

### **ELABORATION OF THREE STEP LACTOSE CONVERSION PROCESS TO D-MANNITOL AS NOVEL PREBIOTIC CARBOHYDRATE**

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Lactose intolerance represents a permanent digesting problem for an increasing part of the human population, which is originated from the decreased activity of the intestinal  $\beta$ -galactosidase enzyme. Lactose is widely occurred and main sugar moiety of various dairy products which consumption can cause digesting dysfunctions for patients suffering lactose malabsorption or lactose intolerance. The main objective of the study was to elaborate a three step enzymatic and fermentation procedure to hydrolyze and transformation of lactose as a dairy industry side-products to D-mannitol with prebiotic activity. The first step is a hydrolysis of lactose to D-glucose and D-galactose and it was carried out using  $\beta$ -galactosidase enzyme (*Kluyveromices lactis*). The formed D-glucose was isomerized to D-fructose using glucose isomerase enzyme in the second process. The obtained fructose-glucose mixture was fermented using heterofermentative lactic acid bacteria (*Leuconostoc citreum*, *Leuconostoc pseudomesenteroides*, *Lactobacillus brevis*). Several chromatography methods were developed to monitor the applied enzymatic and fermentation process. High-performance liquid-chromatography methods coupled with evaporative light scattering detection (HPLC-ELSD) was used to follow lactose hydrolysis and glucose isomerization processes. Gas-chromatography with mass spectrometry detection (GC-MS) was applied to study fermentation process resulted D-mannitol formation. Based on the obtained results, an almost complete enzymatic lactose hydrolysis was achieved, while the glucose isomerization efficiency was varied between 30-35%. The D-fructose and D-glucose mixture was transformed to D-mannitol with the application of microbiological strains with 27-48% efficiency.

Keywords: D-mannitol, fermentation, enzymatic efficiency, heterofermentative lactic acid bacteria

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