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Building a fructan LC–MS^2 library and its application to reveal the fine structure of cereal grain fructans

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ABSTRACT

A liquid chromatography-mass spectrometry (LC–MS) library is presented containing the relative retention times of 28 fructan oligomers and MS^2 spectra of 18 of them. It includes the main representatives of all fructan classes occurring in nature and with a degree of polymerization between three and five. This library enables a rapid and unambiguous detection of these 18 fructan structures in any type of sample without the need for fructan purification or the synthesis of fructan standards. Its wide applicability is demonstrated by the analysis of fructans in a set of cereal flour samples. Marked differences were observed in the types of fructans present in oat, barley, rye, spelt and wheat flour. A putative link between the accumulation of certain fructan types and cereal phylogeny is described.

1. Introduction

Fructans are defined as fructose-based oligomers and polymers (Lewis, 1993) and are accumulated by a diverse array of algae, flowering plants and bacteria (Hendry, 1993). They vary widely in chemical structure and five classes of structurally different fructans are known to occur in nature. Inulin-type fructans and levan-type fructans have a linear fructose backbone consisting mainly of Fruf(β2-1) and Fruf(β2-6) units, respectively (Fig. 1).

When a terminal glucose is present in inulin-type or levan-type fructans, the anomeric carbon of glucose is linked to the anomeric carbon of fructose as in sucrose (Fruf(β2-1α)Glc). When fructans are branched with both Fruf(β2-1) and Fruf(β2-6) units in one molecule, they are classified as graminan-type fructans. Fructans with an internal glucose unit belong to the neo-inulin and neolevan-type fructans and have mainly Fruf(β2-1) and Fruf(β2-6) units, respectively (Van den Ende, 2013).

Fructans have been studied intensively as they can serve multiple functions in plants (Van den Ende, 2013) and because their consumption affects human health (Roberfroid et al., 2010). Fructans are dietary fibers and inulin-type fructans are recognized as prebiotics (Roberfroid et al., 2010). However, for some sensitive individuals, fructan consumption causes unwanted gastro-intestinal side effects as well (Kelly, 2009). Patients suffering from irritable bowel syndrome appear to benefit from following a diet low in FODMAPs (fermentable oligo-, di- and monosaccharides and polyols), a specific group of carbohydrates that includes fructans (Gibson, 2017). FODMAPs are defined as poorly digested short-chain carbohydrates and polyols that are rapidly fermented in the colon (Gibson & Shepherd, 2005). Fructan structure is important in this context because it affects the fermentation rate (Hernot et al., 2009; Stewart, Timm, & Slavin, 2008). Insight in fructan structure is also useful from a food technological point of view as it affects the extent of fructan degradation during food processing (Verspreet, Dornez, Van den Ende, Delcour, & Courtin, 2015). In addition, fructan structure is under scrutiny in fundamental research aiming to comprehend the role of fructans in plants under stressful conditions (Hincha et al., 2007; Peshev, Vergauwen, Moglia, Hideg, & Van den Ende, 2013). Structural analysis is hence becoming instrumental in the understanding of the nutritional effects of fructans, their fate during food processing and their physiological role in plants.

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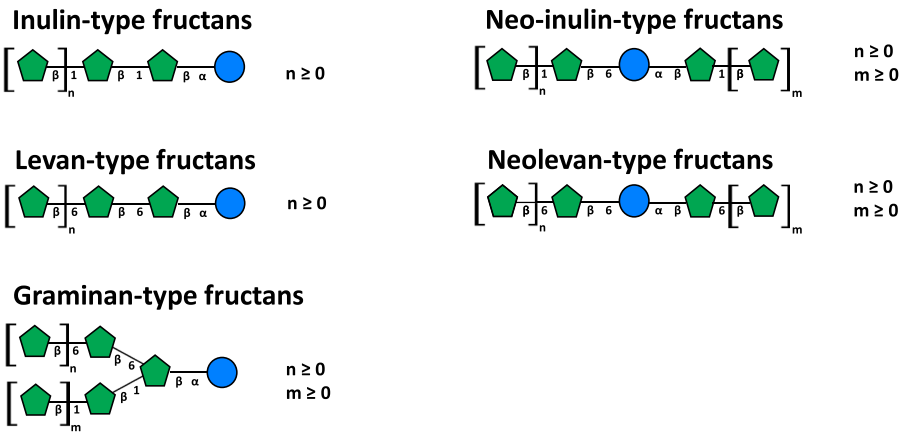

Fig. 1. Schematic representation of fructans of the five different fructan classes. Blue circles and green pentagons represent glucose and fructose units, respectively. Numbers (1 or 6) indicate the position of the carbon atom involved in the glycosidic linkage and Greek letters (α or β) are used for anomeric carbons. Graminan-type fructans are depicted with a branch point at the fructose unit of sucrose only, but branches can also occur at other fructose residues. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The most promising technique to study fructan structure is liquid chromatography – mass spectrometry (LC–MS). LC–MS can provide detailed structural information of individual fructan structures even when present in complex mixtures. It furthermore allows high-throughput screenings as no preceding purification or derivatization steps are required (Verspreet, Hansen, Dornez, Courtin, & Harrison, 2014). Dr. Harrison was one of the first to apply this technique to study the chemical structure of fructans and described the MS^2 fragmentation of inulin-type, levan-type (Harrison, Xue, Lane, Villas-Boas, & Rasmussen, 2012) and neo-inulin-type fructans (Harrison, 2012). More recently, the MS^2 fragmentation of graminan-type fructans was documented (Verspreet, Hansen, Dornez, Harrison, & Courtin, 2014), leaving the neolevan-type fructans as the only remaining fructan class for which the MS^2 fragmentation has not yet been described.

The first aim of this study is to analyze the MS^2 fragmentation of neolevan-type fructans. Their MS^2 fragmentation will be compared with that of fructans from other structural classes and all data combined to establish a fructan LC–MS^2 library. We hypothesize that creating such a library will pave the way for rapid structural identification of fructans in any type of sample. The second aim of this study is to demonstrate the wide applicability of the LC–MS^2 library by characterizing fructans in a set of cereal flours. Although cereal grains are staple foods worldwide, their fructan structure has not yet been closely examined. Only the structure of wheat grain fructans was recently investigated (Verspreet, Hansen et al., 2015).

2. Materials and methods

2.1. Materials

All chemicals, solvents and reagents were purchased from Sigma-Aldrich (Bornem, Belgium) and were of analytical grade unless specified otherwise. 1-Kestotriose (1-K) was purchased from Megazyme (Bray, Ireland) and 1,1-kestotetraose (1,1-KT) along with 1,1,1-kestopentaose (1,1,1-KP) from Elicityl (Crolles, France). Sucrose labeled at specific positions with ^{13}C isotopes was bought from Campro Scientific (Veenendaal, The Netherlands). Universally labelled (UL) sucrose ([UL-$^{13}C_6$ Fru] sucrose) contains a fructose unit with six ^{13}C isotopes while [1-$^{13}C_1$ Fru] sucrose contains fructose with a ^{13}C isotope on the first carbon. Production of 6G-kestotriose (6G-K) by *Xanthophyllomyces dendrorhous* was based on the work of Kritzinger, Kilian, Potgieter, and du Preez (2003). X. *dendrorhous* cells were washed with sodium phosphate buffer (50 mM, pH 6) and incubated (5 g/L) in the same buffer with sucrose (1 M) at 20 °C. The mixture was centrifuged (4000 *g*, 5 min) once formation of larger oligomers (DP > 3) started. The resulting supernatant was heated (90 °C, 10 min), dialyzed and purified by gel filtration chromatography (Verspreet, Dornez, Delcour, Harrison, & Courtin, 2015). *Dactylis glomerata* leaves were extracted to obtain levan-type fructans (Maleux & Van den Ende, 2007). Branched fructans produced by incubation of 1-K and sucrose with a heterologously expressed sucrose:fructan 6-fructosyltransferase (6-SFT) from *Pachysandra terminalis* (Verspreet, Hansen, Dornez, Harrison et al., 2014) served as a standard for graminan-type fructans with a 1-K core. The recombinant 6-SFT can transfer a fructose unit from sucrose to 1-kestotriose or another fructan acceptor and form a β(2-6) fructose-fructosyl linkage (Van den Ende et al., 2011).

Commercial wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.) and oat (*Avena sativa* L.) flour were obtained from Dossche Mills (Deinze, Belgium), Koopmans (Leeuwarden, The Netherlands) and Flahavan's (Kilmacthomas, Ireland), respectively. Barley (*Hordeum vulgare* L.) flour (cultivar Explorer, harvest 2015) and spelt (*Triticum aestivum* ssp *spelta* L.) grains (cultivar Cosmos, harvest 2015) were donated by the Walloon agricultural research center (Gembloux, Belgium). Spelt grains were tempered to a moisture content of 16.5% prior to milling by a CD1 mill (Chopin, Villeneuve-la-Garenne, France).

2.2. Methods

2.2.1. Synthesis of fructan standards

Heterologous expression of barley 6-SFT (Sprenger, Bortlik, Brandt, Boller, & Wiemken, 1995) and fructan:fructan 6G-fructosyltransferase (6G-FFT)/fructan:fructan 1-fructosyltransferase (1-FFT) from perennial ryegrass (*Lolium perenne* L.) (Lasseur et al., 2006) was performed in *Pichia pastoris* using the procedure of Schroeven, Lammens, Van Laere, and Van den Ende (2008) with small adaptations. The methanol incubation step for 6G-FFT/1-FFT and 6-SFT expression was not performed at 24 °C but at 30 °C and 15 °C, respectively. The recombinant enzymes were purified by $(NH_4)_2SO_4$ precipitation (Schroeven et al., 2008) and used to produce fructan standards. Graminan-type fructans with a 1,1-KT base were produced by incubating barley 6-SFT with sucrose (0.3 M) as donor substrate and 1,1-KT (0.3 M) as acceptor substrate for 6 days at 10 °C in sodium acetate buffer (100 mM, pH 5.5, 0.02% NaN_3). This reaction mixture was used as a reference in the study of cereal fructans. The production of neolevan-type fructans starting from either native sucrose (0.3 M), [UL-$^{13}C_6$ Fru] sucrose (0.3 M) or [$^{13}C_1$ Fru] sucrose (0.3 M) as donor