Qualitative and quantitative analysis of crop syrup containing enzymatically produced Isomaltooligosaccharides

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Isomaltooligosaccharides (IMOs) are non-digestible oligosaccharides, considered as prebiotics and therefore aim to selectively feed probiotics indigenous to the human colon. Recent data obtained in human subjects, support the involvement of dietary oligosaccharides in physiological processes in the different intestinal cell type and also outside the gastrointestinal tract (e.g. hormone production, lipid and carbohydrate metabolism).

IMOs consists of glucose monomers linked by at least one α-1-6, or in a lower proportion α -1-3 (nigerose family) or α -1-2 (kojibiose family) glucosidic linkages. In our case they are produced enzymatically from corn starch. It results in a very complex mixture with molecules characterized at the same time by their DP value (from 2 to ~20), linkages types (α-1-2, 3 or 6) and the proportion and position of each type of linkage (only α -1-6 or combined types). The challenge of this study was to find a qualitative and quantitative method to characterize the syrups. In a subsequent study, every unidentified peak could be determined by NMR or Mass spectrometry. Three different chromatographic methods have been tested and compared over their selectivity, sensibility, robustness, applicability and their quantitative power.

The HPLC-RID (Refractive Index Detector) used with a Prevail Carbohydrate column (Alltech), adapted for carbohydrates with a certain value of DP, appear to be poorly selective due to the obligation of working in isocratic conditions.

The HPLC-ELSD (Evaporating Light Scattering Detector) used with the same column gave a very good separation upon the DP as the Prevail accepts a wide range of solvant changing and even a separation of the different DP 2 molecules. The sensibility was also improved due to the stability of the baseline. The quantitative analysis is a bit more awkward due to the relatively limited linear portion of the calibration curve.

High-performance Anion Exchange Chromatography coupled with Pulsed Amperometric Detection (HPAEC-PAD) appeared to deserve a special mention, since it provides selective separation and sensitive detection. In fact, this technique is the only chromatographic one to separate with effectiveness homologue molecules having a difference only in their DP (up to high DPs) or linkage types. This is due to the separation mode based on the difference in ionic force of the sugars in alkaline conditions (mainly due to the DP and the structure of the molecule). However, during the detection, the electrochemical behavior of carbohydrates can be affected by molecular weight as well as by structural differences giving rise to different response factors; as a consequence, HPAEC-PAD is not generally considered as suitable for quantitative studies of complex mixtures of oligosaccharides. In this work a methodological approach is presented in order to get a quantitative response through a rigorous methodology for our IMOs. It also opens the way to the theoretical determination of the response factor for the homologue molecules in relation with their structure and DP.