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Structural analysis of linear mixed-linkage glucooligosaccharides by tandem mass spectrometry

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ABSTRACT

Dextrans and glucooligosaccharides (GLOS) are produced by lactic acid bacteria (LAB) during sourdough fermentation. The dextrans can act as hydrocolloids in sourdough bread, while the GLOS may have anti-staling and prebiotic properties, depending on their structure. Development of high-throughput methods for screening the structural properties of dextrans and GLOS produced by different LAB in varying fermentation conditions is therefore of interest. In this study we explored the possibility of using electrospray ionisation tandem mass spectroscopy (ESI-MS/MS) to unequivocally determine the structures of underivatised GLOS. The emphasis was on linear mixed linked model GLOS, especially those containing (1 → 3) linkages that are common in dextrans. After evaluation of the model GLOS, the ESI-MS/MS method was used to determine the linkage positions of two mixed-linked tetrasaccharides obtained by hydrolysis of *Weissella confusa* and *Leuconostoc citreum* dextrans. In positive mode, only the reducing end linkage could be determined because isomeric fragment ions, present in subsequent MS^n cycles, hindered assignment of the remaining linkages. By contrast, it was possible to unambiguously assign all the linkages in each GLOS using the negative mode spectra. The present study thus shows that negative mode is the preferred method for ESI-MS/MS structural analysis of underivatised GLOS. In combination with liquid chromatography this method will enable rapid profiling of the structural variation of dextrans and prebiotic GLOS.

1. Introduction

Lactic acid bacteria (LAB) are used as starter cultures in the production of various fermented food products. In addition to acidity, flavour and aroma development, the LAB have been utilised for *in situ* production of exopolysaccharides (EPS) that improve the rheological and textural properties of fermented food (Welman, 2009). In sourdough bread, EPS (produced by *Leuconostoc* and *Weissella* strains) have been shown to be high potential hydrocolloids (Korakli, Rossmann, Ganzle, & Vogel, 2001; Lacaze, Wick, & Cappelle, 2007; Tieking, Korakli, Ehrmann, Gänzle, & Vogel, 2003; Katina et al., 2009). The most common EPS produced by these strains in sourdough is dextran which is an α-glucan produced using sucrose as a glucosyl donor (Jeanes et al., 1954; Naessens, Cerdobbel, Soetaert, & Vandamme, 2005). Based on structure, dextrans are divided into three classes: Class 1 dextrans containing α-(1 → 6) linkages in the main chain and α-(1 → 2), α-(1 → 3) or α-(1 → 4)-linked branches, Class 2 dextrans (alternans) that have alternating α-(1 → 3) and α-(1 → 6) linkages in the main chain with α-(1 → 3)- and α-(1 → 6)-linked branches, and Class 3 dextrans (mutans), which contain α-(1 → 3) linkages in the main chain with α-(1 → 6) side chains (Naessens et al., 2005; Robyt, 1986). The dextrans are known to improve bread volume, shelf life, softness and crumb texture (Bohn, 1961; Vandamme, Renard, Arnaut, Vekemans, & Tossut, 1997).

In situ production of dextrans during sourdough fermentation is usually accompanied by a concomitant production of glucooligosaccharides (GLOS). Though the GLOS may originate from enzymatic hydrolysis of starch and β-glucan during fermentation, studies show that the dominant GLOS in sourdough arise from the grafting of glucosyl units onto maltose by dextransucrases (Katina et al., 2009; Schwab, Mastrangelo, Corsetti, & Gänzle, 2008). Maltose and other small oligosaccharides, such as raffinose and gentiobiose, are known to act as acceptors for dextransucrases, which leads to the synthesis of a series of GLOS at the expense polymeric dextran (Robyt, Yoon, & Mukerjea, 2008). The GLOS formed from these acceptor reactions are currently being studied as potential prebiotics (Côté, Dunlap, & Vermillion, 2009; Monchois, Willemot, & Monsan, 1999; Sanz, Côté, Gibson, &

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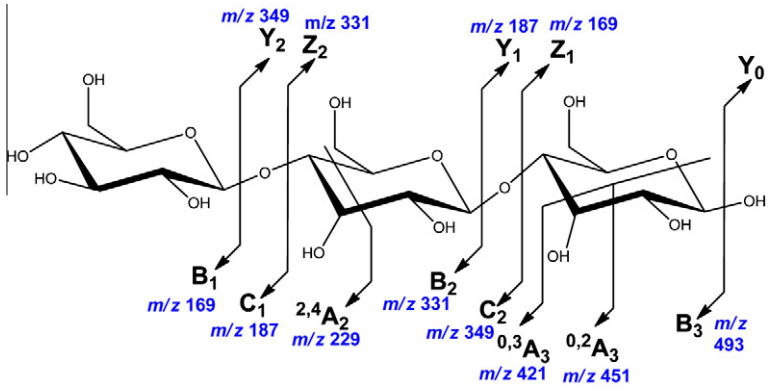

Fig. 1. Schematic representation of glucotriose, illustrating the nomenclature of fragment ions observed in the MS/MS spectrum according to Domon and Costello (1988). The *m/z* values of lithium adduct fragment ions are also shown.

Rastall, 2006). The impact of these GLOS on the properties of sourdough bread is still not known. They may, for example, have antistaling properties, as previously suggested (Katina et al., 2009).

Since the functional properties of dextrans in food applications and the prebiotic properties of the GLOS depend on their structural characteristics, development of quick methods to screen their structural properties is of interest. Structural analysis, using mass spectrometry, is suitable for this purpose and, in combination with liquid chromatography, it would enable rapid analysis of GLOS mixtures. For polymeric dextrans, MS can be used in combination with dextran-hydrolysing enzymes which have been shown to produce enzyme-resistant GLOS that vary, depending on the structure of the dextran (Katina et al. 2009; Maina, 2012). Current developments in hydrophilic interaction liquid chromatography (HILIC) for the separation of oligosaccharides have significantly simplified online ESI-MS detection through the use of MS-compatible eluents (Wuhrer, de Boer, & Deelder, 2009). Thus, HILIC–ESI-MS is a highly favourable option for LC-MS analysis of the GLOS.

MS-based structural analysis of oligosaccharides involves the evaluation of structure-diagnostic fragment ions in the tandem MS (MS/MS) spectra. The recorded MS/MS spectra contain two types of fragment ions: glycosidic cleavage and cross-ring cleavage, named according to the formal nomenclature proposed by Domon and Costello (1988) (Fig. 1). In structure elucidation, the cross-ring cleavages (A-type fragment ions) of the reducing end residue are the most informative, as they depend on the glycoside bond. The mechanisms for formation of these cross-ring cleavages have been demonstrated in various studies (Domon & Costello, 1988; Hofmeister, Zhou, & Leary, 1991; Spengler, Dolce, & Cotter, 1990). The glycosidic cleavages only provide information concerning the sequence, branching and size of the monosaccharide building blocks (Geyer & Geyer, 2006). Therefore, at each MS/MS stage, the diagnostic cross-ring cleavages of the reducing end units of the isolated product ion are used to establish the glycosidic linkage (Chai, Piskarev, & Lawson, 2001; Garozzo, Giuffrida, Impallomeni, Ballistreri, & Mon taudo, 1990; König & Leary, 1998).

Numerous studies have focussed on the structural analysis of glycoprotein and glycopeptide-associated glycans by MS, and several reviews have addressed this topic (Geyer & Geyer, 2006; Harvey, 2001; Zaia, 2004). Additionally, MS has been used to determine the structures of oligosaccharides obtained after hydrolysis of plant polysaccharides such as wheat arabinoxylans (Fernandez, Obel, Scheller, & Roepstorff, 2003; Quéméner, Ordaz-Ortiz, & Saulnier, 2006) and glucuronoxylans (Reis, Domingues, Domingues, Ferrer-Correia, & Coimbra, 2003). Few studies, however, have addressed the challenges in the analysis of mixed-linked linear GLOS by MS/MS. Comprehensive studies involving GLOS have mainly involved disaccharides (Garozzo et al., 1990; Hofmeister et al., 1991; Jiang & Cole, 2005; Spengler et al., 1990; Zhang, Brokman, Fang, Pohl, & Yeung, 2008) or larger molecules with one type of glycosidic linkage, α/β-(1 → 4) or α-(1 → 6), or both α-(1 → 4) and α-(1 → 6) linkages (Pasanen, Jänis, & Vainiotalo, 2007; Usui et al., 2009; Yamagaki & Sato, 2009; Čmelík & Chmelík, 2010).

The aim of this study was to explore the possibilities of using ESI-MS/MS to determine the structure of GLOS that have mixed-linkages, especially those containing (1 → 3) linkages that are common in dextrans. The samples analysed included commercial GLOS produced from the hydrolysis of barley β-glucan. Two commercially available galactooligosaccharides, originating from seaweed carrageenan, were also included, to increase the variety of oligosaccharides with (1 → 3) linkages in different positions. The structures of two tetrasaccharides obtained by enzymatic hydrolysis of *Weissella confusa* E392 and *Leuconostoc citreum* E497 dextrans, that have a potential application in sourdough baking, were then analysed with the ESI-MS/MS method. Both negative and positive ionisation were utilised and their ability to unequivocally determine the structure of the di-, tri- and tetrasaccharides evaluated.

2. Materials and methods

2.1. Materials

Reagents used included HPLC-grade methanol and isopropanol, formic acid, lithium acetate, and ammonium chloride from Merck (Darmstadt, Germany). The model disaccharides used to determine the typical fragmentation patterns of different linkages and model tri- and tetrasaccharides are listed in Table 1. Additionally, tetrasaccharides obtained by enzymatic hydrolysis of dextrans produced by *W. confusa* VTT E-90392 (=DSM 20194, NCDO 1975) and *L. citreum* VTT E-93497 were analysed. Isolation of the tetrasaccharides, after enzymatic hydrolysis of the dextrans, was carried out as previously reported (Maina, Virkki, Pynnönen, Maaheimo, & Tenkanen, 2011).

2.2. Methods

2.2.1. Mass spectrometry analysis

Mass spectrometry was carried out with electrospray ionisation (ESI-MS) in positive and negative mode. The mass spectrometer used was an Agilent 1100 Series LC/MSD Ion Trap XCT Plus with an electrospray ion source (Agilent Technology, Palo Alto, CA, USA). Prior to MS/MS analysis, 2–10 μl of each oligosaccharide (1–2 mg/ml) were mixed with 400 μl of MeOH:water:formic acid