MONITORING THE COMPOSITION OF FRUTO-OLIGOSACCHARIDES DURING TRANSFRUCTOSYLATION REACTIONS BY MEANS OF INFRARED SPECTROSCOPY

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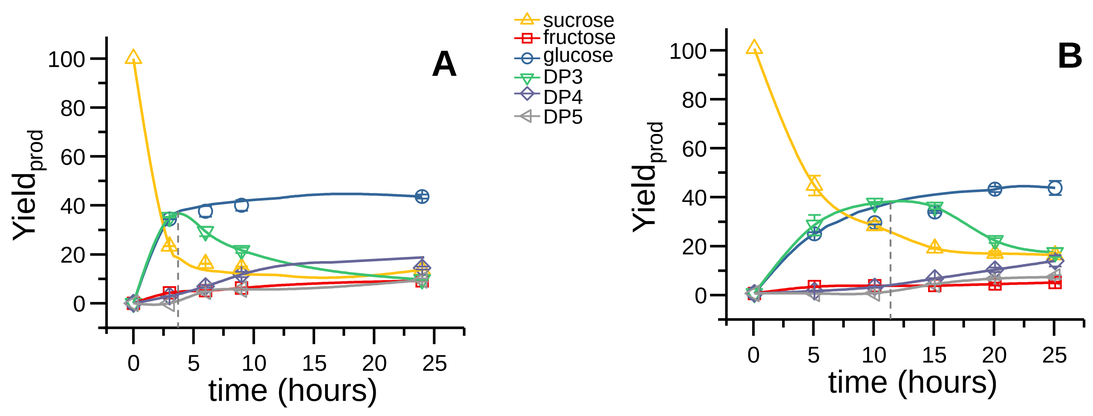
INTRODUCTION

Fructo-oligosaccharides (FOS) are well recognized prebiotics, that is, substances selectively used by host microorganisms conferring a health benefit [1]. Their beneficial properties include the modulation of colonic microflora, improvement of the gastrointestinal physiology and immune functions, bioavailability of minerals, metabolism of lipids, control of diabetes, reduction of uremia and prevention of colonic carcinogenesis [2].

From a chemical point of view, FOS are composed of fructose units linked by (2→1)-β-glycosidic bonds and a single D-glucosyl unit at the non-reducing end of the chain. In most cases, FOS are mixtures of oligosaccharides with degree of polymerization (DP) from 3 to 6. They can be obtained by transfructosylation reactions using sucrose as substrate. Considering that the composition of FOS determines their prebiotic effect on human health, and this composition is in turn determined by the conditions of synthesis, the goal of this work was twofold: from one side, to obtain FOS of different compositions by adjusting the synthesis conditions; from the other side, to define a model based on multivariate analysis to determine the composition of FOS directly from the FTIR spectra.

RESULTS

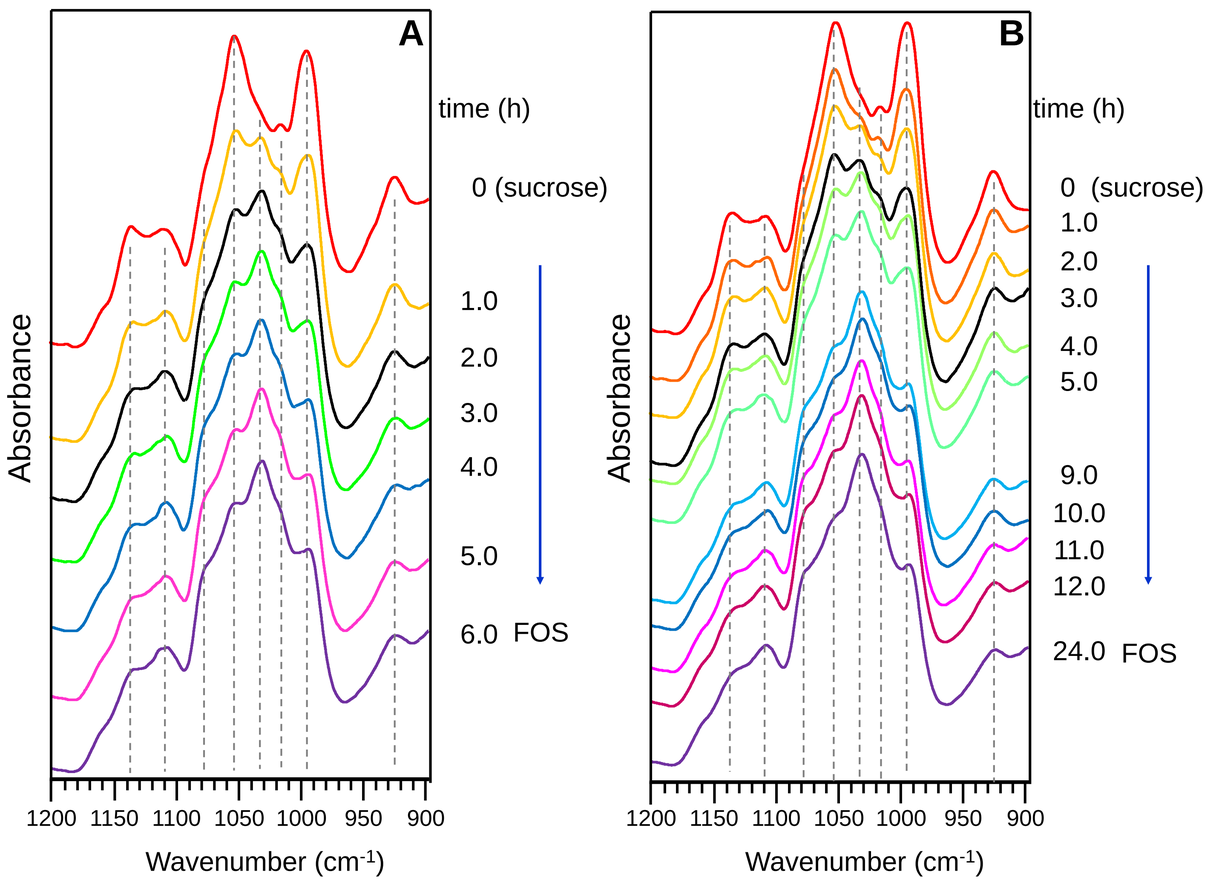
Fig. 1. Yield (Y) of products *vs* time of synthesis. Yield (Y) is expressed in grams of product/100 g sucrose. A: 30% w/v sucrose (substrate); B: 60% w/v sucrose (substrate).



DP3 was the oligosaccharide produced with the highest efficiency no matter the initial sucrose concentration. The lower the initial sucrose concentration, the earlier the max Y_{DP3} was reached (3.5 h for 30% w/v sucrose and 11 h for 60% w/v sucrose).

DP5 was the FOS produced in lowest concentrations and in none of the conditions overpassed 10 g DP5/100 g sucrose.

Fig 2. Evolution of the FTIR spectra throughout the synthesis of FOS in the 1200-900 cm^{-1} region. Dash lines indicate the bands increasing and decreasing throughout the synthesis. A: 30% w/v sucrose; B: 60% w/v sucrose.



Noticeable changes in the fingerprint region of sugars were observed throughout the enzymatic synthesis. Typical bands of sucrose decreased as well as new wide bands, arising from the mixture of FOS with different DP, were detected.

MATERIALS AND METHODS

Synthesis of FOS

Syntheses were carried out at 50°C using *Viscozyme L* as biocatalyst and sucrose (30 and 60 %w/v) as substrate. Transfructosylation reactions were performed for 24 hours at 50±1°C. The progress of the syntheses was followed by taking samples at regular intervals. The composition of oligosaccharides was analyzed by HPLC (reference method) and FTIR. For each sample, eight FTIR spectra were registered in ATR mode (4000-500 cm^{-1} range) by co-adding 16 scans with 4 cm^{-1} spectral resolution.

- ***Yield (Y_{prod})*** represents the percentage of initial sucrose converted to FOS (DP3, DP4 or DP5) at a given time during the reactions (Equation 1): $Y_{prod} = DP(n)/ s_0 \times 100$ (1)
where ***DP(n)*** represents the mass of short chain FOS, glucose, or fructose produced, and s_0, the initial mass of sucrose.

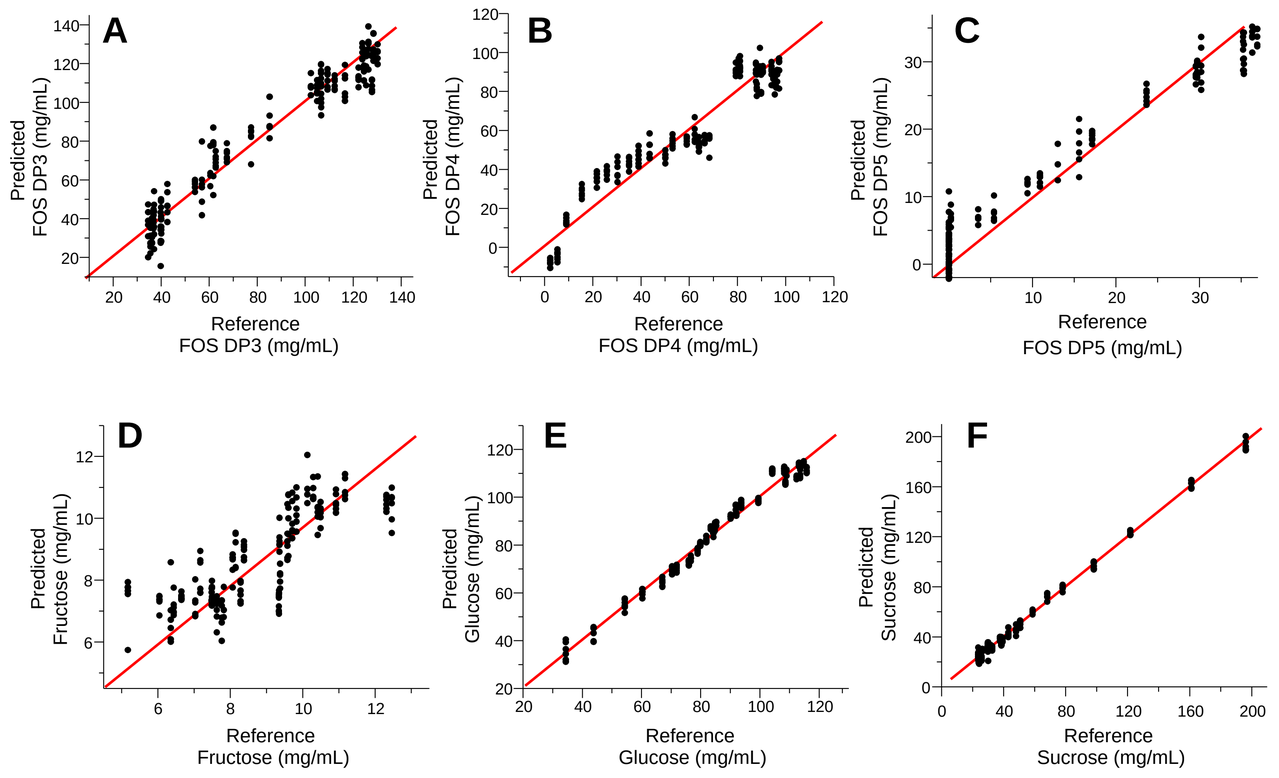
HPLC analysis

HPLC analysis was performed on a Perkin-Elmer Series 200 chromatographer, equipped with a Benson Polymeric BP-100 Ag+ (300x7.8 mm) column. The samples were filtered through a 0.45 μm filter prior to injection and eluted with milli-Q water (mobile phase) at a flow-rate of 0.4 mL/min. Chromatograms were integrated using Total Chrom software (version 6.3.1).
External standards of fructose, glucose, sucrose, and FOS were used to determine their retention times and check the linear range of the measurements.

Multivariate analysis

Multivariate analysis was carried out on the FTIR spectra using The SOLO 8.6.1 version of PLS Toolbox software from Eigenvector. Six different PLS models were calibrated to determine the concentration of DP3, DP4, DP5, glucose, fructose and sucrose in the mixtures.
A group of 181 FTIR spectra obtained from ca. 40 samples was used to calibrate each of the six models. The composition of FOS obtained by HPLC was used as a reference to define the PLS models. A set of 66 spectra collected independently from those used for calibration were used to validate the models. The reliability and robustness of the calibrated models were determined as a function of their correlation, R-square and their calibration and prediction errors (RMSEC and RMSEP).

Fig. 3. Calibration of a PLS regression method to obtain the concentrations of each product of the enzymatic synthesis of FOS from their ATR-FTIR spectra: predicted (FTIR) *vs* reference (HPLC) values of the enzymatic reactions´ products. A: DP3; B: DP4; C: DP5; D: fructose; E: glucose; F: remaining sucrose.



The means of predicted values fitted nicely the results obtained by HPLC (R^2 greater than 0.91 in all cases), thus supporting the use of the PLS model to investigate unknown samples.

Table 1. Statistic parameters of the PLS regression method for the ATR-FTIR 1180-970 cm^{-1} region.

	fructose	glucose	sucrose	DP3	DP4	DP5
RMSEC	1.023	2.679	2.981	8.872	9.189	3.579
RMSECV	1.075	2.757	3.105	9.233	9.450	3.644
R^2 calibration	0.678	0.983	0.995	0.938	0.911	0.932
R^2 validation	0.650	0.982	0.994	0.933	0.906	0.929

CONCLUSION

This work integrates two complementary aspects related to the production of FOS: a rationalization of their enzymatic synthesis and a high-throughput method to quantify them. Understanding the effect of initial sucrose concentration on the final composition of FOS and the evolution of the different species along synthesis enables the possibility of engineering FOS according to the pursued goals. FTIR provided a quick, reliable and environmentally friendly tool to both monitor the enzymatic production of FOS and perform quality control analysis, in a much simpler way than traditional methods such as HPLC.

In the context of the importance of the functional foods' market, this work represents a useful and valuable tool both in the academia and in the food industry.

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