CHAPTER V

CONCLUSIONS

1. Both isoamylase and pullulanase activities of DBE were found in parenchyma of cassava tuber cv. KU50. During development of tubers, both activities increased to the highest level in 9 months old tubers.

2. Crude DBE from parenchyma of 9 months old tuber were partially purified by 60% ammonium sulfate precipitation, DEAE-Sepharose and Sephacryl S-200 columns.

3. Isoamylase activity was eluted from DEAE-Sepharose column at 0.1 M NaCl. Final purification by Sephacryl S-200 column yielded 14.6 folds purified ISA with 2.3% yield.

4. Pullulanase activity was eluted from DEAE-Sepharose column at 0.15 M NaCl. Final purification by Sephacryl S-200 column yielded 20 folds purified pullulanase with 8% yield.

5. The native molecular weight of isoamylase was determined to be 98 kDa by gel filtration on Sephacryl S-200. The molecular weight from SDS-PAGE were 41 and 34 kDa.

6. The native molecular weight of pullulanase was determined to be 175 kDa by gel filtration on Sephacryl S-200. The molecular weight from SDS-PAGE were 54, 46 and 41 kDa.

7. The optimum pH of both isoamylase and pullulanase activities was 6.0. The optimum temperature of isoamylase and pullulanase were 70°C and 50°C, respectively. DBE was stable at 4-37°C for 24 hours.

8. IAA and NEM showed inhibitory effect on DBE while DTT, GSH and β-mercaptoethanol showed activating effect on the DBE.

9. Divalent metal ion, Cu^{2+} strongly inhibited isoamylase and pullulanase. Co^{2+} and Mn^{2+} at low concentrations activated pullulanase. Co^{2+} activated isoamylase.

10. The K_m and V_{max} of isoamylase were 21.14 mg/ml and 52.10 nmol maltose/min. The K_m and V_{max} of pullulanase were 39.49 mg/ml and 35.31nmol maltose/min.

11. Pullulanase was highly specific to pullulan and can moderately hydrolyze amylopectin, soluble starch and amylose. Isoamylase was specific to amylopectin and had no activity on pullulan.