Production of Chito-Oligosaccharides With Chitinase Immobilized on SOTCN-beads

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Recent advances of glicobiology indicate several new functions until recently unknown to oligosaccharides derived from chitin and chitosan, depending on the composition and molecular weight. The chemical hydrolysis of chitin and chitosan do not provide products with regular molecular weights, and this is a problem that can be resolved by enzymatic hydrolysis. In this work, a chitinase produced by *Thrichoderma asperellum* was immobilized on totally cinnamoylated D-sorbitol (SOTCN) beads and used for chitin hydrolysis. **Methods:** The SOTCN beads were prepared by adding a sorbitol cinnamate solution (15g dm^{-3}) to glass beads (0.6-1mm), at room temperature for 10 min, following by filtration, drying and UV-irradiation for 15 min to produce the cross-linking cinnamate. The immobilizations were tested varying reaction time (30 min-20h) in absence/presence of 0.5 M Na_2SO_4. Immobilized chitinase (SOTCN-chitinase) was analyzed concerning thermal stability (45-60^oC, 1-3 h incubation), repeated use and storage stability. **Results:** Immobilization in presence of salt resulted in production of 104µg of NAG against 46µg NAG without salt after 2h of immobilization. After optimization, 32% of total enzyme offered was immobilized, with 100% of binding efficiency, measured as a relation between protein and enzyme immobilized (initial specific activity = 45.3; final specific activity = 50.3). Free and SOTCN-chitinase presented very similar kinetics (V_{max} reached at 90 min reaction). Thermal stability of both free and SOTCN-chitinase was similar, with losses in activity after 55^oC. Moreover, free and SOTCN-chitinase retained 100% activity after 3h incubation at 55^oC. SOTCN-chitinase was used in a bath-wise reactor during 11 cycles, producing 1,123 mg of NAG without any loss of activity.

Keywords: immobilization; chitinase; SOTCN-chitinase.