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Heat and pH stability of prebiotic arabinoxylooligosaccharides, xylooligosaccharides and fructooligosaccharides

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ABSTRACT

The heat and pH stability of prebiotic non-digestible wheat bran-derived arabinoxylooligosaccharides (AXOS), xylooligosaccharides (XOS)-and chicory root inulin-derived fructooligosaccharides (FOS) were compared. Heat stability (at 100 and 121 °C) and shelf-life measurements (at 4 and 37 °C) revealed decomposition at alkaline pH (pH 11.0) for all three preparations tested. The short chain oligosaccharides, XOS and FOS (apparent peak molecular mass of 400 and 800 Da, respectively), were more sensitive to alkaline decomposition than were the longer chain AXOS (apparent peak molecular mass of 1500 Da), the latter being the result of the higher abundancy of reducing ends in short chain oligosaccharide preparations. At pH 2.0 and 3.0, hydrolysis of oligosaccharide linkages took place, with FOS being the most acid-sensitive component.

1. Introduction

During the past decade, the use of prebiotics, i.e. non-digestible food ingredients that selectively stimulate growth and/or activity of particular gut microbiota that are considered beneficial to health, such as bifidobacteria and lactobacilli (Gibson & Roberfroid, 1995; Holzapfel & Schillinger, 2002), has grown rapidly (Crittenden & Playne, 1996; Sako, Matsumoto, & Tanaka, 1999; Voragen, 1998; Voragen, 2000). While some peptides, proteins and certain lipids are potential prebiotics, non-digestible carbohydrates, in particular non-digestible oligosaccharides (NDO), have received most attention (Gibson & Roberfroid, 1995; Ziemer & Gibson, 1998). The physiological properties of NDO, such as their potential to reduce blood cholesterol levels and to improve mineral absorption (Hirayama, 2002; Tungland & Meyer, 2002), are typically the main focus of research. Much less effort has been devoted to the study of their physicochemical properties (Sako et al., 1999; Tungland & Meyer, 2002).

To date, the physicochemical characteristics of the NDO inulin and oligofructose have been studied most intensively. Both NDO consist of a linear backbone of β-(2 → 1)-linked fructose units with an α-D-glucose or β-D-fructose moiety, respectively, at the end of the backbone (Crittenden & Playne, 1996; Kaur & Gupta, 2002; Niness, 1999; Roberfroid & Delzenne, 1998; Tungland, 2000). They possess a number of interesting technological properties and are, therefore, used in different foods and beverages (Franck, 2002). However, to achieve certain properties, considerable levels of NDO have to be added to food and/or feed systems, frequently up to 10% by weight (Doerr, Ritter, & Ter Meer, 2002). In addition, the stability of NDO must be guaranteed, which is not always the case when they are stored for a long time in the liquid state (Suwa et al., 1999) or under acidic or low temperature conditions (Aldrich, Hanger, & Ritter, 2004; Benkeblia, Onodera, & Shiomi, 2004; Sangeetha, Ramesh, & Prapulla, 2005). As a result, there is an interest in NDO with excellent stability characteristics. In this respect, Yamada, Itoh, Morishita, and Taniguchi (1993) showed that (arabino)xylooligosaccharides [(A)XOS] with low degree of polymerization (DP) show good resistance to both acids and heat. The objective of the present study was to compare the temperature and shelf-life stability of AXOS, XOS and fructooligosaccharides (FOS) preparations.

2. Materials and methods

2.1. Materials and chemicals

The XOS preparation 'Xylo–oligo 95P' was a commercial product from Suntory Limited (Tokyo, Japan) containing at least 95% XOS. The main components of this product were xylobiose and

Abbreviations: A, arabinose; (A)XOS, (arabino)xylooligosaccharides; Dm, dry matter; DP, degree of polymerization; DS, degree of substitution; FOS, fructooligosaccharides; HPAEC-PAD, high-performance anion-exchange chromatography with pulsed amperometric detection; MM, molecular mass; NDO, non-digestible oligosaccharides; X_1, xylose; X_2, xylobiose; X_3–X_6, XOS with a DP of 3–6.

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xylotriose. The chicory root inulin-derived FOS additive 'Raftilose P95' was obtained from Orafti (Tienen, Belgium) and consisted mainly of oligofructose [with some traces of fructose, glucose and sucrose, adding up to 5% of dry matter (dm)] with an average DP of five (Raftilose P95 Product sheet). All chemicals and reagents were of at least analytical grade and supplied by Sigma–Aldrich (Bornem, Belgium) unless otherwise specified. Shodex standard P-82 pullulans were purchased from Showa Denko K.K. (Tokyo, Japan) and XOS standards with a DP of 3–6 (X_3–X_6) from Megazyme (Bray, Ireland).

2.2. Preparation of wheat bran-derived arabinoxylooligosaccharides

AXOS were derived from commercial wheat bran, as described by Swennen, Courtin, Lindemans, and Delcour (2006). Production of the latter implied enzymic removal of starch and proteins to yield an arabinoxylan (AX)-enriched destarched, deproteinized wheat bran fraction, and further incubation of the latter with a *Bacillus subtilis* xylanase (Grindamyl H640, Danisco, Copenhagen, Denmark), converting arabinoxylan into AXOS. The AXOS containing solution was further concentrated by evaporation and spray-drying to yield wheat bran AXOS. Because approximately 15% of this material is insoluble (Swennen et al., 2006), an additional purification step was performed. Wheat bran AXOS were suspended in deionized water (1:10 w/v), followed by shaking (2 h, 18 °C), centrifugation (10,000*g*, 20 min, 18 °C), filtration and freeze-drying. The obtained powder was ground, sieved (250 μm sieve), and is further referred to as AXOS.

2.3. Preparation of purified arabinoxylooligosaccharides

A purified AXOS sample was obtained by treating a suspension of AXOS (1:25 w/v) with active carbon (0.75 g/g AXOS). After stirring (1 h, 18 °C) and removal of the active carbon fraction by centrifugation (10,000*g*, 30 min, 18 °C), the residual supernatant was freeze-dried, and is further referred to as purified AXOS.

2.4. Standard analyses

Moisture and ash contents were analyzed according to AACC methods 44–19 and 08–01, respectively (AACC, 2000). Protein contents were determined using the Dumas combustion method, an adaptation of AOAC official method 990.03 (AOAC, 1995) to an automated Dumas protein analysis system (EAS varioMax N/CN, Elt, Gouda, The Netherlands), using 5.7 as a factor for conversion from nitrogen to protein content.

2.5. Analysis of non-cellulosic sugar and reducing end sugar content

Non-cellulosic sugar contents of NDO preparations were determined by gas–liquid chromatography of alditol acetates, as described by Courtin, Van den Broeck, and Delcour (2000). NDO-containing samples, approximately 15 mg (accurately weighted) or 2.5 ml, were hydrolyzed at 110 °C for 1 h with 2.0 N (5.0 ml) or 4.0 N (2.5 ml) trifluoroacetic acid, respectively. The resulting monosaccharides were reduced and converted to alditol acetates which were separated on a Supelco SP-2380 column (30 m × 0.32 mm ID, 0.2 μm film thickness) (Supelco, Bellefonte, PA, USA) with helium as carrier gas in a Agilent 6890 series chromatograph (Agilent, Wilmington, DE, USA) equipped with autosampler, splitter injection port (split ratio 1:20), and flame-ionization detector. Separation was at 225 °C with injection and detection temperatures at 270 °C. *β-D*-Allose was used as internal standard and calibration samples, containing the expected monosaccharides, were included with each set of samples. The AXOS content was calculated as 0.88 times the sum of the monosaccharides, arabinose and xylose, while the term average degree of substitution (DS) was used for the arabinose to xylose ratio. In the particular case of low molecular mass (MM) AXOS (DP ⩽ 10), the AXOS content was calculated as

$$\text{AXOS content}(\%) = \left\{\left(\frac{132}{150}\right) \times \text{Arabinose}\%\right\} + \left\{\left(\frac{132 \times (DP-1)+150}{150 \times DP}\right) \times \text{Xylose}\%\right\}$$

For FOS, the reduction of fructose released in the hydrolysis step gives rise to both mannitol and sorbitol as intermediates in the derivatisation procedure. Therefore, for FOS quantification, the sum of both mannose and glucose was used.

Analysis of reducing end sugar contents was very similar to that of non-cellulosic sugar contents. Samples [approximately 50 mg (accurately weighted) or 2.5 ml] were reduced prior to hydrolysis and acetylation to alditol acetates (Courtin et al., 2000). Combined with non-cellulosic sugar analysis, the results allowed calculation of the average DP of AXOS-rich materials as the sum of the total xylose and arabinose contents divided by the level of reducing end xylose.

2.6. High-performance size-exclusion chromatography

Apparent MM distributions were determined using high-performance size-exclusion chromatography on a high-performance liquid chromatography system (Kontron Instruments, 325 pump systems, Kontron, Milan, Italy) equipped with auto-injection. Aliquots of samples (1.5 mg/ml) were filtered (0.45 μm membrane filter) and injected (20 μl) on a Shodex SB 800P guard column (50 mm × 6 mm) (Showa Denko K.K.) attached to a Shodex SB 806 HQ HPSEC column (300 mm × 8 mm, separation range 100 Da–20 MDa). Elution was with deionized water (0.5 ml/min, 30 °C) and monitored with a refractive index detector (VSD Optilab, Berlin, Germany). MM markers were Shodex standard P-82 pullulans (2.0 mg/ml) with a MM of 78.8×10^4, 40.4×10^4, 21.2×10^4, 11.2×10^4, 4.73×10^4, 2.28×10^4, 1.18×10^4, 0.59×10^4 Da and glucose (180 Da).

2.7. High-performance anion-exchange chromatography with pulsed amperometric detection

High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was performed with a Dionex DX-500 chromatography system (Sunnyvale, CA, USA) equipped with an ED-40 electrochemical detector, a GP-50 gradient pump and an AS-3500 autosampler. Aliquots of samples (1.5 mg/ml) were filtered (0.20 μm membrane filter) and injected (25 μl) on a CarboPac PA-100 guard column (25 × 4 mm) attached to a CarboPac PA-100 anion-exchange column (250 × 4 mm). Elution (1.0 ml/min) was with a linear gradient of 0–250 mM sodium acetate in 100 mM sodium hydroxide for 30 min, followed by a linear gradient of 250–400 mM sodium acetate in 100 mM sodium hydroxide for 15 min. The gradients were ended by 5 min of washing with 100 mM sodium hydroxide. The elution was monitored using the ED-40 detector in the pulsed amperometric detection mode with following potentials and time periods: E_1, +0.05 V (t_1 = 400 ms); E_2, +0.75 V (t_2 = 200 ms); E_3, −0.15 V (t_3 = 400 ms). Arabinose (A), xylose (X_1), xylobiose (X_2) and XOS standards with a DP of 3–6 (X_3–X_6) were used as references.

2.8. Stability measurements

2.8.1. Heat stability measurements

Heat stability measurements of the AXOS, XOS, and FOS preparations were carried out according to procedures by Suwa et al.

(1999) and Yamada et al. (1993). The preparations were dissolved in universal buffers with pH values of 2.0, 3.0, 7.0 and 11.0 to obtain solutions having NDO concentrations of 0.15% (w/v). The buffers were prepared from a stock solution of citric acid (31 mM), potassium dihydrogen phosphate (29 mM), trihydrogen borate (29 mM) and diethylbarbituric acid (31 mM) in deionized water (1.0 l). Aliquots (20 ml) of this stock solution were either adjusted with 2.0 M HCl or 2.0 M NaOH to obtain the desired pH. Each of the NDO solutions was maintained in water at 100 °C and in a heating block at 121 °C for different time periods (0, 5, 10, 15, 20, 30 and 60 min). Thereafter, the solutions were cooled and non-cellulosic sugar and reducing end sugar contents were determined. At each pH value, the decomposition, i.e. the molecular breakdown of individual sugar residues, percentage of the FOS and (A)XOS preparations, was calculated with formulae (1) and (2), respectively. The percentages of glycosidic linkages lost by hydrolysis at pH values of 2.0, 3.0 and 7.0, were calculated with formulae (3) and (4), respectively. In the particular case of (A)XOS preparations, the percentages of xylose and arabinose linkages lost by hydrolysis, were calculated with formulae (5) and (6), respectively.

(1) $\%\ \text{decomposition}_{FOS} = 100 \times \{[(Glc + Man)_{time\ 0} - (Glc + Man)]/(Glc + Man)_{time\ 0}\}$

(2) $\%\ \text{decomposition}_{(A)XOS} = 100 \times \{[(Ara + Xyl)_{time0} - (Ara + Xyl)]/(Ara + Xyl)_{time\ 0}\}$

(3) $\%\ \text{hydrolyzed linkages}_{FOS} = 100 \times \{[(Glc_{RS} + Man_{RS}) - (Glc_{RS} + Man_{RS})_{time\ 0}]/(Glc_{RS} + Man_{RS})_{MAX\ in\ solution}\}$

(4) $\%\ \text{hydrolyzed linkages}_{(A)XOS} = 100 \times \{[(Ara_{RS} + Xyl_{RS}) - (Ara_{RS} + Xyl_{RS})_{time\ 0}]/(Ara_{RS} + Xyl_{RS})_{MAX\ in\ solution}\}$

(5) $\%\ \text{hydrolzsed linkages}_{Xyl} = 100 \times \{[(Xyl_{RS}) - (Xyl_{RS})_{time0}]/(Xyl_{RS})_{MAX\ in\ solution}\}$

(6) $\%\ \text{hydrolzsed linkages}_{Ara} = 100 \times \{[(Ara_{RS}) - (Ara_{RS})_{time0}]/(Ara_{RS})_{MAX\ in\ solution}]\}$

with Ara, Xyl, Glc and Man the concentrations (mg/ml) of total arabinose, xylose, glucose and mannose at a certain time; Ara_{RS}, Xyl_{RS}, Glc_{RS}, Man_{RS} are the concentrations (mg/ml) of reducing arabinose, xylose, glucose and mannose at a certain time; and $(Ara_{RS})_{MAX\ in\ solution}$ and $(Xyl_{RS})_{MAX\ in\ solution}$ are the maximum amounts of reducing arabinose and xylose, respectively, that can theoretically be released from the oligosaccharides into the solution.

2.8.2. Shelf-life stability measurements

Shelf-life stability measurements were, like heat stability measurements, carried out by dissolving NDO preparations in universal buffers of pH 2.0, 3.0, 7.0 and 11.0. All the solutions were stored at 4 and 37 °C and, at regular time intervals (0, 8, and 18 weeks for 4 °C and 0, 1, 3, 7, 14, 30 and 60 days for 37 °C), samples were withdrawn for non-cellulosic sugar and reducing end sugar analysis. At each pH value, decomposition percentages or percentages of glycosidic linkages lost by hydrolysis in the three NDO preparations were calculated with formulae 1–6.

3. Results

3.1. Composition of non-digestible oligosaccharide preparations

Table 1 presents the chemical composition of the (A)XOS preparations. (A)XOS content was approximately 68% and 82% dm for AXOS and XOS preparations, respectively. The remaining part of the samples consisted mainly of protein. (~1–5% dm) and mannose (~0.1% dm) as well as of glucose (~1–7% dm) and galactose (~0.3–1% dm) polymers. In addition, it can be expected that the samples contained ash, and esterified ferulic and/or glucuronic acids.

Table 1
Protein contents, monosaccharide compositions, AXOS contents, average degrees of substitution (DS), apparent peak molecular masses (MM) (Da) and average degrees of polymerization (DP), as determined by gas–liquid chromatography, of XOS, AXOS and purified AXOS

	XOS	AXOS	Purified AXOS
Protein content (% dm)	1.2	4.9	5.2
Monosaccharide composition (% dm)			
Arabinose	7.3	18.7	21.3
Xylose	82.6	58.8	68.3
Mannose	0.1	0.1	0.1
Galactose	0.3	0.9	0.8
Glucose	6.5	1.3	0.9
AXOS content (% dm)[a]	82.4[d]	68.2	79.3
DS[b]	0.09	0.32	0.31
MM	400	1500	1500
DP[c]	3	15	15

[a] AXOS = 0.88 × (Arabinose + Xylose).
[b] DS = Arabinose to Xylose ratio.
[c] DP = (Arabinose + Xylose)/reducing end Xylose.
[d] AXOS = {(132/150) × Arabinose} + {[{132 × (DP−1) + 150}/(150 × DP)] × Xylose} as DP ⩽ 10.

While AXOS were used for stability measurements, purified AXOS were used for sensory analysis, as off-flavours, resulting from the production process (Swennen et al., 2006), could influence sensory evaluation. The purification protocol did not impact the AXOS population.

Table 1 shows that the NDO preparations contained different structures. While the (A)XOS preparations showed an apparent peak MM, varying between 400 Da (for XOS) and 1500 Da (for AXOS), the FOS product had an apparent peak MM of approximately 800 Da (Raftilose P95 Product sheet). In addition, XOS and AXOS had arabinose substituents (average DS 0.09 and ~0.32, respectively) whereas FOS is a linear NDO (Raftilose P95 Product sheet).

3.2. Stability measurements

3.2.1. Heat stability measurements

The three NDO preparations were kept at different pH values (pH 2.0, 3.0, 7.0 and 11.0) at 100 °C and 121 °C for different time periods. Because it is generally accepted that AX are stable between pH 3 and 11, pH values in this range were omitted, except for pH 7, which acted as a control. Temperature conditions were chosen to reflect boiling and sterilization processes. As shown in Fig. 1, at 100 °C, none of the NDO preparations underwent substantial decomposition at low or neutral pH (pH 2.0, 3.0 or 7.0). Only low decomposition levels (~1–10% w/w) (if any) could be detected, in particular in the case of the FOS preparation (Fig. 1c). At alkaline pH (pH 11.0), all three NDO preparations underwent decomposition. The effect was most clear for the XOS product (Fig. 1b), of which 73% (w/w) was decomposed after 60 min of incubation at 100 °C. The latter was also observed in the HPAEC-PAD profile of the XOS preparation (Fig. 2). The profile showed arabinose (A), xylose (X_1) and xylobiose (X_2), as well as a well separated range of peaks of XOS (X_3 and X_4) and unidentified (A)XOS components, of which the levels, in particular those of X_2, X_4 and unidentified (A)XOS, decreased progressively with increasing incubation time, indicating that xylose residues in the XOS preparation were decomposing. The decomposition of the XOS preparation at alkaline pH is of the 'alkaline peeling' type, which leads to cleavage of glycosidic bonds at the reducing end of carbohydrate backbones (Cancilla, Penn, & Lebrilla, 1998;

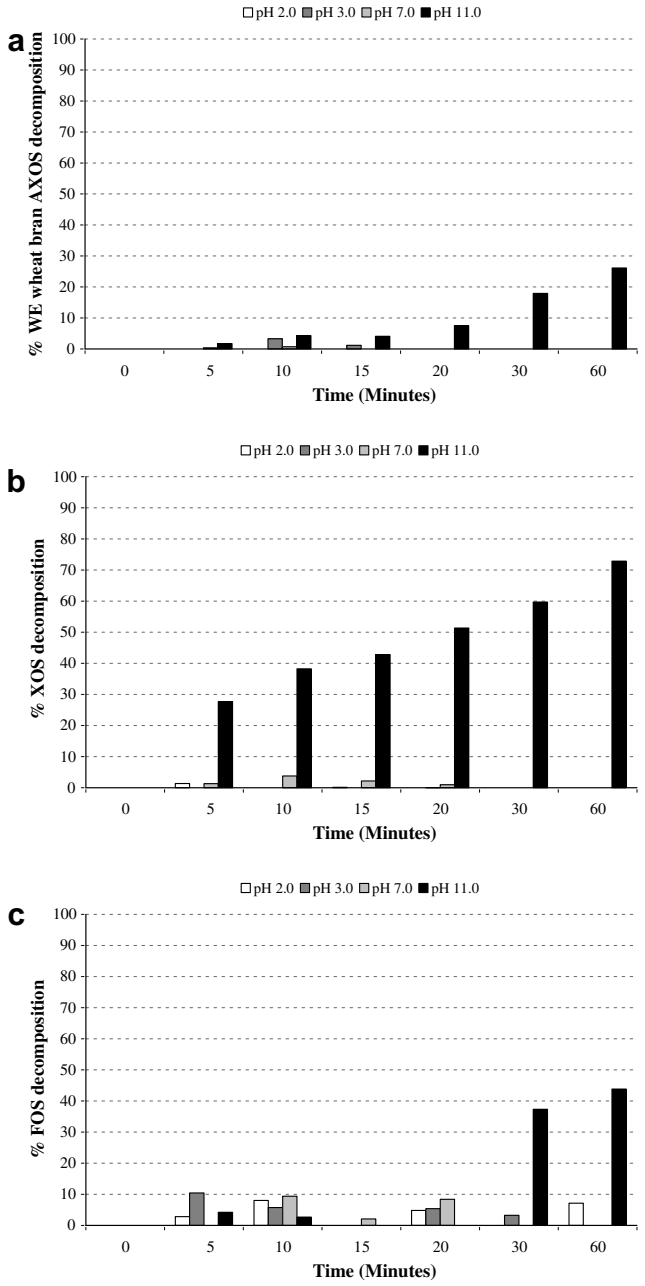

Fig. 1. Percentages of decomposition in the AXOS (a), XOS (b), and FOS (c) preparations when incubated for different time periods (0, 5, 10, 15, 20, 30 and 60 min) at pH 2.0, 3.0, 7.0 and 11.0 at 100 °C.

Knill & Kennedy, 2003; Whistler & BeMiller, 1958). The reaction occurs at elevated temperatures (60–100 °C) and generally results in formation of organic acids and an NDO chain which is shortened by one carbohydrate unit of the chain. As the formed NDO chain carries a new reducing end group, it can undergo alkaline peeling again (Cancilla et al., 1998; Phongkanpai, Benjakul, & Tanaka, 2006; Sartori et al., 2003; VanLoon & Glaus, 1997). Therefore decomposition of the xylobiose- and xylotriose-rich XOS preparation results in formation of xylose, which, with time, by itself undergoes alkaline peeling, as it carries a reducing end group. Indeed, in Fig. 2 the level of X_1 increases slightly from 0 to 30 min after which it further decreases.

For AXOS, the arabinose substituents probably affect the stability of the components and slow down the alkaline peeling reaction. As observed earlier for xylose in the HPAEC-PAD profile (Fig. 2), the

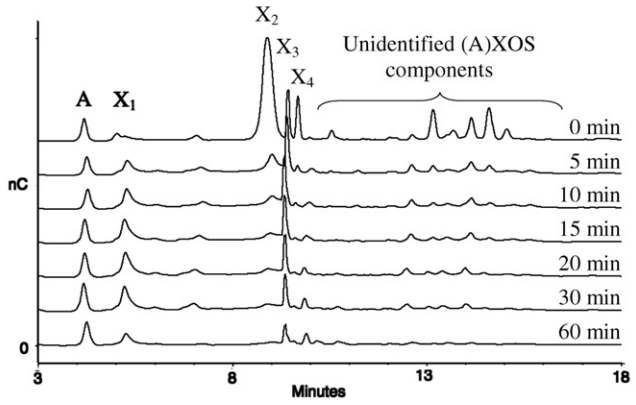

Fig. 2. HPAEC-PAD profiles of the XOS preparation after incubation during 0, 5, 10, 15, 20, 30 and 60 min at pH 11.0 and 100 °C. Arabinose (A), xylose (X_1), xylobiose (X_2), xylotriose (X_3), xylotetraose (X_4), and unidentified (A)XOS components are indicated.

level of arabinose increases from 0–30 min after which it slightly decreases. Arabinose substituents, which have no reducing end when linked to xylose residues in the XOS backbone, are first cleaved off, yielding free arabinose moieties with a reducing end, before they undergo alkaline decomposition.

In comparison to the XOS preparation, the AXOS and FOS preparations had a greater alkaline stability as their decomposition percentage, were only 26 and 44% (w/w), respectively, after 60 min of incubation at 100 °C (Fig. 1a and c, respectively). The higher sensitivity of the former preparation to alkaline peeling was not surprising as short chain NDO, per weight basis, have a higher abundancy of reducing ends than have longer chain NDO and are thus more prone to alkaline peeling.

Of the three NDO preparations tested, the glycosidic linkages in the FOS product were clearly the most sensitive to hydrolysis at low pH (Fig. 3). At pH 2.0 and 3.0, 67 and 60% (w/w), respectively, of the β-(2 → 1)-linkages between fructose units were hydrolyzed after 60 min at 100 °C (Fig. 3c). This feature was also seen in the HPAEC-PAD profile of the FOS preparation (Fig. 4). The levels of free monosaccharides, which are released when glycosidic linkages between fructose units are hydrolyzed, were clearly higher at pH 2.0 and 3.0 than at pH 7.0. The glycosidic linkages, including both the arabinose and xylose linkages, in the AXOS and XOS preparations, were clearly more stable than were those in the FOS preparations (Fig. 3). In the AXOS preparation, 15 and 2% (w/w) of all the glycosidic linkages present were hydrolyzed at pH 2.0 and 3.0, respectively (Fig. 3a), whereas, in the XOS product, 21 and 9% (w/w), respectively were cleaved (Fig. 3b). Arabinose linkages were clearly more susceptible to acidic conditions than were xylose linkages. Indeed, incubation of the XOS and AXOS preparation at pH 2.0 for 60 min at 100 °C caused hydrolysis of 73 and 56% (w/w) of all arabinose linkages, respectively (Fig. 5a and c), but only hydrolysis of 14 and 4% (w/w) of all the xylose linkages, respectively (Fig. 5b and d). Similar, but less intense results were found at pH 3.0.

All the products formed at 100 °C, were also formed at 121 °C. However, a faster decomposition and loss of glycosidic linkages by hydrolysis was observed at this temperature (results not shown).

3.2.2. Shelf-life stability measurements

Shelf-life stability measurements between 4 °C and 37 °C, temperatures which reflect storage at refrigerated and suboptimal (high) temperatures, respectively, showed that none of the NDO preparations tested underwent substantial decomposition at low

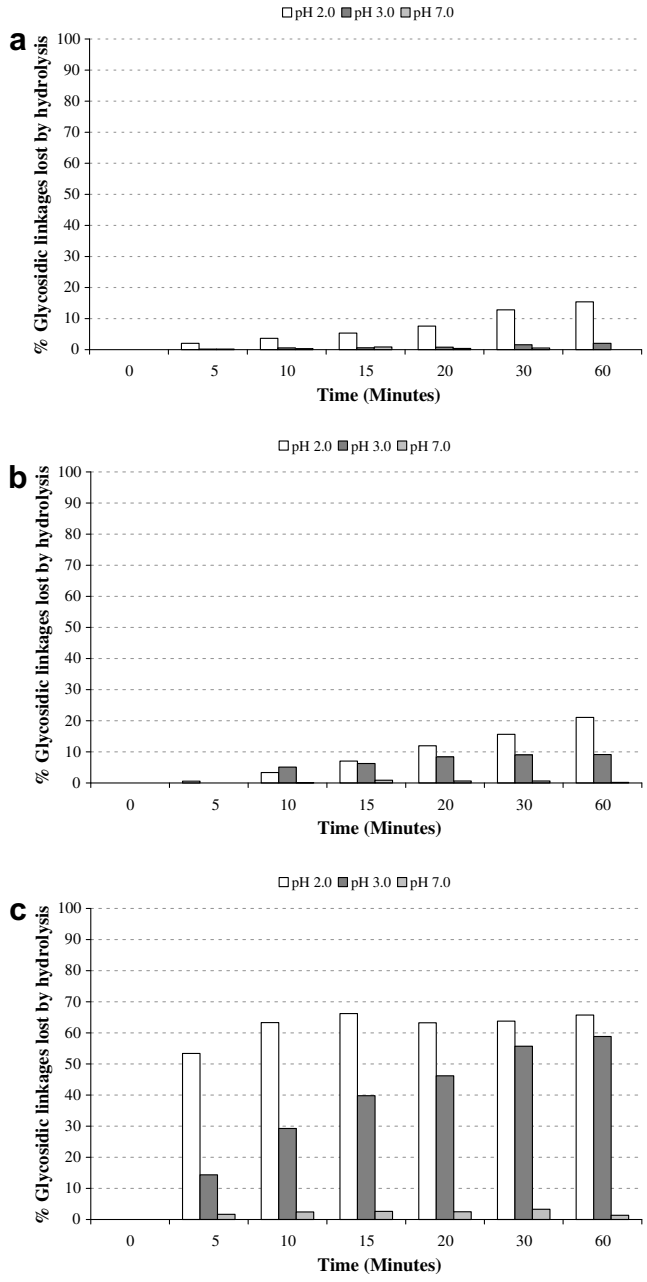

Fig. 3. Percentages of glycosidic linkages lost by hydrolysis in the AXOS (a), XOS (b), and FOS (c) preparations when incubated for different time periods (0, 5, 15, 20, 30 and 60 min) at 100 °C.

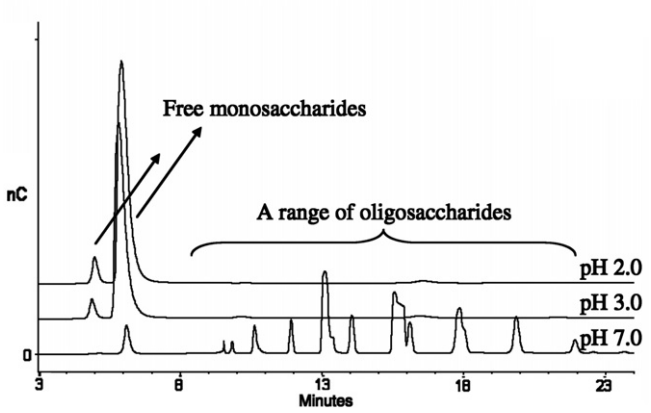

Fig. 4. HPAEC-PAD profiles of the FOS preparation after incubation during 60 min at pH 2.0, 3.0 and 7.0 at 100 °C. Free monosaccharides and a range of oligosaccharides present in the FOS preparation are indicated.

or neutral pH (pH 2.0, 3.0 or 7.0) (results not shown), while alkaline peeling was observed at pH 11.0. As was the case in the heat stability measurements, the latter was greatest for the XOS preparation. Decomposition percentages between 67 and 16% (w/w) were noted after 56 days of storage at 37 °C and 18 weeks of storage at 4 °C, respectively (Table 2). The AXOS product was again the most alkali-stable with only 34 and 7% (w/w) of decomposition after 56 days of storage at 37 °C and 18 weeks of storage 4 °C, respectively (Table 2).

Of the NDO preparations tested, the glycosidic linkages in the FOS product were the most sensitive to hydrolysis at low pH. At pH 2.0, 63 and 38% (w/w) of the linkages were hydrolyzed after 56 days of storage at 37 °C and 18 weeks of storage at 4 °C, respectively (Table 2). For the XOS and AXOS products, comparable levels of hydrolyzed linkages, 12 and 11% (w/w), respectively, were observed after 56 days at pH 2.0. When stored at 4 °C, only negligible levels of linkages were hydrolyzed at pH 2.0 for both NDO preparations. All the above results were also observed at pH 3.0, although they were less clear. Again, arabinose linkages were more susceptible to acidic hydrolysis than were xylose linkages (Table 3), a feature also confirmed by HPAEC-PAD analysis (results not shown).

4. Discussion

This study clearly illustrates that stability and sensory properties of NDO are strongly dependent on their molecular structure. Heat stability and shelf-life measurements showed that the short chain NDO preparations, XOS and FOS (average DP of 3 and 5, respectively), were more sensitive to alkaline decomposition than were longer chain AXOS (average DP of 15). The higher sensitivity of the former to alkaline peeling is not surprising, as short chain NDO, per weight basis, have much more reducing ends than have their longer chain analogues.

Hydrolysis of NDO linkages took place much more easily at acidic pH than at neutral pH. Among three NDO preparations tested, FOS was the most acid-sensitive. The sensitivity of FOS linkages to acid was already earlier reported (Blecker, Fougnies, Van Herck, Chevalier, & Paquot, 2002; L'Homme, Arbelot, Puigserver & Biagini, 2003) when analyzing the fate of FOS in food products subjected to different pH and temperature conditions. The fructose furanosyl residues in FOS are probably more prone to acid hydrolysis than are the pyranosyl units. This feature, which was also observed by Voragen (1998), was also reflected in the percentage of hydrolysis of arabinofuranosyl and xylopyranosyl units in the (A)XOS preparations. In addition, it should be noted that β-linkages, such as those in xylan, are much more stable than are α-linkages, which link arabinose substituents to the xylan backbone (Voragen, 1998).

In general it can be concluded that, among the three NDO preparations tested, AXOS showed the most interesting stability properties, especially under extreme pH and temperature conditions. However, applications involving low or high pH, high temperature processing or prolonged storage under ambient conditions will (to some extent) decompose NDO structures, resulting in a loss of nutritional and physicochemical properties. The extent to which these phenomena take place, evidently differs greatly for various classes of NDO, depending on the sugar residues present, their ring form, anomeric configuration and linkage type.

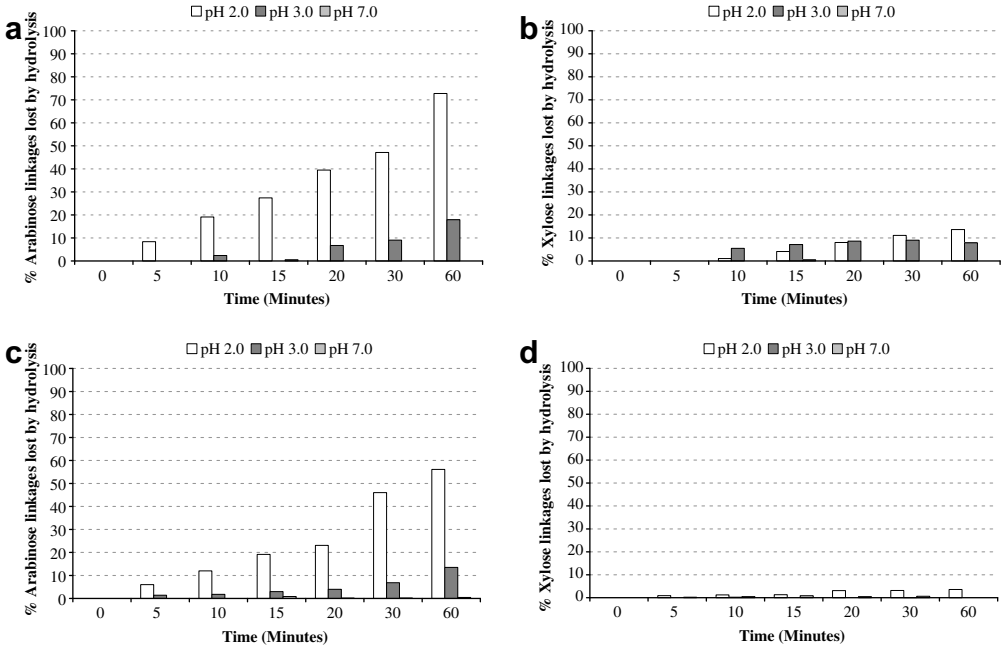

Fig. 5. Percentages of arabinose (a) and (c) and xylose (b) and (d) linkages lost by hydrolysis in the XOS (a) and (b) and AXOS (c) and (d) preparations when incubated for different time periods (0, 5, 15, 20, 30 and 60 min) at 100 °C.

Table 2
Percentages of decomposition at pH 11.0 and percentages of glycosidic linkages lost by hydrolysis at pH 2.0 in the XOS, AXOS and FOS preparations after storage at 4 and 37 °C during 0, 8 and 18 weeks and 0, 1, 3, 7, 14, 28 and 56 days, respectively

	% Decomposition (pH 11.0) (w/w)			% Glycosidic linkages lost by hydrolysis (pH 2.0) (w/w)		
	XOS	AXOS	FOS	XOS	AXOS	FOS
Storage (4 °C)						
Start	0	0	0	0	0	0
Week 8	5	3	5	0	0	19
Week 18	16	7	10	0	0	38
Storage (37 °C)						
Start	0	0	0	0	0	0
Day 1	10	3	1	0	1	32
Day 3	15	5	3	2	2	54
Day 7	22	8	11	4	3	58
Day 14	26	11	19	4	6	58
Day 28	32	12	23	8	10	60
Day 56	67	34	59	12	11	63

Table 3
Percentages of arabinose and xylose linkages lost by hydrolysis at pH 2.0 in the XOS and AXOS preparations after storage at 4 and 37 °C during 0, 8 and 18 weeks and 0, 1, 3, 7, 14, 28 and 56 days, respectively

	% Arabinose linkages lost by hydrolysis (pH 2.0) (w/w)		% Xylose linkages lost by hydrolysis (pH 2.0) (w/w)	
	XOS	AXOS	XOS	AXOS
Storage (4 °C)				
Start	0	0	0	0
Week 8	1	1	0	0
Week 18	2	2	0	0
Storage (37 °C)				
Start	0	0	0	0
Day 1	2	2	0	1
Day 3	7	5	2	1
Day 7	16	12	2	1
Day 14	29	23	0	1
Day 28	49	40	2	1
Day 56	74	51	3	0

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