TITLE: Covalent immobilization of recombinant GH28 endopolygalacturonase from *Stereum purpureum* on chitosan and glutaraldehyde functionalized ferromagnetic nanoparticles: Characterization of immobilized enzyme

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ABSTRACT: Endopolygalacturonase are a group of enzymes that break down homogalacturonan of wall cell vegetal into small molecules of galacturonic acids. These enzymes can be applied in a variety of industrial processes, especially the juice, textile and biofuel industries. However, the difficulties in enzyme reusing limit the application of native pectinases in industrial process, mainly due to the high cost of enzymes. Enzymes immobilization provide many advantages in relation to the use of free enzymes, including repeated or continuous reuse, easy separation of the product from reaction media, easy recovery of the costly enzyme. This work aimed to evaluate the biochemical and kinetic characteristics of a covalently immobilized recombinant endopolygalacturonase (EndoPG) on chitosan and gluteraldehyde functionalized nanoparticles and the results were compared with those obtained for free enzyme in solution. The immobilization yield was approximately 3% and the specific activity was similar to that observed for free enzyme. In addition, the immobilized EndoPG retained about 50% of the specific activity after 8 cycles of reaction with citrus pectin substrate at 60 °C. Both the free and immobilized EndoPG displayed an optimal enzymatic activity at 60ºC and pH 4.5. Immobilized enzyme hydrolyzed citrus pectin with V_{max} = 5250.60 ± 315.59 U mg^{-1} and K_M = 2.93 ± 0.3 mg mL^{-1}, resulting in a catalytic efficiency (k_{cat}/K_M) of 2178.84 ± 10.2 mL mg^{-1} s^{-1}. These values were similar to that observed for free EndoPG, indicating that the conformational flexibility of pectinase was maintained even after immobilization. Immobilized EndoPG retained about 50% of its initial activity after incubation at 70 °C for 30 min, showing more stable than the free enzyme, which presented about 25% of the initial activity under the same conditions. These results confirmed that covalent immobilization on chitosan and gluteraldehyde functionalized nanoparticles was a good strategy for EndoPG immobilization with good reusability and improved thermostability of the enzyme, characteristics highly appreciated for application in industrial processes.

Keywords: Endopolygalacturonase, Covalent immobilization, ferromagnetic nanoparticles.
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