Purification and characterization of Catechol oxidase from Tadela (*Phoenix dactylifera L*) date fruit

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Abstract

Catechol oxidase (PPO) was extracted and purified from Tadela (*Phoenix dactylifera* L.) date fruit ,by a procedure that included $(NH_4)_2SO_4$ precipitation followed by dialysis, Q-Sepharose bb ion-exchange chromatography and HPLC gel filtration chromatography .Some of its biochemical characteristics were studied. The purification rate and the yield were 80% and 20%, respectively. The Tadela date fruit catechol oxidase exhibited a molecular weight of 90 kDa using SDS-PAGE.The catechol oxidase showed only o-diphenolase and triphenolase activities while no monophenolase activity was detected. A better affinity was observed using catechol as substrate (Km = 35 mM) with thus, a higher Vmax /Km ratio (80U/mM.mL).This enzyme is thermostable in the temperature range (30-60 ° C) with optimum activity in acidic range of pH. Four inhibitors were used for the control of enzymatic browning, of which sodium metabisulfite was the most potent (IC_{50}= 0,11mM). The values of K_I and mechanism of inhibition were also determined. No significant change on enzyme activity was noticed in the presence of metal ion and detergents. Therefore, thermal inactivation was studied in the temperature range between 60 and 80 °C using catechol as substrate. Their kinetic (K, D.t1/2 ,Zt,Ea) and thermodynamic (ΔH, ΔG and ΔS) parameters were also estimated.

Keywords: Tadela catechol oxidase; purification and biochemical characterization; enzymatic browning.