Title: Fructo-oligosaccharides: production, characterization and purification

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The consumers' interest in healthy and high nutritional food has significantly increased in the recent years. This trend towards the adoption of healthier lifestyles has been the main driver for the great demand of functional ingredients, such as the prebiotics fructo-oligosaccharides (FOS). Industrially, FOS are produced from sucrose through purified enzymes, in two-step bioprocesses, with low theoretical yields (0.50-0.55 $g_{FOS}.g_{Sucrose}^{-1}$) and purities (50-55%). Downstream steps are therefore needed to remove the non-prebiotic sugars and enable the incorporation of these FOS mixtures in diabetic, dietetic and healthy foods.

In the last ten years, we have been investigating new strategies to produce FOS with higher contents, purities and differentiated functionalities. We have been exploring *Aureobasidium pullulans* and *Aspergillus ibericus* as FOS producers, in one-step fermentation processes, using the whole cells of the microorganisms instead of the isolated enzymes. This strategy proved to be efficient, fast and economic, yielding 0.64 $g_{FOS}.g_{Sucrose}^{-1}$. The FOS mixtures produced were able to stimulate the growth of probiotic strains and were simultaneously resistant to hydrolysis along the gastrointestinal system confirming their health claims as prebiotics. The probiotic strains preferentially metabolized the FOS mixture synthesized by *A. ibericus*, followed by the one from *A. pullulans* and lastly the commercial FOS.

The purification of FOS is not straightforward due to the physicochemical similarities between the different oligosaccharides and the smaller saccharides. To increase the FOS purity, we have been exploring different strategies including microbial treatments and downstream treatments as activated charcoal and ion-exchange chromatography.

As microbial treatments, we studied the use of a *Saccharomyces cerevisiae* strain, able to metabolize the small saccharides without FOS hydrolyse, in co-culture with the FOS microorganism producer or in a two-step fermentation, in which FOS are firstly synthesized and then purified by the *S. cerevisiae*. Fermentations in two-steps were found to be more efficient than the co-culture ones and purities of 82% (w/w) in FOS were obtained [1]. To avoid competition by the subtract in the co-culture, we are now evaluating the use of a *S. cerevisiae* strain with the SUC2 gene for invertase expression repressed. Using this strategy, FOS are being produced with yields of 0.64 $g_{FOS}.g_{Sucrose}^{-1}$ and purities up to 93% (w/w).

As downstream treatment we optimized an adsorption/desorption process of sugars using activated charcoal and ethanol as eluent. Mixtures containing 50.6% (w/w) of FOS were purified to 92.9% (w/w) with a FOS recovery of 74.5% (w/w) and some fractions were obtained with purities up to 97% (w/w) [2].

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References

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