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## Title: One-step fructo-oligosaccharides production and purification

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Fructo-oligosaccharides; co-culture; integrated process; Aspergillus ibericus; Saccharomyces cerevisiae, one-step fermentation

### Background

Growing consumer awareness on high nutritional value ingredients and their impact in health has been raising the interest in functional food including prebiotics. Fructo-oligosaccharides (FOS) are prebiotic sugars that have been industrially produced via fermentation, by several microorganism enzymes, in two-step bioprocess. Due to enzyme activity inhibition by the high amount of glucose released during fermentation, the maximum theoretical yield obtained ranges between 55 to 60% of sucrose converted into FOS, with less than 60% of pure FOS [1]. To obtain high-content FOS we explored the use of a co-culture of *Aspergillus ibericus* MUM 0.349 as FOS producer strain, with *Saccharomyces cerevisiae* YIL162W (a yeast with the gene responsible for sucrose hydrolysis disrupted) as small saccharides removal.

#### Method

The whole-cell microorganisms were used in a one-step bioprocess. Fermentation conditions were optimized through a central composite design (CCD) in shaken-flask. Substrate used was 200 g.L<sup>-1</sup> of sucrose. Parameters such as initial yeast concentration, inoculation time, fermentative broth composition, temperature and pH were optimized. Sugars were analysed by HPLC. Finally, fermentation was scaled-up to a lab bioreactor size.

#### **Results and conclusions**

Fermentations conducted in shaken-flasks using a single-culture of *A. ibericus* yielded 65  $\pm$  5% of FOS with a purity of 60.4  $\pm$  0.4% (w/w). The simultaneous inoculation of the coculture reached the highest purity in FOS. The CCD model showed that the yeast extract concentration and the temperature were the most significant factors affecting FOS purity. Fermentations run under 30°C, initial pH of 6.0 and 17 g.L<sup>-1</sup> yeast extract led to FOS mixtures with 97.4  $\pm$  0.2% (w/w) purity. After model validation, fermentations were carried out in the bioreactor. The scale-up fermentations yielded 64  $\pm$  2% of FOS, after 141 h, with a FOS content of 118  $\pm$  5 g.L<sup>-1</sup> and a purity of 93.0  $\pm$  0.5% (w/w). The one-step fermentation using the specific co-culture studied showed to be more efficient,

economical and fast than the conventional two-step bioprocesses, thus avoiding the need of the conventional downstream treatments.

# References and Acknowledgements

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[1] Nobre, C. et al. (2015) Critical Reviews in Food Science and Nutrition,55(10):1444-1455.