

Thesis title Trypsin inhibitors from three varieties of legumes:
characterization and proteolytic inhibition in fish muscle system

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Degree Master of Science (Food Technology)

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ABSTRACT

Trypsin inhibitor was isolated from navy bean (*Phaseolus vulgaris*), red kidney bean (*Phaseolus vulgaris L*) and adzuki bean (*Vigna angularis*) from the Royal Project Foundation, Thailand. Extraction of navy bean and red kidney bean with 0.02 M NaOH showed the highest recovery of trypsin inhibitor while water was the best extractant for adzuki bean. The extraction time significantly affected the recovery of trypsin inhibitor ($p < 0.05$). Two hours of extraction provided the best yield of trypsin inhibitor for three types of legumes. Fractionation of trypsin inhibitor was conducted by using heat treatment (70°C for 10 min), ammonium sulfate (AS) precipitation (60-80% saturation) and three phase partitioning (TPP) methods. The AS precipitation with 60-80% saturation resulted in 41, 89 and 34-fold of the purity and 74, 349 and 53% of inhibitor recovery for navy bean, red kidney bean and adzuki bean, respectively. TPP provided the purity of 5, 14, and 7-fold with 315, 441, and 228% recovery for navy bean, red kidney and adzuki bean, respectively. The SDS-PAGE revealed the major inhibitor band of navy bean with the molecular weight (MW) of 132 kDa. Meanwhile, the protein bands with the MW of 118 and 13 kDa were observed in red kidney bean and adzuki bean. The fractions from navy bean and adzuki bean showed higher pH stability compared to that of the red kidney bean and have the

optimum pH range of 7 to 9. The highest relative inhibitory activity of the fractions of navy bean and red kidney was found at 50°C and all fractions were relatively stable at 90°C for 60 min.

Increasing the concentration of salt (up to 3% w/v) did not significantly decrease the inhibitory activity of all fractions ($p>0.05$). The trypsin inhibitor fractions were capable to inhibit proteolysis of Nile tilapia. However, the autolysis of Pacific whiting and arrowtooth flounder was not inhibited by trypsin inhibitor fractions.

Keywords: adzuki bean / legume / navy bean / proteolytic / red kidney bean / three phase partitioning / trypsin inhibitor

