Functional Foods in Europe
International Developments in Science and Health Claims
9-11 May 2007, Malta

Experimental approach for prebiotic oligosaccharides fractionation and chemical characterization

***Duarte Torres*[1,2*], Ana Dominguez[2], Lígia Rodrigues[1,2], Isabel Rocha[1,2], Susana M. Cardoso[3], Artur M. S. Silva[4], M. Rosário M. Domingues[4], Orlando Rocha[2], Clarisse Nobre[2], Eugénio Ferreira[2], José A. Teixeira[2]**

[1] BIOTEMPO – Biotechnology Consulting, Lda. Braga, Portugal.
[2] Center of Biological Engineering, University of Minho, Braga, Portugal
[3] Agrarian Superior School, Institute Polytechnic of Bragança, Bragança, Portugal.
[4] Department of Chemistry, University of Aveiro, Aveiro, Portugal

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 effect is attributed [1]. From a fundamental and practical perspective, it is desired a better understanding of the structure-function relationships of prebiotic oligosaccharides [2]. Following these purposes, during the development of the BIOLIFE project (PRIME03/347) “Production of ingredients for functional foods application”, it has been established a set of experimental techniques that allow the fractionation and chemical characterization of prebiotic oligosaccharides produced by fermentation of different microorganisms. The fractionation, at lab or semi-preparative scale, was obtained using reverse-phase high performance liquid chromatography techniques (RP-HPLC). Subsequently, the collected fractions were analyzed in a hybrid mass spectrometer Q-TOF 2 (Micromass), with an Electrospray source (ESI). Product ion spectra of $[M+Na]^+$ and $[M+Li]^+$ ions observed in the ESI-MS spectra were obtained showing glycosidic cleavage that confirmed its composition on monosaccharides, and cross ring fragmentations typical of the different types of linkage. Additional ^{13}C-nuclear magnetic resonance (^{13}C-NMR) and 2D-NMR-spectroscopy assays allowed the clearly identification of the monomeric composition and of the anomeric carbon configurations of these prebiotic compounds.

This work presents a successful application of these experimental techniques to the fractionation and characterization of a mixture of galacto-oligosaccharides produced by fermentation. Further work on the different fractions obtained will be conducted in order to elucidate the mechanisms underlying its functional activity.

[1] Aggett PJ *et al*, Eur J Nutr 2005;44(Suppl 1):I/1-I/30
[2] Rastal RA and Maitin V, Curr Opin Biotech 2002;13:490-96

* Corresponding author e-mail: dtorres@biotempo.com