

CHAPTER 5

CONCLUSION

1. The proteinaceous amylase inhibitor from hydroxyapatite column was partially purified by native-PAGE verification. A single protein band with MW 36.3 kDa by native-PAGE and 47.7 kDa by Sephadex G-100 filtration. The inhibitors have 3 subunits of 15.5, 17 and 18.2 kDa by SDS-PAGE.

2. The nonproteinaceous amylase inhibitor have $R_f = 0.581$, which was between the R_f of standard glucose (G1) and maltose (G2) of molecular weight 180 Da and 360.3Da, respectively.

3. The optimum temperature for the inhibition of human salivary α -amylase by the nonproteinaceous inhibitor was in the range of 40-50 °C. The inhibitory activity was decreased at 50 °C and ceased at 80 °C due to the deterioration of α -amylase.

4. The nonproteinaceous amylase inhibitor was stable after being kept at the temperature range from 4-50 °C for 30 min but its ability in inhibiting salivary α -amylase was dropped seriously above 50 °C.

5. The nonproteinaceous amylase inhibitor has inhibitory against human salivary α -amylase at optimum pH 7.0.

6. The nonproteinaceous amylase inhibitor was stable at pH 6.0 and sharply decreased its inhibitory activity against human salivary α -amylase at pH 6.9-9.0.

7. Addition of salts either NaCl, KCl, CaCl₂ or MgSO₄, enhanced the inhibitory activity of the inhibitor as following: MgSO₄ > CaCl₂ > NaCl = KCl > No salt.

8. Kinetic inhibition of the nonproteinaceous amylase inhibitor on human salivary α -amylase is a mixed noncompetitive type with K_i and K_i' values (K_i

1.571 mg/ml; K_i' 36.968 mg/ml). These results revealed that the inhibitor tend to bind enzyme-substrate complex better than free enzyme (K_m 1.056 mg/ml).

9. Kinetic inhibition of acarbose human salivary is a mixed noncompetitive type K_i and K_i' values (K_i 0.578 mg/ml; K_i' 3.133 mg/ml). This revealed that the inhibitor tend to bind enzyme-substrate complex better than free enzyme (K_m 1.007 mg/ml).

10. *In vitro* study showed that the crude extract had a power in inhibiting the activity of on human salivary α -salivary > porcine pancreatic α -amylase > yeast maltase but not inhibited yeast sucrase.

The porteinaceous amylase inhibitor had a power in inhibiting the activity of on human α -salivary more than porcine pancreatic α -amylase, but not inhibited yeast maltase and sucrase.

The nonproteinaceous amylase inhibitor has the power in inhibiting the activity of on porcine pancreatic α -amylase more than human α -salivary, but not inhibited yeast maltase and sucrase.

11. *In vivo* study showed that the fed substances *i.e.* nonproteinaceous α -amylase inhibitor, crude extract, crude extract at IC_{50} , and nonproteinaceous α -amylase inhibitor at IC_{50} could significantly ($p < 0.05$) reduce blood glucose level when compared with the control group after feeding to the rats for 15 days. However, the proteinaceous inhibitor solution and its dilution at IC_{50} did not show any effect on the reduction of blood glucose level during the feeding time.

Result indicated of this study also that the test substances did not affect the growth of the rats in consideration to the normal increasing of their weight along the 15 days of treatment.

12. Result of maltose, sucrose and starch tolerance tests did not support the effect of amylase inhibitor in slowing starch digestion in the rat intestinal lumen although *in vitro* study gave a positive result of the inhibitor samples in inhibiting the activities of salivary and pancreatic α -amylase. This study a fixed dose of the fed inhibitor samples were used. A positive expected result may be obtained if the fed inhibitor samples are varied corresponded to the rat weight.