OPTIMIZATION AND BIOCHEMICAL CHARACTERIZATION OF BETA-FRUCTOFURANOSIDASE PRODUCED BY Aspergillus versicolor

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β-D-fructofuranoside fructohydrolase (FFase - EC 3.2.1.26) is an enzyme that irreversibly hydrolyzes the β,1-4 linkage of disaccharide sucrose to produce glucose and fructose. Microbial FFase is used in calf feed preparation and also for the manufacture of inverted sugars as nutrients for honeybees. The aim of this study was to optimize the β-D-fructofuranosidase production of Aspergillus versicolor using Central Composite Design, purification and biochemistry characterization were also performed. A. versicolor was cultured under static conditions for 12 days at 28 °C in Czapek medium supplemented with 3% (w/v) apple pomace as a carbon source. The extracellular crude extract of A. versicolor was previously precipitated with ammonium sulfate at 75% and applied in chromatography DEAE-Sephadex column. The assays were carried out with 0.2 M sucrose in 50 mM sodium acetate buffer (pH 4.5) at 60ºC for 10 min, and the reducing sugar was determined by the dinitrosalicylic acid. Two activity peaks were obtained from ion exchange chromatography, named β-fructofuranosidase- I and II, with yields of 22.57% and 33.08%, respectively. The partially purified fructofuranosidase I showed maximum activity at pH 6.0, and it was stable in the range of pH 3.0 – 6.0, whereas the optimum temperature for enzyme activity was 55°C and thermal stability for 40-60°C. The FFase-I activity was inhibited with 1mM Hg²⁺ (88.31%) but enhanced with 1mM β-mercaptoethanol (47.81%) and 5mM Fe²⁺ (27.32 %). Km and Vₘₐₓ for sucrose were 26.71 mM, e 56,980 μmol.min⁻¹, respectively. These biochemical characteristics of the β-fructofuranosidase from A. versicolor reveal the potential application of this enzyme in the ethanol production industry, which require high temperature stability and acidic pH.

Keywords: Invertase, filamentous fungi, statistical optimization

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