CHAPTEH 6

CONCLUSIONS

1. Method of Grant *et al*. (1995) without heating was used for extracting α-amylase inhibitor from the pericarp of *P. spesiosa* in this study. Detail of methodology comprises of 1) stirring fine powder of fresh pods in 0.02 M sodium phosphate buffer pH6.9 containing 0.15 M NaCl at 4°C for 16 hours 2) removing unwanted precipitate and collecting the crude extract containing α-amylase inhibitory activity.

2. Amylase inhibitor was partial purified by precipitation with 95 % methanol and the 95%MeOH supernatant (Aq 95%MeOH) was further purified by Sephadex G-75 column eluted with 0.02 M phosphate buffer pH 6.9 containing 0.01 M NaCl. Fractions with α-amylase inhibitory activity were pooled, concentrated, redissolved with distilled water to a desired volume and used in further studies.

3. The optimum temperature for the inhibition of α-amylase by α-amylase inhibitor in the from of crude extract or Sephadex G-75 fraction were in the range of 4-37°C and gradually decreased from 40°C and ceased at 90°C.

4. The inhibitory activity of the inhibitor either in the form of crude extract or Sephadex G-75 fraction was stable after being kept at the temperature range from 4 to 60 °C for 30 min but its activity was gradually decreased at 50-60 °C and further dropped seriously at 80-90 °C.

5. The inhibitor has inhibitory activity against α-amylase at optimum pH 7.

6. The inhibitor was quite stable at pH 5-7 and sharply decreased it activity against α-amylase at pH 8-9.

7. Addition of salts *i.e.* NaCl, KCl, $CaCl_2$ or $MgSO_4$ did not affect on inhibitory activity of the inhibitor against α-amylases.

8. Kinetic inhibition of the inhibitors on human salivary α-amylase is a mixed noncompetitive type. K_i and K_i' values (K_i 29.29 mg/ml; K_i' 66.36 mg/ml for Sephadex G-75 fraction and K_i 0.24 mg/ml ; K_i' 0.51 mg/ml for crude extract) revealed that the inhibitor tend to bind free enzyme more than enzyme-substrate complex.

9. Kinetic inhibition of the inhibitors on yeast maltase and yeast sucrase is a mixed noncompetitive type. K_i and K_i' values of yeast maltase (K_i 69.35mg/ml ; K_i' 47.50 mg/ml for Sephadex G-75 fraction and K_i 0.54mg/ml ; K_i' 0.46 mg/ml for crude extract) and sucrase (K_i 269.94 mg/ml ; K_i' 124.62 mg/ml for Sephadex G-75 fraction and K_i 21.78 mg/ml ; K_i' 4.21mg/ml for crude extract) revealed that the inhibitor tend to bind enzyme-substrate complex better than free enzyme.

10. The inhibitor has the power in inhibiting the activity of on human salivary α-amylase, yeast maltase, porcine pancreatic α-amylase, porcine intestinal maltase and yeast sucrase from high to low, respectively.

11. The inhibitors could inhibit amylase of *S. orysea* and *C. chinensis* but did not inhibit *C.maculatus*. The inhibitor showed no effect on the growth of *C. chinensis* and *C. maculatus* when mixed its in artificial beans or coated on skin of mung been seeds.

12. Identification of Sephadex G-75 fraction by Folin reagent, IR absorption spectra and TLC of its acid hydrolysate suggested that a nonproteinaceous amylase inhibitor in the fraction contains a structure of phenolic compound with a carboxylic acid and hydroxyl functional groups in its molecule.