

**A novel aqueous two phase system composed of a thermo-separating polymer and an organic solvent for purification of thermo-acidic amylase enzyme from red pitaya (*Hylocereus polyrhizus*) peel**

ABSTRACT

The purification of thermo-acidic amylase enzyme from red pitaya (*Hylocereus polyrhizus*) peel for the first time was investigated using a novel aqueous two-phase system (ATPS) consisting of a thermo-separating copolymer and an organic solvent. The effectiveness of different parameters such as molecular weight of the thermo-separating ethylene oxide-propylene oxide (EOPO) copolymer and type and concentration of organic solvent on the partitioning behavior of amylase was investigated. In addition, the effects of phase components, volume ratio (VR), pH and crude load of purification factor and yield of amylase were evaluated to achieve the optimum partition conditions of the enzyme. In the novel ATPS method, the enzyme was satisfactorily partitioned into the polymer-rich top phase in the system composed of 30% (w/w) EOPO 2500 and 15% (w/w) 2-propanol, at a volume ratio of 1.94 and with a crude load scale of 25% (w/w) at pH 5.0. Recovery and recycling of components was also measured in each successive step of the ATPS process. The enzyme was successfully recovered by the method with a high purification factor of 14.3 and yield of 96.6% and copolymer was also recovered and recycled at a rate above 97%, making the method was more economical than the traditional ATPS method.

**Keyword:** Novel aqueous two phase system; Thermo-separating copolymer; Organic solvent; Thermo-acidic amylase; Recycling of phase components; Partition coefficient; Purification fold; Yield