CHAPTER V

CONCLUSION

Three clones of full coding sequence of *Tribolium cataneum* α-amylase gene were obtained. Each clone contains 1,470 bp open reading frame encoding a predicted protein of 490 amino acids starting from the first ATG. The amino acid sequences of these clones show almost 99% identities to the *Tribolium castaneum* α-amylase when compared to NCBI nucleotide database (NCBI Reference Sequence: NM_001114376.1), and show 98 scores of sequence alignment. The GeneBank accession numbers of our *Tribolium cataneum* α-amylase genes are KF247314, KF247315 and KF247316. The first seventeen amino acids represent the signal peptide. The catalytic site residues of *Tribolium cataneum* α-amylase are Asp204, Glu241 and Asp303.

In addition, the thirteen recombinant clones of 1,650 bp cDNA containing *Tribolium castaneum* α-amylase sequences were also obtained. However, when compared to the NCBI nucleotide database (NCBI Reference Sequence: NM_001114376.1), these clones contain four extra regions of, 50 bp, 38 bp, 46 bp and 47 bp along with an abnormal stop codon within, assuming that they probably are pseudo-genes.

Tribolium cataneum α-amylase gene was expressed in *E.coli*strain BL21 (DE3) pLysS using pET32a (+) vector. The expressed protein was analyzed by SDS-PAGE and visualized by Coomassie blue R250 staining. The protein band at approximately 72 kDa was observed (TcAm: 53.3 kDa + Thioredoxin/Hitidine tag from vector: 18.7 kDa). The activity of *Tribolium castaneum* alpha-amylase was determined by Zymogram and measured by 2-Chloro-4-nitrophenyl-α-D-maltotrioside substrate. *Tribolium cataneum* α-amylase was found to active in the pH range of 5.5 – 9.0, with the optimum at pH 7. The optimal temperature of TcAm was at 50 °C, with wide range of high activity between 30 °C – 80 °C. The purified recombinant TcAm showed 190.98 U activities and 4338.41 U/mg specific activities to 2-Chloro-4-nitrophenyl-α-D-maltotrioside as the substrate. The thermal stability of TcAm decreased when the protein was exposed at 60-70 °C for 15 min.

Furthermore, various metal ions and reagents were tested to determine the possibility of inhibitors and activators. The enzyme activity was significantly enhanced with 10 mM of $CaCl_2$, $MgCl_2$, $MnCl_2$ and Imidazole in optimal condition. On the other hand, the activity was reduced with NaCl, EDTA and KCl.

This study provides fundamental knowledge that could lead to further regulation of insect α-amylase expression, structure/function and inhibition. The molecular specification between TcAm and the inhibitors should be further studied, in order to design the inhibitors specific to other insect pest amylases, which could initiate work for developing of transgenic rice expressing such an inhibitor.