CHAPTER I
INTRODUCTION

1.1 Rationale and background

Amylases are amylolytic enzymes that catalyze the hydrolysis of alpha-1,4-glycosidic linkage in polysaccharide such as starch and are divided into two main groups: alpha-amylase and beta-amylase. Alpha-amylase (alpha-1,4-glucanglucanohydrolase, E.C.3.2.1.1) is the endoamylase that randomly hydrolyzes alpha-1,4-glycosidic linkage in starch, amylose and amylopectrin to yield limit dextrin, glucose and oligosaccharide (maltotriose and maltose). Beta-amylase (alpha-1,4-glucan-maltohydrolase, E.C.3.2.1.2) is the exoamylase that hydrolyzes at nonreducing ends to produce maltose and limit dextrin. They are found in animal organs, higher plants, germinated seeds of cereals and microorganism, etc. (Brena et al., 1996) Amylases constitute a class of industrial enzymes, sharing approximately 25% of the enzyme market and benefiting several applications in bread and baking industries, starch liquefaction and saccharification, textile, paper and detergent industry, analysis in medical and clinical chemistry, including food and pharmaceutical industries (Aiyer, 2005; Souza and Perola, 2010). They are also used in biotechnological applications such as thinning starch in the liquefaction process of sugar, brewing industries and alcohol, etc. (Brena et al., 1996) In addition, amylases are important to produce bioethanol, as renewable energy obtained by hydrolyzing plant starch found in cassava and corn into sugar. Then, it was converted to alcohol by yeast fermentation. Cassava, which is the economical source and easily available raw material, was currently used for producing fuel alcohol in Thailand.

Although amylases have been used in many industries, their stability and activity are limited. In addition, they are always imported with high cost. However, in the present research, new sources of amylase with high activity and stability from natural resources such as microorganisms, animals, especially in plant and fruits are under investigation. For banana, which contain up to 20% starch by weight at the mature green stage, possesses less than 1% at the fully ripened stage. Amylase is the

major enzyme that hydrolyzes starch into sugar, causing sweetness in fruits (Hulme et al., 1970). Edna et al. (1978) found the parallel to the increase in amylase and a decrease in the starch content of the avocado fruit pulp. In the same way, the increase of total sugars in the aril also increased concomitantly with the decrease in starch content in durian fruit during ripening. (Ketsa and Deuangkanit,1998). Fuch et al. (1980) studied changes in amylase activity, starch and sugar contents in mango fruit pulp. The results showed that amylase activity increased parallel to the increase in fruit weight. Moreover, during ripening, there was a decrease in starch content and an increase in the reducing and nonreducing sugars. Later, Luize et al. (2001) found the climacteric raising in mango fruit which is marked by an appreciable increase in the activity of amylase, reducing and nonreducing sugar contents and decrease in the starch content. Mao et al. (2003) studied amylase activity in ripening banana. The result shows that α-amylase activity was about 15-20 units per milligram protein. Kanwal et al. (2004) purified and characterized α-amylase from apples (*Malus pumila*). The purified amylase showed 5.025 U/mL and specific activity 38.95 U/mL with 20-fold purification, pH optimum of 6.8, temperature optimum 37 °C. Noman et al. (2006) purified and studied some properties of α-amylase from post-harvest *Pachyrhizus erosus* L. tuber. The result showed that α-amylase had molecular weight of 40 kDa, optimum activity at pH 7.3 and temperature optimum 37 °C with an apparent K_m value of 0.29% for starch as a substrate.

However, amylases have not been reported from Thai fruits especially in Mon Thong durians. Mon Thong durians (*Durio zibethinus* Murr. cv. Mon Thong) are important commercial fruit of Thailand. The delicious pulp is golden yellow with unique smell and has sweet taste. Because large amount of starch is stored in fruit and converted into sugar by amylase causing them to become sweeter during ripening. This ripening process might be expected from amylase activity. Therefore, Mon Thong durians are interested to be a model for research. As it is a favorite fruit among people for a long time, it was considered to be good source for study owing to its availability in the local area. Moreover, it has high possibility to be applied for industry in the future as well. Therefore, the major aim of this study is to purify and characterize amylase in Thai Mon Thong Durians.

1.2 Scope and limitation of this study

Crude durian extract was prepared from ripe Mon Thong Durians (*D. zibethinus* Murr. cv. Mon Thong). Ripening stage was estimated by Iodine test and 3, 5-dinitrosalicylic acid method (DNS method). Protein concentration of crude durian extract was measured by Bradford method. To study protein pattern and activity of amylase of crude durian extract, SDS-PAGE technique and Zymographic method were applied, respectively. Then, 2D-PAGE and LC-MS/MS strategies were used to analyze partial amino acid sequence of amylase. Later, crude durian extract was purified by ammonium sulphate fractionation, Epoxy-activated Sepharose 6B affinity chromatography and DEAE-Toyopearl Anionic Exchange Chromatography, respectively. The purity and molecular mass of purified amylase was checked using SDS-PAGE. Consequently, the purified amylase was partially characterized to obtain the optimum pH and temperature, including the pH and heat stability and effect of metal ions and EDTA.

1.3 Objectives of the study

1.3.1 To study the amylase activity in Mon Thong Durians *(Durio zibethinus* Murr. cv. Mon Thong)

1.3.2 To purify and characterize properties of the amylase in Mon Thong Durians (*Durio zibethinus* Murr. cv. Mon Thong)

1.4 Anticipated outcomes

1.4.1 The amylase activity of Mon Thong Durian (*Durio zibethinus* Murr. cv. Mon Thong) was verified by Zymographic method.

1.4.2 The protein pattern of Mon Thong Durian and partial amino acid sequences of amylase were obtained.

1.4.3 The amylase from crude Mon Thong Durian with high purity, high activity and stability, could be found to be a good choice for various applications in the future.