a polymeric adsorbent capable of binding aromatic compounds (Saulnier, Vigouroux, & Thibault, 1995). Initially, all of the oligosaccharides were retained on the Amberlite XAD-2 column. Application of distilled water to the column led to the elution of oligosaccharides that did not contain ester-linked hydroxycinnamic acids. As shown in Fig. 1, the end products released from WBIDF were xylose and XOS with degree of polymerization range of 2–7 with a purity of 95% (w/w), and xylobiose and xylotriose were the predominant XOS products. XOS was obtained with a yield of approximately 31.2% of WBIDF tested and the arabinose-to-xylose was 0.27 (Table 1). This demonstrated that the xylanases in Sunzymes degraded the main chain of xylan in WBIDF internally in a random manner to release a mixture of various XOS. Maes, Vangeneugden, and Delcor (2004) reported that 41% of water-unextractable arabinoxylan in the destarched, deproteinised wheat bran were easily released by *B. subtilis* endoxylanase, and the solubilisation of the remaining water-unextractable arabinoxylan occurred less efficiently. Yang, Yang, and Liu (2007) reported that 2% xylan extracted from wheat bran was hydrolyzed by extracellular xylanases from *Thermobifida fusca* at 60 °C and pH 7.0 for 10 h, and the xylooligosaccharides (degree of polymerization range of 2–4) that accumulated in the broth were about 7.6%. Swennen, Courtin, Lindemans, and Delcour (2006) reported that the destarched, deproteinised wheat bran was incubated with pure *B. subtilis* endoxylanase to convert wheat bran arabinoxylan into arabinoxyloligosaccharides with a purity of 72% and a yield of 6%.

3.3. The stability of XOS during pasteurization and autoclave sterilization

It is important that NDOs used in food production are resistant to unfavorable conditions occurring during the manufacturing process. A factor that determines the stability of chemical compounds present in food to a large extent is thermal processing used mainly

Table 1
Chemical composition (% on dry matter) of wheat bran, WBIDF and XOS.

Sample	Yield	Ash	Protein	Starch	Xyl ^a	Ara ^a	Man ^a	Gal ^a	Glu ^a	AX ^b	A/X ^c
Wheat bran	100	6.98	13.97	14.21	15.66	8.72	0.47	1.42	27.64	21.45	0.56
WBIDF	43.25	1.46	3.78	0.23	42.78	19.45	0.49	1.48	1.53	54.76	0.45
XOS	31.22 ^d	0.21	ND ^e	ND ^e	78.16	21.23	ND ^e	0.14	0.15	87.46	0.27

^a Xyl, xylose; Ara, arabinose; Man, mannose; Gal, galactose; Glu, glucose.

^b AX, arabinoxylan = $0.88 \times (\% \text{ arabinose} + \% \text{ xylose})$.

^c A/X, arabinose to xylose ratio.

^d Expressed as percentage (%) WBIDF.

^e ND, not detected.

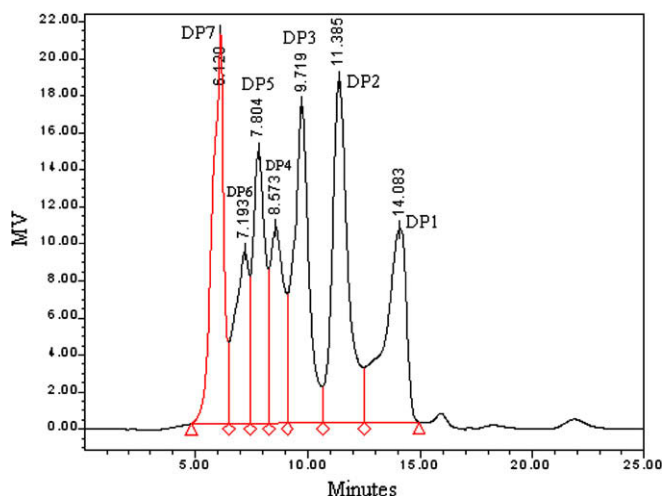


Fig. 1. The HPLC chromatogram of xylooligosaccharides from WBDF hydrolyzed by Sunzymes. Xylose, xylobiose, xylotriose, xylotetraose, xylopentaose and xylohexaose were used as markers with retention times of 13.954, 11.278, 9.597, 8.461, 7.668 and 7.133 min, respectively.

to preserve foodstuffs. In addition, unfavorable conditions are created by a low pH promoting the hydrolysis of saccharides. The process time and temperature depend on the type of food and the final result one wants to achieve, such as retaining a food's nutrients, color, texture and flavor. Thus, in this study, during pasteurization and autoclave sterilization, the process time was deliberately lengthened to a specified time so as to apply extremely unfavorable conditions from the point of view of the stability of saccharides. For the same reason, the pH was at the range of 2.0–4.0.

3.3.1. The stability of XOS during pasteurization

Pasteurization is the process of heating liquid or semi-liquid foods to a particular temperature for a designated period sufficient to kill pathogenic bacteria to make a food safe to eat. It has long been an important step in food processing operations and in most cases identified as a critical control point in a HACCP plan. Today, many foods, such as eggs, milk, juices, spices and ice cream, are pasteurized. In this study, the stability of XOS was compared with that of FOS. It has been reported that considerable amounts of NDOs have to be added to food and/or feed systems, frequently up to 10% by weight, in order to achieve certain properties (Doerr, Ritter, & Ter Meer, 2002). Thus, a 10% (w/w) of XOS or FOS preparation was kept for 30 min at a specified temperature (60–100 °C) during pasteurization at a pH range of 2.0–4.0. As shown in Fig. 2, during pasteurization, none of the XOS preparations underwent substantial decomposition at pH 2.0 and 2.5. Only very low decomposition levels of 0.1–2.8% could be detected. XOS indicated no hydrolysis at pH 3.0–4.0. However, the degradation of FOS was significantly affected by the tested temperatures and pH values. A significant decrease in the amount of remaining FOS with an increase of the temperature of the solution was observed below pH 3.5 and the change are obviously faster at 90–100 °C. There is a slighter effect of pH on the hydrolysis than that of temperature in the tested range. At 60–70 °C, the hydrolysis of FOS was insignificant at pH 3.0–4.0, whereas it was considerable at pH 2.0 and 2.5, the remaining FOS was 65.4–89.2%. The degradation process of oligosaccharides takes place by proton catalysis, so the possibility of cleavage is ensured by the acidic media (L'homme, Arbelot, Puigserver, & Biagini, 2003). The decrease of pH, namely the increase of proton concentration of the solution makes degradation faster. FOS began to be decomposed at pH 3.5, and was completely hydrolyzed at pH 2.0 and 2.5 at 100 °C. FOS only showed good stability at

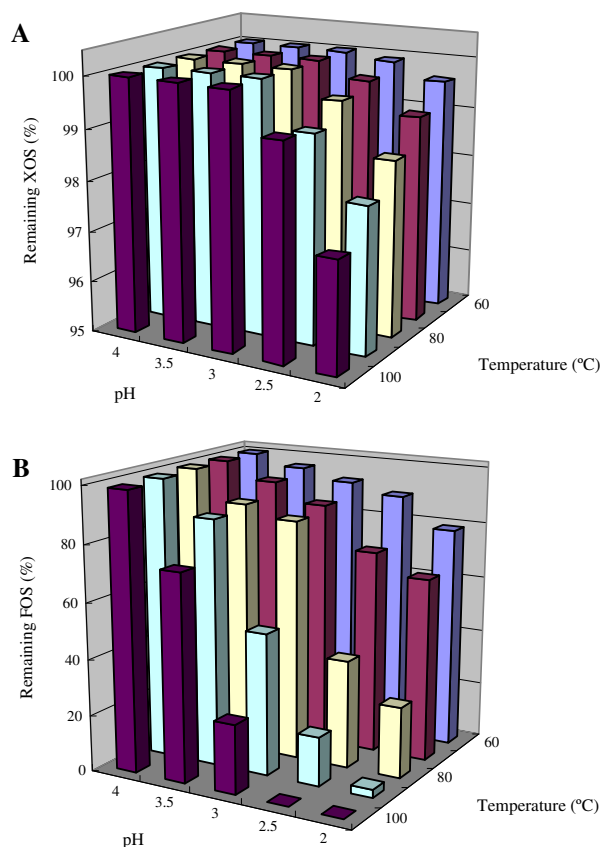


Fig. 2. The remaining percentage of XOS (A) and FOS (B) during pasteurization at various temperatures (60–100 °C) for 30 min at pH 2.0–4.5 (data were the mean values of three replicate determinations).

pH 4.0 at the tested temperature range. The lower stability of FOS at acidic pH could be associated with a protonic activation of the leaving group. The fragility of the fructose–fructose and fructose–glucose C–O osidic bonds involved in the glycosidic linkage in both protonated inulobiose and sucrose are significantly higher than those in the non-protonated forms. Thus, protonated oligosaccharides are more rapidly hydrolyzed at acidic pH than at neutral or basic pH values (L'homme et al., 2003). XOS demonstrated higher stability than FOS in tested conditions which might demonstrate that the glycosidic linkages, including both the arabinose and xylose linkages, in XOS preparations were more stable than those in the FOS preparations.

3.3.2. The stability of XOS during autoclave sterilization

Autoclave sterilization is another type of heat treatment used in the sterilization of food industry, which temperature of treatment is over 100 °C. In the steam autoclave process, microorganisms are killed by heat, and this is accelerated by the addition of moisture. Steam by itself is not sufficient for sterilization, and pressure that is greater than atmospheric is needed to increase the temperature of steam for thermal destruction of microbial life. In this study, the sample solutions containing XOS or FOS were heated at 121 °C for 10–50 min in an autoclave under 1 kg/cm² of pressure during autoclave sterilization. As shown in Fig. 3, the effect of acid and heat on the stability of XOS and FOS during autoclave sterilization was similar to that during pasteurization for 30 min at 100 °C. At pH 3.0–4.0, the percentages of remaining XOS were 100. The minimum remaining XOS of 84.2% was observed during sterilization for 50 min at pH 2.0. However, compared with that during pasteurization for 30 min at 100 °C, a faster decomposition was observed

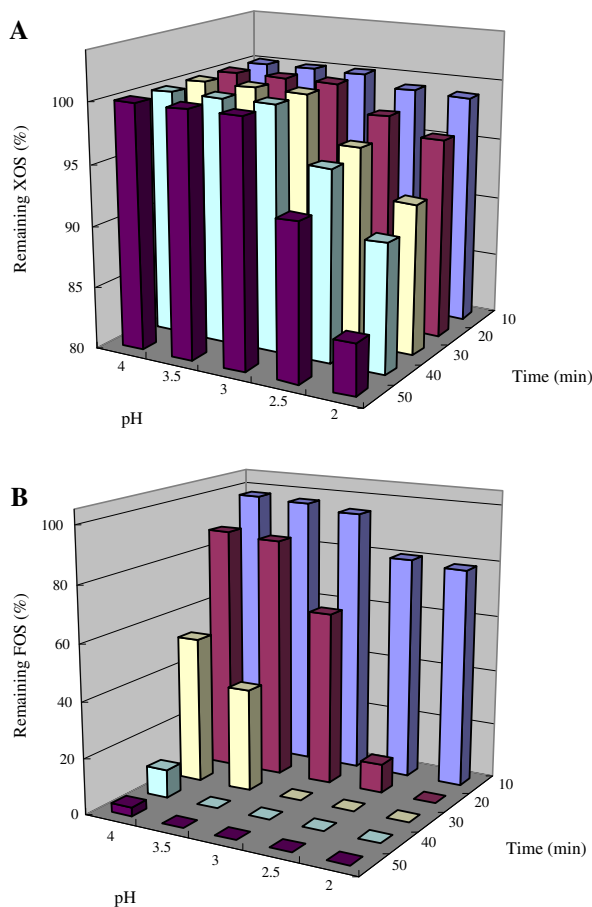


Fig. 3. The stability of XOS (A) and FOS (B) during autoclave sterilization (121 °C at 1 kg/cm² of pressure, 10–50 min) at pH 2.0–4.5 (data were the mean values of three replicate determinations).

at the elevated temperature for FOS during autoclave sterilization. At below pH 3.5, FOS was completely hydrolyzed and the remaining FOS reached zero during sterilization for over 30 min. The thermal stability of oligosaccharide under acidic conditions is an important consideration in food processing, because the proliferation effect for *Bifidobacteria* will be lost if oligosaccharides are degraded to monosaccharides. Therefore, XOS showed advantages over FOS in terms of resistance to both acids and heat, allowing their utilization in low pH liquid or semi-liquid foods.

4. Conclusions

Wheat bran was destarched and deproteinised by well-characterized enzymes to obtain WBIDF, which was enzymatically hydrolyzed. The hydrolysate was further subjected to purification by adsorbent resin. The resulted XOS, with a purity of 95%, 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. XOS showed good thermal stability during pasteurization and autoclave sterilization at low pH compared with FOS. Particularly in lower pH, the percentage of remaining XOS in solutions could be around 100% even at a higher temperature during pasteurization. It is necessary for NDOs to remain their stable physicochemical properties during processing when they were added to liquid or semi-liquid food to develop their beneficial effects. From this point of view, it might be more suitable for use as functional food ingredients.

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References

- Bataillon, M., Mathaly, P., Nunes Cardinali, A.-P., & Duchiron, F. (1998). Extraction and purification of arabinoxylan from destarched wheat bran in a pilot scale. *Industrial Crops and Products*, 8, 37–43.
- Beaugrand, J., Cr  nier, D., Debeire, P., & Chabbert, B. (2004). Arabinoxylan and hydroxycinnamate content of wheat bran in relation to endoxylanase susceptibility. *Journal of Cereal Science*, 40, 223–230.
- Bergmans, M. E. F., Beldman, G., Gruppen, H., & Voragen, A. G. J. (1996). Optimisation of the selective extraction of (glucurono)arabinoxylans from wheat bran: Use of barium and calcium hydroxide solution at elevated temperatures. *Journal of Cereal Science*, 23, 235–245.
- Blakeney, A. B., Harris, P. J., Henry, R. J., & Stone, B. A. (1983). A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydrate Research*, 113, 291–299.
- Bunzel, M., Ralph, J., Marita, J. M., Hatfield, R. D., & Steinhart, H. (2001). Diferulates as structural components in soluble and insoluble cereal dietary fibre. *Journal of the Science of Food and Agriculture*, 81, 653–660.
- Doerr, M., Ritter, G., & Ter Meer, H. -U. (2002). Flavor enhancement of beverages. Patent Application US 0146501 A1.
- Grootaert, C., Delcour, J. A., Courtin, C. M., Broekaert, W. F., Verstraete, W., & Wiele, T. V. (2007). Microbial metabolism and prebiotic potency of arabinoxylan oligosaccharides in the human intestine. *Trends in Food Science & Technology*, 18, 64–71.
- Huebner, J., Wehling, R. L., Parkhurst, A., & Hutkins, R. W. (2008). Effect of processing conditions on the prebiotic activity of commercial prebiotics. *International Dairy Journal*, 18, 287–293.
- Izydorczyk, M. S., & Biliaderis, C. G. (1993). Structural heterogeneity of wheat endosperm arabinoxylan. *Cereal Chemistry*, 70, 641–646.
- Kabel, M. A., Kortenoeven, L., Schols, H. A., & Voragen, A. G. J. (2002). In vitro fermentability of differently substituted xylio-oligosaccharides. *Journal of Agricultural and Food Chemistry*, 50, 6205–6210.
- Karkalas, J. (1985). An improved enzymic method for the determination of native and modified starch. *Journal of the Science of Food and Agriculture*, 10, 1019–1027.
- Klewicki, R. (2007). The stability of gal-polyols and oligosaccharides during pasteurization at a low pH. *LWT-Food Science and Technology*, 40, 1259–1265.
- Kontula, P., von Wright, A., & Mattila-Sandholm, T. (1998). Oat bran β -gluco- and xylio-oligosaccharides as fermentative substrates for lactic acid bacteria. *International Journal of Food Microbiology*, 45, 163–169.
- Lequart, C., Nuzillard, J. M., Kurek, B., & Debeire, P. (1999). Hydrolysis of wheat bran and straw by an endoxylanase: Production and structural characterization of cinnamoyl-oligosaccharides. *Carbohydrate Research*, 319, 102–111.
- L'homme, C., Arbelot, M., Puigserver, A., & Biagini, A. (2003). Kinetics of hydrolysis of fructooligosaccharides in mineral-buffered aqueous solution: influence of pH and temperature. *Journal of Agricultural and Food Chemistry*, 51, 224–228.
- Maes, C., Vangeneugden, B., & Delcor, J. A. (2004). Relative activity of two endoxylanases towards water-unextractable arabinoxylan in wheat bran. *Journal of Cereal Science*, 39, 181–186.
- McIlvaine, T. C. (1921). A buffer solution for colorimetric comparison. *Journal of Biological Chemistry*, 49, 183–186.
- Montan  , D., Nabarlaz, D., Martorell, A., Torn  -Fern  ndez, V., & Fierro, V. (2006). Removal of lignin and associated impurities from xylooligosaccharides by activated carbon adsorption. *Industrial & Engineering Chemistry Research*, 45, 2294–2302.
- Mussatto, S. I., & Mancilha, I. M. (2007). Non-digestible oligosaccharides: A review. *Carbohydrate Polymers*, 68, 587–597.
- Rastall, R. A., & Maitin, V. (2002). Prebiotics and synbiotics: Towards the next generation. *Current Opinion in Biotechnology*, 13, 490–496.
- Rhodes, D. I., Sadek, M., & Stone, B. A. (2002). Hydroxycinnamic acids in walls of wheat aleurone cells. *Journal of Cereal Science*, 36, 67–81.
- Saulnier, L., Vigouroux, J., & Thibault, J. F. (1995). Isolation and partial characterization of feruloylated oligosaccharides from maize bran. *Carbohydrate Research*, 272, 241–253.
- Swennen, K., Courtin, C. M., Lindemans, G. C. J. E., & Delcour, J. A. (2006). Large-scale production and characterisation of wheat bran arabinoxyloligosaccharides. *Journal of the Science of Food and Agriculture*, 86, 1722–1731.
- Thiago, L. R., & Kellaway, R. C. (1982). Botanical composition and extent of lignification affecting digestibility of wheat and oat straw and pastalum hay. *Animal Feed Science and Technology*, 7, 71–81.
- Van Laere, K. M. J., Hartemink, R., Bosveld, M., Schols, H. A., & Voragen, A. G. J. (2000). Fermentation of plant cell wall derived polysaccharides and their

- corresponding oligosaccharides by intestinal bacteria. *Journal of Agricultural and Food Chemistry*, 48, 1644–1652.
- Vázquez, M. J., Alonso, J. L., Domínguez, H., & Parajó, J. C. (2000). Xylooligosaccharides: Manufacture and applications. *Trends in Food Science & Technology*, 11, 387–393.
- Yang, C. H., Yang, S. F., & Liu, W. H. (2007). Production of xylooligosaccharides from xylan by extracellular xylanases from *Thermobifida fusca*. *Journal of Agricultural and Food Chemistry*, 55, 3955–3959.
- Zilliox, C., & Debeire, P. (1998). Hydrolysis of wheat straw by a thermostable endoxylanase: Adsorption and kinetic studies. *Enzyme and Microbial Technology*, 22, 58–63.