**Comparison and Evaluation of Recombinant Brazzein Protein Production With and Without GST Fusion Tag**

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In the past, compounds such as proteins were considered to be tasteless and the idea that such macromolecules could have a taste or particular flavor activities was unexpected but the discovery of the miraculin with the nature of the protein and as a flavor modifying glycoprotein was ended these ideas. Following Miraculin, other types of proteins including Brazzein were identified with intrinsic sweets. The present study was carried out with aim to evaluate and compare the production of Brazzein protein with and without the GST fusion tag by expression vectors pET41a and pET28a in *E.coli* bacteria. The sequence of the gene encoding Brazzein was synthesized and cloned into pET41a and pET28a. the resulted constructs were transformed to expression host E.coli, strain Rosseta (DE3). A significant amount of GST (as a control), GST-His-bra and His-T7-bra proteins was generated with induction of expression of the protein by IPTG. Due to the presence of this GST-tag and the sensitivity of this protein to the presence of a special N-terminal sequence, GST-His-bra protein was digested by protease enterokinase. Success in digestion, separating GST-tag and Brazzein protein size was confirmed by the Tricine-SDS-PAGE gel. Heterologous production of Brazzein with GST fusion tag was significantly more than Brazzein Without this tag but the digestion process and two-steps purification of recombinant GST-His-bra protein, the amount and final concentration of Brazzein was greatly reduced.

Key words: Brazzein, sweet protein, GST fusion tag, enterokinase enzyme