Enzymatic synthesis of lactulose using a novel biocatalyst

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Functional food is defined as 'natural or processed foods that contain known biologically-active compounds which provides a clinically proven and documented health benefit, and thus, an important source in the prevention, management and treatment of chronic diseases of the modern age [1]. Many compounds of animal and plants origin can be used to complement functional foods, but nowadays, the most commonly used are probiotics, prebiotics and antioxidants [2]. Prebiotics are 'substrates that are utilized by host microorganisms conferring a health benefit' [3]. They have been successfully incorporated in a wide variety of food products like breads, baked goods, nutritional bars, meet products, salad dressings, sweeteners and yoghurts [2]. One of the most well-recognized prebiotics is lactulose, a disaccharide derived from lactose. Lactulose is not found naturally so it has to be synthetized through different methods, e.g. chemical or enzymatic synthesis, and electro-activation [4]. Traditional enzymatic synthesis involves the use of β-galactosidases or glycosidases. However, a problem associated with the use these enzymes is an eventual lactulose degradation by the biocatalyst and the simultaneous production of monosaccharides and galacto-oligosaccharides, which compromises the yield and purity of the final product [4]. Recently, the production of lactulose through lactose isomerization catalyzed by cellobiose 2-epimerase was reported [5]. This new strategy is gaining more attention as a preferable methodology for industrial usage due to the noteworthy yields obtained. In this study, we propose a new and promising biocatalyst for lactulose production. The combination of a GRAS producer such as *Saccharomyces cerevisiae* with a production process using only lactose as substrate can be a more economic and attractive approach for the synthesis of lactulose for functional food applications. The biocatalyst was obtained by cloning the cellobiose 2-epimerase gene from *Caldicellulosiruptor saccharolyticus* in the BY4741 *S. cerevisiae* strain. The cellobiose 2-epimerase enzyme was produced in a synthetic media composed by YNB (0,67 g/L), glucose (10 g/L) and amino acids (100 mg/L). After 24h of fermentation, the biomass was disrupted, and the supernatant was used for lactulose production. Following an optimization of several reaction parameters (reaction buffer, pH, time and substrate concentration), the best condition led to a prebiotic yield of 20.8% and a productivity of 8,83 g/Lh^{-1} after 1h of reaction. Based on this result, it is possible to conclude that this approach could be a promising and safe strategy for lactulose production, since the biocatalyst was obtained in only 24h and it was able to reach a good prebiotic yield and productivity, being competitive with the reported ones involving the cellobiose 2-epimerase enzyme produced by *E. coli* that reaches 57% of prebiotic yield after 4h reaction [6]. Additionally, the GRAS status of *S. cerevisiae* confers another great advantage to the process. Furthermore, when comparing the results with the β-galactosidase enzyme, higher yields and productivity were obtained in shorter fermentation and reaction time. A study using β-galactosidase reports the production of lactulose in an enzymatic membrane reactor reaching a maximum yield of 5,47% [7]. Afterwards, the potential of this biocatalyst for the synthesis of lactulose was studied and it was demonstrated that it could be a sustainable and safe approach to produce lactulose suitable for food application.

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