



## **Extraction, Purification and Characterization of $\beta$ -Galactosidase from Apricot (*Prunusarmeniacakaisa*) Fruit for lactose intolerance treatment**

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**Abstract:**  $\beta$ -Galactosidase ( $\beta$ -D-galactoside-galactohydrolase, EC 3.2.1.23) was extracted by eight solutions from apricot fruit (*Prunusarmeniacakaisa*), purified in several protein purification steps and characterized biochemically. It was found that 10% sodium chloride pH 5 was the best solution for extraction the enzyme and that 20-60% ammonium sulfate saturation was the best method for partially purification of enzyme with a purification fold 4.31 and enzymatic recovery 61.6%. After that, it was purified by ion-exchange chromatography column using DEAE-Cellulose (Diethyl amino ethyl Cellulose) and gel filtration column with Sephadex G-100 as a final purification step with 2000U/mg proteins specific activity, 4.5 purification fold and 40.7% enzymatic yield. The molecular weight of the enzyme was estimated to 165.95KDa by gel filtration using SephadexG-100 column and the isoelectric point for enzyme was 4.4. Carbohydrate concentration of enzyme was found to be 25.9% by employing phenol-sulfuric acid method. Optimum enzymatic activity was found at 50°C and pH 5 and its activity was stable at 25-55 °C and pH 5-6 for 15 min. Activation energy ( $E_a$ ) was estimated by Arrhenius equation to be 7.56kcal/ mol, while deactivation energy was found to be 48.46kcal/mol. The existence of metal ions like  $\text{Na}^+$ ,  $\text{Mg}^{+2}$  and  $\text{Mn}^{+2}$  at 0.1 and 1.0mM concentrations had an positively effect on enzyme activity, on the other hand, enzyme activity was inhibited when incubated with  $\text{Cd}^{+2}$ ,  $\text{Ca}^{+2}$ ,  $\text{Fe}^{+2}$ ,  $\text{Pb}^{+2}$  and  $\text{Hg}^{+2}$ . The average values of Michalis-Menten constant ( $K_m$ ), maximum velocity ( $V_{max}$ ), as well as, catalytic constant ( $K_{cat}$ ) were evaluated to be 1.43mg/ml, 1.36U/min and  $13.6\text{min}^{-1}$ , respectively using ONPG as substrate. In addition to the ability of purified enzyme to hydrolyze 24.5% of lactose after one hour and about 89.6% after four hours.

**Key Words:**  $\beta$ -Galactosidase, Apricot, Lactose Intolerance, Purification.