Fractionation of fructo-oligosaccharides by gel filtration

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Nowadays, production of fructo-oligosaccharides (FOS) has gained large commercial interest due to its beneficial properties in the human health as prebiotics. Fermentative processes appear to be a good technique for large scale production of FOS, namely kestose (GF2), nistose (GF3) and fructo-furanosilnistose (GF4). However, the sugars mixture that results from the fermentations contains high levels of fructose (F), glucose (G) and sucrose (GF) which have to be eliminated. Thus, the aim of the present study was to separate FOS from those components. The separation was performed by gel filtration chromatography using Bio-Gel P2 polyacrylamide. The elution of sugars from the column was based on the molecular weight with the largest sugars eluting first and the smallest later. The molecular weights of sugars present in the fermentative broth are respectively: 180 (F, G), 342 (GF), 504 (GF2), 666 (GF3) and 825 g/mol (GF4). Bio-gel P2 appears to be an efficient gel for the purification of these sugars since it allows the fractionation of molecules with molecular weight between 100 and 1800 g/mol. Two different samples were used in this study; one from the end of fermentation (Sample 1) consisting of 16.1 (F), 47.0 (G), 64.84 (GF), 84.37 (GF2), 13.18 (GF3) and 0 g/L (GF4); and the other (Sample 2) previously purified in a charcoal column to concentrate FOS consisting of 0.92 (F), 1.64 (G), 2.68 (GF), 18.06 (GF2), 46.02 (GF3) and 7.96 g/L (GF4).

Two hundred microliters of sample were loaded onto the Bio-Gel P2 column (100 X 1.6 cm) and eluted with distilled water at a flow rate of 0.3 mL/min. Fractions were collected and analysed by ELSD (*Evaporative Light Scattering Detector*) and HPLC. Results achieved from the fractionation of sample 1 showed that it is possible to obtain pure kestose, and that sucrose and monosaccharides (G/F) are also well separated. Nevertheless, and although its presence was detected by ELSD (elution volume: 104-112 mL) it was not possible to quantify nistose by HPLC as it was much diluted. Kestose and nistose were obtained from the fractionation of sample 2. As the concentrations of fructo-furanosilnistose, sucrose and monosaccharides in the initial sample were very low it was not possible to quantify them by HPLC. However, the presence of GF4 (elution volume: 92 - 102 mL) and sucrose (elution volume: 125-132 mL) was detected by ELSD.

In sum, although gel filtration with Bio-Gel P2 provides a good fractionation of FOS from fermentative broths, the process is time consuming (8h) and the amounts of final product achieved are very small. Therefore, unless the final product has a high commercial value in its fractionated form, the implementation of this procedure at an industrial scale is not economically viable. However, the achieving of pure sugars makes the chemical, clinical and nutritional characterization of each single sugar possible.

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