Introduction

Rice is the staple food of over half the world's human population. Since the 1960's, the green revolution has transformed rice production in developing countries with the breeding and introduction of high yielding varieties (HYVs). Widespread utilization of the HYVs, associated with the application of chemical fertilizers, herbicides and insecticides, in addition to technical guidance and provision of agricultural credit to farmers, has brought about remarkable growth in the world's rice production.

Demand for rice will continue to grow in the future with the increasing population and rising consumers' incomes. However, in respect to consumers' incomes, the demand for better quality is likely to be more elastic than the demand for quantity. As consumers' incomes grow, the quantity of rice consumtion does not always increase proportionately due to a shift to better quality rice or other foods (Damardjati and Made, 1989).

In rice production, the quality is an important factor in determining the income of farmers. Improving grain quality could increase rural incomes and the welfare of urban consumers (Juliano and Duff, 1989). Furthermore, improved rice grain quality can also improve national welfare by allowing more efficient competition in the world rice market. Quality improvements may allow rice exporters to obtain better prices or to expand exports in premium rice quality markets. Nutritional qualities of rice are defined by protein content, the available mineral content, such as iron and zinc, and other components such as vitamins. Milled rice is the principal source of dietary energy (50%) and protein (35-40%) in tropical Asia (FAO, 1984).

In the market, the consumers may pay twice or more for a higher quality rice compared with a lower one. For example, in the Philippines, traditional rice varieties receive only about half the price of modern rice varieties such as Cisadane and IR64. Growth in consumers' income increases the demand for rice with taste characteristics that are considered to be 'superior' compared to other varieties (Directorate of Food Crops Economic, 1988).

Previous studies have investigated some factors affecting rice yield and quality. These factors include genetic (Jongkaewwattana, 1990; Nangju and De Datta, 1970), crop and post-harvest management (De Datta, 1986; Fagade and Ojo, 1977; Yoshida, 1981), and environmental conditions during the growing period (Henderson, 1954; Yoshida and Hara, 1976). Environmental factors include location, soil fertility, water regime, season, as well as time and rate of N fertilizer application (Juliano and Duff, 1989). Genotype, environmental conditions during ripening, time of harvesting, and post-harvest operations can also influence rice quality (Ikehashi and Khush, 1979; Juliano, 1985a).

Unlike wheat, barley and maize, rice is purchased by consumers in a relatively unprocessed state, except for the milling and polishing purposes. Thus, rice quality characteristics are determined largely by its 'natural' appearance (Damardjati and Made, 1989). Head rice recovery, that is rice kernels which remain as three-fourths or more of their normal length after milling (Simpson *et al.*, 1965; USDA,1995), is widely used to determine the milling quality of rice. Prices that farmers receive for their rice also vary considerably because of rice quality, even among rice grown from the same variety such as KDML105 in Thailand. The first factor is moisture content, which can influence the quality of milled rice as well as the actual quantity of the harvest. The moisture content can now be precisely measured with portable moisture meters in many rice growing countries, including Thailand. After moisture, it is less clear how prices paid to farmers are determined. Therefore, this study set out to examine the relationship between rice quality characteristics and the price paid to farmers.

Milled rice is graded and priced according to the proportion of whole grain, termed head rice (Kaosa-ard and Juliano, 1989). Previous studies have reported that nitrogen fertilization increased head rice recovery. For example, in IR8, application of 75 kg N ha^{-1} increased head rice by 7% over the no N plots (Fagade and Ojo, 1977). The effect of N supply on head rice recovery differs among rice varieties. An application of N fertilizer up to 120 kg N ha^{-1} in the dry season increased the percent head rice of chalky varieties IR8, IR5 and Sigadis, but not for a non-chalky variety, C4-63 (Nangju and De Datta, 1970). Jongkaewwattana *et al.* (1993) showed that N fertilization was positively correlated with head rice recovery. Thus, local farming practices such as rate and timing of N fertilizer affect rice grain breakage (Dilday, 1989). Furthermore, head rice recovery also depends on grain moisture content at harvest. McNeal (1950) reported that in a long grain variety, Rexark, the grain moisture content at harvest for good head rice yield was between 16 to 22%, but in a medium grain variety, Zanith, was between 17 to 24%. Previous studies (Nangju and De Datta, 1970) have suggested that the relationship between head rice recovery and grain moisture at harvest varies with genotype during milling. However, effects of N fertilization and grain moisture content at harvest on the milling quality of widely planted Thai rice cultivars remains unknown, and therefore are explored experimentally in this thesis. Many studies have shown that N application increase protein content in rice grain (De Datta *et al.*, 1972; Gomez and De Datta, 1975; Perez *et al.*, 1996). Nangju and De Datta (1970) and del Rosario *et al.* (1968) postulated that N fertilization may decrease rice milling breakage by increasing the density of protein bodies that occupy the space between starch granules to act as a binder which strengthen the grain against breakage. This sounds plausible, but no data are yet available to support the hypothesis. Furthermore, anatomical studies are conducted to describe the internal grain structure of commercial Thai varieties. Studies which have been undertaken so far focus specifically on how the distribution of protein and starch in the endosperm is related to the applications of N fertilization and therefore how N concentration in the endosperm relates to grain breakage during milling. An understanding of the effect of N fertilization on grain breakage during milling may assist Thai farmers to better manage N fertilization in rice production systems to achieve good milling quality and more income. The work reported in this thesis is based on the following three hypotheses:

1. Head rice is the main factor for determining rice price in Thailand.
2. Nitrogen fertilizer management can improve head rice yield recovery after milling.
3. Increasing storage protein in rice endosperm can reduce grain breakage during milling.

The structure and scope of this study is show in the following diagram (Figure 1).

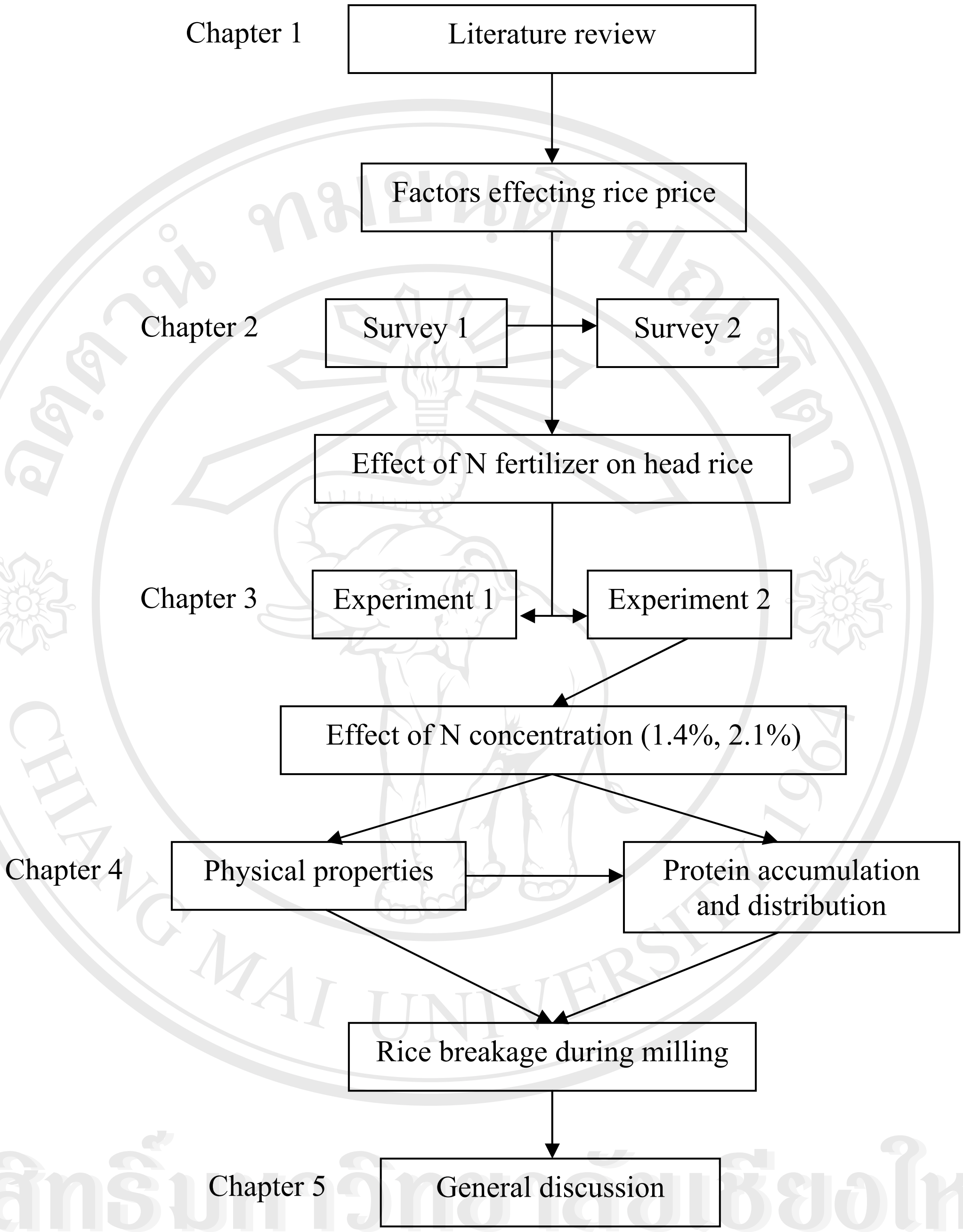

Figure 1 Diagram of structure and scope of this study.

Chapter 1

Literature Review

1.1. Rice quality characteristics

The quality of rice can be defined in many aspects including milling (physical) quality, cooking and eating (physicochemical) quality, and nutritional quality. The physical characteristics are percentage of head rice, grain size and shape, chalkiness, vitreousness, whiteness, and translucency. The physicochemical characteristics are amylose content, gel consistency and gelatinization temperature. Nutritional qualities are protein content, and mineral content such as iron, zinc and iodine (Blakeney, 1996; Juliano, 1985b; Juliano and Gonzales, 1989).

1.1.1. Milling quality

In rice marketing, milling quality is the most important factor for determining rice price. Consumers purchase milled rice on the basis of physical appearance and variety designation made by retailers. The assumption is that price differences are mainly due to quality. In many parts of Asia, aromatic rice has a premium price.

1.1.1.1. Head rice

The market value of rough rice is mostly based upon its milling quality and milling yield. Milling quality is defined as the head rice recovery after milling and milling yield is the total milled rice that is produced in the milling of rough rice (Brorsen *et al.*, 1984; Jongkaewwattana, 1990). Head rice is defined as rice kernels which remain as three-fourths of their normal length or more after milling (Simpson *et al.*, 1965; USDA,1995). The rice that has more broken rice is considered as a low grade rice. Percent grain breakage after milling is influenced by genotype, environment and management (Khush and Juliano, 1984). Many reports pointed out that rice grain breakage during milling might be associated with chalkiness, sun fissures or cracks and immature grains (Huysmans, 1965; Mores *et al.*, 1968; Kunze and Prasad, 1978).

1.1.1.2. Chalkiness

The chalkiness, also called white core, is caused by layers of endosperm cells with loose packing of starch granules and are influenced by genetic and environmental factors, but immature grains cause generally chalky grain (Blakeney, 1996; del Rosario *et al.*, 1968). Lisle *et al.* (2000) reported that the international rice markets will not accept rice that contains more than 2% chalky grains. The chalky areas are not as hard as the translucent areas and the grain with chalkiness are more sensitiveness to breake during milling (Khush *et al.*, 1979). Chalky portions are observed in the endosperm of some non-waxy varieties. Factors affecting chalkiness were quoted and caused by genotype and environment (Blakeney, 1996). For example, Ebata and Nagota (1967) reported that chalkiness might be induced by environmental factors such as high night temperature. Lisle *et al.* (2000) found that rice grown at high temperature, 38/21 °C (day/night temperature) had more chalky grains. It was observed that grains in the interior position of the panicle were more susceptible to be chalky than those in the superior position. Furthermore, grain harvested from the dry season tended to be less chalky than those from the wet season (Attaviriyasook, 1983).

1.1.1.3. Whiteness

Whiteness is the coloration of the surface of milled rice after milling. Whiteness is correlated with the degree of milling, the index describing the extent to which bran has been removed from brown rice by polishing. The maximum whiteness is affected by the inherent color of the variety and by chalkiness (Ikehashi and Khush, 1979). Whiteness is one criterion for determining the grade of rice at the mill. For example, 100% rice in the Thai grading system, is described as having well milling degree, and complete polishing to remove all the bran (Kaosa-ard and Juliano, 1989).

1.1.1.4. Grain size and shape

Milled rice size and shape are determined by grain length and the length/width ratio, respectively. A grain length of more than 7.50 mm is classified as extra long grain, 6.61-7.50 mm as long grain, 5.51-6.50 mm as medium grain and less than 5.50 mm as short grain (Juliano, 1985a). A length to width ratio of more than 3.0 is classified as slender, 2.0-3.0 medium, 1.1-2.0 bold and less than 1.1 round (Jennings *et al.*, 1979). Grain size and shape are under genetic control (Oka, 1988).

1.1.2. Cooking and eating quality

Rice cooking and eating quality vary by culture of the country or regions within the same country. People from different regions have different tastes and preferences. Japanese prefer the soft and relatively sticky short-grain japonica rice.

By contrast, Thais tend to favor well-milled, long grain indica rice, which is soft but flaky when cooked. In addition, many traditional rice markets such as India, Pakistan and Thailand, fragrant rice varieties receive the highest prices. In non-traditional markets, such as those in the West, fragrant rice is considered as spoiled or contaminated rice (Efferson, 1985). However, Juliano and Duff (1989) suggested that variety is the major factor influences cooking and eating qualities of rice. The characteristics determining rice cooking and eating quality are amylose content, gelatinization temperature, gel consistency and aroma (Juliano, 1985a).

1.1.2.1. Amylose content

Amylose content is a major or a principal determinant of cooking and eating quality. It directly affects water absorption and volume expansion during cooking (Juliano *et al.*, 1965). Kaosa-ard and Juliano (1989) reported that rice of high amylose content usually exhibits high volume expansion (though not necessarily elongation) and a high degree of flakiness. The tenderness and stickiness of cooked rice are inversely correlated with amylose content. When cooked, rice grains of high amylose content are relatively separate, firm texture and become hard after cooling.

1.1.2.2 Gelatinization temperature

The temperature at which the starch granules begin to swell irreversibly in hot water is known as the gelatinization temperature. An alkali test (Little *et al.*, 1958) is used to measure the gelatinization temperature. This property is expected to be increasingly important if quick cooking is desired, e.g. in the production of instant or minute-rice. Most of the recommended varieties of Thai rice have a low gelatinization temperature. U.S. long grain rices generally have intermediate gelatinization temperatures while the medium and short grain varieties have low gelatinization temperatures (Kaosa-ard and Juliano, 1989). Juliano *et al.* (1989) suggested that cooking time, the period required for the grain core to be gelatinized (absence of opaque center) in boiling water, is affected directly by gelatinization temperature and protein content.

1.1.2.3 Gel consistency

Gel consistency is using to determine the texture or tenderness of cooked rice which has a similar amylose contents (Kaosa-ard and Juliano, 1989). Varieties with soft gel consistency are more preferable than rice varieties which have similar amylose contents. For example, Khush *et al.* (1979) found that IR5 and IR8 had similar amylose contents but IR5 was preferred over IR8 due to IR5 having a soft gel consistency.

1.1.2.4 Aroma

Aromatic rice, which has the volatile oil 2-acetyl-1-pyroline (Butterly *et al.*, 1983b), is preferred for consumers in some Asian countries such as Bangladesh, India, Pakistan, the Philippines, Indonesia and Thailand. Aromatic rice receives premium prices in some international markets in South and Middle East Asia (Dela Cruz and Khush, 2000). There are a few relatively well known aromatic varieties including Basmati rice from India and Pakistan and Khao Dawk Mali 105 from Thailand (Kaosa-ard and Juliano, 1989). Most of traditional rice varieties produce different aroma flavors. Over 114 compounds have been identified as components of flavor

(Buttery *et al.*, 1983a; Tsuzuki *et al.*, 1981). Although all the aromatic rice traits are genetically controled (Singh *et al.*, 2000), but their expressions are very much dependent on environmental and management conditions such as temperature (Julino, 1972; Mann, 1987), soil factors e.g. salinity (Bocchi *et al.*, 1997; Sarkarung *et al.*, 2000), fertilizer application (Suwanarit *et al.*, 1996, 1997), time of transplanting and harvesting (Canellas *et al.*, 1997) and grain storage conditions (Rohilla *et al.*, 2000).

1.1.3. Nutritional quality

Nutritional qualities of rice are defined by protein content and available mineral content of components such as iron, zinc and vitamins. Milled rice is the principal source of dietary energy (50%) and protein (35-40%) in tropical Asia (FAO, 1984). Protein content is the main index of nutrition value in milled rice (Eggum and Juliano, 1973). Milled rice contains about 7.3% protein (Juliano and Villareal, 1993). Nanda and Coffman (1979) suggested that increasing rice protein by 2% (i.e. from 7 to 9%) would increase protein intake in the Asian diet by 10% to 20%. Rice has the lowest protein content of all the major cereals but rice protein is one of the best nutritionally. Most of the other cereals, the main storage protein is prolamin, while in rice is glutelin, which is relatively rich in lysine (Pomeranz *et al.*, 1973). Lysine is a limiting essential amino acid for humans who consume rice as staple food (Juliano, 1985a). Juliano *et al.* (1973) reported that increasing the protein content increased the nutritionally value in rice by increasing lysine content. Unfortunately, protein content has a poor heritability and is significantly affected by environmental factors such as growing season, plant spacing, application of N fertilizer and growth duration (Khush and Juliano, 1984).

1.2. Rice quality and price relationship

Rice quality has become an important issue that affects both domestic consumption and international trade. In the world rice markets, milling quality is the most important factor that determines rice prices. Head rice yield and milling degree are the quality characteristics that rice buyers use to determine the price of rough rice and mills use for grading milled rice.

However, moisture content is the primary price determinant for rough rice. Empty and mixed grains, grain appearance, green and broken grains are other factors reported to be used in pricing rough rice (Made *et al.*, 1989). On the basis of the pattern of domestic and international trade, physical properties appear to be the major determinant of domestic and international demand for grain quality. This is followed by physicochemical characteristics, which although not visually observable, are implied by the choice of variety made by customers in the domestic trade and by the importer's choice of country for the purchase of quality grain. Rice growers and exporters are known to establish their own criteria in order to grade their commodities. The most common criteria appear to involve physical properties, such as length of grain, degree of milling, percent of broken grain, proportion of damaged grain, grain color, moisture level and impurities (Kaosa-ard and Juliano, 1989). In Philippine urban markets, modern variety prices are affected by head rice content, translucency, grain shape, impurities and gel consistency while in rural markets, important characteristics included whiteness, translucency, shape and alkali spreading value (Maranan *et al.*, 1989). Thai rice exporters, on the other hand, have a reputation for dependable quality as measured by grain composition (head rice and broken rice) and low amount of materials such as foreign matters, chalky grains, red rice, or damaged and immature grains (Attaviriyasook, 1983).

1.2.1. Definition of rice quality in different markets

Rice quality denotes different properties to different rice sectors, farmers, processors, millers and culture and tradition of consumers. Definition of 'good quality' for rice differs in different cultures and different methods of cooking. So there are the short, round grain and low amylose content of Japanese rice, fragrant Basmati with extra long and slender grain for South Asia and the Middle East, special rice for Spanish pilaf to Italian risotto. Good quality glutinous rice for Laos and northern and northeastern Thailand, as well as Japanese sushi rice, must retain its soft texture after it has been cool for many hours after cooking.

Many studies have reported that the preferred rice also differs in the international markets. For examples, in Hong Kong, indica rice is preferred and higher prices are paid for relatively long grain, a high percentage of head rice, and grain which is flaky but soft in texture (Unnevehr *et al.*, 1992). In Italy, consumers like the characteristics of japonica rice, i.e. chalky grain and a relative harder gel consistency (Kaosa-ard and Juliano, 1989). In Germany, only physical quality, such as percentage of head rice, has an impact on rice price, as consumers are not well-informed about eating quality (Kaosa-ard and Juliano, 1989; Unnevehr *et al.*, 1992). These reports indicated that rice quality characteristics are the consideration of consumers, and the preferred cooking quality for different regions is influenced by different tastes and preferences.

Juliano and Villareal (1993) showed that most people prefer rice that, after it is cooked, is soft but not very sticky, all grain size and shape types are still represented, except for round grain. Long slender grain is important in the Americas and in some exporting countries such Thailand, Vietnam and Pakistan. Medium grain has plurality in Africa, but grain size differs widely among the countries sampled. Medium and short grains are important in Asia and Europe: medium grain in Cambodia, India, Indonesia, Laos, West Malaysia, Nepal, Pakistan, the Philippines, and Turkey; short grain in Bangladesh, Bhutan, China, Japan, Republic of Korea, Taiwan and Vietnam; and both types in Sri Lanka. In Europe, long grain is the major type in Hungary; medium grain in France, Greece, Italy and Portugal; and short grain in Bulgaria, Russia and Spain.

1.2.2. Rice quality in Thailand

In Thailand, two main groups of rice, non-glutinous and glutinous rice, are consumed domestically, the choice depending on ethnic and regional preferences (Sriswasdilek *et al.*, 1989). Non-glutinous rice is separated into aromatic and non-aromatic rice. This group has long and slender grain, few breakages and intermediate amylose content (Unnevehr *et al.*, 1992).

Thai glutinous rice is mostly consumed in the north and northeast part of Thailand. Glutinous rice is an opaque grain and has virtually no amylose content. For the price, consumers pay a premium for fewer broken grains.

One of the major factors for determining rice price in Thailand is rice variety. Thai Jasmine or Thai Hom Mali is Thailand's special quality rice for which local consumers and export markets are willing to pay a premium price, but the price varies with production areas. For examples, in the retail market in Chiang Mai, in 2002, milled rice (special grade, 100% head rice) from Phao, retailed at 22 baht kg^{-1} (43 bath

= 1 US$); from Sanpatong, at 20 baht kg^{-1}; but from Mae Chan, in Chiang Rai market price was 16 baht kg^{-1}. Milled rice from Phao with 5% broken grain was priced at 17 baht kg^{-1}. The different prices may vary due to other quality characteristics, such as grain moisture content, head rice, chalkiness, translucency and vitreousness. Sriswasdilek *et al.* (1989) reported that broken rice and chalkiness adversely affect rice price.

1.3. Structure and development of the grain

The main anatomical structures of the mature rice grain are the caryopsis coat, aleurone layer, embryo and endosperm (Figure 1.1). Both endosperm and embryo are enclosed by the aleurone layers, which lies beneath the tegumen (Hinton, 1948). Varieties differ in the thickness of their aleurone layers. Short grain varieties tend to have more cell layers than slender, long grain varieties. The aleurone layer reacts positively to stains for protein (Harris and Juliano, 1979), hemicellulose and cellulose (Little and Dawson, 1960). Upland varieties have more aleurone cell layers on the dorsal and ventral sides than those in lowland varieties (Hoshikawa, 1967b). Rice endosperm cells are thin-walled parenchyma cells, usually radially elongated and packed with amyloplasts containing starch granules and some protein bodies. The two outermost cell layers (sub-aleurone layer) are rich in protein and lipid and have smaller amyloplasts and compound starch granules than those in the inner endosperm. The starch granules are polyhedral and usually 3 to 9 μm in size, with unimodal distribution (Azhakanandam *et al.*, 2000; Juliano, 1993). The embryo is small and is located on the ventral side of the caryopsis and is enclosed on the outer side by the aleurone layer. Most of the reserve materials are stored in the embryo.

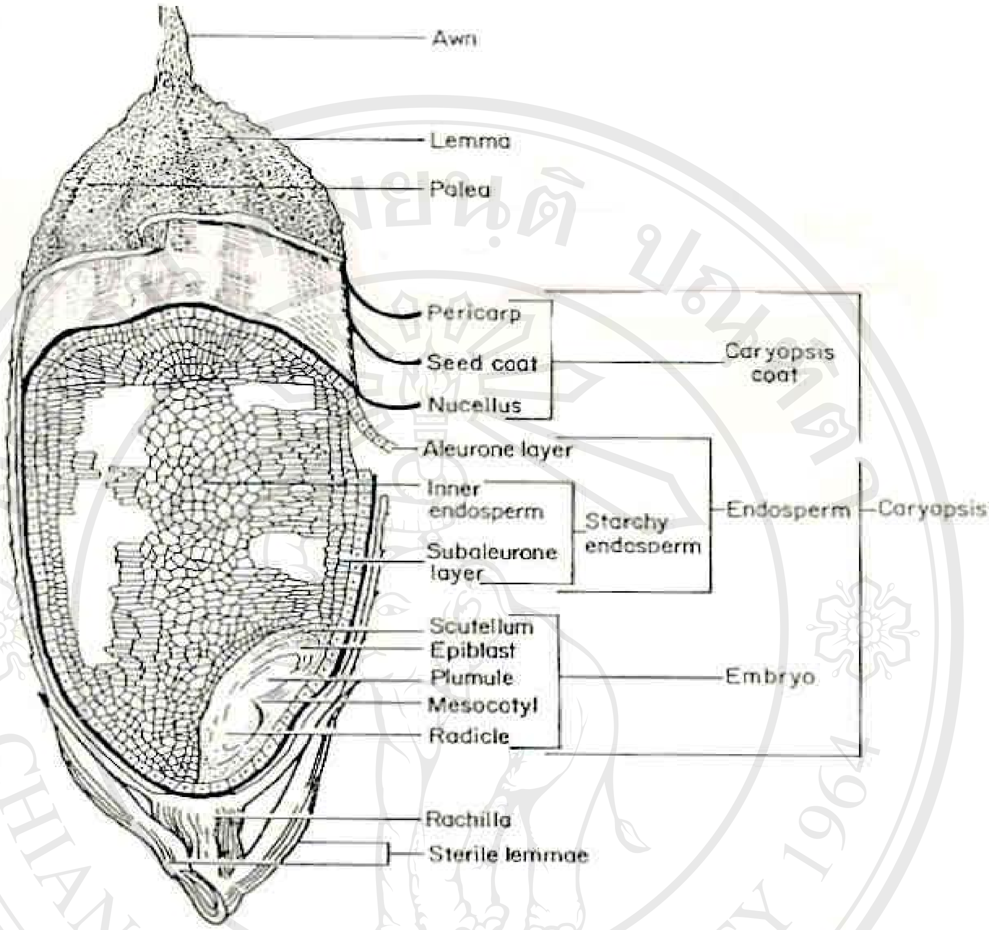

Figure 1.1 Structure of the rice grain (De Datta, 1981).

The development of the young seed following fertilization has been described in detail by Matsuo and Hoshikawa (1993). Following, the main stages are briefly summarized. After fertilization, the endosperm nuclei divide rapidly forming an embryo sac lacking cell walls. The endosperm nuclei form an orderly single layer especially at the surface of the embryo sac, thus forming the peripheral layer of the endosperm. The peripheral layer gradually multiplies and expands toward the antipodal pole. After that, the endosperm near the embryo simultaneously begin to form cell membranes and then cell walls are laid down. Cellularization proceeds centripetally towards the central cavity of the embryo sac (Matsuo and Hoshikawa, 1993). The outermost peripheral cell layer of the endosperm tissue differentiates into the aleurone layer. The aleurone layer differs in morphology and function from the starchy endosperm (Juliano, 1985a). Hoshikawa (1967b) described the aleurone layer as being composed of one to two layers of cells on the ventral side, one layer on the lateral side and three to six layers on the dorsal side. The aleurone layer completely surrounds the rice grain and the outer side of the embryo (Juliano, 1985a). Bechtel and Pomerez (1977) noted that there are two storage structures in the aleurone layer: the aleurone grains (protein bodies) and lipid bodies (Figure 1.2)

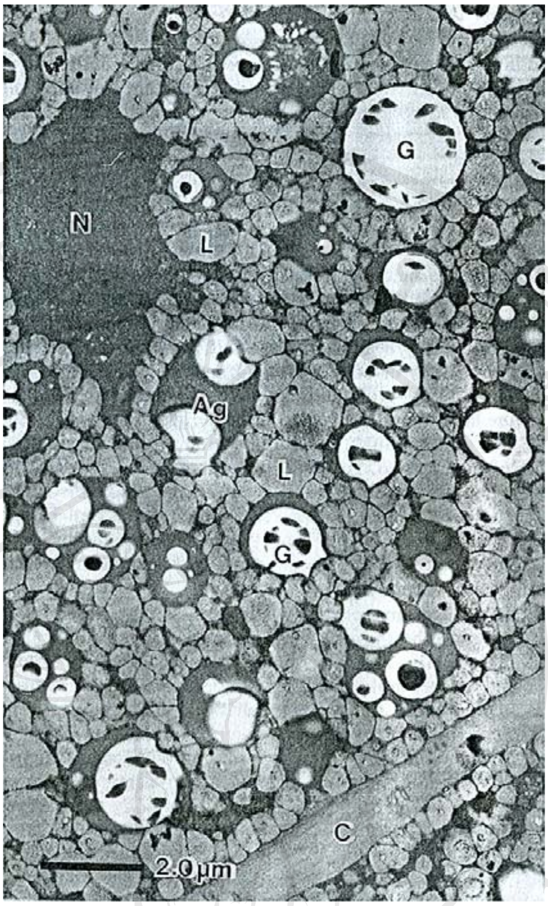

Figure 1.2 Cross section of the aleurone layer of rice var. Coloro, showing numerous lipid droplets (L) and aleurone protein bodies (aleurone grains, Ag) containing globoids (G) that have been fractured. The nucleus (N) is centrally located, and the cytoplasm lightly oppressed against the cell wall (C) (Juliano and Bechtel, 1985).

1.4. Transportation route of seed reserves into the brown rice grain

The supply route for the development of the embryo, endosperm and for substances stored in the endosperm extends from the vegetative organ to the panicle, and then enters the racilla via the rachis-branch vascular bundle, branching out from the rachis vascular bundle. The vascular bundle arises from the base of the ovary along the dorsal side and reaches its furthermost extend at the upper part of the ovule (Hoshikawa, 1975a). Major conduction to the embryo and endosperm is preformed through the vascular bundle on the dorsal side of the ovary. At the early ripening stage, the vascular bundle on the dorsal side also elongates and develops. Many studies (Sato, 1964; Hoshikawa, 1972; Hoshikawa, 1973; Kawahara *et al.*, 1977; Oparka and Gates, 1981), have reported that the reserve substances are transported to the endosperm from the conducting vascular bundle via the nucellar projection and then to the starch storage parenchyma via the dorsal aleurone tissue of the endosperm (Figures 1.3, 1.4).

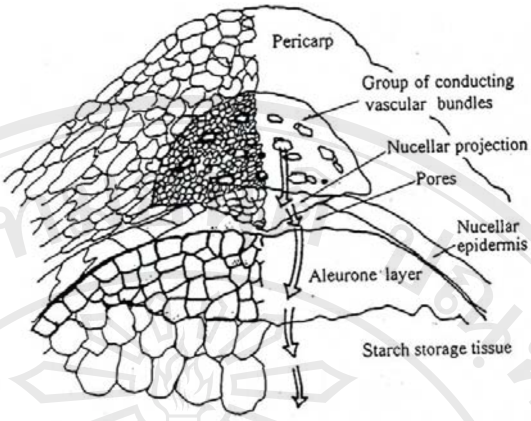

Figure 1.3 Cross section of the dorsal portion of a brown rice grain (Hoshikawa, 1993).

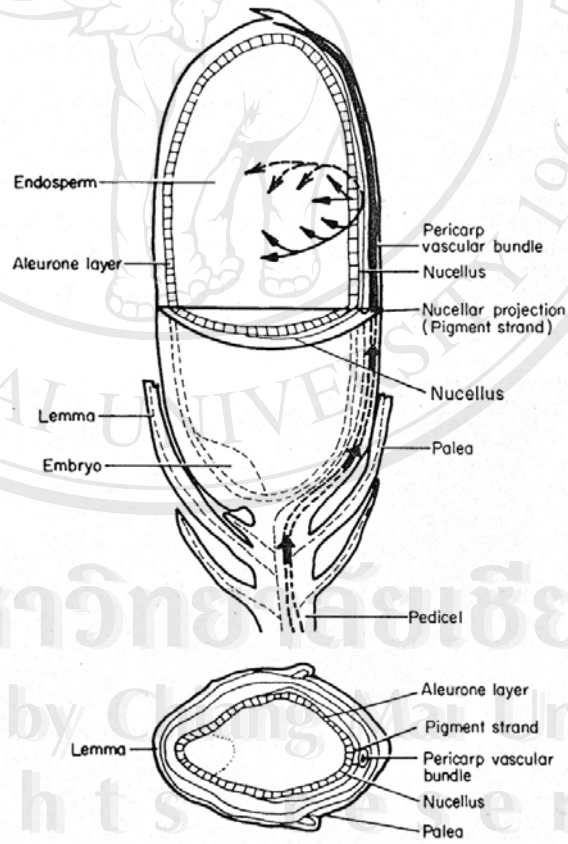

Figure 1.4 Translocation pathways of developing rice grain (Adapted from Sato, 1964; Hoshikawa, 1973; Kawahara *et al.*, 1977; and Oparka and Gates, 1981).

1.5 Formation and accumulation of reserve substances

1.5.1. Starch formation and accumulation

The deposition of starch, which is the predominant reserve substances in the endosperm tissue, starts around 5 days after flowering. Small, white colored amyloplasts containing starch have been observed as early as 5 days after flowering, and are about 1 μm in diameter (Hoshikawa, 1968a). Seven days after flowering, the starch granules increase in size. At 17 days after flowering, the starch granules grow larger and perform the shape of a turtle shell, which is their final shape and structure (Tanaka *et al.*, 1995).

Starch granule accumulation begins at the center of the developing grain and gradually spreads into the peripheral cells (Hoshikawa, 1975b). Cell division and proliferation of endosperm cells are complete in 9-10 days after flowering (Hoshikawa, 1967a). Singh and Juliano (1977) reported that starch deposition reaches a maximum at around 20 days after flowering, and thereafter it becomes constant (Figure 1.5). By contrast, the amount of free sugars increases until 9 days after flowering, then decreases and reaches a constant level thereafter. The content of free sugars is extremely small as compared to the amount of starch deposited. When the grain is mature, free sugars are converted into high molecular starch and practically no free sugar remains. Starch of non-glutinous rice is composed of about 20% amylose and 80% amylopectin (Juliano, 1979) compared with glutinous rice which contains mostly amylopectin. Like starch, amylopectin increases linearly from around 5 days after flowering, and reaches a maximum approximately 20 days after flowering. By contrast, amylose accumulates more slowly but also reaches a maximum level at around 20 days after flowering. Starch accumulation, adjacent to the aleurone layer, is completed around 30 days after flowering.

The major path of starch synthesis depends on ADP-glucose starch synthase (Murata *et al.*, 1964). This enzyme consists of two types; one is a starch granule bonding type and the other is a soluble type. In the case of non-glutinous rice, the bound type is the main component, while glutinous rice, the soluble form is in a majority (Murata and Akazawa, 1966). The activity of this enzyme in the non-glutinous rice during the ripening stage indicates that soluble starch synthase increases until around 8 days after flowering, 4 n moles ADP glucose/min, and drops to 2 n moles ADP glucose/min at 10 days after flowering and then remains constant until 21 days after flowering (Figure 1.6). The precursor of starch is sucrose, which is translocated into the endosperm, but is not used as a direct substrate for starch synthesis. Sucrose is first converted into ADP- (or UDP-) glucose and fructose by the action of sucrose synthase localized in the cytoplasm of the endosperm cell (Murata *et al.*, 1966). Thereafter, ADP- (or UDP-) glucose biosynthesized from glucose-1-P is used as the substrate for starch synthesis (Murata *et al.*, 1964). The activity of sucrose synthase increases linearly from immediately after flowering, reaching a maximum level approximately 10 days after flowering and then decreases.

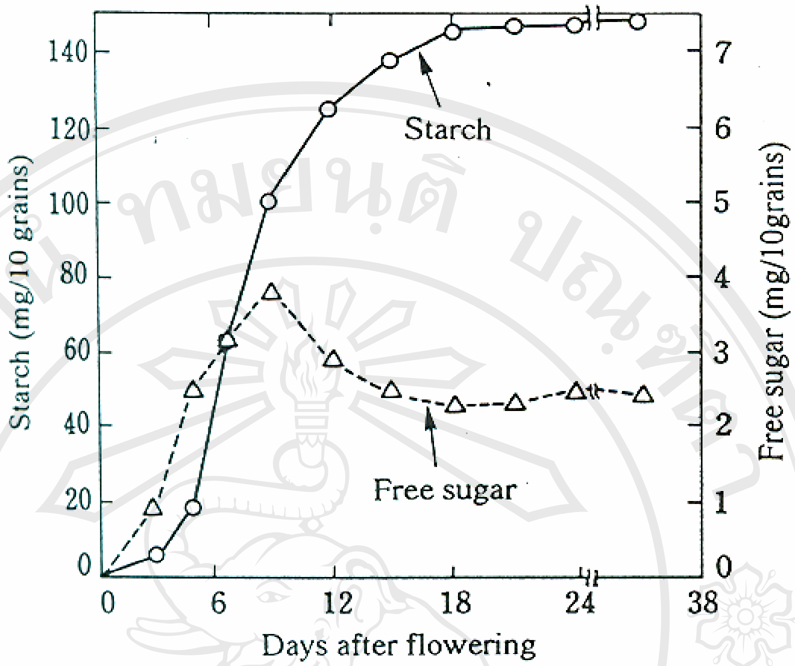

Figure 1.5 Changes in the deposition of starch and free sugars in a rice grain during the ripening stage (Singh and Juliano, 1977).

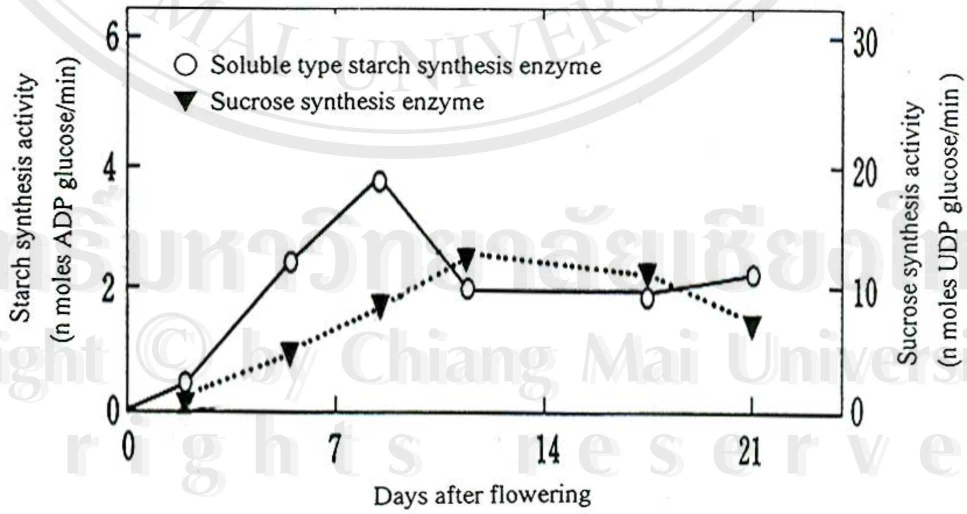

Figure 1.6 Changes in the soluble starch and sucrose synthesis activity (adapted from Juliano, 1985a).

All enzymes involving in the biosynthesis of starch occur in the amyloplast, the site of starch deposition. However, sucrose synthase and invertase, which are both associated with the decomposition of sucrose, as well as hexokinase, which participates in the conversion of glucose into glucose-6-P, exist only in the cytoplasm of the endosperm cell (Echeverria *et al.*, 1988). In other words, the enzymes, which convert sucrose into the substances readily absorbed by amyloplasts, occur in the endosperm cytoplasm, while the enzyme system participating in starch synthesis are compartmentalized in the amyloplast.

1.5.2. Protein formation and accumulation

Protein accumulation accelerates from 5 days after flowering, and reaches a maximum at 20 days after flowering (Figure 1.7). Thereafter until full maturity, the amount decreases slightly, while its concentration is almost constant at about 200 μg N $grain^{-1}$ (Juliano, 1985a). The content of free amino acid is extremely low throughout the ripening period. This means that the translocated N is converted into protein and stored. This pattern is similar to the case of sugar, described above, which is rapidly converted into a high molecular substance and stored (Matsuo *et al.*, 1995). Glutelin is the primary protein of the grain, and increases rapidly from 4 to 6 days after flowering (Figure 1.8). The amount of albumin and globulin start to increase gradually from 5 days after flowering. On the other hand, the prolamin content shows little increase until 5-10 days after flowering and then it begins to increase (Luthe, 1983; Tanaka *et al.*, 1995; Yamakata *et al.*, 1982). However, all protein contents reach a maximum level around 30 days after flowering, thereafter becoming constant.

The above indicates that deposition of prolamin proceeds with a time lag after the deposition of glutelin (Tanaka *et al.*, 1995).

Storage proteins are localized mainly in a proteinaceous organelle called a protein body (PB). In cereal, PBs present in endosperm (and also in aleurone and embryo) serve as the main accumulation site of storage protein. The rice endosperm protein is localized mainly in two types of protein bodies: spherical (PB-I) and irregular-shaped (PB-II). The irregular-shaped PB contained glutelin and globulin, whereas prolamin is localized in the spherical PB (Ogawa *et al.*, 1987; Tanaka *et al.*, 1980). The major storage proteins of rice endosperm are glutelin, while prolamin is regarded as a minor storage protein (Juliano, 1985b). In contrast, prolamin is the predominant storage protein in most of the other major cereals. The two types of protein differ in density, shape and protein composition. The PB-II (2-3 μm in diameter) is larger than PB-I and can be stained uniformly with osmium tetroxide, uranyl acetate and lead citrate (Bechtel and Juliano, 1980; Tanaka *et al.*, 1980). PB-I is formed from rough endoplasmic recticulum, while PB-II is derived from vacuoles (Yamagata and Tanaka, 1986). Protein bodies appear in the sub-aleurone layer at 7 days after flowering. Most protein bodies in rice endosperm are type PB-II, with high electron density (Tanaka *et al.*, 1995).

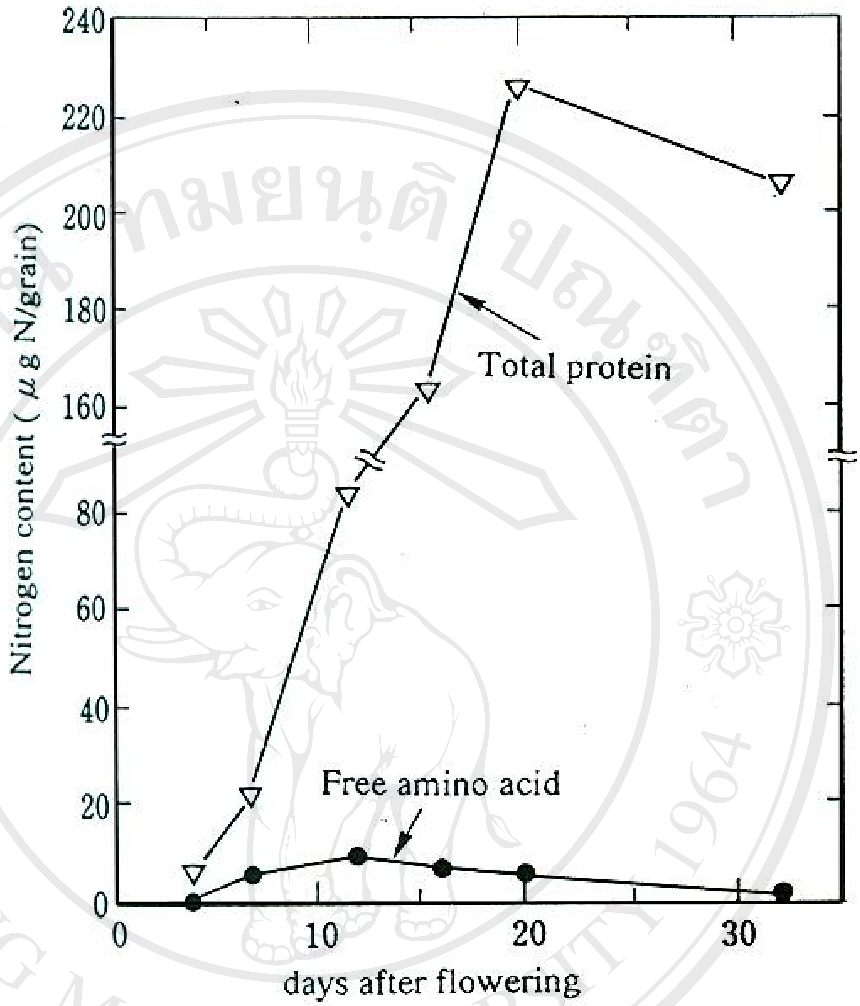

Figure 1.7 Changes in the deposition of protein and free amino acid in rice grain during the ripening stage (Juliano, 1985a).

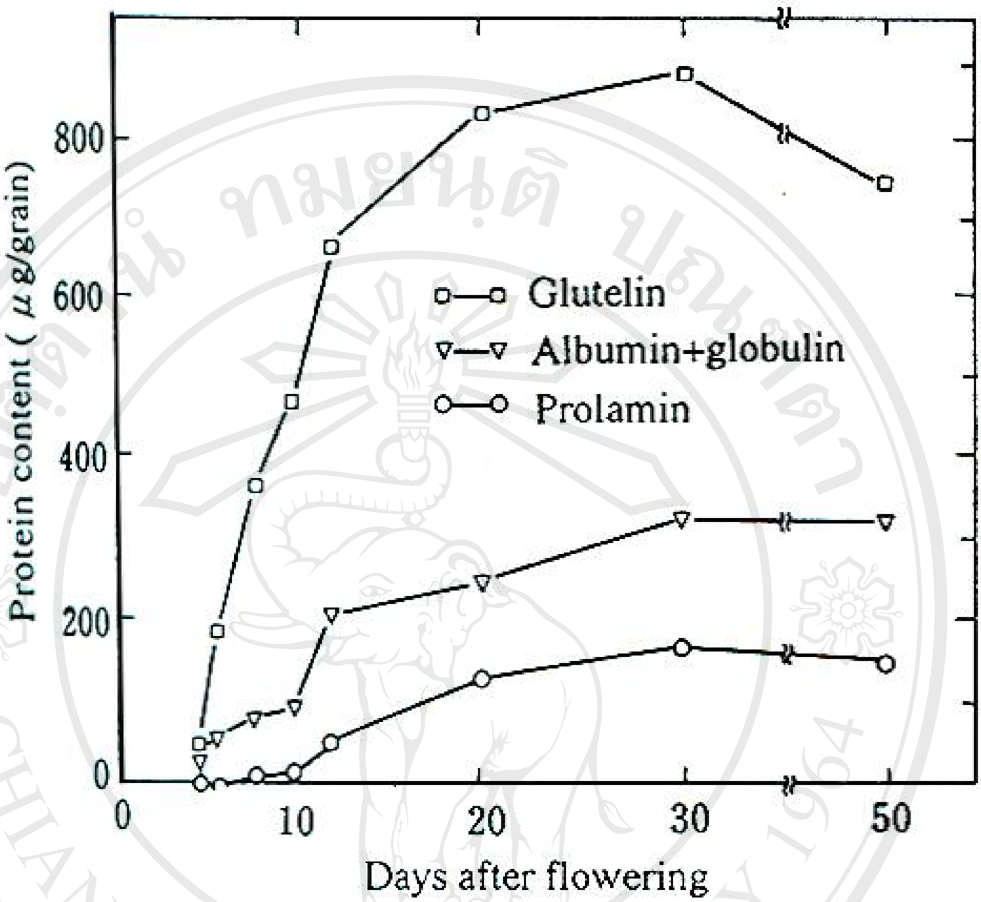

Figure 1.8 Changes in protein fractions extracted from rice grain during the ripening period (Tanaka *et al.*, 1995).

Note: Albumin-globulin extracted by 0.5 M NaCl solution; Prolamin extracted by 60% propanol solution; Glutelin extracted by 1% lactic acid solution.

In term of solubility properties, rice storage proteins can be divided into four classes: albumins are water soluble, globulins are salt soluble, prolamin is soluble in aqueous alcohol solutions and glutelin is soluble in alkali or acid (Juliano, 1985b; Shotwell and Larkins, 1989). The prolamin polypeptide, PB-I, has a molecular weight of 13 kDa. The molecular weights of the glutelin subunits of PB-II are 20 and 40 kDa. Other than these, there exist 3 main kinds of globulin (13, 16 and 26 kDa polypeptides). Polypeptide 26 kDa probably exists in PB-II, but the location sites of other globulins are presently unknown. In addition to the reserve protein in the starchy endosperm, a reserve protein mainly composed of albumin and globulin also accumulates in the aleurone layer and the scutellum (Juliano, 1985a). This reserve protein in the aleurone particles and its molecular origins are completely different from those of glutelin, prolamin and globulin in the starchy endosperm (Matsuo *et al.*, 1995).

In rice endosperm, protein, lipid and other mineral contents generally decrease with distance from the surface to the center of the grain (in both low and high protein rice). Starch and amylose contents of starch increase progressively from the surface to the center of the grain and are lower in high-protein grain. However, high protein rice has more protein fractions in the sub-aleurone layer than low protein rice. Protein and protein bodies of the sub-aleurone layer and the inner endosperm have similar aminograms and electrophoretic patterns using analytical and SDS-polyacrylamide disc gels (Resurreccion *et al.*, 1979).

1.5.3. Aleurone particle formation and accumulation

Aleurone particles occur in the aleurone layer. An aleurone particle seems to be derived from the vacuole, but internally it is composed of plural numbers of membrane systems. Commonly, an aleurone particle contains a phytin globoid. The phytin glodoid typically consists of macronutrients (N, P, K and Mg) and micronutrients (Fe, Mn and Zn). With grain ripening, the phytin globoid develops and occupies the entire vacuole (Matsuo *et al.*, 1995).

1.5.4. Lipid accumulation

The deposition sites of lipids are localized in the aleurone layers and scutellum tissues, but not the starchy endosperm where starch and protein accumulate. The deposition site of lipid is in the spherozome, and neutral lipid constitutes its main part. Lipid deposition appear from around 4 days after flowering and reach a maximum at 12 days after flowering, then become constant thereafter. Hence, lipid deposition begins immediately after the clear differentiation of the aleurone layer and scutellum, and is completed in a very short period. The site of lipid synthesis is considered to be the plastids, but the synthesis mechanism and the storage of neutral lipid in the ripening grain has not been clarified. The deposition of phospholipids and glycolipids occurs earlier than that of neutral lipids, but their contents are extremely small (Matsuo *et al.*, 1995).

1.6. Factors affecting head rice yield

1.6.1. Genotype

In general, head rice recovery is dependent upon grain size, shape and appearance. Varieties and breeding lines with long or bold grains and those having chalky grains perform low head rice yield. Khush *et al.* (1979) reported that head rice recovery could vary from as low as 35% to as high as 92%. The tested IR rice varieties, IR8 and IR5, which have bold and chalky grains, give the lowest head rice yield (55%), whereas IR20, IR26, IR36 and IR42 provide very high head rice yield (90%) (Khush *et al.*, 1979).

1.6.2. Nitrogen fertilization and protein

Time and method of N application also influence nutrient utilization and grain yield. Growth and grain yield can be altered greatly by varying the application time of N fertilizer (Sims *et al.*, 1967). Nitrogen applied during the early reproductive growth phase is more effectively utilized to form grain than N applied during the vegetative lag phase (Wells and Johnston, 1970; Yoshida, 1981). Sims *et al.* (1967) found that delaying mid-season N application increased grain yields. Rice grain yield increased with the addition of 30 kg N ha^{-1} at booting by about 5 and 25% during the wet and dry seasons, respectively (Wopereis-Pura *et al.*, 2002).

Previous studies have shown that nitrogen fertilizers have an adverse affect on the milling quality of rice (Cheaney and Wyche, 1955). Japanese Food Agency (1998) suggested that apply N fertilizer around booting will enhance photosynthetic capacity during the grain filling period and bring to an increase the percentage of head rice. For examples, applying N fertilizer at booting in IR1529 and Sahel180, the milling recovery increased by 3% and head rice ratio increased by 12% in the wet season and by 24% in the dry season. Furthermore, application of 30 kg N ha^{-1} at booting stage increased the N content of the grain by 30% (Wopereis-Pura *et al.*, 2002). Wells and Johnston (1970) and Yoshida (1981) reported that N applied during the early-reproductive growth phase was more effectively utilized to form grain than N applied during the vegetative-lag phase.

Nangju and De Datta (1970) suggested that application of nitrogen up to 60 kg N ha^{-1} increased the head rice yield only of Sigadis. Addition of up to 120 kg N ha^{-1} in the dry season improved the milling quality of the chalky varieties, IR8, IR5 and Sigadis, but not in C4-63, a non-chalky variety. In addition, Seetanun and De Datta (1973) reported that IR20 and RD1 were able to produce higher percentage of head rice when applied N at flowering than applyed at panicle initiation or transplanting.

Head rice recovery was significantly positive correlation with milled rice protein (Perez *et al.*, 1996). The higher grain protein concentration resulted in reduced number of chalky kernels and greater recovery of head rice after milling through reduced breakage (Nangju and De Datta, 1970; Seetanum and De Datta, 1973). However, it seemed that head rice yield responded to grain N content varied greatly with rice genotypes. For example, head rice of Lemont was positively related with grain N content but only slightly in Newbonnet and not in Starbonnet (Borrell *et al.*, 1999). Nangju and De Datta (1970) asserted that the reasons of increasing head rice was probably due to the binding of protein bodies with starch granules resulting in increasing resistance of the rice grain to breakage during milling. This suggests that by increasing N supply to the grain it may be possible to reduce the cracking of the grain, and therefore, the percentage of broken rice will be decreased during milling.

This hypothesis has not yet been tested experimentally. Barlow *et al.* (1973) reported that the starch and storage-protein interface differs between hard and soft varieties in wheat. The entire areas between starch granules are filled with material staining as protein. Water-soluble proteins are confined to a position immediately surrounding starch granules, and this area is capable of rapid swelling on hydration. The total water-soluble material appears to play the role of cementing substances between starch granules and storage protein.

1.6.3. Moisture content at harvest

The optimum grain moisture content for providing the maximum percent head rice is widely affected by variety and environment (Kunze and Prasad, 1978). For example, McNeal (1950) reported that the harvest moisture content for a long grain variety, Rexark, was between 16 to 22% but in a medium long grain variety, Zanith, was between 17 to 24%. Grain moisture at harvest is a factor that can influence kernel breakage. Calderwood *et al.* (1980) found that grain yields tended to decrease by delaying the harvest. The percent head rice of Brazos and Lebonnet varieties increased when harvested at grain moisture content of 20%, but this figure was 16% in Nato and Labelle. Head rice yield of Brazos and Lebonnet varieties decreased by 38% when the grain moisture was reduced from 18% to 15% but not in Labelle variety. The average head rice yields in the dry season were slightly higher than in the wet season, 42 and 37%, respectively. Generally, total milled rice yields averaged from 65 to 68% depending on year and season (Attaviriyasook, 1983).

Harvesting either immature or over-mature crop will decrease the grain yield and milling quality of rice (Huysmans, 1965; Mores *et al.*, 1968). Dilday (1989) suggested that percentage of broken rice increased and head rice recovery decreased significantly as the moisture content of the grain at harvest decreased. Berrio and Cuevas-Perez (1989) also reported that delaying the harvest 2 weeks past 20-25% grain moisture content reduced head rice yield about 18% for 16 tested varieties. Delay in harvest is a common stress that affects milling yield evaluation. Nangju and De Datta (1970) suggested that milled and head rice yields increased to maximum as the moisture content decreased up to the time of optimum yield. The optimum time of harvest for maximum grain and head rice yields was 28-34 days after heading (19-22% wb) in the dry season and 32-38 days after heading (18-21% wb) in the wet season.

In Arkansas and Taxas, rice harvested at 23 to 28% grain moisture resulted in maximum grain yields after drying and the greatest percentage of head rice (Smith *et al.*, 1983). Mores *et al.* (1967) reported that total grain yield of Caloro increased as the moisture content dropped to about 20% and the percentage of head rice peaked at harvest moisture contents of 30 to 28%. In Muda Agricultural Development Authority, Malaysia, in 1985, IR42 had extremely poor head rice yields (38%), when harvested late. IR42 gives excellent head rice yields when harvested at optimum maturity, but readily fissures when over dried or subjected to moisture adsorption stress (IRRI, 1986; 1987).

Huysmans (1965) reported that cracking, referred to as sun-checking, results from rapid fluctuation in atmospheric humidity during the ripening process and late harvest that results in low grain moisture content. The cause appears to be mechanical, fluctuations in temperature and moisture content causing the outer portion of the grain to expand more quickly than the center, resulting in the formation of cracks along endosperm cell walls. Kunze and Prasad (1978) suggested that a crack is a nature cleft in an organ and cracking of whole kernel results in broken kernels of decreased economic value when the mechanical stresses of threshing and milling are imposed. Low moisture content kernels will crack due to moisture adsorption. Such conditions occur when kernels are exposed to high relative humidity or rainfall after rice is physiologically mature. Chalky and immature grains also break easily during milling.

1.6.4. Other factors

Addition to the previous factors, head rice yield has been found to differ with rice growing locality. For example, in the Philippines, IR 42 produces an average head rice of 58% (Khush and Juliano, 1984), but only reaches 38% in Malaysia (Anonymous, 1985). Water management is another factor that is documented to influence head rice yield (Reddy and Hussaini, 1984). At the productive growth stage, a large amount of water is consumed, if drought occurred from panicle initiation to flowering can impair yield and reduce milling quality. Furthermore, high temperature and humidity during ripening as well as post-harvest handling operations can also influence grain breakage during milling (Khush *et al.*, 1979).

Concluding remarks

In domestic and international rice markets, rice quality is more and more an issue of concern. From the literature review, a number of studies have concluded that N fertilizer has a positive correlation with head rice yield recovery after milling, but how N fertilization affects rice grain breakage is still unknown. Furthermore, most of works related on grain physical properties and internal structure have been reported in japonica rice, and quite limited studies on indica rice, especially, Thai rice varieties. An understanding of N fertilizer affect on physical properties and internal structure of rice grain will be useful to elucidate how N fertilizer is able to prevent grain breakage during milling. The proposed study is aimed to investigate the effect of N fertilization on rice grain N concentration and storage protein distribution and accumulation in rice endosperm that may be associated with reduced grain breakage during milling (Chapters 3, 4). This study also examines the quality characteristics that the mill's buyer uses to price the farmer's rough rice (Chapter 2). An understanding of quality characteristics that determine the rough rice price will be useful for farmers to manage N fertilization or harvest time in order to maximize grain quality.