

Optimization of conditions for the single step IMAC purification of miraculin from *Synsepalum dulcificum*

ABSTRACT

In this study, the methods for extraction and purification of miraculin from *Synsepalum dulcificum* were investigated. For extraction, the effect of different extraction buffers (phosphate buffer saline, Tris-HCl and NaCl) on the extraction efficiency of total protein was evaluated. Immobilized metal ion affinity chromatography (IMAC) with nickel-NTA was used for the purification of the extracted protein, where the influence of binding buffer pH, crude extract pH and imidazole concentration in elution buffer upon the purification performance was explored. The total amount of protein extracted from miracle fruit was found to be 4 times higher using 0.5M NaCl as compared to Tris-HCl and phosphate buffer saline. On the other hand, the use of Tris-HCl as binding buffer gave higher purification performance than sodium phosphate and citrate-phosphate buffers in IMAC system. The optimum purification condition of miraculin using IMAC was achieved with crude extract at pH 7, Tris-HCl binding buffer at pH 7 and the use of 300 mM imidazole as elution buffer, which gave the overall yield of 80.3% and purity of 97.5%. IMAC with nickel-NTA was successfully used as a single step process for the purification of miraculin from crude extract of *S. dulcificum*.

Keyword: *Synsepalum dulcificum*; Miraculin; Nickel-NTA; Imidazole; Purification