Parichart Kitsongsermthon 2007: Covalent and Entrapment Immobilization of Protease on Chitosan for Use in Seasoning Sauce Production. Master of Engineering (Chemical Engineering, Major Field: Chemical Engineering, Department of Chemical Engineering. Thesis Advisor: Assistant Professor Nanthiya Hansupalak, Ph.D. 82 pages.

This work studied protease enzyme immobilized on chitosan by two methods; covalent bond and entrapment for use in seasoning sauce production. Particularly, for covalent bond method, effects of glutaraldehyde concentration, activated time and the effects of temperature and pH on immobilized protease were investigated. The activity of immobilized enzyme by covalent bond and entrapment compared with free enzyme and the distribution of enzyme on chitosan surface were confirmed with scanning electron microscopy. The stability against temperature and pH of the highest activity immobilized enzyme was tested. In addition, the immobilized enzyme obtained from optimal conditions was used to study the reusability for seasoning sauce production.

The results obtaind from the experiment showed that the immobilized enzyme on chitosan by covalent bond gave the highest activity of 2,898 unit/g protein and 11,930 µg in protein coupling, when the glutaraldehyde concentration used is 0.1 %w/v and activated for 2 hours 30 minutes at 30 °C then immobilized with protease in phosphate buffer solution that have ionic strength 0.05 M, pH 7 at 40 °C for 3 hours. The immobilized enzyme on chitosan by entrapment at 37 °C gave the highest activity of 2,189 unit/g protein and 8,467 µg in protein coupling. The activity of free protease was also found to be 15,450 unit/g protein. From the study of protease enzyme immobilized by two methods, the immobilized enzyme was stable and active at approximately 50°C and pH 7. The amounts of nitrogen from amino acid obtained from the immobilized enzyme by covalent bond and entrapment methods for use in seasoning sauce production after four cycles are 1,800 and 706 microgram, respectively.

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