Abstract

The lactase or -galactosidase (EC 3.2.1.23) is an exclusively intracellular enzyme in Kluyveromyces marxianus ATCC 8554, so that, to obtain it, there are several methods to the extraction. The objective of this work consisted on optimizing the process of extraction of this enzyme, and it can be its used in the dairy industry. The enzyme production was induced while yeast growth in a means with lactose as the one carbon source for 9 hours, where it reached the phase logarithmic end of growth. The physiochemical parameters combination was evaluated: temperatures 30; 37 and 42°C; pH 6.5; 8.5 and 10 and time 5; 10 and 20 hours using the extraction method with toluene to 2% in buffer phosphate 0.1M. The enzymatic activity of the lactase was determined with the use of cromogen substrate the or-nitrophenyl -D-galactopyranoside (ONPG) on the extracts free of cells. The quantity of total proteins was determined by the method of Lowry that allowed the valuation of the specific activity. The results were analyzed using the statistical package Statgraf version 7.0, applying variance trifactorial analysis with a single effect, LSD, Tukey test. The analysis for TEM of the cells of K. marxianus revealed that the toluene produced a permeabilizant effect, liberating the enzyme without rupture of the cellular integrity. It was found that there was a significant reduction in the necessary time to the extraction of the -D galactosidasa of the cells of K. marxianus, being the best combination: 37°C, pH 6.5 and 5 hours, generating in this time the biggest quantity in ONP: 0.4937 µmoles/mL/10 min. and the biggest specific activity 34.2095 U-gal/mg proteins

Keywords

Lactase, Kluyveromyces marxianus, extraction