A STUDY OF TRYPSIN INHIBITORS AND PHYTOHAEMAGGLUTININS IN WINGED BEAN SEED

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ABSTRACT

A STUDY OF TRYPSININHIBITORS AND HAEMAGGLUTININS IN WINGED BEAN SEEDS

Two anti-nutritional factors, trypsin inhibitors and haemagglutinins present in the winged bean, Psophocarpus tetragonolobus were studied with respect to concentration and purification.

Optimum conditions for the assay of trypsin were established using the non-specific substrate casein and specific substrate Bensoyl-DL-arginine-p-nitroanilide (BAPA).

The trypsin inhibitor and haemagglutinin activities present in the winged bean seed flour extracted in phosphate buffered saline (0.1M, pH 7.6) were 20.4 mg and 0.42×10^5 per gram seed flour respectively.

Stepwise fractional purificaton of the extract by trichloroacetic acid (at 1, 2 and 3 percent concentration) revealed that,

i. 1% TCA solution (pH 5.0) removed the nuclear proteins and particulate materials; the clear supernate retained 94% of trypsin inhibitor and 96% of haemagglutinin activities of the crude extract.

ii. 13% of trypsin inhibitor and 47% of haemagglutinin activities were lost during 2% acid precipitation.

iii. 48% of trypsin inhibitor and 82% of haemagglutinin activities were lost during 3% acid precipitation.

When the supernate obtained from 1% TCA extraction was subjected to $MgSO_4.7H_2O$ precipitation at 50% level, 93 percent of trypsin inhibitors and 96 percent of the haemagglutinins were present in the precipitate. Trypsin inhibitors and haemagglutinins were purified to 2.0 and 2.1 fold respectively at this stage.

Heat treatment studies on the residue (50% $MgSO_4.7H_2O$ precipitate) solubilised in phosphate buffered saline showed that,

i) both the antinutritional factors were denatured at 80°C, within 5 minutes, and

ii) at 70°C, trypsin inhibitor and haemagglutinin proteins were purified to 4.9 and 3.1 folds respectively.

DEAE cellulose column chromatography of the heat treated (70°C) supernate resulted in 8 and 5 fold purification of the trypsin inhibitors and haemagglutinins respectively. The proteins consisting of 536 mg trypsin inhibitor and 7×10^5 haemagglutinating units per gram of proteins were eluted in one fraction at 0.08M, phosphate buffer, pH 7.6. The column fractionation of the crude extract showed four fractions (at 0.01, 0.02, 0.04 and 0.08 molarities) containing trypsin inhibitor activity and five fractions (at 0.01, 0.02, 0.04, 0.08 and 0.1 molarities) containing haemagglutinating activities.

It is concluded from this study that there are more than one trypsin inhibitor and haemagglutinin proteins present in winged bean.