Partial purification and characterization of urease from black-eyed pea (Vigna unguiculata ssp unguiculata L.)

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Abstract	Urease is one of the most important enzymes in the industry. The aim of this research was to isolate and to partial-purity urease from black-eyed pea including urease characterization. Research begins by germinating the black-eyed pea. Germinated black-eyed peas were extracted using phosphate buffer pH 7 and separated by centrifugation to obtain a crude extract of urease. The crude extract of urease was further fractionated using acetone at concentrations of 20, 40, 60, and 80%. The fraction which has highest specific activity then determined by molecular weight using SDS PAGE method and characterized including the influence of temperature, pH, substrate concentration, and metal addition to urease activity. The urease activity is determined by the Nessler method. The specific activity increased during the fractionation phase and specific activity is obtained amount 428.59 mU/mg with a purity level of 2.2 times in FA 80. The results of electrophoresis analysis showed that FA 80 estimated to have four polypeptides with a molecular weight of about 15, 17, 35 and 55 kDa. The result of characterization was obtained the optimum FA 80 urease activity at temperature 30 degrees C, pH 7, substrate concentration 0.125% (w/v) with K-M value 17.8 mM. Urease FA 80 from black-eyed pea was classified as a metaloenzyme. The addition of CaCl2, NaCl, NiCl2 and CuCl2 metals at various concentrations decreased the urea activity of FA 80. The higher metal concentration was added then the FA 80 urease activity decreased further.
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