Experimental approach for prebiotic oligosaccharides fractionation and chemical characterization

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BACKGROUND
Prebiotic oligosaccharides can be produced in transglycosylation reactions catalyzed by glycosidases. Glycosidases from different biological sources have specific ability to catalyze the formation of oligosaccharides with particular chain lengths (usually DP < 7) and predominant glycosidic linkages. Despite the range of commercially available oligosaccharides mixtures (mainly fructo and galacto-oligosaccharides), very few studies are focused on the mechanisms behind the prebiotic activity of particular oligosaccharides. Probably this lack is due to the unavailability of well characterized oligosaccharide fractions for prebiotic function assessment.

From a legal perspective, the first criterion for the scientific substantiation of claims is the characterization of the food or food component to which the claimed prebiotic function assessment.

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AIMS
Fractionation and chemical characterization of prebiotic oligosaccharide mixture produced by enzymatic transglycosylation during fermentation.

EXPERIMENTAL APPROACH
Materials
Crude mixture of galacto-oligosaccharides (GOS) was provided by BIOTEMPO, Biotechnology Consulting Lda. and was produced by enzymatic transgalactosylation (β-galactosidase) during yeast growth in a lactose containing medium.

Methods
Reverse Phase High Performance Liquid Chromatography (RP-HPLC)
The chromatographic analysis was carried out in an analytical HPLC unit (Jasco) equipped with a refractive index detector.

Electrospray Mass Spectrometry (ESI-MS and MS/MS)
The ESI-MS and ESI-MS/MS were carried out on a Micromass (Manchester, UK) Q-TOF2 hybrid tandem mass spectrometer. The cone voltage was set to 35 V, and capillary voltage was 203 allowed to confirm the presence of a trisaccharide composed by three hexoses. The cross ring fragments observed in the MS/MS spectra allowed to infer the type of linkage present in the disaccharide [4]. 1→4 linked saccharides fragment by loss of C₂H₄O₂ (0,2An or 2,4An, -60 Da), and C₅H₉O₄ (0,2An or 2,4An plus 2,4Xₙ, -2x60Da = -120Da).

RESULTS

Figure 1 - i) HPLC chromatogram of the oligosaccharide mixture; ii) MS spectra ESI-MS/MS spectrum of the collected compound D.

The cleavage of the glycosidic linkages (yielding Y ions) with the formation of the ions at m/z 365 and 203 allowed to confirm the presence of a trisaccharide composed by three hexoses. The cross ring fragments observed in the MS/MS spectra allowed to infer the type of linkage present in the disaccharide [4]. 1→4 linked saccharides fragment by loss of C₂H₄O₂ (0,2An or 2,4An, -60 Da), and C₅H₉O₄ (0,2An or 2,4An plus 2,4Xₙ, -2x60Da = -120Da).

Figure 3 - (1H,13C) gHMBC spectrum of tube D; Carbon and proton resonances of the sugar units of the trisaccharide D (δ, ppm). The resonances assignments were based on the 13C-NMR, (1H, 13C) NMR, (1H, 13C) HMBC and C1. (1H, 13C) HSQC.

The 1→4 linkage between T-β-Galp (residue A) and 4)→β-β-Galp (residue B) was confirmed by two cross peaks in the gHMBC spectrum: one between the H-1 of residue A and the C-4 of residue B, and the other, between the H-4 of residue B and the anomic carbon of residue A. The 1→4 linkage between 4)→β-Galp(1→ residue B) and Glucose was confirmed in the same spectrum, by the correlation peak between the anomeric carbon of residue B and the C-4 of β-Glcp and/or α-Glcp (81.2 ppm).

CONCLUSIONS
This work presents a successful application of chromatography, ESI-MS and RMN to the fractionation and characterization of a mixture of galacto-oligosaccharides produced by fermentation. The main saccharide obtained from the mixture was identified as β-D-galactopyranosyl(1 →4)-β-D-galactopyranosyl(1 →4)-D-glucopyranose. The others saccharides were also characterized (results not shown).

Further work on the different fractions obtained by semi-preparative HPLC will be conducted in order to elucidate the mechanisms underlying the functional activity of individual oligosaccharides.

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Nuclear Magnetic Resonance (NMR)
NMR spectra were recorded on a Bruker Avance 500 spectrometer operating at 500.13 and 75.48 MHz for 1H and 13C, respectively. The sample was dissolved in 0.5 mL of D2O and chemical shifts values were given in ppm relative to TSS.

BIBLIOGRAPHY

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