Isolasi dan purifikasi fosfatase asam dari air kelapa (cocos nucifera linn)

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Abstrak

Fosfatase asam merupakan enzim yang dapat digunakan untuk teknik ELISA - untuk mengukur kadar suatu zat dalam cairan tubuh yang jumlahnya sangat kecil. Salah satu sumber enzim tersebut adalah air kelapa (Cocos nucifera Linn). Penelitian yang dilakukan oleh Sadavisan (1951), Wilson et al. (1952), Anna Istiqomah (1994) dan Emilia Aisjah (1996) menunjukkan adanya aktivitas enzim fosfatase dalam daging buah dan air kelapa.

Penelitian ini adalah mengisolasi dan pemurnian enzim fosfatase asam dari air kelapa. Untuk pemurnian enzim digunakan kromatografi gel dengan Sephadex G-75 dan kromatografi afinitas dengan Gel immobilized p-aminobenzyl phosphonic acid sepharose. Kemurnian enzim diperiksa dengan elektroforesis gel poliakrilamid. Aktivitas enzim secara kuantitatif ditentukan dengan spektrofotometer, dan secara kualitatif dapat dilihat dengan pewarnaan substrat. Kadar protein diukur dengan spektrofotometer pada panjang gelombang 280 nm.

Hasil.penelitian ini menunjukkan bahwa dalam air kelapa terdapat enzim fosfatase asam yang ditunjukan oleh satu pita pada elektroforesis gel poliakrilamid.
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Isolation And Purification Of Acid Phosphatase From Coconut Water (Cocos nucifera Linn)Acid phosphatase was detected in coconut. More than fourty years ago, early investigators found this enzyme solely in coconut milk. It is not until recently that the enzyme was also found in coconut water. Recent studies also reported some characteristics of the enzyme from both sources.

The purpose of this study is to isolate and to purity acid phosphatase from coconut water. Firstly, the enzyme was precipitated with etanol. After the dialysis in a cellophane bag, the precipitate was redissolved in 0,9% NaCl. The enzyme was separated with Sephadex 0-75, using 0,9% NaCl as eluent. Fractions of 5 mL were collected and each was analysed for the protein contents and for acid phosphatase activities. The fractions of high enzymes activity were pooled and purified, using an affinity chromatography technique with benzoyl phosphonic acid, a competitive inhibition, as a stationary immobilized ligand. Retained enzymes were eluted with 0,2 M NaHHPO4 and fractions of 2 mL were collected.

During the study, protein contents were measured with A 280 technique and enzymes activities were assayed with p-nitrophenyl phosphate (p-NPP) as a substrate. In both steps, the purity of the pooled fractions were analysed with a polyacrylamide gel electrophoresis, stained with Coomassie Blue as well as p-NPP-ammonium sulfide, lead acetat for revealing the enzyme.It is found that acid phosphatase could be isolated and purified, as indicated by increasing specific activity and by PAGE analysis, from coconut water, by gel

filtration and affinity chromatography technique.