

## **Enhanced physicochemical stability and efficacy of angiotensin I-converting enzyme (ACE) - inhibitory biopeptides by chitosan nanoparticles optimized using Box-Behnken design**

### **ABSTRACT**

Bromelain-generated biopeptides from stone fish protein exhibit strong inhibitory effect against ACE and can potentially serve as designer food (DF) with blood pressure lowering effect. Contextually, the DF refer to the biopeptides specifically produced to act as ACE-inhibitors other than their primary role in nutrition and can be used in the management of hypertension. However, the biopeptides are unstable under gastrointestinal tract (GIT) digestion and need to be stabilized for effective oral administration. In the present study, the stone fish biopeptides (SBs) were stabilized by their encapsulation in sodium tripolyphosphate (TPP) cross-linked chitosan nanoparticles produced by ionotropic gelation method. The nanoparticles formulation was then optimized via Box-Behnken experimental design to achieve smaller particle size (162.70nm) and high encapsulation efficiency (75.36%) under the optimum condition of SBs:Chitosan mass ratio (0.35), homogenization speed (8000rpm) and homogenization time (30min). The SBs-loaded nanoparticles were characterized for morphology by transmission electron microscopy (TEM), physicochemical stability and efficacy. The nanoparticles were then lyophilized and analyzed using Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD). The results obtained indicated a sustained in vitro release and enhanced physicochemical stability of the SBs-loaded nanoparticles with smaller particle size and high encapsulation efficiency following long period of storage. Moreover, the efficacy study revealed improved inhibitory effect of the encapsulated SBs against ACE following simulated GIT digestion.

**Keyword:** Physicochemical stability; ACE; Biopeptides; Box-Behnken design