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Effect of sucrose concentration on the composition of enzymatically synthesized short-chain fructo-oligosaccharides as determined by FTIR and multivariate analysis

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ABSTRACT

Fructo-oligosaccharides (FOS) are mixtures of oligosaccharides composed of fructose and glucose units. As their composition is determined by the synthesis conditions, the goals of this work were: (a) to engineer FOS of different composition by adjusting the sucrose concentration used as initial substrate; (b) to define partial least square (PLS) based-models to quantify all the sugars present in the reaction medium directly from the FTIR spectra. The yield of each reaction was calculated as the percentage of initial sucrose converted to each oligosaccharide, as monitored by HPLC. In parallel, the reactions were followed by FTIR. Six different PLS models aiming to determine the concentration of each carbohydrate present in the reaction medium were calibrated and independently validated. The means of predicted values fitted well to those obtained by HPLC. Determining FOS composition directly from the FTIR spectra represents a useful tool to monitor enzymatic synthesis, with strong impact at both an academic and an industrial level.

1. Introduction

Fructo-oligosaccharides (FOS) are small chain oligosaccharides composed of fructose units linked by (2→1)-β-glycosidic bonds and a single D-glucosyl unit at the non-reducing end. In most cases, FOS are mixtures of short chain oligosaccharides, namely 1-kestose [degree of polymerization (DP) equal to 3], nystose (DP4) and 1^F-fructofuranosylnystose (DP5) (Crittenden & Playne, 2009).

FOS are well recognized prebiotics, that is, non-digestible food components that beneficially affect the host health by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Gibson & Roberfroid, 1995). They have a great economic importance as they are used in infant formula and other functional food products (Romano, Tymczyszyn, Mobili, & Gómez-Zavaglia, 2015). FOS are also low-calorie and non-cariogenic sweeteners, and effective protectants of biological structures during dehydration processes (Romano et al., 2014; Schwab, Vogel, & Gänzle, 2007). This latter property has been ascribed to their capacity to interact with lipid membranes, which in turn is dependent on their structure and on their DP (Hincha, Popova, & Cacela, 2006; Hincha et al., 2007).

Short chain FOS (*i.e.*: DP3, DP4, DP5) are generally produced from sucrose by transfructosylation reactions using fructosyltransferases (β-fructofuranosidase, EC 3.2.1.26 or β-D-fructosyltransferase, EC 2.4.1.9) as biocatalysts (Vega & Zuniga-Hansen, 2011, 2014; Vega-Paulino & Zuniga-Hansen, 2012). The composition of the FOS obtained can be modulated by adjusting the reaction parameters

Abbreviations: FOS, fructo-oligosaccharides; DP, degree of polymerization; DP3, degree of polymerization equal to three; DP4, degree of polymerization equal to four; DP5, degree of polymerization equal to five; HPLC, high performance liquid chromatography; FTIR, Fourier transform infrared spectroscopy; PLS, partial least square; FU, fructosyltransferase units; *X*, sucrose conversion; X_{max}, maximum sucrose conversion; *Y*, yield; MSC, mean centering correction; EMSC, extended multiplicative scatter correction; RMSEC, root mean square error of calibration; RMSEP, root mean square error of prediction; SEC, standard error of calibration; SEP, standard error of prediction; Glu, glucose; Fru, fructose; Tg, glass transition temperature.

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(*i.e.*, time, temperature, pH, enzyme source and substrate concentrations).

Commercial enzyme preparations have economic and technical advantages, including low price, versatility and stability under reaction conditions of industrial processes. Most of them have both transfructosylase and hydrolase activities, thus to synthesize short chain oligosaccharides, preparations with high transfructosylase activity are those to be selected. *Viscozyme L* from *Aspergillus aculeatus* (Novozyme, Denmark) is an adequate enzymatic preparation because it has both high transfructosylation activity and high transferase/hydrolase ratio (Lorenzoni, Aydos, Klein, Rodrigues, & Hertz, 2014; Lorenzoni et al., 2015; Vega-Paulino & Zuniga-Hansen, 2012).

The economic importance of FOS requires on quick and reliable methods to monitor their synthesis. Even though the usefulness of high performance liquid chromatography (HPLC) in determining the composition of sugar mixtures is unquestionable, Fourier transform infrared spectroscopy (FTIR) is nowadays a trustworthy technique to ascertain structural and physical properties of carbohydrates. As no exogenous chemical reagents are needed, samples require almost no preparation and analytical testing does not generate hazardous waste, FTIR is definitely a useful tool to determine the composition of complex oligo- and polysaccharides in a quick and environmentally friendly way (Anjos, Campos, Ruiz, & Antunes, 2015; Coimbra, Gonçalves, Barros, & Delgadillo, 2002; Santos, Gerbino, Tymczyszyn, & Gomez-Zavaglia, 2015). The use of FTIR in tandem with multivariate analysis has enabled an expeditious determination of the oligo and polysaccharide composition of different products, including commercial sugars (Kačurakóvá & Wilson, 2001), cellulose, pectins (Fellah, Anjukandi, Waterland, & Williams, 2009), starch, hemicelluloses, carrageenans, hyaluronates (Fellah et al., 2009; Kačurakóvá & Wilson, 2001), fruits (Bureau et al., 2009), cereals (Cozzolino, Roumeliotis, & Eglinton, 2014), honey (Anjos et al., 2015) and wine extracts (Coimbra et al., 2002). Moreover, different enzymatic reactions including sugars either as substrates or as products have been monitored using FTIR (Baum et al., 2013; Chiş, Fetea, Taoutaou, & Socaciu, 2010; Schindler, Le Thanh, Lendl, & Kellner, 1998).

With regard to FOS, FTIR and multivariate analysis were used to qualitatively characterize FOS in strawberries (Blanch, Goñi, Sanchez-Ballesta, Escribano, & Merodio, 2012), barley (Cozzolino et al., 2014) and agave (Mellado-Mojica & López, 2015). Trollope, Nieuwoudt, Görgens, and Volschenk (2014) used FTIR to quantify the consumption of sucrose as an indicator of the activity of β-fructofuranosidases from different origins. More recently, they developed partial least square (PLS) calibration models based on FTIR spectra for the screening of β-fructofuranosidases libraries generated by random mutagenesis (Trollope, Volschenk, Görgens, Bro, & Nieuwoudt, 2015).

Considering that the composition of FOS determines both their prebiotic properties and their capacity to interact with lipid membranes, and that this composition is in turn determined by the conditions of synthesis, the goal of this work was twofold: (a) to obtain FOS of different composition by adjusting the initial sucrose concentration; and (b) to define models based on multivariate analysis to determine the composition of FOS throughout the synthesis, directly from the FTIR spectra.

2. Materials and methods

2.1. Materials

Viscozyme L was donated by Blumos SA-Chile. 1-kestose (DP3), nystose (DP4) and 1^F-fructofuranosylnystose (DP5) standards were purchased from Wako Chemicals (Richmond, VA, USA). Sucrose, glucose, fructose and other reagents were obtained from Sigma Chemical (St. Louis, MO, USA).

2.2. Methods

2.2.1. Synthesis of FOS

Sucrose solutions within 10% and 60% w/v prepared in distilled water were used as substrate for the enzymatic synthesis. The pH was adjusted to 5.5 with 2 M NaOH and 4% v/v Viscozyme L (56 FU/mL; FU: fructosyltransferase units) was used as biocatalyst, according to Vega and Zuñiga-Hansen (2014). The volume of the reaction mixture was 15 mL. Transfructosylation reaction was performed for 6 h at 50 ± 1 °C in 25 mL Erlenmeyer flasks with stirring (100 rpm). One FU was defined as the amount of enzyme required to transfer 1 μmol of fructose per minute at 55 °C, pH 5.5 and stirred at 100 rpm.

The progress of each enzymatic reaction was followed by taking samples at regular intervals (every 30 min the first 3 h and every 1 h afterwards, up to a total of 6 h). The reactions were stopped after 6 h by heating at 100 °C for 2 min. A scheme representing the enzymatic production of short chain FOS is presented as Supplementary material (Scheme S1).

The composition of oligosaccharides was analyzed by HPLC and FTIR (see Sections 2.2.2 and 2.2.3 below). The following parameters were considered:

- *Sucrose conversion (X):* Represents the percentage of the initial sucrose consumed in the reaction (Eq. (1)):

$$X = [(S_0 - S)/S_0] \times 100 \quad (1)$$

  where S_0 and S represent the initial and final concentration of sucrose, respectively.
- *Yield [$Y_{DP(n)}$]:* Represents the percentage of initial sucrose converted to FOS (DP3, DP4 or DP5) at a given time during the reactions (Eq. (2)). It was evaluated every 30 min the first 3 h of reaction and every 1 h afterwards.

$$Y_{\mathrm{DP}(n)} = \mathrm{DP}(n)/s_0 \times 100 \quad (2)$$

  where DP(n) represents the mass of short chain FOS produced, (n) indicates their DP (3, 4 or 5), and s_0, the initial mass of sucrose.

The yield was also determined for glucose (Y_{glu}) and fructose (Y_{fruct}), which are byproducts of transfructosylation.

2.2.2. HPLC analysis

The composition of carbohydrates obtained throughout the syntheses (Section 2.2.1) was determined by HPLC in a Perkin-Elmer Series 200 equipment (Massachusetts, USA) with refractive index detector and autosampler. The chromatographic column used was a BP-100 Ag+ (300 × 7.8 mm) for carbohydrate analysis (Benson Polymeric, Reno, NV, USA). The column is composed of a stable high cross-linked styrene–divinylbenzene copolymer resin in the silver form that can resolve saccharides as large as DP-7.

Column and detector temperatures were maintained at 50 °C and 40 °C, respectively. The progress of each enzymatic reaction was followed by taking samples at regular intervals according to Section 2.2.1. Once collected, reactions were stopped by heating at 100 °C for 2 min, and samples diluted, filtered through 0.22 μm Millipore Durapore membranes (Billerica, MA, USA) and eluted with milli-Q water (mobile phase) at a flow-rate of 0.4 mL/min. Chromatograms were integrated using PeakFit software (version v4.12).

The composition of samples was determined by assuming that the area of each peak was proportional to the weight percentage of the respective sugar of the total sugar mass (Boon, Janssen, &