

# Utilization of Acid Whey Lactose Using Commercial and Innovative Biocatalysts for the Production of Galactooligosaccharides

<sup>1</sup>Athanasios Limnaios, <sup>1</sup>Nausika Korialou, <sup>1</sup>Elena Tsika, <sup>2</sup>Anastasia Zerva, <sup>1</sup>Maria Tsevdou, <sup>2</sup>Evangelos Topakas and <sup>1</sup>Petros Taoukis

<sup>1</sup>Laboratory of Food Chemistry and Technology, School of Chemical Engineering, National Technical University of Athens, Greece

<sup>2</sup>Laboratory of Biotechnology, School of Chemical Engineering, National Technical University of Athens, Greece

## Abstract

Greek yoghurt is a dairy product with high nutritional characteristics and quality that has gained significant popularity by consumers worldwide, over the past few years. However, its production is related to certain environmental issues, since during the straining procedure large amounts of acid whey with high Biological Oxygen Demand (BOD) and low pH value are produced, turning its management by waste treatment facilities problematic. As a result, innovative processes for whey valorization have to be explored. In this context, galactooligosaccharides (GOS), i.e. oligomers of lactose with prebiotic properties, could be enzymatically synthesized using commercially available or novel  $\beta$ -galactosidases, utilizing the high lactose content of acid whey. In this research, the production of GOS catalyzed by two commercial (from *Aspergillus oryzae* and *Kluyveromyces lactis*) and one novel, in-house produced (from *Thermothielavioides terrestris*, heterologously expressed in *Pichia pastoris*)  $\beta$ -galactosidases was studied, taking into account several parameters, e.g. lactose concentration, enzyme load, pH value, temperature, and reaction time. The products were analyzed through chromatographic methods. Results showed that the production of GOS is significantly dependent on the origin of the biocatalyst used. Due to the different microbial origin, the three enzymes exhibited different optimal reaction conditions, regarding mainly the pH value and the reaction temperature. For the quantification of the results, maximal GOS yield, expressed as the percentage of total GOS concentration to the initial lactose concentration, was used. Maximum GOS yield of 25.4% was achieved after 9 h using 2.4 U/mL of  $\beta$ -galactosidase from *A. oryzae* at 45 °C and pH 4.5. Similarly, a maximum GOS yield of 37.0% was achieved after 2 h using 0.052 U/mL of  $\beta$ -galactosidase from *K. lactis* at 37 °C and pH 7.0. In both cases, acid whey with initial lactose concentration of 15% w/v was used. When the enzyme from *T. terrestris* was used, a maximum GOS yield of 14.8% was achieved after 5 h at 50 °C and pH 4.5, using acid whey with initial lactose concentration of 10% w/v. Although  $\beta$ -galactosidase from *K. lactis* seems to lead to the

highest GOS yield, it was observed that this enzyme also lead to hydrolysis of the formed oligomers and lactose precipitation, due to the requirement for pH adjustment to the optimum value, resulting in inefficient process control. Therefore, the optimization of lactose oligomerization is a key factor in order to maximize production yields of high nutritional value ingredients, in the framework of circular economy.

**Keywords:** acid whey, galacto-oligosaccharides,  $\beta$ -galactosidase, prebiotics

**Acknowledgments:** This research has been co-funded by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH - CREATE - INNOVATE (project code: T2EDK- 00783).