CHAPTER VI
CONCLUSION

The aim of this work is to purify and characterize amylase in Mon thong durian. Firstly, Iodine test and DNS method were used to estimate ripe stage of such sample. The results showed 1.1% starch of durian pulp and 0.17% reducing sugar of fresh weight in crude durian. Protein concentration in crude durian extracted was measured by Bradford method and showed 0.97 mg protein/ g fresh weight. After SDS-PAGE analysis, the protein pattern or crude durian with molecular weight between 28-97 kDa was observed, showing major bands around 38-55 kDa in range. The zymogram of durian amylase showed clear transparent amylase band at 45 kDa with nonreducing condition. Therefore, 2D-PAGE and LC-MS/MS strategies were used to obtain partial amino acid sequence of amylases. The total of 40 excised spots were picked and analyzed by LC-MS/MS, 27 spots were identified and showed high homology with known proteins. Then, they were organized into 9 groups, concerning carbohydrate, protein, lipid and secondary metabolism. Some are predicted to be protein in folding process, ripening process, antioxidant enzyme, cell wall hydrolysis and other (not identified). Moreover, we found interesting protein such as glutathione reductase, isoflavone reductase and α-amylase. Especially, α-amylase in crude durian was identified with molecular weight of 45 kDa and pI 6.51. The partial amino acid sequence was "IATVLPDK". In purification procedures, crude durian was precipitated with 70% (w/v) ammonium sulphate in the first step and showed the highest specific activity (1.39 Unit/mg). And then the precipitated protein was applied into Epoxy-activated Sepharose 6B affinity column in the second step. The fractionated amylase activity was eluted with 10 mg/ml β-cyclodextrin solution. The result showed that the durian amylase was purified more than 313.04 folds with specific activity of 216.00 Unit/mg. Finally, the fractions containing amylase activity were pooled, concentrated and loaded into DEAE Toyopearl Anion Exchange column. Proteins which display high amylase activity were eluted at 0.16 M NaCl. The result of final purification step showed

340.36 purification folds and 234.85 U/mg of protein of specific activity with 0.06% recovery. In order to characterize durian amylase, the optimum pH, optimum temperature, pH stability, heat stability, effect of metal ions and EDTA were performed using DNS method and Zymogaphic method. Crude durian showed maximum activity at pH 7.0 and 40 °C. Contrary for partial purified amylase showed maximal activity at pH 7.0 and showed highly active at widely temperature from 30 to 70 °C. Interestingly, the activity of durian amylase still remained activity at 90 and 100 °C. Thus, such enzyme may be as thermostable enzyme. Moreover, The enzyme was stable and highly active over a wide range of pH from 6 to 10 and over a wide range of temperature from 30 °C to 60 °C. The metal ion (Ca^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+}, Na^{+} and K^{+} ions) did not effect on amylase activity. However, amylase activity was almost completely abolished by 5 mM EDTA indicating that amylase of Mon thong durian is an α-type metalloenzyme.