

SELECTIVE ISOLATION OF TRYPSIN INHIBITOR OF SOYBEAN WHEY BY CHITOSAN

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Chitosan is a high molecular weight polysaccharide linked by β -1, 4 glucoside. It is composed by *N*-acetyl-glucosamine and glucosamine.[1] In acidic solution, amine groups of chitosan are protonated resulting in a positively charged polymer providing chitosan with novel adsorption properties utilized in many applications. These properties include reaction with polyanions by electrostatic interactions between $-\text{COO}^-$ or $-\text{SO}_3^-$ and $-\text{NH}_3^+$ leading to the formation of polymeric complexes [2,3]. These reactions can be used to facilitate the sedimentation of suspended solids [4]. The purpose of this research is to isolate trypsin inhibitor selectively with different type of chitosan from soybean whey.

β -Chitin was prepared by acidic and alkaline treatment from squid pens. Chitosan was prepared by hot alkaline deacetylation from β -chitin and the degree of deacetylation and average molecular weight were 97% and 197 kDa respectively.

The chitosan (in acetic acid solution, powder, and bead) added to soybean whey protein solutions, subsequently adjust pH of the protein solutions in the pH 4.0-6.5, then positive chitosan molecule formed complex with negative trypsin inhibitor protein by electrostatic interaction, and the complex was centrifuged and collected (Fig. 1). The adsorbed trypsin inhibitor was de-adsorbed from the complex in solution of pH 9.0, subsequently the dissolved chitosan flocculated and precipitated due to deprotonation of the amino group.

The trypsin inhibitor suspension was analyzed by HPLC. We got the great separation by modified mobile phase B (0 min-5%, 8 min-16%, 12.5 min-20%, 13.5 min-24%, 17.5 min-40%, 22.5 min-45%, 26.5 min-50% 27 min-95%) (Fig. 2).

At the capability of selectively isolated trypsin inhibitor, chitosan bead was better than chitosan powder. Around 35-49% of soybean trypsin inhibitor was selectively isolated from soybean whey protein solution by chitosan beads in the pH 4.0-5.5.

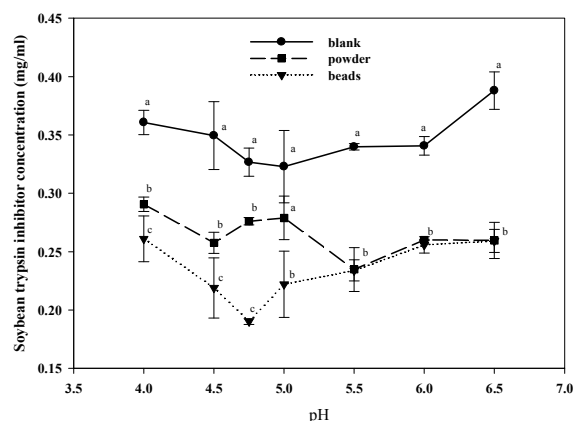


Fig. 1. Effect of solution pH on the concentration of soybean trypsin inhibitor (mg/ml) treated with chitosan in the supernatant. Vertical bars represent standard deviation values (n=3). Different superscripts (a-c). for each pH are significantly different ($p < 0.05$).

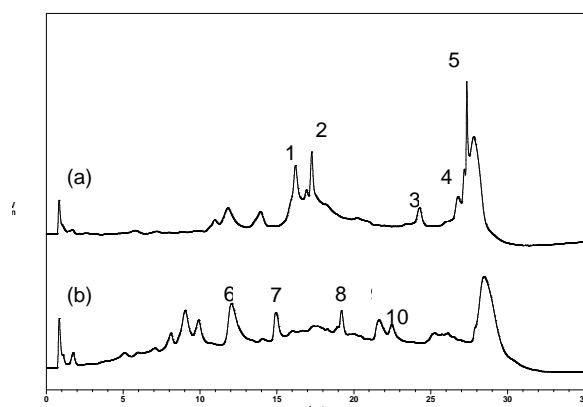


Fig. 2. Reverse-phase HPLC chromatograms of soybean whey protein. (a) mobile phase B: refer to Castro-Rubio et al., 2007 [5]; (b) modified mobile phase B. (1-3 and 6-8: soybean trypsin inhibitor, STI; 4-5 and 9-10: Lectin).

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