عنوان مقاله:

Extraction, Purification and Characterization of Lipoxygenase from Aspergillus niger

محل انتشار:

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نویسندگان:

AL-Hashimi alaa-gazi - *Dept. of Food Science, College of Agriculture, University of Basra, Basrah, Iraq*

al-asady amal kadhim - *Dept. of Food Science, College of Agriculture, University of Basra, Basrah, Iraq*

al-temime noora taha - *Dept. of Food Science, College of Agriculture, University of Basra, Basrah, Iraq*

خلاصه مقاله:

Lipoxygenase was extracted from a local isolate of Aspergillus niger that was obtained from maize. The isolate was grown and biomass was harvested after 5days of incubation at 30°C for detecting enzymatic activity. Three steps were used to purify lipoxygenase: concentration by using ammonium sulfate with (30-90%) saturation , then ion exchange chromatography by using DEAE Sephadex A-50 with NaCl (0-1)N. Two peaks appeared at the washing step and four peaks appeared at the elution step, Only one peak had specific activity ,which was 592.14 u/mg, That peak was chosen for the third step of purification, using gel filtration chromatography with Sephadex G-100. Only one peak showed specific activity (1069.7u/mg). The purity of enzyme was tested by gel electrophoresis using polyacrylamide, The enzyme was show to be purified by appearance as band. The characteristics of purified lipoxygenase were then studied: The molecular weight was104 KDa as shown by SDS- polyacrylamide gel electrophoresis, The optimum temperature and pH of enzyme activity were 35 Ĉand 6.5 respectively. Heat stability and optimum pH of stability were (0-45) Ĉ and (7-6) respectively. The effect of some mineral ions on enzymatic activity, had been noticed that CaCl2, KCl, NiCl2 , MgCl2 and MnCl2 increased the activity while the enzymatic activity was decreased when using ZnCl2,FeCl3 , and CuCl2. EDTA inhibited the enzyme activity. Studying of kinetic constants showed that Michaelis constant (Km) and maximum velocity (Vmax) of the enzyme were 0.2 mg/mLand 45 µm/mL/min respectively. The .activation energy was 8 kcal/mole, and the denaturation energy was 54 kcal/mole

کلمات کلیدی:

Lipoxygenase, Extraction, Purification, Characterization, Activity, Stability