CHAPTER II

LITERATURE REVIEW

Rice (*Oryza sativa L.*)

Rice (*Oryza sativa*) is one of the most important food crops of the world's living populations, especially, in Asia, Middle East, Latin America and the West Indies (Chun-hong, et al., 2010). *Oryza sativa* is comprises three subspecies based on geographic conditions; *O. sativa* indica, *O. sativa* javanica and *O. sativa* japonica.

Oryza sativa indica is the major type of rice cultivated in the tropics and subtropics. They grow mostly in Thailand, Philippines, India and Pakistan. Indica grains are long, slender, flat and contain 23-31% amylose (International Rice Research Institute, 2007).

Figure 1 The different of indica, javanica and japonica rice

Source: http://www.knowledgebank.irri.org/ericeproduction/0.5_Rice_races.htm

Components of rice seed

The most abundant component of rice seed is starch (carbohydrate) by found that milled rice contains starch about 89.05% to over 90%. The approximate other

compositions of rice are 0.37% of ash, 1.46% of lipids, 8.20% of protein, 0.89% of fiber, minerals and other constituents. (Nellie Wong and Chow Yang Lee, 2011; German Research Centre for Food Chemistry, 1991).

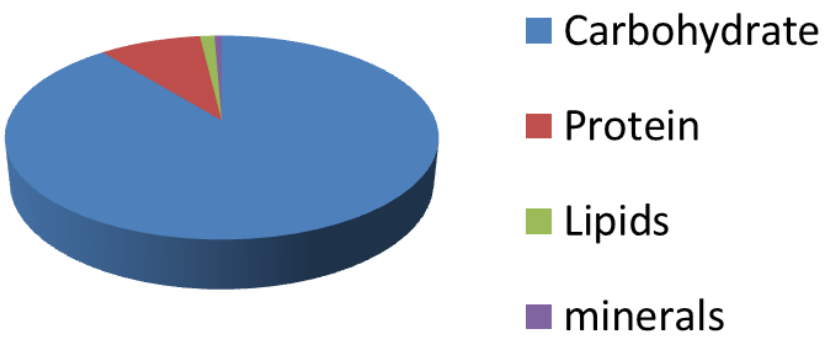

Figure 2 The components of rice seed

Source: http://www.food-allergens.de/symposium-vol.(4)/data/rice/rice composition.htm

Starch in rice seed

Starch consists of two types of glucose polymers, amylose and amylopectin (Figure 3). Amylose is a linear α-1,4-linked glucan, whereas amylopectin has various branch points formed by α-1,6 linkages joining linear chains (Yao, Zhang and Ding, 2002; Samuel, 1991). Although, the starch in rice is a mixture of amylose and amylopectin molecules, the proportion is various in each rice variety (International Rice Research Institute, 2007).

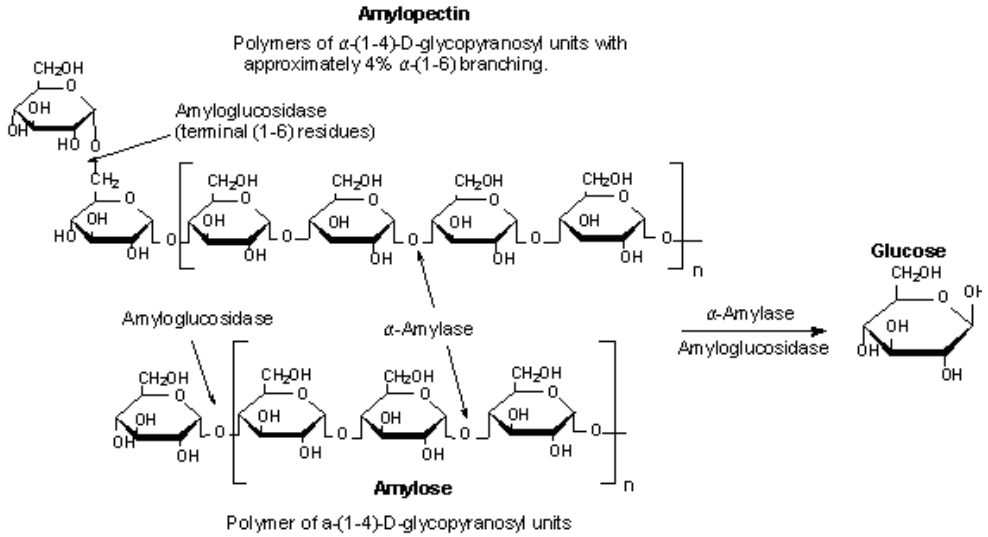

Figure 3 Structures of starch consist of amylopectin and amylase

Source: http://www.sigmaaldrich.com/life-science/metabolomics/enzymatic-kits.html

Rice damage in cultivation process

Annually, there are numerous of rice cultivation problems that could reduce the yield and quality of rice grains such as diseases, animals, weeds and insect pests (Ministry of Agriculture and Cooperatives, 2005).

1. Rice diseases

Rice diseases consist of fungal, bacterial and viral diseases (Franco, et al., 2002). Moreover, the mycoplasma and nematode can also damage the cultivated rice (Nagaraju, et al., 1988; Safdar A. Anwar, et al., 2011). Therefore, several methods have been employed in the rice diseases management such as host resistance, cultural management and chemical control (Mongkol Chan, 1993).

Figure 4 Rice diseases. 1) Yellow orange leaf disease: Virus, 2) Grassy stunt disease: Virus, 3) Bacterial leaf blight: Bacteria, 4) Blast: Fungi

Source: 1) http://www.brrd.in.th/rkb/disease%20and%20insect/index.php.htm
2) http://plantpro.doae.go.th/diseasegroup/rice/leaf_blight/r_leaf_bligth.htm
3) http://www.pmc04.doae.go.th/Myweb%202553/33%20%20warning%20Aug53/warningAug.htm

2. Rice animal pests

Rice animal pests found in Thailand are rice field crab, roof rat, ship rat, rice field rat, spotted munia, lesser bandicoot, great bandicoot and golden apple snail. The golden apple snail (*Pomacea canaliculata Lamarck*) (Figure 4) is a major rice pest in a number of Asian countries, including Taiwan, Japan, the Philippines, Indonesia and Thailand. It causes devastative damage to rice crops incurring huge loss to farmers. The adult snails cut the young paddy seedlings or tillers that are less than 21 days old. Attacks mainly take place during the night. The signs of the attack are the cut rice tillers floating in the water. Attacks tend to be worse in areas where the water is more than 1 cm deep, or in stagnant water.

Controlling the snails is difficult and costly. The snails are prolific and females may lay 2,000 – 3,000 eggs per year. The natural predator is unknown and the use of pesticides may kill other types of beneficial snails and aquatic organisms. Current measures to control the snails are quarantine action, collecting, destroying

eggs and adults, as well as introducing ducks to feed on the snails. Sometimes the snails aregathered in the field, crushed and fed to penned ducks (Zubir Bidin, 2002).

Figure 5 Rice animal pests: spotted munia, rice field rat, rice field crab and golden apple snail

Source: 1) http://www.go4get.com/add_go4board.php?id=628
2) http://gasertintree.blogspot.com/2010/04/blog-post_17.html
3) http://bigdd.igetweb.com/index.php?mo=59&action=page&id=382073
4) http://123ee.blogspot.com/2012/05/blog-post_06.html

3. Rice weeds

Weed infestation continues to be a serious problem in rice cultivation, which causes grain yield losses of 50–91% (Elliot, et al., 1984). There are various species of weeds found in paddy field such as *Echinochloa* spp., *Ageratum conyzoides* L., *Paspalum distichum* L., and *Cyperus difformis* L. Some weeds compete more with cereals because of their similar growth condition and nutrient requirements (Manandhar, Shrestha and Lekhak, 2007). Considerable progress in weed control system has been achieved with various measures such as ensuring the purity of rice seed, proper selection of cultivar and seeding rate, proper planting method, land preparation and water management, hand weeding and chemical weed control and crop rotation used together in a system of integrated weed management (Samuel, 1991). Weed control is important to increasing rice productivity. Herbicides are

considered to be an alternative. However, the development of new, improved herbicides for dry-seeded rice is also needed (Gupta, et al., 2003).

Figure 6 Rice weeds 1) Indian heliotrope 2) Water clover 3) Umbrella sedge 4), 5) Crowfoot grass

Source: 1) http://www.oknation.net/blog/surasakc/2012/04/13/entry-1
2) http://www.fernsiam.com/FernWorld/Taxonomy/Marsileaceae/
3) http://blog.yahoo.com/_MEE62TMU4X4ZGO676BKI6EY3ME/articles/228233
4), 5) http://www.brrd.in.th/rkb/weed/index.php-file=content.php&id=16.htm

4. Rice insect pests

Generally, there are various insects found in cultivated rice field. Only a few insects are considered as the rice pests. Insect pests damage rice by feeding on the leaves, stems, roots, and grain (University of Arkansas, 2010). The rice insect pests can be classified into 2 groups, the insect pests that attack all stages of plant growth and the insect pests of stored-product.

The insect pests that attack all portions of the rice plant and all stages of plant growth such as orange leafhopper *Thaia subrufa*, green leafhoppers *Nephotettix* spp., and brown planthopper *Nilaparvata lugens* are observed on the ratoon crop rather than on the main crop. Other insects present on both main and ratoon crops are whorl maggot *Hydrellia griseola*, paddy gall midge *Orseolia oryzae*, leaffolder *Cnaphalocrocis medinalis*, thrips *Baliothrips biformis*, stem borers *Tryporyza incertulas* and *T. innotata (A.K. Nagaraju, Chakravarthy, and M. Mahadevappa*, 1988). The examples ofthe stem borers such as yellow stem borer, is a predominant monophagous pest of rice, which causes 5% to 30% loss of the rice crop (Herdt RW, 1991). The larvae feed rapidly on stem parts and complete their entire growth and development within the rice stem and hence are not easily to sprayed pesticides (Sharma, 2009).

The examples of the insect pests of stored-product are angoumois grain moth, rice moth, lesser grain borer and rice weevil.

Figure 7 Yellow stem borer and Bagnall

Source: 1) http://visualsunlimited.photoshelter.com/search?I_DSC=Yellow+stem+borer&I_DSC_AND=t&_ACT=search

2) http://kkn-rsc.ricethailand.go.th/rice/pest/insect/rice_thrips.html

Postharvest loss and damage of rice product

Postharvest losses in rice product are physical losses from shattering, spillage, waste and quality losses from delays in post-harvest operations and improper storage. Both quantitative and qualitative losses occur in rice production between harvest and

consumption. Qualitative losses, such as loss in edibility, nutritional quality, and consumer acceptability of the rice products, are much more difficult to assess than quantitative losses (A.A. Kader, 2005). In South and Southeast Asia, physical losses are 10–25% and quality losses can discount prices by up to 30% (International Rice Research Institute, 2013).

Stored product insect pests

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). Rice weevil *Sitophilus oryzae*, maiz weevil *Sitophilus zeamais* andred flour beetles *Tribolium castaneum. Tribolium castaneum* (Coleoptera: Tenebrionidae) are the serious pests of the stored rice grains in Thailand (Hayashi, et al., 2004) that cause extensive the stored grains lose their weight, culinary value, seed viability and commercial value after the pest infestation. Moreover, the alimentary tracts of insect pests contained symbiotic bacteria and fungi which are potential hygiene problems to consumers.

Red flour beetles: *Tribolium castaneum* (Coleoptera: Tenebrionidae)

The red flour beetle, *Tribolium castaneum*, found wherever grains or other dried foods are stored, has a highly evolved kidney-like cryptonephridial organ to survive such extremely dry environments. It has demonstrated resistance to all classes of insecticides used against it. Like all beetles, *Tribolium* has elytra (wing covers) that coordinate precisely with folding wings, allowing flight while providing protection (Figure 8) (*Tribolium* Genome Sequencing Consortium, 2008).

The beetles are the most abundant and injurious insect pest of flour. They are not direct health hazard but infesting flour and grains causes a sharp odor and moldy flavor (Smith, et al., 1971; Rajendran, 2005). Worldwide, the 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. Both adults and larvae feed on grain dust and broken kernels, but not the undamaged whole grain kernels. These beetles often hitchhike into the home in infested flour and can multiply into large populations. Some

survive on food accumulations in cabinet cracks, crevices, and furniture**.** 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)

Figure 8 Red flour beetles: *Tribolium castaneum* (Coleoptera: Tenebrionidae)

Source: Photo, Identified by National biological control research center Naresuan University

Identification

Red flour beetles can be divided into two types: red (rust) flour beetle *Tribolium casteneum* and confused flour beetle *Tribolium castaneum* (Thailand Junior Encyclopedia, 1993). Rust and confused flour beetles are similar in appearance. Both red flour beetles are flat and approximately 3 - 4 mm in length, 1.8 mg body weight (Armstrong and Newton, 1985), shiny, reddish-brown, and elongated (Bonneton, 2008). Antennae segments of the confused flour beetle increase in size gradually from the base to the tip to form a club of four segments; in the rust flour beetle, the last segments at the tip of the antennae are abruptly larger than the preceding ones, forming a three-segmented club. Also, the confused flour beetle has a straight-sided thorax, while the thorax of the rust flour beetle has curved sides.

Biology and life cycle of red flour beetle: *Tribolium castaneum*

The red flour beetle is a polyphagous, cosmopolitan pest in flour mills and wherever cereal products and other dried foods are processed or stored. It is often the most common species in the pest complex attacking stored wheat. Although its

pest status is considered to be secondary, requiring prior infestation by an internal feeder, it can readily infest wheat or other grains damaged in the harvesting operation. The beetle has a highly evolved kidney-likecryptonephridial organ to survive such extremely dry environments.

The life cycle of red flour beetles consists of four stages (Figure 9). The eggs, larvae, and pupae are similar to other beetles. Female beetles lay averagely about 450 eggs directly into flour or other starch food during a period of five to eight months (2 - 3 eggs per day). Eggs are colorless and approximately 80 μm in size and adhere with food particles surface. Brown-headed larvae are cream to yellow, slender and reaching a length of 2.5 mm. Larvae have six legs and two-pointed or forked projections from the last rear body segment. Pupae are white to light brown. Fully grown larvae transform into naked pupae without any form of protection (Figure 10). At the optimum temperature of 35^{o}C, the development times for each stage are about 3 days for hatching, 16 days for larval growth and 4.5 days for pupae at relative humidity 60-80% (Beckett, 1994).

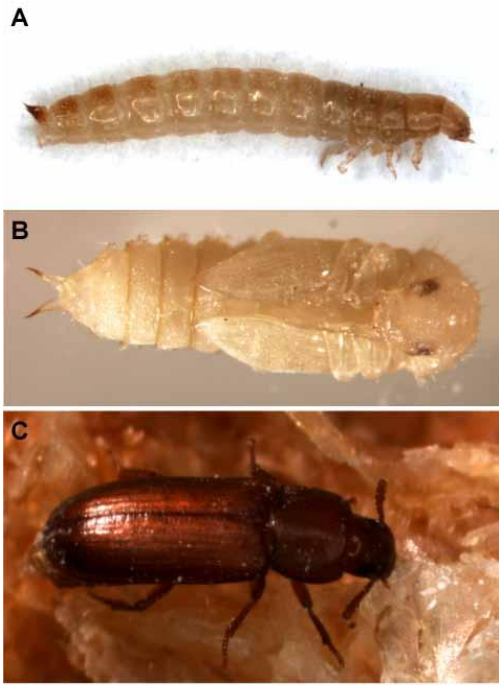

Figure 9 Life cycle of red flour beetle 1 (A) larva, (B) pupa and (C) adult

Source: http://old.padil.gov.au/pbt/index.php?q=node/23&pbtID=201

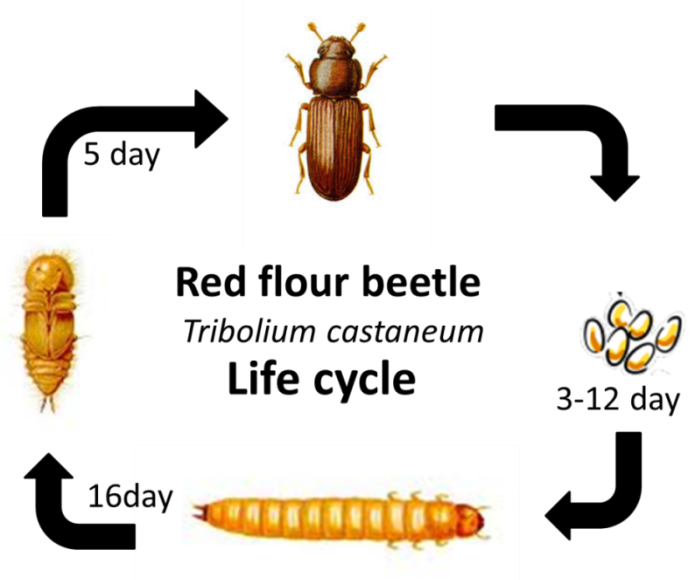

Figure 10 Life cycle of red flour beetle 2

Note: adapted from: *Tribolium confusumStages of Development*.

Source: http://old.padil.gov.au/pbt/index.php?q=node/23&pbtID=201

Amylase

Amylases (E.C: 3.2.1.0) are enzyme that hydrolyse starch molecules to release diverse products including dextrins and progressively smaller polymers composed of glucose units. Amylases were studied in many research groups. In 1930, Ohlsson divided starch degrading enzyme by catalyzes patterns into; α-amylases and β-amylases. Amylases are prevalent enzymes being found in all organisms, which have a vital role in carbohydrate metabolism (Wanderly, et al., 2004; Tripathi, et al., 2007). They can be derived from several sources such as plants, animals, insect (Zverlov, Holl and Schwarz, 2003) and micro-organisms, for example, *Bacillus* species and *Aspergillus* species (Gouda and Elbahloul, 2008; Pandey, et al., 2000). Previous study, amylases can be classified into two categories, which are endoamylase and exoamylase. Endoamylases are alpha-amylase to catalyse hydrolysis in a random manner in the interior of the starch molecule producing linear and branched oligosaccharides of various chain lengths. Exoamylases are beta-amylase and glucoamylase act from the non-reducing end successively resulting in short end

product (Reddy, Nimmagadda and Sambasiva, 2003; Pandey, et al., 2000). Then they can specifically cleave the O-glycosidic bonds in several types of starch source such as in the crops or rice seed. Amylases are wide spread used for several industrial processes such as the preparation of glucose syrups, textile, food, bread making, brewing and distilling industries. Furthermore, the property of amylase enzyme has been applied in other fields such as clinical, medicinal and analytical chemistries (Kumar, Singh and Rao, 2005; Pandey, et al., 2000).

Alpha-amylase

α-Amylases (α-1,4-glucan-4-glucanohydrolases) belong to a glycoside hydrolase family 13, which is a family of endo-amylases that catalyzes the hydrolysis of α-(1,4) glycosidic linkages in starch component, glycogen and other carbohydrates (Janecek, 1993). This enzyme plays an important role in carbohydrate metabolism of humans, animals, plants and microorganisms (Strobl, et al. 1998). In humans, it is found mostly in salivary glands and the pancreas as different isoforms while plant α-amylases are important for plant germination (Grossi De Sa and Chrispeels, 1997; Oliveira-Neto, et al., 2003; Kekos and Macris, 1983). The structures of these α-amylases generally compose of three domain, (Domain A) a structurally conserved $(\beta/\alpha)_8$-barrel domain first observed in triose phosphate isomerase, an additional domain inserted within Domain A (Domain B) and the C-terminal domain (Domain C). All known α-amylases include calcium ions that contribute to stabilization of the structures (Nonaka, et al., 2003).

Alpha-amylase in animals

For alpha-amylase of animals, it is found in saliva, pancreatic juice and other tissues (Brayer, Luo and Withers, 1995). This enzyme is a digestive enzyme and is calcium metalloenzymes, completely unable to function in the absence of calcium., α-amylase enzyme of animal breaks down long-chain carbohydrates, ultimately yielding maltotriose and maltose from amylose, or glucose, maltose and "limit dextrin" from amylopectin by acting at random locations along the starch chain. For example, homogeneous chicken pancreas α-amylase consists of two main isozymes, which have been isolated by ion exchange chromatography. They show similar kinetic behaviour

and inhibition patterns by two protein inhibitors from wheat (Buonocore, et al., 1984). The crystal structure of porcine pancreatic α-amylase (PPA) has been solved at 2.9 Åresolution by X-ray crystallographic methods. The enzyme contains three domains. The larger,in the N-terminal part, consists of 330 amino acid residues (G.Buisson, et al., 1987). α-Amylase has been purified and characterized from the muscle and intestines of the parasitic helminth of pigs, *Ascaris suum* (Zoltowska, 2001). Several α-amylases also have been cloned in fish species, such as winter flounder, *Pseudopleuronectes americanus* (Douglas, et al., 2000); barramundi (sea bass), *Lates calcarifer* (Ma, et al., 2004); and the spotted green puffer fish, *Tetraodon nigroviridis* (Bouneau, et al., 2003).

Human α-amylase

In humans, α-amylase is present in both salivary and pancreatic secretions and exhibits a high level of structural similarity. The human pancreatic α-amylase structure has been determined using X-ray diffraction techniques. This enzyme is a 56 kDa protein consisting of 496 amino acids in a single polypeptide chain and is found to be containing of three structural domains (Figure 10), along with the locations of each of the bound calcium and chloride ions (Brayer, Luo and Withers, 1995).

Human salivary *α*-amylase (HSAmy) is an important enzyme in the oral cavity carrying out several functions (Scannapieco, et al., 1995). HSAmy consists of a single polypeptide chain of 496 amino acids (Ramasubbu, et al., 1996) and exists in several isoforms in the salivary secretions. Human salivary *α*-amylase (HSAmy) has three different functions relevant to oral health: hydrolysis of starch, binding to hydroxyapatite and binding to bacteria (e.g. viridans streptococci). Oral bacteria utilize the starch hydrolyzing activity of HSAmy to derive their nutrients from dietary starch. The structure of saliva α-amylase composes of three domains like human pancreatic α-amylase (Chandran Ragunath, 2008).

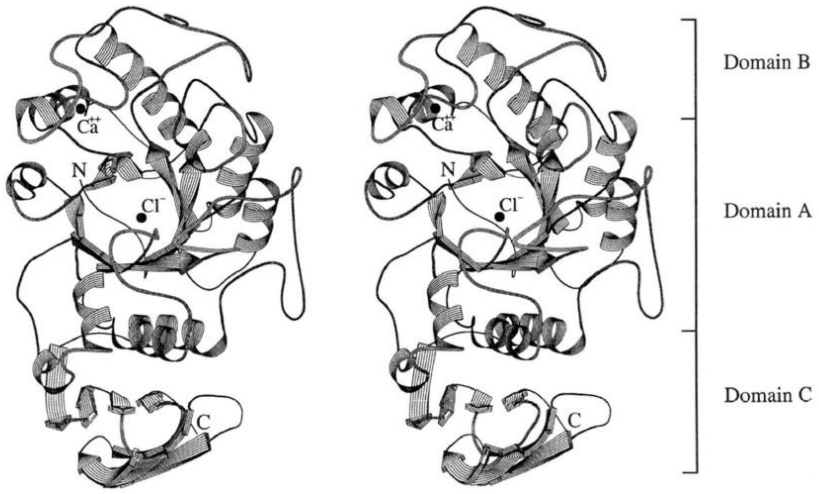

Figure 11 **Stereo drawing of a schematic representation of the polypeptide chainfold of human pancreatic α-amylase. Also indicated is the relative positioning of the three structural domains present in this protein**

Source: Brayer, Luo and Withers, 1995

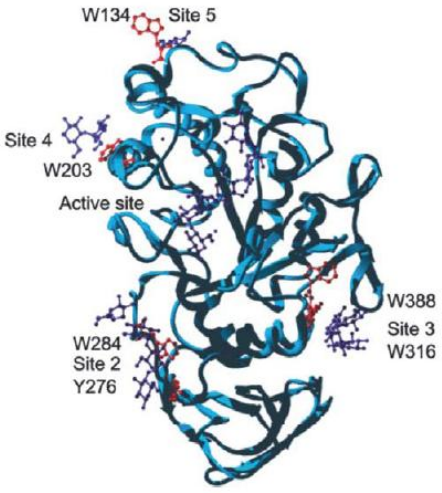

Figure 12 **Ribbon diagram of HSAmy showing the active site and secondary saccharide binding sites**

Source: Chandran Ragunath, 2008

Alpha-amylases in microorganisms

The alpha-amylases are found in several species of microorganism, which have been studied extensively from various aspects including structure, function, secretion and industrial application. Bacterial α-amylases used as an anti-staling agent were effective in baked food, which were realized in the middle of the 20th century (Miller, Johnson and Palmer, 1953). A significant increase in amylase production and utilization occurred in the early 1960s when *Bacillus subtilis* α-amylase and *Aspergillus niger* glucoamylase were used to replace acid catalysis in dextrose production from starch. Development of genetic engineering tools in 1970s successfully paved the way for the production of cloned amylases (*Bacillus stearothermophillus*) in 1980s and 1990s for industrial applications (Brumm, Hebede and Teegue, 1991; Zemen and McCrea, 1985). Fungal amylases have been widely used for the preparation of oriental foods. In spite of the wide distribution of amylases, microbial sources, namely fungal and bacterial amylases, are used for the industrial production due to advantages such as cost effectiveness, consistency, less time and space required for production and ease of process modification and optimization (A. Burhan, et al., 2008)

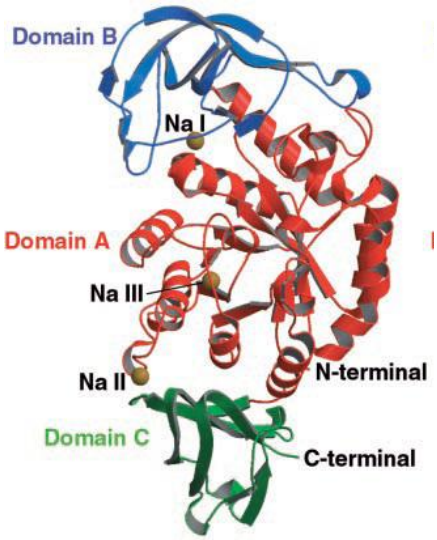

Figure 13 Stereo view of the ribbon model of the *Bacillus*sp. α-amylase structure

Source: Nonaka, et al., 2003

Cereal α-amylases

Cereal α-amylases play a key role in the starch metabolism by hydrolyzing starch in the germinating seeds and in other tissues. These highly expressed enzymes are synthesized under the influence of plant growth hormones such as gibberellic acid (GA3) and they exist in multiple forms (MacGregor, 1977; Mitchell, 1972).

Cereal amylases are divided into two groups based on their chemical, physical and immunochemical properties (Kruger and Marchylo, 1985). Group-I is characterized by a pI close to 5.8 and is calcium independent. It appears early at the onset of germination in the presence of gibberellic acid. Group-II has a pI value close to 4.5 and represents 60% of the total α-amylase activity. This group of enzymes requires calcium for their activity during the germination induced by gibberellic acid (MacGregor, 1983).

In numerous research studies, the presence of α-amylase activity during rice, barley, wheat and oat seed maturation (Meredith and Jenkins, 1973), as well as during seed germination (Hill and MacGregor, 1988) has been extensively examined. The enzyme is approximately 30% of the total protein synthesized during germination.

The α-amylase gene family in rice has at least eight members classified into three subfamilies, Amy1, Amy2 and Amy3 (Huang, et al., 1990; Mitsui, et al., 1993; Ranjhan, et al., 1991). The peptide sequences encoded by these α-amylase genes possess four highly homologous domains containing conserved catalytic residues (Nakajima, et al., 1986). The α-amylase isoforms I-1 and II-4 are found in rice grains during ripening, which isoform II-4 is the most predominant isoform. In the recent study, the increase of α-amylase activities in transgenic rice can inhibit the accumulation of reserve starch and lower the grain quality of rice (Makoto Hakata, et al., 2012; Asatsuma, A., et al., 2006).

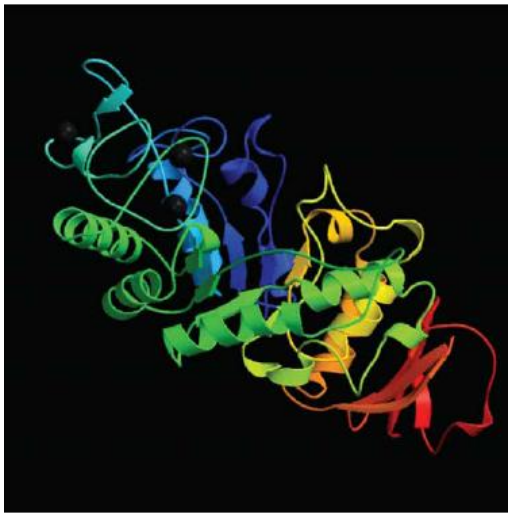

Figure 14 Ribbon diagram of barley α-amylase

Source: Kadziola, et al., 1994

Insect α-amylase

The α-amylase plays a key role in the carbohydrate metabolism of animals, plants and microorganisms. Several insects feeding on starchy seeds during larval and adult stages such as seed weevils and rice weevils depend on their α-amylases for survival. The carbohydrate digestion of bruchid weevils such as the Mexican bean weevil (*Zabrotes subfasciatus*) and the cowpea weevil (*Callosobruchus maculatus*) mainly appear in the lumen of the midgut since high enzymatic activities against starch, maltose, maltodextrins and galactosyl oligosaccharides were found in the luminal fluid (Sliva, et al., 1999). In the yellow mealworm (*Tenebrio molitor*), α-amylases are synthesized in anterior midgut cells and packed in the golgi area into secretory vesicles that undergo fusion prior to migrating to the cell apex.

The different forms of α-amylases in the insect midgut lumen have been observed in *C. maculatus* and *Z. subfasciatus* (Slivaet, et al., 1999; Campos, et al., 1989). The α-amylase expression patterns vary in *Z. subfasciatus* fed on different diets, apparently in response to the presence of antimetabolic proteins such as α-amylase inhibitors, rather than as a response to structural differences in the starch granules.

The Mexican bean weevil larvae have the ability to modulate the concentration of α-glucosidases and α-amylases when reared on different diets (Sliva, et al., 1999). The enzymatic mechanism of insect α-amylases has not yet been completely elucidated. However, α-amylases from different sources have a similar mechanism of action with catalytic residues conserved among all the enzymes (Svensson, 1994; MacGrehor, 2001),for example, three acidic side chains in PPA (Asp197, Glu233 and Asp300) (Brayer, 1995) corresponding to Asp185, Glu222 and Asp287 in TMA (Figure 13) (Strobl, et al., 1998) are directly involved in catalysis (Machius,1996).

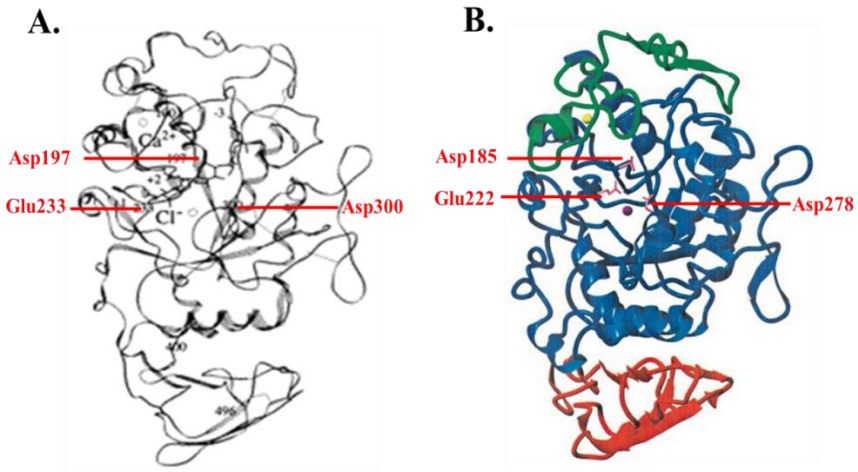

Figure 15 Three acidic side chains in (A) PPA: Asp197, Glu233 and Asp300 corresponding to (B) TMA: Asp185, Glu222 and Asp287

Source: (A) Brayer, et al., 2000 and **(B)** Strobl, et al., 1996

The study of insect digestive enzymes seems to make sense in the realization that the gut is the major interface between the insect and its environment. There are significant differences of the α-amylase activity in different *Tribolium castaneum* larval stages. Thus, the understanding of digestive enzyme function is essential for the development of insect controlling method such as the use of enzyme inhibitors and transgenic plants to control insect pests.

Beta-amylase

Beta-amylase (α-1, 4-glucan maltohydrolase, E.C.3.2.1.2) is an exohydrolases that catalyses the non-reducing ends of starch molecules, producing β-maltose and β-limit dextrin as product (Douglas, Stanley and Laurens, 1982; Ziegler, 1999).

Gamma-amylase (Glucoamylase)

Gamma-amylase (EC.3.2.1.3) is a group of exoamylase that catalyses the hydrolylsis of α-1, 4-linkages and α-1, 6-linkages in starch. It has been found in several microorganisms like bacteria and fungi.

Alpha-amylase inhibitor

The enzyme inhibitors act on key insect gut digestive enzymes, the α-amylases and proteinases. Several types of α-amylase and proteinase inhibitors, present in seeds and vegetative organs, act to regulate numerous phytophagous insects (Konarev, 1996; Chrispeels, et al., 1998; Gatehouse, et al., 1998).

A number of α-amylases inhibitors (α-AI) were isolated and identified in several research studies. They have been classified into two groups: non-proteinaceous inhibitors and proteinaceous inhibitors.

Non-proteinaceous inhibitors class contains diverse types of organic compounds such as acarbose, isoacarbose, acarviosine-glucose, hibiscus acid and the cyclodextrins. The two hibiscus acid forms, purified from Roselle tea (*Hibiscus sabdariffa*), the acarviosine-glucose, the isoacarbose and α-, β- and γ-cyclodextrins are highly active against porcine and human pancreatic α-amylase (Kim, et al., 1999; Hansawasdi, 2000; Nahomum, 2000; Qian, 2001). The inhibitory activity of these compounds against α-amylases is due to their cyclic structures, which resemble α-amylase substrates binding to α-amylase catalytic sites.

Proteinaceous α-amylase inhibitors are generally found in microorganisms, plants and animals (Ryan, 1990; Silano, 1987; Franco, et al., 2000; Iulek, et al., 2000). In plants, proteinaceous inhibitors are not only present in cereals such as wheat *Triticum aestivum* (Silano, 1987; Petrucci, et al., 1976; Feng, et al., 1996), barley *Hordeum vulgareum* (Abe, et al., 1993), sorghum *Sorghum bicolor* (Bloch Jr., et al., 1991), rye *Secale cereale* (Iulek, et al., 2000, Gracia-Casado, et al.,1994) and rice

Oryza sativa (Yamagata, 1998), but also in leguminosae such as pigeonpea *Cajanus cajan* (Giri, et al.,1998), cowpea *Vigna unguiculata* (Melo, et al.,1999) and bean *P. vulgaris* (Young, 1999). Different plant α-amylase inhibitors elucidate different specificities against α-amylases from diverse sources. Therefore, α-amylase inhibitors are effective candidates for the control of seed weevils as these insects are highly dependent on starch as an energy source (OctaÂ vio L. Franco, 2002). The specificity of inhibition is the important step towards the discovery of an inhibitor that could be helpful for generating insect-resistant transgenic plants. The proteinaceous α-amylase inhibitors can be divided into six different classes: lectin-like α-amylase inhibitors, knottin-like α-amylase inhibitors, cereal-type α-amylase inhibitors, Kunitz-like α-amylase inhibitors, c-purothionin-like α-amylase inhibitors and thaumatin-like α-amylase inhibitors (Richardson, M., 1990).