

# CHAPTER I

## INTRODUCTION

### **Rational for the study**

In rice cultivation processes, there are various factors that could reduce the quality of rice grains such as diseases, animals, weeds and insect pests. Naturally, most stored grain products are often infested with insect pests such as *Corcyra cephalonica* (Coelho, et al., 2007), *Rhyzopertha dominica* (Smriti, et al., 2010), *Sitophilus oryzae*, *Sitophilus granarius* and *Tribolium* spp. (Stoll, 2000). In Thailand, the most serious pests of the stored rice grains are *Sitophilus zeamais* and *Tribolium castaneum* (Hayashi, et al., 2004).

*Tribolium castaneum* (Coleoptera: Tenebrionidae), Commonly known as red flour beetles, causes extensive damage of more than 30% loss of stored rice grains, especially, if the rice grains are stored for a long period of time. The adult beetles can secrete malodorous fluid that could enhance mold growth. Moreover, beetles can excrete chemical compound named hydroxyquinone leading to product contamination, which is an important factor of food product quality losses (Assie, 2007)

The  $\alpha$ -amylases ( $\alpha$ -1,4-glucan-4-glucanohydrolases), a glycoside hydrolase family 13, which is a family of endo-amylases that catalyzes the hydrolysis of  $\alpha$ -(1,4) glycosidic linkages in starch component, glycogen and other carbohydrates (Janecek, 1997) plays an important role in carbohydrate metabolism of human, animals, plants and microorganisms. Insects feeding on grain products rich in starch depend on  $\alpha$ -amylase for survival. The  $\alpha$ -amylase found in insect digestive systems such as *Tribolium castaneum* was demonstrated to be important for growth and development. The  $\alpha$ -amylase genes and biochemical properties of  $\alpha$ -amylase have been studied in several insects such as *Tenebrio molitor* (Strobl, et al., 1998), *Callosobruchus maculatus* and *Zabrotes subfasciatus* (Silva, et al., 1999), *Rhyzopertha dominica* (Priya, et al., 2010), *Bombyx mori* (Ngernyuang, 2010) and *Sitophilus oryzae* (Lertkaeo, 2011). The damage is from the utilization of  $\alpha$ -amylases of the insects to digest starch in rice grains as their nutritious source. Understanding the insect

$\alpha$ -amylase biochemical characteristic could be important to control insect growth and thereby protect stored rice grain products. The gene encoding *Tribolium castaneum*  $\alpha$ -amylase should be studied for elucidation of the difference of enzyme properties. The goal of this study is to clone *Tribolium castaneum*  $\alpha$ -amylase genes, and study their biochemical properties.

### **Objectives of the study**

1. To clone and analyse nucleotide sequence of  $\alpha$ -amylase genes from Red beetle flour (*Tribolium castaneum*).
2. To investigate biochemical properties of  $\alpha$ -amylases from Red beetle flour (*Tribolium castaneum*).

### **Scope of the study**

1. The beetle used in this experiment was only adult stage of Red beetle flour (*Tribolium castaneum*)
2. The RNA was isolated from Red beetle flour (*Tribolium castaneum*).
3. The cDNA was cloned by TA cloning and nucleotide sequences were determined by DNA sequencing.
4. The *Tribolium castaneum*  $\alpha$ -amylase gene was cloned into expression vector and corrected positive clones
5. The *Tribolium castaneum*  $\alpha$ -amylase expression and biochemical properties were investigated.

### **Hypotheses of the study**

The red beetle flour causes extensive damage of more than 30% loss of stored rice grains by  $\alpha$ -amylase enzyme that hydrolyses alpha (1, 4) glycosidic bonds of amylose and amylopectin in rice grains. Understanding the insect  $\alpha$ -amylase biochemical characteristic could be important to control insect growth and there by protect stored rice grain products. The gene encoding *Tribolium castaneum*  $\alpha$ -amylase should be studied for elucidation of the difference of enzyme properties. The goal of this study is to clone *Tribolium castaneum*  $\alpha$ -amylase genes, and study their biochemical properties.

**Anticipated outcomes**

1. To clone and provide nucleotide sequences of red beetle flour  $\alpha$ -amylase (*Tribolium cataneum*) and information for studying  $\alpha$ -amylase of other insect.
2. To study biochemical properties of red beetle flour  $\alpha$ -amylase.