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Identification and structural analysis of cereal arabinoxylan-derived oligosaccharides by negative ionization HILIC-MS/MS

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ABSTRACT

Recent works provide evidence of the prebiotic potential of arabinoxylan-derived oligosaccharides (A)XOS. In this study, we developed a structural analysis for cereal-derived (A)XOS by negative ionization HILIC-MS/MS. Initially, we assessed twelve (A)XOS samples of known structures with different linkage positions and branching points by direct-infusion negative ESI-MS^n. We subsequently developed the negative ion HILIC-MS/MS with a post-column addition of ammonium chloride. The selected (A)XOS represented both linear (arabinofuranosyl residue linked to the non-reducing end of xylooligosaccharide) and branched structures. Each (A)XOS sample produced a specific spectrum in negative ion ESI-MS^n. By analyzing cross-ring fragment ions, we determined the linkage positions of linear (A)XOS. The presence or absence of diagnostic ions in the MS^3 allowed us to detect different branches (*O*-2- or/and *O*-3-linked arabinofuranosyl with/or without *O*-4-linked xylopyranosyl at the non-reducing end). Furthermore, we could identify all analyzed samples by HILIC-MS/MS, based on the formed spectral library and chromatographic retention times.

1. Introduction

Arabinoxylan (AX) is a major polysaccharide found in the cell walls of cereal grain endosperm (Saulnier, Guillon, Sado, & Rouau, 2007). AX degrades to (arabino)xylooligosaccharides [(A)XOS] by enzymatic hydrolysis in the colon by means of intestinal microbiota or during food processing, such as brewing (Broekaert et al., 2011). Both AX and (A)XOS are considered as dietary fibers (Mendis & Simsek, 2014). (A)XOS include both xylooligosaccharides (XOS) and arabinoxylooligosaccharides (AXOS). Since recent studies have shown evidence of the prebiotic effects of AX and (A)XOS in the colons of humans and animals, interest in them has increased. AX and (A)XOS are associated with many health benefits, including immunomodulatory activity and attenuation of type II diabetes (Mendis & Simsek, 2014). (A)XOS have also been reported to attenuate serum cholesterol, triglyceride, and glucose levels (Broekaert et al., 2011).

Cereal grain AX consists of β(1 → 4)-linked xylopyranosyl residue (Xyl*p*) backbone with arabinose mono- and di-substituents, as reviewed by Biliaderis and Izydorczyk (2007), Izydorczyk and Biliaderis (1995) and Saulnier et al. (2007). Arabinose substituents are α(1 → 2)- or α(1 → 3)-linked furanosyl residues (Ara*f*) or both. Small amounts of β-D-Xyl*p*-(1 → 2)-α-L-Ara*f*-(1 → 3)-sidechains have also been found. Acidic AXs are present in the outer tissues of cereal grains. They carry α(1 → 2)-linked glucopyranosyluronic acid or 4-*O*-methyl-glucopyranosyluronic acid substituents. Feruloyl residues linked to Ara*f* residues are also found. The number of substituents is known to vary in different cereals, tissues, and even in tissue layers (Saulnier et al., 2007). Since structural features are related to physicochemical properties, such as water solubility, viscosity, and gelation (Saulnier, Sado, Branlard, Charmet, & Guillon, 2007), identification of the structure of AX is important if it is added to baking products or exploited in other industrial applications.

The structural analysis of large polysaccharide molecules is challenging; therefore, AXs are commonly hydrolyzed into (A)XOS prior to analysis. Nuclear magnetic resonance (NMR) spectroscopy (Duus, Gotfredsen, & Bock, 2000), high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Pastell, Tuomainen, Virkki, & Tenkanen, 2008, Pastell, Virkki, Harju, Tuomainen, & Tenkanen, 2009), and gas chromatography-mass spectrometry (GC–MS) analysis of methylated (A)XOS (Ciucanu, 2006) have been popular techniques for the structural characterization of (A)XOS. The last two decades have seen an increased interest in using mass spectrometry (MS) and tandem mass spectrometry (MS/MS) for the structural analyses of carbohydrates (Mischnick, 2011). One specific benefit of MS and MS/MS analyses is that they require only small amounts of a sample to achieve a very sensitive analysis. Matrix-

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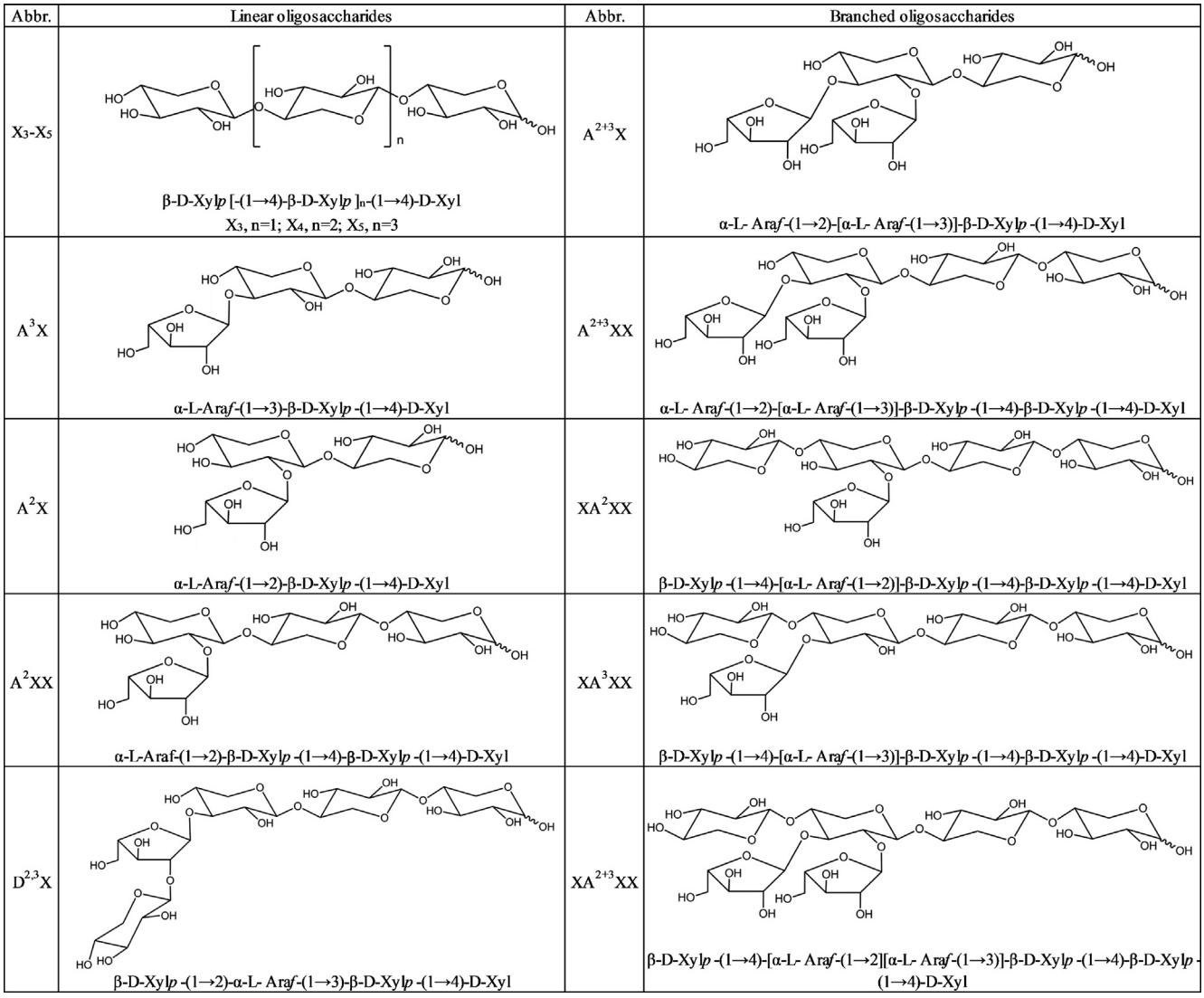

Fig. 1. The structural models and abbreviations of xylooligosaccharide and arabinoxylooligosaccharide samples (XOS and AXOS). Abbreviations of AXOS by Fauré et al. (2009).

assisted laser desorption ionization mass spectrometry (MALDI-MS) has been shown to be an efficient tool for determining the molecular weight distribution of (A)XOS. Reverse-phase high-performance liquid chromatography (RP-HPLC) combined with MS/MS has been used for the structural characterization of derivatized (A)XOS (Bowman, Dien, Vermillion, & Mertens, 2014). Recent and novel stationary phases, such as porous graphitized carbon (PGC) and hydrophilic interaction liquid chromatography (HILIC) phases, have enabled the HPLC-MS or HPLC-MS/MS analysis of oligosaccharides without derivatization (Everest-Dass, Kolarich, Campbell, & Packer, 2013, Everest-Dass et al., 2013, Hernandez-Hernandez, Calvillo, Lebron-Aguilar, Moreno, & Sanz, 2012, Leijdekkers, Sanders, Schols, & Gruppen, 2011, Leijdekkers et al., 2011, Liu, Kisonen, Willför, Xu, & Vilaplana, 2016, Liu et al., 2016, Pu, Zhao, Xiao, & Zhao, 2017).

Positive ion MS/MS can determine linkage positions and sequences of different oligosaccharides (Asam & Glish, 1997, Hofmeister, Zhou, & Leary, 1991, Mischnick, 2011). However, in MS/MS, positively charged oligosaccharide ions fragment from both ends, and, if the oligosaccharides consist of the same mass monomers, such as (A)XOS, identification of isobaric fragment ions is difficult and sometimes even impossible. Therefore, derivatization techniques, such as derivatization by per-*O*-methylation (Ciucanu, 2006, Matamoros Fernández, Obel, Scheller, & Roepstorff, 2003) and labeling the reducing end, for example, with ^{18}O isotope (Hofmeister et al., 1991) or reagents (Suzuki, 2013), have commonly been used to differentiate the reducing end and non-reducing end fragment ions.

However, some studies have indicated that derivatization may not be needed if negative ionization is used. Negatively charged oligosaccharides have been reported to fragment mainly from the reducing end towards the non-reducing end and produce non-reducing end consisting ions (A-, B-, and C-ions) (Carroll, Willard, & Lebrilla, 1995, Chai, Piskarev, & Lawson, 2001, Harvey, 2005, Maina et al., 2013, Pfenninger, Karas, Finke, & Stahl, 2002a). Fragmentation in one direction can facilitate the analysis of cross-ring fragments from middle residues (Maina et al., 2013). By analyzing the cross-ring fragments, the linkages have been determined from negatively charged deprotonated (Harvey, 2005, Pfenninger et al., 2002a) or anion adduct (Guan & Cole, 2008, Zhu & Cole, 2001) disaccharides and oligosaccharides.

Although negative ionization MS/MS methods have been widely developed for the structural analysis of oligosaccharides, to our knowledge, only two MS/MS studies of AXOS using negative ionization have been published. Quemener, Ordaz-Ortiz, and Saulnier (2006) characterized the structure of neutral deprotonated AXOS by electrospray ionization quadrupole time-of-flight mass spectrometry (ESI-Q-TOFMS) and electrospray ionization ion trap mass spectrometry (ESI-ITMS). They showed that mono- or di-substituted AXOS (*O*-3-