

Abstract

Application of pectinase in food and beverages urge the scientists to look for its purified form. In order to attain this objective pectinase was produced from Penicillium notatum MAIIB-33, purified and characterized. Biosynthesis of pectinase (15.4±0.03 U/ml/min) was carried out for 120 h under pre-optimized conditions employing solid state fermentation. It was purified using ammonium sulphate precipitation, anion exchange chromatography and SDS-PAGE and 2.6 folds purification with increased specific activity i.e. 41.6U/mg was achieved. Furthermore, molecular weight of purified enzyme was determined as 38 kDa. Optimum temperature and pH value for activity assay of purified pectinase was determined as 30°C and 6, respectively. Kinetic parameters V_{max} and K_m were estimated as 4.51 U/ml/min and 7.4mg/ml, respectively. Among metal ions, mercury inhibited the enzyme activity at all concentrations while iron had no effect on enzyme activity. In the presence of all organic solvents (DEE, toulene, acetone, methanol and ethanol) and inhibitors SDS, BME, Triton X, Tween-80 and L-cysteine), enzyme exhibited reduced activity. For better storage stability, purified enzyme was immobilized by calcium alginate beads and nanoemulsions. Nanoemulsion based pectinase showed better stability at -20°C, 4°C and 25°C compared to alginate beads. Pectinolytic effect of purified pectinase was also observed on clarification of fresh apple juice and complete clarification was obtained after 4 h of incubation.