

## **Purification and characterization of membrane-bound polyphenoloxidase (mPPO) from Snake fruit [*Salacca zalacca* (Gaertn.) Voss].**

### **ABSTRACT**

Membrane-bound polyphenoloxidase (mPPO) an oxidative enzyme which is responsible for the undesirable browning reaction in Snake fruit (*Salacca zalacca* (Gaertn.) Voss) was investigated. The enzyme was extracted using a non-ionic detergent (Triton X-114), followed by temperature-induced phase partitioning technique which resulted in two separate layers (detergent-poor phase at the upper layer and detergent-rich phase at the lower layer). The upper detergent-poor phase extract was subsequently fractionated by 40–80% ammonium sulfate and chromatographed on HiTrap Phenyl Sepharose and Superdex 200 HR 10/30. The mPPO was purified to 14.1 folds with a recovery of 12.35%. A single prominent protein band appeared on native-PAGE and SDS-PAGE implying that the mPPO is a monomeric protein with estimated molecular weight of 38 kDa. Characterization study showed that mPPO from Snake fruit was optimally active at pH 6.5, temperature 30 °C and active towards diphenols as substrates. The  $K_m$  and  $V_{max}$  values were calculated to be 5.46 mM and 0.98 U/ml/min, respectively, when catechol was used as substrate. Among the chemical inhibitors tested, l-cysteine showed the best inhibitory effect, with an  $IC_{50}$  of  $1.3 \pm 0.002$  mM followed by ascorbic acid ( $1.5 \pm 0.06$  mM), glutathione ( $1.5 \pm 0.07$  mM), EDTA ( $100 \pm 0.02$  mM) and citric acid ( $186 \pm 0.16$  mM).

**Keyword:** Snake fruit (*Salacca zalacca*); Membrane-bound polyphenoloxidase; Extraction; Purification; Characterization.