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PHYSICAL AND CHEMICAL PROPERTIES OF MULTIPLE VARIETIES OF NERICA, INDICA AND JAPONICA TYPES OF RICE FOR ASSESSING AND ENHANCING QUALITY

米の品質評価と品質向上のための ネリカ米インディカ米ジャポニカ米の複数の品種の物理化学特性

Hokkaido University
Graduate School of Agricultural Science
Division of Environmental Resources
Doctor Course

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This research is dedicated to

my son, Finnian Olivares Thorne, in gratitude for his gift of inspiration.

SUMMARY

Rice (*Oryza sativa*), due to its adaptability and high caloric value, feeds more than half of the world population. More than 90% of the world's rice is produced and consumed in developing countries in Asia and Africa. However, in Southeast Asia, postharvest losses of rice equate to approximately 37% of the production market value, and to 50% in Sub-Saharan Africa. These losses are mainly caused by deficiencies in technologies for postharvest processing, which is one of the biggest constraints to the rapid expansion of New Rice for Africa (NERICA) type of rice. On the other hand, in Japan, while per-capita consumption has been decreasing, nearly 66% of Japanese prefer to consume high quality and palatable rice compared to cheaper rice. Appropriate analysis and understanding of physical and chemical properties are used for both reducing losses in postharvest processing and predicting rice quality. I, therefore, focused my attention on both physical and chemical properties as basic indicators for assessing and enhancing rice quality. The overall objectives of this research were 1) to provide information for improving the deficiency of technologies for postharvest processing of rice in developing countries and 2) to contribute to the enhancement of the quality and palatability of rice to meet Japanese consumer requirements.

Both moisture content and thickness affect the physical properties of rice. The physical properties of NERICA, Indica and Japonica types of rice were therefore assessed and compared considering different levels of the moisture content of rough rice and different thickness fractions of milled rice. Dimensional, mass and frictional characteristics were measured for each level of moisture content and each thickness fraction. The results obtained provided information that can be useful for improving the deficiency of technologies for postharvest processing. The NERICA and Indica types were found to be similar in the kernel dimensions of rough rice and in the dimensional, mass and frictional characteristics of milled rice. This result could be applied to developing technology-transfer strategies in countries where NERICA production is expanding. The information obtained in this study can contribute to a reduction in postharvest losses, thereby relieving constraints to NERICA expansion, increasing production and enhancing grain quality in developing countries.

Amylose content and protein content are essential to the high palatability and good taste demanded by Japanese consumers. In previous studies, both constituents have been found to influence the physicochemical properties of Japonica varieties produced in the Kyushu and Honshu areas of Japan during the growing period. This study examined the relationship between physicochemical properties and kernel maturity of rice produced in Hokkaido. The analysis was carried out while the brown rice was being processed by a color sorter machine and during processing in a grain elevator. Samples comprised mostly of translucent sound whole kernels, which were classified as mature, were thicker, heavier and had a higher percentage of amylose content and a lower percentage of protein content. This result suggested a relationship of amylose content to physical properties and protein content. Also, samples in the mature level, by contrast to samples in the immature level, indicated the lowest percentage of protein content and were comprised of the thickest and lightest kernel among the levels of maturity within each thickness fraction. The level of protein content also decreased as thickness fraction increased. Consequently, and based also on the fact that higher thickness fractions indicated a range of protein content similar to that associated with high palatability and good taste for Japanese consumers, this suggested that rice could potentially be sorted according to protein content by thickness fraction. As a result, rice of high quality with high palatability and good taste could be obtained.

At grain elevators in Japan, when rice is received from farmers, protein content, moisture content and sound whole kernel rate are automatically inspected using a near-infrared (NIR) spectrometer and a visible light segregator. Ideally, amylose content would be added to the set of properties to predict rice quality. But NIR spectroscopy at grain elevators is not accurate enough at determining amylose content. Based on the relationship between amylose content and physicochemical properties of Japonica non-waxy rice varieties produced in Hokkaido, I developed a calibration model using NIR spectroscopy and physicochemical information. Physicochemical properties were found to improve the accuracy of the calibration model for assessing rice amylose content by NIR spectroscopy. Consequently, the calibration models developed, which are named dual-step calibration models, enable grain quality screening to be done in accordance with rice amylose content at grain elevators. The assessment of rice quality at grain elevators when rice is received from farmers could be improved, contributing to the high palatability and good taste demanded by Japanese consumers.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. CHAPTER I: GENERAL INTRODUCTION

1.1 An introduction to rice

1.1.1 What is rice?

Rice is an edible starchy cereal grain and also the plant by which it is produced (Encyclopedia Britannica, 2016).

The cultivated rice plant belongs to the genus *Oryza*, of the tribe *Oryzeae*, of the family *Poaceae* or *Gramineae*. *Oryza* contains approximately 22 species of which only *Oryza sativa* and *Oryza glaberrima*, are cultivated; wild rice *Zizania palustri* is not related to *Oryza Sativa*. *Oryza sativa* is cultivated globally, including in Asian, North, Central and South American, European Union, Middle Eastern and African countries. Meanwhile, *Oryza glaberrima L* grows mostly in African countries. The *Oryza sativa* and *glaberrima-sativa* cross, commonly known as NERICA (New Rice for Africa), is replacing *O. glaberrima L* in many parts of Africa due to higher production yields (Vaughan and Morishima, 2003).

1.1.2 The importance of rice to mankind

Archaeological evidence in China, Southeast Asia, and the Indus Valley indicated that rice could be approximately eight thousand years old (Bhattacharya, 2011a). Despite its possible late development and continuous cultivation, rice has played an important role in the progress of mankind for thousands of years (Chang, 2003).

Due to its rich genetic diversity, rice is grown and eaten in more than a hundred countries distributed across a wide range of locations and with varying climatic conditions. For that reason, rice is the primary staple for more than half the world's population and is also the major caloric source for a large portion of the human race (Chang, 2003; Bhattacharya, 2011a; Childs, 2017).

The world population currently stands at nearly 7.6 billion but is expected to reach 8.6 billion in 2030, 9.8 billion in 2050 and pass 11.2 billion in 2100. Food demand is projected to increase by between 59% and 98% by 2050 (Elferink and Schierhorn, 2016). Population growth is estimated to be higher in developing countries of Asia and Africa (Elferink and Schierhorn, 2016; United Nations News Service, 2017) where over half of the world’s undernourished people (578 million) reside (Htwe et al., 2017) and where more than 90% of world rice is produced (Childs and Raszap, 2017).

In addition, approximately 900 million of the world’s poorest people—those who have a daily income of less than 1.25 US dollars—depend on rice either as producers or as consumers (Larson et al., 2010). In order to feed the growing population, rice production will have to increase by 26% by the year 2025 (Fujita et al., 2013).

1.1.3 Rice classification

Rice can be classified in various ways. The most common classifications are according to types, levels of production, and grain size and shape.

As has been shown, rice can be classified into *Oryza sativa* and *Oryza glaberrima L* according to its type, group or class. Within *Oryza sativa,* there are the Indica and Japonica types of rice. The Indica type of rice is originally from the tropics. It is short or long grain, is generally slender, has high amylose content, and has a firm and non-sticky texture when cooked. The Japonica type of rice is cultivated in temperate regions. This rice is rather short and round, low in amylose and has a soft and sticky texture when cooked. *Oryza glaberrima L*, on the other hand, is originally from Africa and is well adapted to the African environment (Bhattacharya, 2011d).

The other type of rice, NERICA, which was first developed in West Africa by crossing *Oryza glaberrima L* and *Oryza sativa* in the 1990s, is obtained by conventional crossbreeding and is therefore not genetically modified (Tollens et al., 2013). NERICA combines the high yields of the Asian parent (*Oryza sativa L.*) with the ability to grow in difficult environments of the African parent (*Oryza glaberrima Steud.*) (Fukuta et al., 2012; GRiSP, 2013). The NERICA type of rice is slender and has a wide range of amylose content (Bhattacharya, 2011d).

Rice can also be categorized according to its form after being processed: as harvested from the field, rice is called rough or paddy rice; after the husk is removed, it is known as brown or husked rice; and after the bran layer and germ have been removed it is called milled rice or white rice (Bhattacharya, 2011c).

Rice grain can also be classified according to its size and shape. The size is related to the grain length, while the shape is related to the length-to-width ratio. For brown rice, the scale for size is as follows: extra-long, >7.50 mm; long, 6.61 to 7.50 mm; medium, 5.51 to 6.60 mm; and short, <5.50 mm. Grain shape is characterized as slender, >3.0; medium, 2.1 to 3.0; bold 1.1 to 2.0; and round, < 1.1. While for milled rice, the scale for size is classified as extra-long, >7.0 mm; long, 6.0 to 7.0 mm; medium, 5.0 to 5.59 mm; and short, <5.0 mm. Grain shape is characterized as slender, >3.0; quasi-slender, 2.4 to 3.0; bold 2.0 to 2.39; and round, < 2.0 (Bhattacharya, 2011d).

1.1.4 World rice production and utilization

Rice (milled equivalent), with an average production of nearly 480 million metric tons (MMt), recently ranked number three among the most produced grains in the world (Office of Global Analysis USDA, 2017) (Figure 1.1).

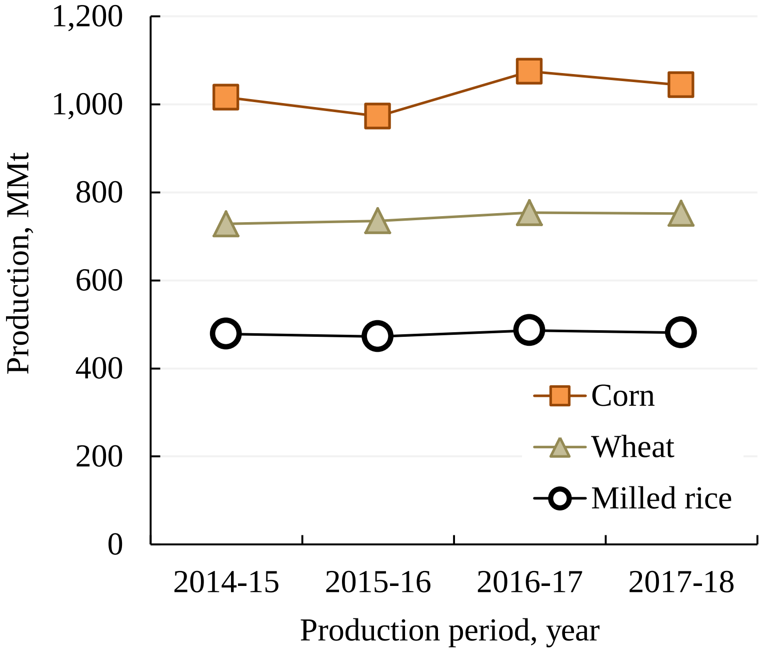

Figure 1.1 Most produced grains in the world
Source: Self-processed from USDA statistics data

Since the beginning of the Green Revolution, global rice production has increased by almost 140% (Mohanty et al., 2013) to reach the more than 715 million metric tons (rough basis) harvested, transported, dried, milled, stored, processed, converted into different products, marketed and consumed in various forms throughout the world in 2016-17 (USDA, 2017). Rice production enables a world rice utilization of around 481 million metric tons of milled equivalent of which about 84% goes to human consumption, 4% to animal feed and 12% to other uses, including seeds, industrial non-food uses, and post-harvest losses (FAOSTAT, 2017).

However, production and utilization (including food, seed, industrial, feed, and waste) are non-uniform; it is focused in Asia, where approximately 90% of the world’s rice is produced and utilized (right-hand plot) (Figure 1.2). In the 2016-17 period, China had the biggest production and utilization (both categories over 140 million metric tons of milled equivalent) in the world, followed by India (both categories approximately 100 million metric tons) (Childs and Raszap, 2017).

Other regions in the world which had a high impact on world production and utilization are North America, South America, and Sub-Saharan Africa (left-hand plot) (Figure 1.2). In

North America, rice production (over 7 million metric tons of milled equivalent) is focused on the United States, while in South America, the highest production and consumption (both categories nearly 8 million metric tons) was reported in Brazil. In Sub-Saharan Africa, the highest production (over 3.5 million metric tons) and highest consumption (approximately 6 million metric tons) was reported by Nigeria (Childs and Raszap, 2017).

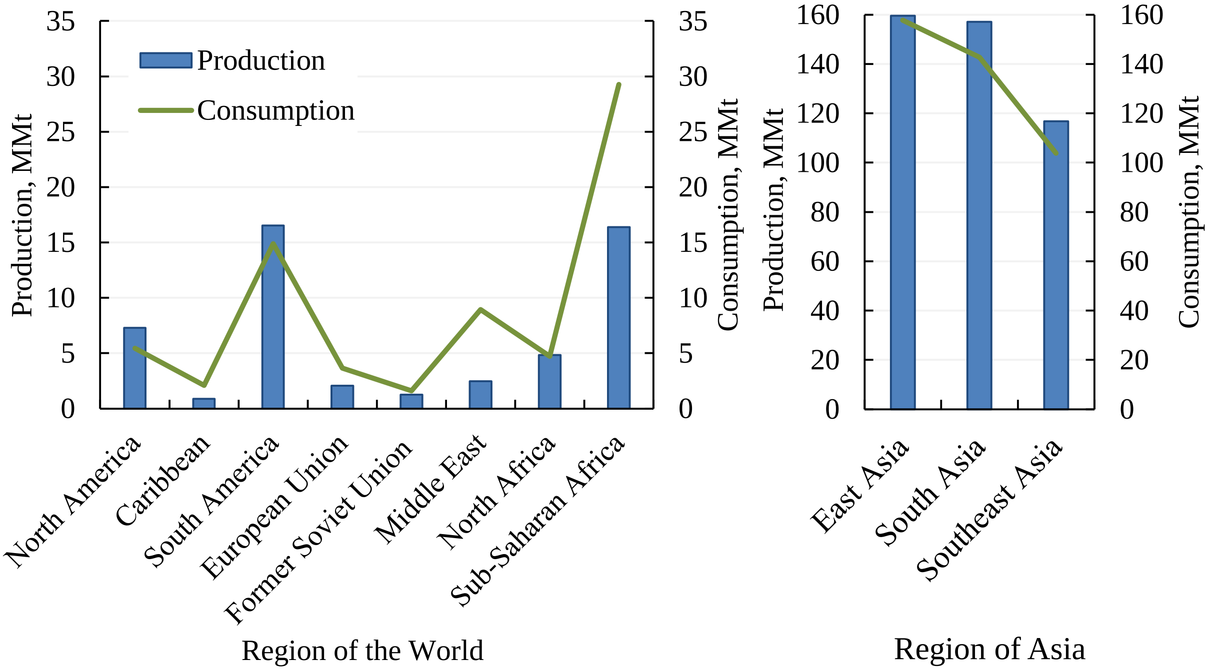

Figure 1.2 World rice production in 2016-17 period
Source: Self-processed from USDA statistics data

1.1.5 Sub-Saharan Africa rice production and utilization

In addition, in Sub-Saharan Africa, more than 70% of poor people (daily income of 1.90 US dollars) depend on agriculture in rural areas for their food. Rice is the staple food and represents the largest sources of food energy in the region (Arouna et al., 2017). However, even though rice production has been increasing in recent decades (over 16 million metric tons within 2016-17 period), levels of consumption still exceed production (over 29 million metric tons in the 2016-17 period). Consequently, the gap between production and consumption is imported (over 13.5 million metric tons in the 2016-17 period), which represents billions of US dollars (USDA, 2017) (Figure 1.3).

Rice production in Sub-Saharan Africa has been increasing in recent decades due to the contribution of NERICA rice (Tollens et al., 2013). NERICA, which has been improving rice production and reducing levels of poverty in the region, have been spreading out in the African continent mostly due to its highly drought resistant and high yielding characteristics (Yokouchi and Saito, 2016).

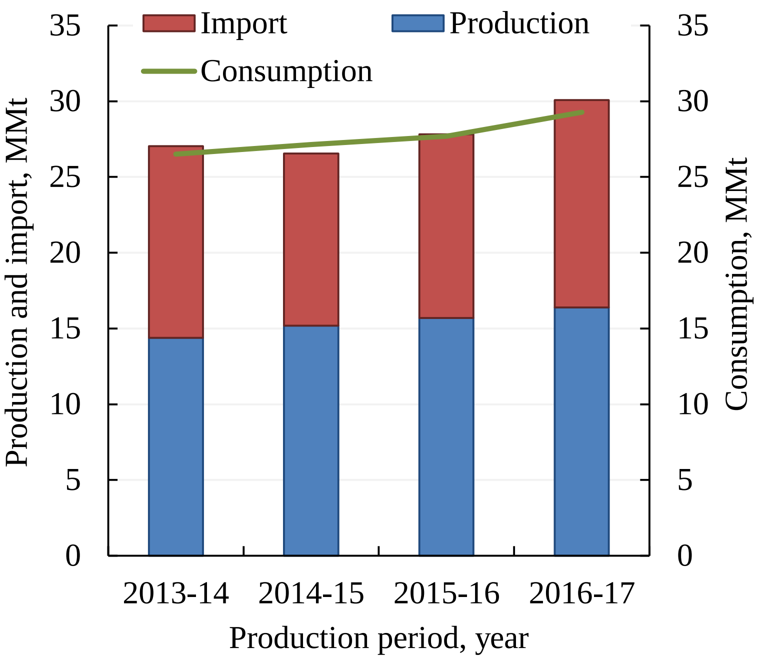

Figure 1.3 Rice production, consumption, and imports in Sub-Saharan Africa
Source: Self-processed from USDA statistics data

However, the deficiency of technologies for rice farming causes a high percentage of losses in the postharvest process and is one of the biggest constraints to the rapid expansion of NERICA (Ndindeng et al., 2016).

1.1.6 Japan rice production and utilization

On the other hand, in Japan, rice production has remained constant (approximately 8 million metric tons of milled equivalent), although per-capita consumption has been decreasing in recent years (USDA, 2017) (Figure 1.4).

Because of the decrease in rice consumption, stocks have exceeded 2 million tons in recent years. Consequently, the Japanese Ministry of Agriculture, Forestry and Fisheries (MAFF), started to subsidize feed rice production, ensuring farmers an income similar or higher than for food rice, resulting in a doubling of feed rice production and decrease of 0.4 million tons in food rice production as well as stocks (Fujibayashi, 2017).

Japanese are consuming more rice snacks and ready-to-eat foods and eating more at restaurants, resulting in an increase in the share of rice eaten in food service establishments (37% of total consumption) and a decrease in cooked rice consumption at home (63% of total consumption) (Fujibayashi, 2017). However, nearly 66% of Japanese prefer to consume palatable rice to cheaper rice. Therefore, since Japan has recently become a more wealthy society, consumers have been demanding higher quality and more palatable rice grains (Hori et al, 2016; Ohtsubo and Nakamura, 2017).

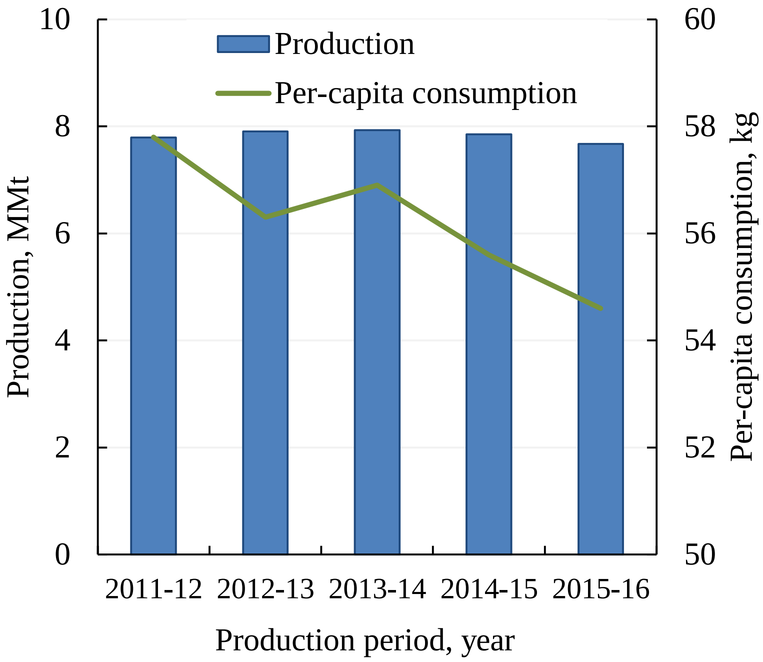

Figure 1.4 Relationship between rice production and per-capita consumption in Japan
Source: Self-processed from USDA statistics data

1.1.7 Hokkaido rice production and characteristics

Hokkaido, the northern island in Japan (42–45°N latitude), is recognized within Japan for having the highest production volume and quality of various agricultural and livestock products including rice, corn, wheat, potatoes, and milk.

However, because the island is large and rich in water, large-scale rice paddies are operated in various areas of Hokkaido. Its rice cultivation area (average of approximately 110 thousand tons) and production weight (average of approximately 600 thousand tons) have ranked number one or two in recent years, competing with Niigata prefecture (Hokkaido Rice, 2017a).

Since Hokkaido is considered to be at the northern limit for rice cultivation, rice varieties in the island must be able to adapt to low temperatures and long natural day length during the growing period. Consequently, Hokkaido rice cultivars, which were created to be produced on the island, are characterized as having extremely low photoperiod sensitivity (Fujino and Sekiguchi, 2005). The most commonly known and consumed varieties include *Yumepirika*, *Nanatsuboshi*, *Kirara*-397, and *Fukkurinko*. Because of its high palatability, *Yumepirika* is particularly well known among Japanese consumers: it is familiar to over 90% of the population of Tokyo, Osaka, and Nagoya, the three largest cities in Japan (Hokkaido Rice, 2017b). This popularity has pushed the average price of production (brown rice 60 kg) up to nearly 17,000 yen in recent years (MAFF, 2017).

1.2 Rice grain structure and constituents

1.2.1 Rice grain structure

In all rice varieties, rice kernel (rough or paddy) weight is composed of approximately 19-22% rice husk (lemma and palea) and 80% brown rice or caryopsis, which is in turn composed of 10% bran layer and embryo, and 69% starchy endosperm or total milled rice (Bhattacharya, 2011a; Muthayya et al., 2014) (Figure 1.5).

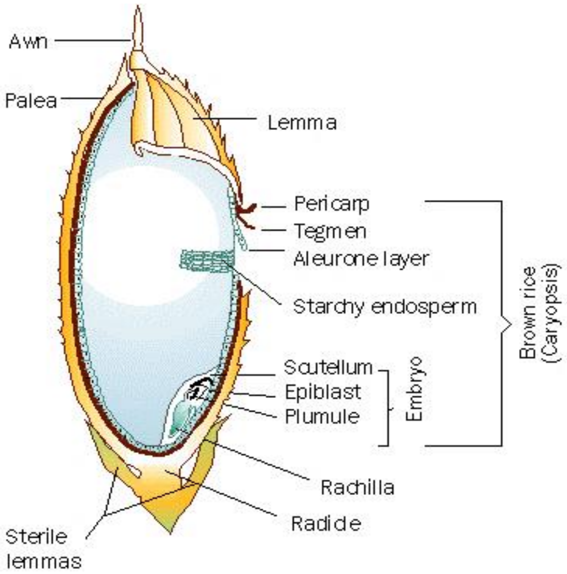

Figure 1.5 Structure of the rice kernel
Source: Reprinted from (Herbert, 2007)

The starchy endosperm forms the large proportion of the grain weight. It is the principal food source for humans and animals and also stores the germinating and growing seedling reserve (Champagne et al., 2004). It is comprised of non-metabolic tissue in the mature grain, which is mostly filled with compound starch granules and protein bodies. At the endosperm boundary, which is the subaleurone layer, the cells are small and the protein portion is higher than starch. Toward the center of the endosperm, however, cells are larger, protein decreases and starch increases (Champagne et al., 2004; Patindol et al., 2015).

1.2.2 Rice endosperm constituents

After removal of the bran layers and embryo, rice is comprised totally of milled rice endosperm. Milled rice endosperm is comprised of starch content of approximately 78% (weight basis) or 90% (dry weight) (Champagne et al., 2004; Li et al., 2017; Silva et al., 2017). The concentration of starch increases from the surface to the center of the endosperm. Protein is the second rich constituent of milled rice, comprising approximately 7-10% of the grain (Katsube-Tanaka et al., 2016; Silva et al., 2017). The concentration of protein, by contrast to starch, is highest toward the surface and decreases toward the center of the endosperm (Patindol et al., 2015). Other constituents of the milled rice endosperm are crude fiber (0.2 to 0.5%), lipid content (0.3 to 0.5%), minerals such as phosphorus and potassium, and vitamins such as niacin, inositol, and choline (Champagne et al., 2004).

Starch is one of the most important agricultural products for the world population, representing nearly 80% of daily calorific intake in some culture. It is also used in non-food applications such as adhesives, coatings, pharmaceuticals, fillers, viscosity modifiers and others. Each application, however, requires properties of starch defined by the macro, micro, and ultrastructure of the starch (Fitzgerald, 2004).

As its definition reflects, milled rice kernel endosperm is mostly comprised of starch granules. Therefore, both the molecular composition and structure of rice starch influence the kernel's visual, textural, nutritional and taste characteristics (Fitzgerald et al., 2009; Biselli et al., 2014). The starch molecular level is composed of two types of glucose polymers: the branched amylopectin and the mostly linear amylose (Biselli et al., 2014; Patindol et al., 2015). The interaction between starch and other components of the rice endosperm such as protein, lipids, and moisture defines both physical and cooking characteristics of rice (Fitzgerald, 2004). In addition, starch quality is determined by aspects such as the structure of both amylose and amylopectin fractions, the amylose and amylopectin ratio, the solubility of each fraction, and the shape and size of starch granules (Fitzgerald, 2004; Li et al., 2017). Amylopectin is essential for the starch synthesis and accounts for the higher fraction of starch. However, amylose content or apparent amylose content, which is the percentage of amylose relative to total starch in rice kernel, is a required indicator of rice cooking and processing characteristics (Fitzgerald et al., 2009; Hori et al., 2016; Ohtsubo and Nakamura, 2017).

Rice amylose content

Amylose content or apparent amylose content, which is the percentage of amylose relative to total starch in rice kernel, is synthesized in the endosperm during the grain-filling period. The enzyme granule-bound starch synthase 1 (*GBSS-1*), which is encoded by a gene called Waxy, is responsible for this synthesis (Chun et al., 2015; Li et al., 2017). Amylose content in rice could range from 0% to 33% (Patindol et al., 2015). Rice varieties are classified according to amylose content as waxy (0–2%), very low-amylose (3–12%), low-amylose (13–20%), intermediate-amylose (21–25%), and high-amylose (over than 25%) (Patindol et al., 2015). Commercially, varieties are classified as low (less than 20% amylose), medium (21–25%) and high (26–33%) (Biselli et al., 2014).

Rice protein content

Rice protein, which is the second major constituent of rice (Arendt and Zannini, 2013), is an important source of nutrition and energy for those who consume rice as a staple food (Shih, 2004) and in developing countries in Asia (Katsube-Tanaka et al., 2016). Due to its hypoallergenic characteristics, rice protein is widely used in processed foods (Bagchi et al., 2016). In addition, rice protein is an essential constituent for determining the texture, pasting capacity and sensory characteristics of rice (Shih, 2004).

Rice endosperm protein, which comprises 7 to 10% of the endosperm (Katsube-Tanaka et al., 2016; Silva et al., 2017), is mostly comprised (95%) of storage discrete particles called protein bodies (PB) (Shih, 2004). Rice endosperm accumulates storage protein as a nitrogen source for germination and the early growth of the next generation (Katsube-Tanaka et al., 2016; Sasou et al., 2016). Rice storage proteins were classified according to solubility into albumin (water-soluble), globulin (salt-soluble), prolamin (alcohol-soluble), and glutelin (alkaline-soluble) (Shih, 2004). Protein bodies are divided into protein body type I (PB-I) and protein body type II (PB-II). PB-I comprises 20% of milled rice protein and is rich in prolamin, while PB-II comprises 60% of milled rice protein and contains mostly glutelins (Wani et al., 2012; Katsube-Tanaka et al., 2016; Sasou et al., 2016).

1.2.3 Factors influencing rice constituents

Factors including production management, soil type and fertility, air temperature, solar radiation, day length, relative humidity, panicle characteristics and kernel maturation, genetic differences, and processing have reported an effect on both amylose and protein contents of rice. Ambient air temperature during grain filling or ripening and kernel maturation (location in the panicle) indicated the most negative impact on agronomic yield of rice and thus quality of the rice grain (Champagne et al., 2004; Siebenmorgen et al., 2013; Chun et al., 2015a; Patindol et al., 2015; Kinoshita et al., 2017; Ma et al., 2017).

Effect of temperature during the endosperm development

In general, high temperatures during the grain filling period decrease the levels of amylose content and amylopectin (Chun et al., 2015a). However, the elevated temperature can result in an increase or no variation in amylose content in some cultivars, indicating that the effect is cultivar-specific (Patindol et al., 2015). Elevated day/night temperatures cause premature termination of grain filling in some cultivars, reduced amylose content, thousand-kernel weight, and kernel thickness, and an increase in protein content and the number of chalky kernels. Day temperature above the average significantly increases the number of chalky kernels caused by poor starch packing during the grain filling (Siebenmorgen et al., 2013; Patindol et al., 2015). Cool temperatures (approximately 18°C) during grain filling cause an increase in amylose content and protein content (Kinoshita et al., 2017a). Thus, high air temperature during grain filling has an enormous negative impact on agronomic yield of rice such as rice milling quality; appearance (higher percentage of chalky kernels), viscosity, gelatinization temperatures, and proximate concentrations, resulting in poorer rice grain quality (Siebenmorgen et al., 2013; Patindol et al., 2015; Kinoshita et al., 2017).

Location in the panicle

The flowering period of spikelets within the rice panicle is long and the flowering date of an individual spikelet is dependent on its location on the panicle. Also, the grain-fill happens from the top of the panicle downward. These facts point to differences in levels of protein and amylose, as well as in growth, weight, maturity and quality, among the kernels located in the upper and lower part, as well as the kernels located in primary and secondary rachis branches, of a rice panicle during rice grain development (Matsue et al., 1994; Myers McClung, 2004; Ma et al., 2017).

Kernels located in primary rachis branches and kernels located in the upper part have reported higher percentages of amylose content (Matsue et al., 1994). This result is caused by the higher activity of GBSSI and hence a higher accumulation of amylose in the starch per kernel (Umemoto et al., 1994; Umemoto et al., 2002; Biselli et al., 2014). By contrast, kernels located in primary rachis branches and kernels located in the upper part of the rice panicle have reported lower percentages of protein content compared to kernels located in secondary rachis branches and the lower part (Umemoto et al., 1994). This is because glutelin molecular weight decreases with the grain-fill (Shih, 2004).

Moreover, the maximum range of variation within a rice panicle of amylose content has reported 3.3% while that of protein content has reported 1.2% (Matsue et al., 1994).

In addition, differences in palatability have been found between grain position in superior and inferior grains based on significant differences in amylose content, protein content and viscosity characteristics. Hokkaido rice varieties have indicated differences in viscosity characteristics compared to Japanese northeastern rice (Ma et al., 2017).

1.3 Rice postharvest losses

1.3.1 What are rice postharvest losses?

Rice postharvest losses are interpreted as physical and quality losses that reduce the rice’s economic value or make it inappropriate for human consumption (Kiaya, 2014).

The physical loss means a reduction in weight of the final usable product from the harvestable paddy. Quality loss means a reduction in value of the usable product due to physical and chemical changes such as changes in grain size, appearance, taste, aroma, cleanliness and other factors that will cause consumers or standards authorities to undervalue or degrade the grain, resulting in low demand, rejection and rice being declared unsuitable for human or animal consumption (Lantin, 1999; Kiaya, 2014).

Postharvest losses commonly occur in processing operations such as harvesting, threshing, cleaning, drying, storage, transportation and milling (Lantin, 1999).

1.3.2 Rice postharvest losses in developing countries

Postharvest losses in Asia, Latin America, the Caribbean and Africa account for 30% of the total weight production. Those losses are caused by spillage and grain loss in all postharvest processes, losses to animals and pests, and inefficiency in rice processing technologies. They result in a 10-30% reduction in the market price of milled rice (IRRI, 2017).

In Southeast Asia, losses in rice have been reported to be in the range of 10 to 37%, while in China, the world highest rice producer, losses have been estimated to be in the range of 8 to 26%. By contrast, India has reported the lowest losses of approximately 3.5% (Kumar and Kalita, 2017).

In Sub-Saharan Africa, rice production constraints cause postharvest losses which account for between 15 to 50% of the market value of production (Abdoulaye, 2007). In Nigeria alone, the highest producer and consumer in Africa, postharvest losses have been reported at approximately 25% of total production, representing an estimated 157 million US dollars (56.7 billion Nigerian Naira) (Kumar and Kalita, 2017). The deficiency of technologies for rice farming and the high percentage of losses in the postharvest process are the biggest constraints to the rapid expansion of NERICA (Wiredu et al., 2014).

Consequently, since the increase in rice production in developing countries has resulted in higher volumes of rice to handle in postharvest processes, improvement of technologies for postharvest processes is required. Such improvements will bring about reduced losses and improved quality, resulting in higher income for farmers (IRRI, 2017). In addition, since NERICA has been produced recently, more detailed information about the type is still required in order to overcome these limitations (Wiredu et al., 2014).

1.4 Rice quality

Rice quality can be described as a combination of properties, mostly physical and chemical, demanded a precise use by a specific user. The more mature the rice grain, the higher its quality (IRRI, 2017).

Rice, in its thousands of varieties, can be produced across a wide range of different latitudes and climate conditions. It is therefore consumed by different cultures in various ways, and different populations have developed different rice preferences (Bhattacharya, 2011a). Rice is also handled and passes through processes such as drying, storage, and milling before consumption. It is therefore difficult to define rice quality with a precise standard, but it can be predicted based on the following parameters: sensory properties such as appearance, texture, taste, and aroma, and physical attributes including size, shape, volume, density and porosity, as well as chemical properties, milling characteristics, nutritive values, among others, which vary from variety to variety (Fitzgerald et al., 2009; Bhattacharya, 2011a; Siebenmorgen et al., 2013; IRRI, 2017). Rice eating quality, based on each of those parameters, will determine rice market and consumer acceptance (Champagne et al., 2010; Hori et al., 2016).

In Japan, rice quality is evaluated by sensory testing and physicochemical measurements. It is based on a method that estimates indirectly the eating quality based on the chemical compositions, cooking quality, gelatinization properties, and physical properties of cooked rice (Ohtsubo and Nakamura, 2017).

1.5 Physical and chemical properties of rice as quality indicator

1.5.1 Physical and chemical properties of rice

Physical property is defined as a property (as color, hardness, boiling point) of matter not involving in its manifestation a chemical change (Merriam-Webster Dictionaries, 2017b). Rice physical properties involve all its external and structural characteristics. Thus, since rice is handled, processed and eaten mostly in whole kernel form, its physical properties are essential factors of its quality. Dimensions, weight, bulk density, and both friction and flow properties are included among rice physical properties (Bhattacharya, 2011d). Since rice characteristics, particularly mass and frictional properties, behave differently when analyzed as individual grain and as grains in bulk, the physical properties of rice must be considered for both individual grains and for rice in bulk. However, assessment should not involve other properties such as cooking, chemical and physicochemical properties (Kunze et al., 2004; Bhattacharya, 2011c).

On the other hand, the chemical property is defined as a property of a substance relating to its chemical reactivity (Merriam-Webster Dictionaries, 2017a). Rice chemical properties describe its sensory characteristics after cooking. Amylose and protein content, gelatinization temperature, gel consistency, alkali spreading value, among others are considered chemical properties (Bhattacharya, 2011a; Siebenmorgen et al., 2013; IRRI, 2017).

Therefore, physical and chemical properties are the focus of our attention as basic characteristics for assessing and enhancing rice quality.

1.5.2 Physical properties of rice for loss reduction

Losses can be classified as quantitative and qualitative. A quantitative loss is a physical loss of a substance and is estimated by a reduction in weight or volume. The qualitative loss is defined as damage of various kinds and is mostly estimated through comparison with standards (Szot et al., 2011).

Processes carried out by agricultural tools and machines during harvest and post-harvest operations, and in storing, processing and transporting, usually cause losses and damage to grains, resulting in a deterioration in quality (Mohsenin, 1986a; Gliński et al., 2011). Therefore, appropriate analysis and comprehension of physical properties can support the accurate design of machines used in farming, harvesting, and storage, in processes for producing high-quality food, and to reduce post-harvest process losses (Dobrzański and Stępniewski, 2013).

1.5.3 Physicochemical properties of rice as quality indicators

Physicochemical property is defined as of or being both physical and chemical property (Merriam-Webster Dictionaries, 2017c). Thus, physicochemical properties of rice encompass both physical and chemical properties.

As stated above, the physical properties of rice are related to its external and structural characteristics, and chemical properties are related to its sensory characteristics after cooking. Since rice is processed from paddy to milled rice and is cooked before being consumed, the processing and product-making quality of rice are highly affected by physicochemical properties (Bhattacharya, 2011a).

For that reason, physicochemical properties are considered the most important aspect of rice quality (Bhattacharya, 2011a; Siebenmorgen et al., 2013).

1.6 Amylose and protein content as rice quality indicators

As explained above, amylose and proteins are the major constituents comprising the rice endosperm (Arendt and Zanini, 2013), and amylose and protein content are required indicators of rice processing and cooking characteristics (Shih, 2004; Fitzgerald et al., 2009; Xie et al., 2014; Hori et al., 2016; Ohtsubo and Nakamura, 2017).

Varieties with high amylose content, which cook dry rice with firm and separate grain, are mostly eaten in European and Latino-American countries (Nakaura et al., 2012; Biselli et al., 2014; Hori et al., 2016). Low amylose content rice varieties and rice varieties with lower protein content, which both tends to cook more tender, glossy and sticky grain, are considered to be very palatable in Northeast Asian countries (Kawamura et al., 2013; Ohtsubo and Nakamura, 2017).

Furthermore, for the *Yumepirika* variety produced in Hokkaido, Japan, the best combination for high palatability and good taste for people in Hokkaido was reported for rice with amylose content of < 19 % and protein content of < 7.5 % and/or amylose content of ≥ 19 % and protein content of ≤ 6.8 % (Kawamura et al., 2013).

1.6.1 Traditional methods for assessing amylose and protein contents of rice

Traditional methods for assessing amylose content include iodine-binding, titrimetric, amperometric, size-exclusion chromatography, asymmetric field flow fractionation, marker-assisted selection, potentiometric titration using an autotitrator, and paper-based microfluidic chip. Among them, iodine-binding, also known as iodine colorimetry or amylose-iodine, is the only validated (Biselli et al., 2014) and most commonly used procedure for determining amylose content (Fitzgerald et al., 2009; Kaufman et al., 2015; Ohtsubo and Nakamura, 2017). It involves the absorbance at 620 or 720 nm of the amylose-iodine complex; amylose is computed against a calibration curve (Saenger, 1984; Wang et al., 2011; Hu et al., 2015).

Protein content (also called crude protein content) is traditionally determined from both Kjeldahl and Dumas nitrogen affected by the factor 5.95. This factor is based on the nitrogen content of glutelin, which is the major rice protein (Chang, 2010).

However, traditional methods for assessing amylose and protein contents are labor-intensive, time-consuming, chemical dependent, and vulnerable to random error (Juliano, 1979; Delwiche et al., 1995; Delwiche et al., 1996; Wang et al., 2011; Duan et al., 2012; Biselli et al., 2014; Xie et al., 2014; Hu et al., 2015; Kaufman et al., 2015). To overcome this shortcoming, near-infrared (NIR) spectroscopy has been used in combination with chemometric techniques as an alternative non-destructive method for assessing rice amylose and protein content. This method is rapid (once the calibration model has been developed), chemical-free, easy to use, and non-destructive (Manley, 2014), (Satake, 1988; Villareal et al., 1994; Delwiche et al., 1996; Himmelsbach et al., 2000; Sohn et al., 2004; Cozzolino et al., 2014; Xie et al., 2014; Porep et al., 2015; Bagchi et al., 2016; Kawamura et al., 2017).

1.7 Brief review of near-infrared spectroscopy

Principle of Near-Infrared Spectroscopy

In general, spectroscopy analyzes the interaction between light and matter (Hughes et al., 2015). Thus, the complex chemical composition makes organic molecules in a sample absorb light in the NIR wavelengths and vibrate at a specific frequency. A NIR spectrometer measures the NIR light absorbed by the sample as a function of the reflected or transmitted light. The NIR spectra achieved from the absorption at various wavelengths can be associated to the level of a precise constituent of the sample (Guidetti et al., 2012; Agelet and Hurburgh, 2014; Kawamura and Koseki, 2017).

The NIR region, which ranges from approximately 780 to 2500 nm, is located between the visible region (from approximately 380 to 780 nm) and the mid-infrared (MIR) region (from approximately 2500 to 50,000 nm) of the electromagnetic spectrum (Agelet and Hurburgh, 2014; Manley, 2014; Porep et al., 2015).

The NIR spectrum is comprised of absorption bands related to overtones and combinations of fundamental vibrations observed in MIR region (Ozaki, 2012; Cozzolino, 2014; Manley, 2014; Beć et al., 2016). Because most of the organic compounds are comprised of a complex molecular structure, the NIR spectrum involves broad and numerous overlapping bands resulting in multicollinearity (Cozzolino, 2014; Manley, 2014; Porep et al., 2015). The most distinguished absorptions bands in the NIR region arise from overtones and combination bands of fundamental vibrations of bonds comprising by hydrogen (H) (lightest atoms, therefore shows the largest vibrations) and Carbon (C), Nitrogen (N), Oxygen (O) and Sulphur (S) (other light atoms) such as C-H, N-H, O-H and S-H. Those absorption bands from overtones and combinations of fundamental vibrations of C-H, N-H, O-H and S-H bond arise in the MIR region, although they are still observed in the NIR region but much weaker than the fundamental absorption bands (Blanco and Villarroya, 2002; Bokobza, 2002; Ozaki, 2012; Cozzolino et al., 2014; Porep et al., 2015). Consequently, NIR spectroscopy is ruled by hydrogen (Ozaki, 2012). Since a large range of organic constituents is comprise by Hydrogen atom within their molecules, NIR spectroscopy is an appropriate technique for their determination (Alander et al., 2013; Manley, 2014; Porep et al., 2015).

In addition, because the absorption of overtones and combinations in NIR region is much weaker than the fundamentals in the MIR region (a factor of 10-100), wavelengths in the NIR range from approximately 800 to 1100 nm are able to deeply penetrate through samples that are not excessively thick and opaque (Blanco and Villarroya, 2002; Agelet and Hurburgh, 2014; Porep et al., 2015). Such characteristics allow its application in biomedical areas and to agricultural products such as meat, fish, fruit, and grains (including individual pieces and in bulk) (Ozaki, 2012; Alander et al., 2013).

Near-Infrared Instrumentation

A crucial factor for the consolidation of NIR Spectroscopy as an analytical technique is the development of NIR instrumentation (McClure, 2003). The instrument that operates in the NIR region is called spectrometers (Barton, II, 2016).

A NIR spectrometer is composed of the following pieces: a radiation source, a device for wavelength selection (e.g. a monochromator), a sample chamber, a detector, and a computer or signal processor (Porep et al., 2015; Barton II, 2016). Among them, the radiation source such as a glow bar or a halogen lamp, the device for wavelength selection such as discrete filters, tilting filters, prisms, gratings, diode arrays, acousto-optic tunable filters (AOTFs) or interferometers, and the detector such as silicon, complementary metal-oxide-semiconductor (CMOS), lead sulphide (PbS), lead selenide (PbSe) or indium gallium arsenide (InGaAs) are the basic and most important pieces (Barton II, 2016). Consequently, selection and arrangement of them within a NIR spectrometer defines its wavelength range, the NIR spectrum appearance as well as its performance (Agelet and Hurburgh, 2010; Guidetti et al., 2012; Alander et al., 2013; Barton II, 2016).

Measurement modes for obtaining NIR spectra from a sample are “transmission”, “reflection”, “transflection”, and “interaction”. The difference among the modes is related to the position of both the radiation source and detector around the sample (Guidetti et al., 2012; Alander et al., 2013; Porep et al., 2015). However, the most common modes in agriculture are reflection and transmission. In reflectance mode, a fraction of the initial diffuse reflected radiation is measured after it has penetrated the sample by a certain distance and returned to the sample surface (Agelet and Hurburgh, 2014). Diffuse reflectance is recommended for use in a range of wavelengths between 1200 nm and 2500 nm and for measuring thicker and denser samples (Agelet and Hurburgh, 2010). On the other hand, transmittance mode, which has higher penetration and lower absorption, is recommended for use in a range of lower wavelengths between 700 nm and 1100 nm and for measuring samples in bulk (Agelet and Hurburgh, 2010; Agelet and Hurburgh, 2014).

Moreover, when the light is attenuated by the sample in reflectance mode the optical geometry measurement is stated as reflectance (R); meanwhile, in transmittance mode, it is stated as transmittance (T). And for developing further chemometric analysis, they are used as "log 1/R" and "log 1/T" values (Porep et al., 2015; Kawamura and Koseki, 2017).

Chemometrics for calibration model development

Another essential factor for the consolidation of NIR Spectroscopy as an analytical technique is the development of chemometrics (McClure, 2003). Since NIR spectra of a sample comprise an enormous number of absorptions measured at various wavelengths, data from the NIR spectra can be considered as multivariate (Metrohm AG, 2013). Multivariate data analysis can be seen as the core of chemometrics (Ozaki, 2012).

The objective of multivariate data analysis is to detect the quantitative relationships between NIR absorption spectra data and values of constituents determined by the reference method (Agelet and Hurburgh, 2014; Porep et al., 2015; Kawamura and Koseki, 2017). This process is commonly called the development of the calibration model. Therefore, the developed calibration model should be able to predict accurately the desired constituent using NIR spectroscopy technique, thereby replacing the labor-intensive and time-consuming reference method (Porep et al., 2015). Because aspects such as the type of soil, varieties, and environmental changes are changeable, calibration models related to grains must be updated after a certain period of time (Agelet and Hurburgh, 2014).

Multivariate data analyses include both linear and non-linear methods. Within the linear method, well-recognized techniques such as principal component analysis (PCA), principal component regression (PCR), multiple linear regression (MLR), and partial least squares (PLS) are the most used. Linear discriminant analysis (LDA) and partial least squares discriminant analysis (PLS-DA) are also techniques used for authentication and traceability of cereals (Ozaki, 2012; Cao, 2013; Cozzolino, 2014; Porep et al., 2015). Meanwhile, within the non-linear method, techniques such as support vector machines and neural networks are used (Cao, 2013; Porep et al., 2015).

NIR spectroscopy is reliant on chemical method (reference method) for developing the calibration model and its validation. Thus, the precision and accuracy of the NIR calibration model will be highly affected by the reliability of both reference laboratory and NIR spectra data (Agelet and Hurburgh, 2014). The high signal-to-noise ratio is considered trustworthy NIR spectra data (Manley, 2014). Sample preparation of both reference method and record NIR spectra would account for 60-70% of the overall error of the analysis (Williams, 2001).

What is more, light scattering in solid or opaque liquid samples, variations in temperature, density, and particle size of samples, and spectral noise of the spectrometer produce prejudicial spectral variations and baseline shifts, which usually affect NIR spectra. For that reason, pre-treatment methods such as noise reduction, baseline correction, resolution enhancement, centering, and normalization are carried out to the original spectra before carrying out the calibration model (Ozaki, 2012; Porep et al., 2015; P. Williams, 2001). Methods such as moving-average, Savitzky-Golay, Multiplicative Scatter Correction (MSC), Orthogonal Signal Correction (OSC), and standard normal variate (SNV) are commonly used (Blanco and Villarroya, 2002; Agelet and Hurburgh, 2014; Porep et al., 2015).

Validation of the calibration model is an essential process to evaluate the accuracy of the calibration model to predict new samples as well as to determine its future applications such as in rough screening, screening, quality control, etc. (Cao, 2013; Agelet and Hurburgh, 2014; Manley, 2014; Porep et al., 2015; Westad and Marini, 2015). The most appropriate validation is usually carried out using independent samples, in other words, samples that have not been included in the calibration set (Agelet and Hurburgh, 2014; Westad and Marini, 2015). Bagging and cross-validation are other methods that are used when the quantity of sample is limited. However, statistics from those methods are not always reliable (Agelet and Hurburgh, 2010; Agelet and Hurburgh, 2014; Westad and Marini, 2015).

On the other hand, validation statistics such as coefficient of determination (R^2), systematic error (difference between reference and prediction values (Bias)), standard error of prediction (SEP), and the ratio of SEP to standard deviation of reference data (RPD) are commonly used for determining the robustness of the calibration model (Agelet and Hurburgh, 2010; Cao, 2013; Agelet and Hurburgh, 2014). Lower values of SEP and Bias as well as higher values of R^2 and RPD are desired (Porep et al., 2015). However, the root mean square error of prediction (RMSEP), which is an estimate of the variation of the reference and predicted values of an independent validation set, has been also referred to as a validation statistic (Porep et al., 2015; Westad and Marini, 2015; Sampaio et al., 2017).

1.7.1 Near-infrared spectroscopy for assessing quality indicators

In recent years, various researchers have evaluated quality indicators of rice by NIR spectroscopy.

Quality indicators such as amylose, protein, moisture contents, appearance, milling characteristics, gelatinization temperature, gel consistency, textural properties, and rapid visco analyzer (RVA) related properties have been analyzed by NIR spectroscopy with acceptable accuracy (Satake, 1988; Delwiche et al., 1996; Kawamura et al., 1999; Bao et al., 2001; Kawamura et al., 2002; Meadows and Barton, 2002; Sohnet al., 2004; Natsuga and

Kawamura, 2006; Bao et al., 2007; Wu and Shi, 2007; Bagchi et al., 2016; Ohtsubo and Nakamura, 2017).

Among quality indicators, several researchers have focused on amylose content, the most important constituent of rice. Amylose content has been determined by NIR spectroscopy techniques in milled rice flour (Satake, 1988; Delwiche et al., 1995; Himmelsbach et al., 2000; Sohn et al., 2004; Bao et al., 2007; Wu and Shi, 2007; Sampaio et al., 2017), single-kernel (XIAO et al., 2003; Wu and Shi, 2004, 2007; Bao et al., 2007; Rash and Meullenet, 2010), bulk kernel (Villareal et al., 1994; Shimizu et al., 2003; Rash and Meullenet, 2010; Bagchi et al., 2016; Kawamura et al., 2017; Ohtsubo and Nakamura, 2017) (brown or milled rice), single cooked rice kernel (Okadome et al., 2002). It has been determined using transmittance or reflectance modes as well as NIR Fourier-transform Raman (NIR-FT/Raman) spectroscopy (Himmelsbach et al., 2000). However, in almost all the results, the accuracy obtained was only suitable for application in breeding programs.

1.7.2 Assessing amylose content by near-infrared spectroscopy at a rice grain elevator

Various studies have reported that rice protein content, as well as moisture content, can be determined by NIR spectroscopy with high accuracy. Such accuracy allows NIR spectroscopy to be put to practical for determining both constituents at rice grain elevators. However, the use of NIR spectrometer alone is not sufficient for developing an accurate calibration model for determining amylose content in milled rice (Kawamura et al., 2014).

Based on the influence of amylose content on rice whiteness and the degree of translucency through the grain, the combined analysis of predicted amylose content and color information decreased the SEP and thus enables reasonable non-destructive determination of amylose content of rice at grain elevators (Jo et al., 2015; Kato et al., 2016; Kawamura et al., 2017). Moreover, the use of physical properties of a single kernel of cooked rice from waxy and non-waxy Japonica varieties produced in Hokkaido, Tohoku, Hokuriku, Chugoku, and Kyushu indicated that the accuracy of regression equations for predicting amylose content was higher than using the reference method of determination. Regression equations were accurate using a wide range of amylose content (glutinous and non-glutinous rice 0-30%) as well as a narrow range (non-glutinous rice 15-20%) (Okadome et al., 2002).

On the other hand, at grain elevators in Japan when rice is received from farmers, its quality is inspected automatically based on protein content, moisture content and the percentage of the sound whole kernel of brown rice (Kawamura, 2015). Since amylose content is one of the most important rice quality indicator constituents, it is desirable to include it into the group of properties used to predict rice quality at grain elevators. The desirable outcome is to reach higher accuracy in predicting amylose content of rice by NIR at grain elevators in Japan.

1.8 Objectives of the research

As stated above, increasing rice production in developing countries has led to a higher volume of rice being handled in postharvest processes. However, the deficiency of technologies for rice farming has been producing a high percentage of losses in the postharvest process and a reduction in market price. In addition, despite a decrease in per-capita rice consumption in Japan in recent years, Japanese consumers have been demanding higher quality and more palatable rice grains.

Consequently, the main objectives of this research were 1) to provide information for improving the deficiency of technologies for postharvest processing of rice in developing countries and 2) to contribute to enhancing the quality and palatability of rice to meet Japanese consumer requirements.

Since appropriate analysis and comprehension of physical properties can help reduce post-harvest process losses, and information on physicochemical properties is essential for predicting rice quality, both physical and physicochemical properties were examined as basic indicators for assessing and enhancing the rice quality. To meet the main objectives, 1) the physical properties of NERICA, Indica and Japonica types of rice were assessed and compared considering different levels of moisture content of rough rice and different thickness fractions of milled rice; 2) I examined the relationship between physicochemical properties and kernel maturity of rice produced in Hokkaido; and 3) I analyzed whether, by using the information on physicochemical properties, the accuracy of the calibration model for determining amylose content of rice by near-infrared spectroscopy could be made more accurate and put to practical use.

1.9 Organization of dissertation

This research addressed significant issues in rice production, such as deficiency of technology for postharvest processes in developing countries and enhancement of rice quality to meet Japanese consumer requirements. In this dissertation, I will address the above-stated research objectives according to the following structure.

Chapter two will focus on the deficiency in technology for postharvest processes in developing countries. Consequently, the physical properties of NERICA, Indica, and Japonica types will be assessed and compared to provide information that could be useful for improving the deficiency of technology for postharvest processing of rice. In addition, chapters three, four and five will focus on the enhancement of rice quality to meet Japanese consumer requirements. In the third chapter, information on the diversity of physical and chemical properties of rice varieties produced in the Hokkaido region will be analyzed to reduce the dimensionality and increase interpretability among properties. The fourth chapter deals with the relationship between kernel maturity and physicochemical properties for assessing quality constituents of rice produced in Hokkaido during postharvest processes. The fifth chapter explores whether using information on physicochemical properties could improve the accuracy of the calibration model for determining amylose content of rice by near-infrared spectroscopy. Finally, the sixth chapter presents overall conclusions and suggestions for future studies.

2. CHAPTER II: ASSESSMENT AND COMPARISON OF PHYSICAL PROPERTIES OF NERICA, INDICA AND JAPONICA TYPES FOR ENHANCING RICE QUALITY

2.1 Summary

This chapter focuses on the deficiency of technology for postharvest processes in developing countries. Consequently, the physical properties of NERICA, Indica, and Japonica types will be assessed and compared to provide information that could be useful for improving the deficiency of technology for postharvest processing of rice.

2.2 Introduction

Rice production has been increasing to meet the needs of growing populations of developing countries (United Nations News Service, 2017), most of Asia and Africa, where rice is the primary staple and the major caloric source (Childs, 2017). However, losses in postharvest processes arising from the deficiency of technology account for a high percentage of the total production (Abdoulaye, 2007; IRRI, 2017; Kumar and Kalita, 2017). This is one of the biggest constraints to the rapid expansion of NERICA (Wiredu et al., 2014). Consequently, technology for postharvest processes needs to be improved (IRRI, 2017). In addition, since NERICA has being produced recently, more detailed information about it is still needed in order to support its expansion (Wiredu et al., 2014).

Appropriate analysis and comprehension of physical properties help in the design of machines used in farming, harvesting, and storage, in processes necessary for producing high-quality food, and in reducing post-harvest process losses (Dobrzański and Stępniewski, 2013). Both moisture content and thickness have been reported to affect the physical properties of rice. Moisture content principally affects rice's dimensions, volume, bulk density, and coefficient of friction (Kunze et al., 2004; Bhattacharya, 2011c). Meanwhile, thickness influences drying, processing, and quality (Wadsworth et al., 1982); the head rice yield and degree of milling (Sun and Siebenmorgen, 1993); and the physical properties of rough and brown rice (Edenio et al., 2015).

Since rice is processed from paddy to milled rice before it is cooked to be consumed, the physical properties of NERICA, Indica and Japonica types of rice were assessed and compared considering different levels of the moisture content of rough rice and different thickness fractions of milled rice. The hypothesis was that assessing and comparing the physical properties of the most consumed types of rice at different levels of processing will provide useful information for improving deficiency of technology for postharvest processing of rice, helping to reduce postharvest losses, increase quality, and consequently secure higher incomes for farmers in developing countries.

2.3 Materials and methods

2.3.1 Rice sample

Five fresh-harvested rice varieties produced in 2014 were used to examine the effect of moisture content on physical properties of rough rice: NERICA varieties *NERICA-1* and *NERICA-4*; Indica varieties *IR-28*, *IR-50*; and Japonica variety *Yumepirika*.

Seven varieties of rice produced in 2013 were used to examine the effect of thickness fraction on the physical properties of milled rice: NERICA variety *NERICA-4*; Indica varieties *IR-28*, *IR-50* and *IR-64*; and Japonica varieties *Nanatsuboshi*, *Yumepirika* and *Oborozuki*.

NERICA and Indica varieties were produced in the Japan International Cooperation Agency (JICA) Tsukuba International Centre, Ibaraki Prefecture, Japan. Japonica varieties were produced at the Hokkaido University Farm, Sapporo, Hokkaido, Japan.

2.3.2 Rice sample preparation

Each of the fresh-harvested rice samples was dried to at least five moisture content levels, with moisture content decreasing by gradations of approximately 3%, using a laboratory grain test dryer (Shizuoka Seiki Co., Ltd, Japan). Meanwhile, each of the milled rice samples was divided into 3 thickness fractions using a laboratory thickness grader (SATAKE Engineering Co., Ltd, Japan).

2.3.3 Methods for determining physical properties

Dimensional characteristics

Slenderness (ratio of kernel length to kernel width) *Sl*, was calculated using Equation 2.1 (Mohsenin, 1986). The volume of kernel *Kv* was calculated using Equation 2.2 (Jain and Bal, 1997). Both were determined as a function of length *L*, width *W*, and thickness *T*.

$$Sl = \frac{L}{W} \qquad \text{Equation 2.1}$$

$$Kv = \frac{1}{4}\left[\left(\frac{\pi}{6}\right) L(W + T)^2\right] \qquad \text{Equation 2.2}$$

Length *L*, width *W*, and thickness *T*, of the kernel, were determined by image-analysis software Grain Dimension version 1.6 (Shizuoka Seiki Co., Ltd, Japan).

Mass characteristics

Thousand-kernel weight *TKW* was determined by weighing 1,000 randomly drawn regular rice kernels in an electronic balance (Sartorius Lab Holding GmbH, Germany) and expressed in g (Bhattacharya, 2011b).

Bulk density *BD* was determined using a grain volume-weight tester (Brauer type, Kiya Engineering, Tokyo, Japan) and expressed as g/L (Bhattacharya, 2011b).

Grain fluidity *GF* was determined using a grain fluidity tester and expressed as g/s (Kawamura, 2015).

Frictional characteristics

Static angle of repose θs was determined using a Perspex box (Bhattacharya, 2011b).

The static coefficient of friction was determined using an inclined plane (Bart-Plange and Baryeh, 2003). Rubber material used on the belt conveyor at a grain elevator in Japan was used as the test surface.

Moisture content

Moisture content was determined by the Japanese Society of Agricultural Machinery and Food Engineers (JSAM) standard method: about 10 g of whole grain rice was placed in a forced-air oven at 135°C for 24 h and computed on a wet basis.

Composition analysis

Components of milled rice such as sound whole, broken, chalky, damaged, and discolored kernels were divided by human observation and expressed as a percentage of the weight (Japan Rice Millers Association, 1997).

Statistical analysis

Two-way analysis of variance (ANOVA) and Tukey's test with 99% of confidence was carried out to determine any significant differences among the means of physical properties by moisture content level among varieties and among moisture content levels within each variety.

One-way ANOVA and Tukey's test with 99% of confidence were carried out to determine any significant differences among the means of physical properties among thickness fractions within each variety.

2.4 Results and discussion

Information was collected on physical properties of types of rice used in this research. Given that, in contrast to Indica and Japonica types of rice, relatively little is known about the physical properties of NERICA rice, results were focused on a comparison of physical properties of NERICA with those of Indica and Japonica types of rice.

2.4.1 Effects of moisture content on physical properties of rough rice

In general, dimensional, mass, and frictional characteristics decreased as moisture content decreased. Two-way ANOVA reported that such characteristics were highly affected by moisture content.

Dimensional characteristics of rough rice

The NERICA varieties were closer in length, width, and thickness to Indica variety *IR-28* (Table 2.1), and thus showed similar kernel volume. Two-way ANOVA did not report significant differences in volume among *NERICA-1*, *NERICA-4,* and *IR-28*. Moreover, *NERICA-4* shrank much more in length than it did in width with every decrease in moisture content level. As a result, its slenderness decreased as moisture content decreased (Table 2.1).

Table 2.1 Average value of dimensions of rough kernel by level of moisture content

Variety	Moisture content %, w.b., 135°C	Length mm	Width mm	Thickness mm	Slenderness -[a]	Volume mm^3
	n = 3	n = 200	n = 200	n = 200	n = 200	n = 200
NERICA-1	10.3	8.70 b	2.98 c	2.06 c	2.92 a	29.2 c
	13.4	8.73 b	3.03 b	2.10 b	2.88 ab	30.2 b
	16.6	8.78 b	3.05 b	2.12 ab	2.88 b	30.9 b
	19.6	8.86 a	3.10 a	2.15 a	2.86 b	32.1 a
NERICA-4	10.2	8.98 d	2.93 b	2.08 c	3.07 b	29.6 d
	13.1	9.22 c	2.95 b	2.11 bc	3.13 a	31.0 c
	16.7	9.42 b	2.97 b	2.14 ab	3.18 a	32.3 b
	19.4	9.57 a	3.02 a	2.16 a	3.17 a	33.7 a
IR-28	10.4	9.42 b	2.80 b	2.08 c	3.37 a	29.4 c
	13.3	9.45 b	2.88 a	2.14 b	3.29 b	31.3 b
	16.4	9.52 ab	2.91 a	2.17 ab	3.28 b	32.2 a
	19.6	9.57 a	2.92 a	2.19 a	3.29 b	32.7 a
IR-50	10.5	8.64 b	2.49 d	1.90 d	3.49 a	21.9 d
	13.1	8.67 b	2.55 c	1.94 c	3.41 b	22.9 c
	16.3	8.75 a	2.60 b	1.98 b	3.38 bc	24.1 b
	19.3	8.78 a	2.65 a	2.02 a	3.31 c	25.1 a
Yumepirika	10.2	7.36 b	3.42 c	2.37 c	2.15 a	32.3 b
	13.4	7.45 ab	3.46 c	2.39 bc	2.16 a	33.3 b
	16.2	7.50 a	3.52 ab	2.41 b	2.14 a	34.7 a
	19.6	7.55 a	3.55 a	2.45 a	2.13 a	35.6 a

For each test, the mean followed by the same letter in the column within each type of rice do not differ statistically at 1% probability through the two-way ANOVA and Tukey's simple main effect.

[a] — = non-dimensional

Mass characteristics or rough rice

NERICA-4 also indicated the highest thousand-kernel weight and bulk density (Figure 2.1, Figure 2.2). Additionally, two-way ANOVA did not report a significant difference between *IR-28* and *Yumepirika* in thousand-kernel weight (Figure 2.1), and between *NERICA-1* and *Yumepirika* varieties in bulk density (Figure 2.2).

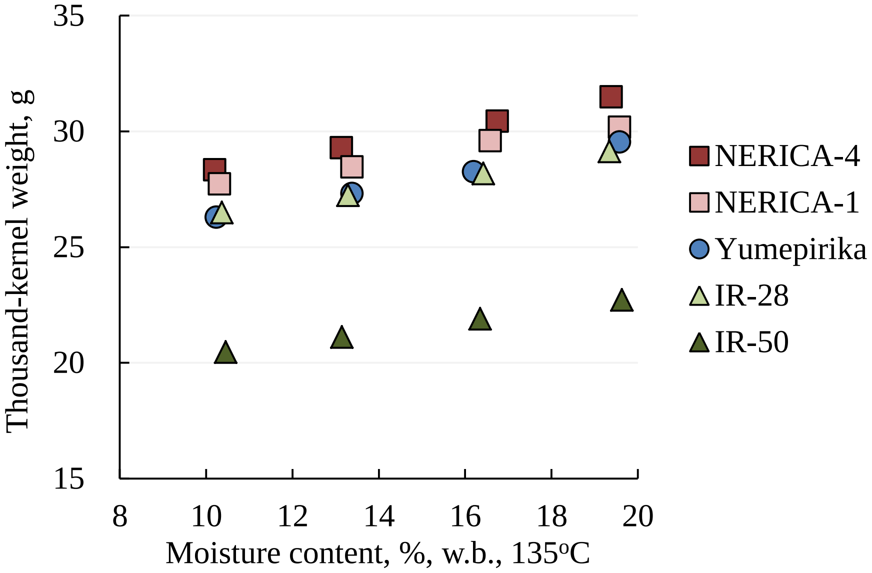

Figure 2.1 Dependency of thousand-kernel weight of rough rice on moisture content

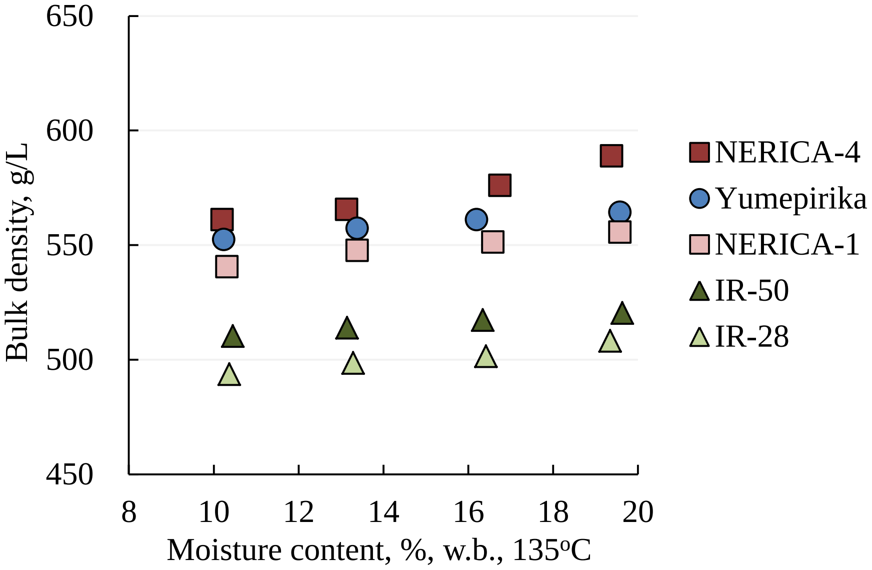

Figure 2.2 Dependency of bulk density of rough rice on moisture content

Frictional characteristics of rough rice

The NERICA varieties showed the highest static angle of repose and static coefficient of friction (Figure 2.3, Figure 2.4). Moreover, two-way ANOVA did not report significant difference among *IR-28*, *IR-50*, and *Yumepirika* in the static angle of repose (Figure 2.3), and among varieties in static coefficient of friction (Figure 2.4).

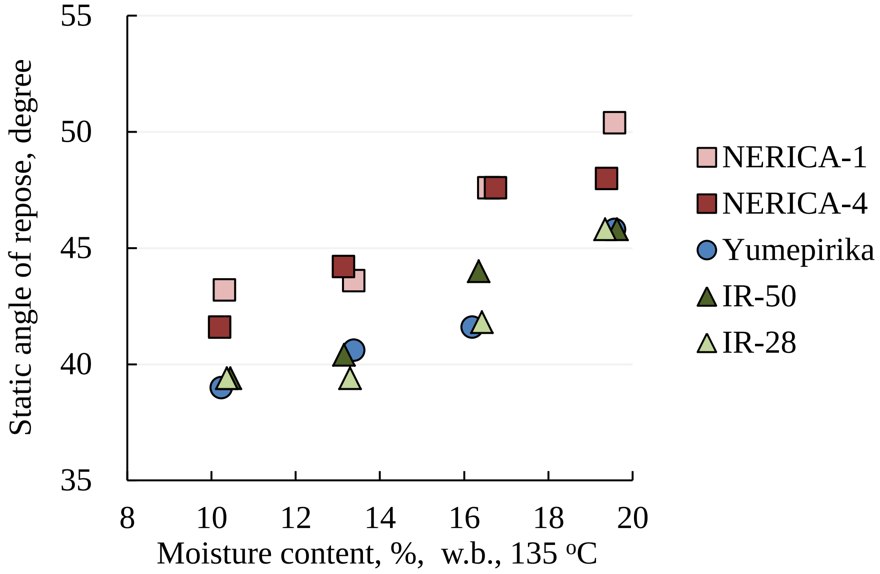

Figure 2.3 Dependency of static angle of repose of rough rice on moisture content

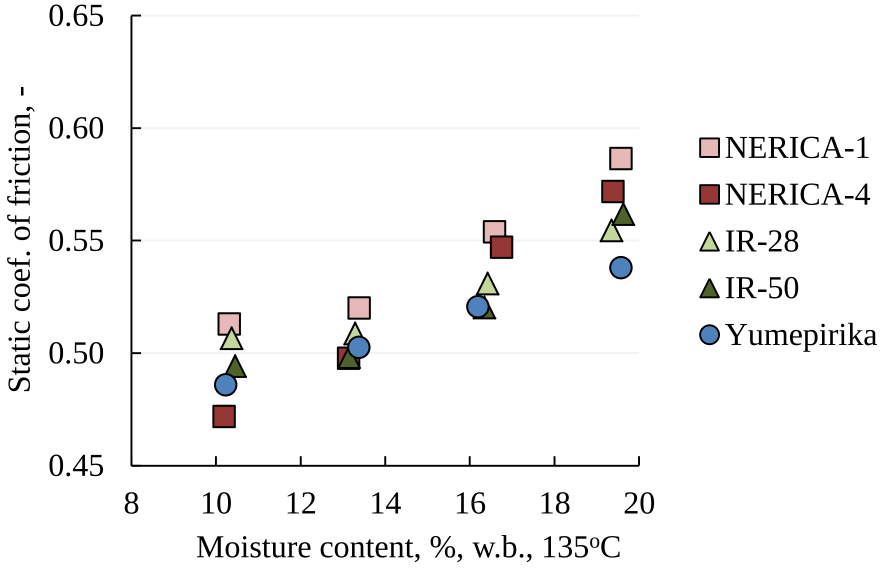

Figure 2.4 Dependency of static coefficient of friction of rough rice on moisture content

2.4.2 Effect of thickness fraction on physical properties of milled rice

In thickness distribution, the NERICA variety *NERICA-4* was closer to the Indica varieties. Japonica varieties indicated the thickest kernels and showed the biggest differences in thickness (0.10 mm each) between thickness fractions. By contrast, Indica and NERICA varieties indicated thinner kernels and showed smaller differences in thickness (0.05 mm each) between thickness fractions (Figure 2.5).

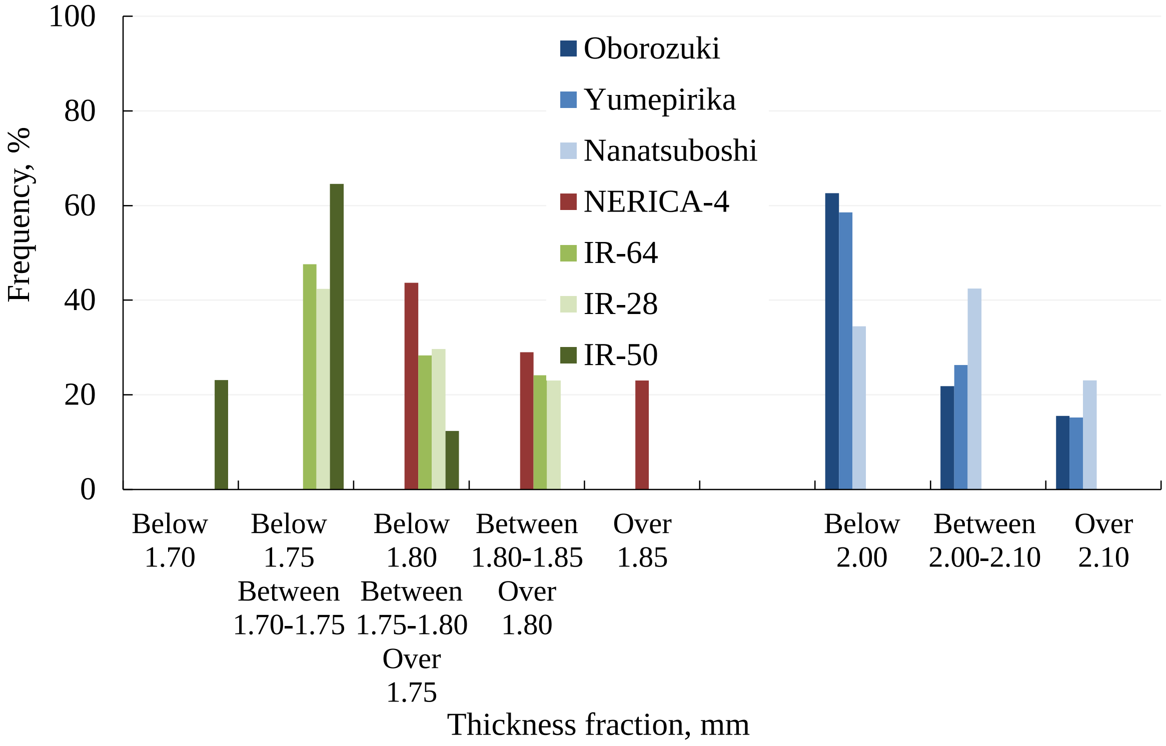

Figure 2.5 Frequency distribution of milled rice by thickness fraction

Composition analysis of milled rice

In general, the highest thickness fraction within each variety contained a higher percentage of sound whole kernels and a lower percentage of broken, chalky, discolored and damaged kernels, and hence contained higher quality samples. The quality of the NERICA variety *NERICA-4* was similar to the Indica varieties. Meanwhile, the Japonica type showed the highest quality and showed the smaller difference in the percentage of sound whole kernel among varieties and thickness fractions (Figure 2.6).

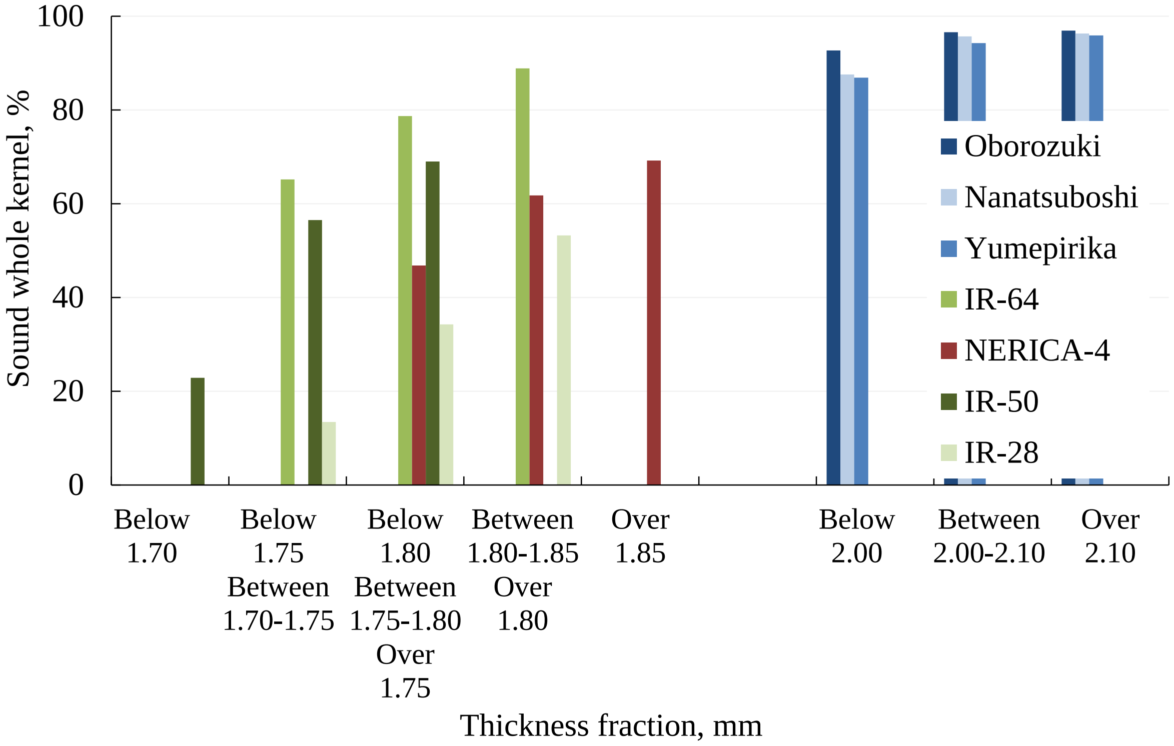

Figure 2.6 Sound whole kernel of milled rice by thickness fraction

In general, dimensional, mass, and frictional characteristics increased as thickness increased. One-way ANOVA reported that such characteristics were highly affected by kernel thickness.

Dimensional characteristics of milled rice

The NERICA variety *NERICA-4* was closer in length, width, and thickness to Indica varieties. The two types of rice thus showed similar slenderness and volume of the kernel of milled rice (Table 2).

Table 2.2 Average value of milled kernel dimensions by thickness fraction

Variety	Thickness fraction mm	Length mm n = 200	Width mm n = 200	Thickness mm n = 200	Slenderness -[a] n = 200	Volume mm^3 n = 200
NERICA-4	Below 1.80	6.16 c	2.34 c	1.74 c	2.64 b	13.5 c
	Between 1.80 - 1.85	6.33 b	2.37 b	1.82 b	2.68 b	14.6 b
	Over 1.85	6.60 a	2.40 a	1.95 a	2.75 a	16.4 a
IR-28	Below 1.75	6.45 c	2.37 a	1.76 c	2.73 b	14.4 c
	Between 1.75 - 1.80	6.56 b	2.38 a	1.78 b	2.77 ab	14.8 b
	Over 1.80	6.68 a	2.40 a	1.93 a	2.79 a	16.3 a
IR-50	Below 1.70	6.22 c	2.07 c	1.67 c	3.00 a	11.4 c
	Between 1.70 - 1.75	6.38 b	2.10 b	1.73 b	3.04 a	12.3 b
	Over 1.75	6.44 a	2.12 a	1.77 a	3.04 a	12.8 a
IR-64	Below 1.75	6.74 c	2.16 a	1.72 c	3.13 b	13.3 b
	Between 1.75 - 1.80	6.91 b	2.17 a	1.76 b	3.19 a	13.9 c
	Over 1.80	7.03 a	2.18 a	1.83 a	3.23 a	14.8 a
Nanatsuboshi	Below 2.00	4.68 c	2.83 c	1.95 c	1.66 a	14.0 c
	Between 2.00 - 2.10	4.80 b	2.89 b	2.10 b	1.66 a	15.7 b
	Over 2.10	4.99 a	3.00 a	2.32 a	1.66 a	18.6 a
Yumepirika	Below 2.00	4.92 b	2.96 c	1.95 c	1.67 a	15.0 c
	Between 2.00 - 2.10	4.99 a	3.00 a	2.01 b	1.67 a	16.5 b
	Over 2.10	5.04 a	3.06 b	2.12 a	1.66 a	17.8 a
Oborozuki	Below 2.00	4.91 c	2.86 c	1.98 b	1.72 a	15.1 c
	Between 2.00 - 2.10	5.09 b	3.01 b	2.02 b	1.71 a	17.1 b
	Over 2.10	5.21 a	3.07 a	2.12 a	1.70 a	18.7 a

For each test, the mean followed by the same letter in the column within each type of rice do not differ statistically at 1% probability through the one-way ANOVA and Tukey’s simple main effect.
[a] — = non-dimensional

Moreover, the behavior shown by thickness distribution was caused by the similarity in kernel dimensions among NERICA and Indica varieties. Consequently, NERICA and Indica varieties were classified as long and medium classes of grain, as their average length was within the range of 6.6-7.5 mm and their slenderness within the range of 2.1-3.0. The Japonica type was classified as short and round, as its length was 5.5 mm or less and its slenderness was less than 2.0 (Bhattacharya, 2011d).

Mass characteristics of milled rice

The NERICA variety *NERICA-4* was closer in weight, density, and fluidity to Indica varieties. However, Japonica varieties were heavier, denser, and flowed faster in volume (Figure 2.7, Figure 2.8).

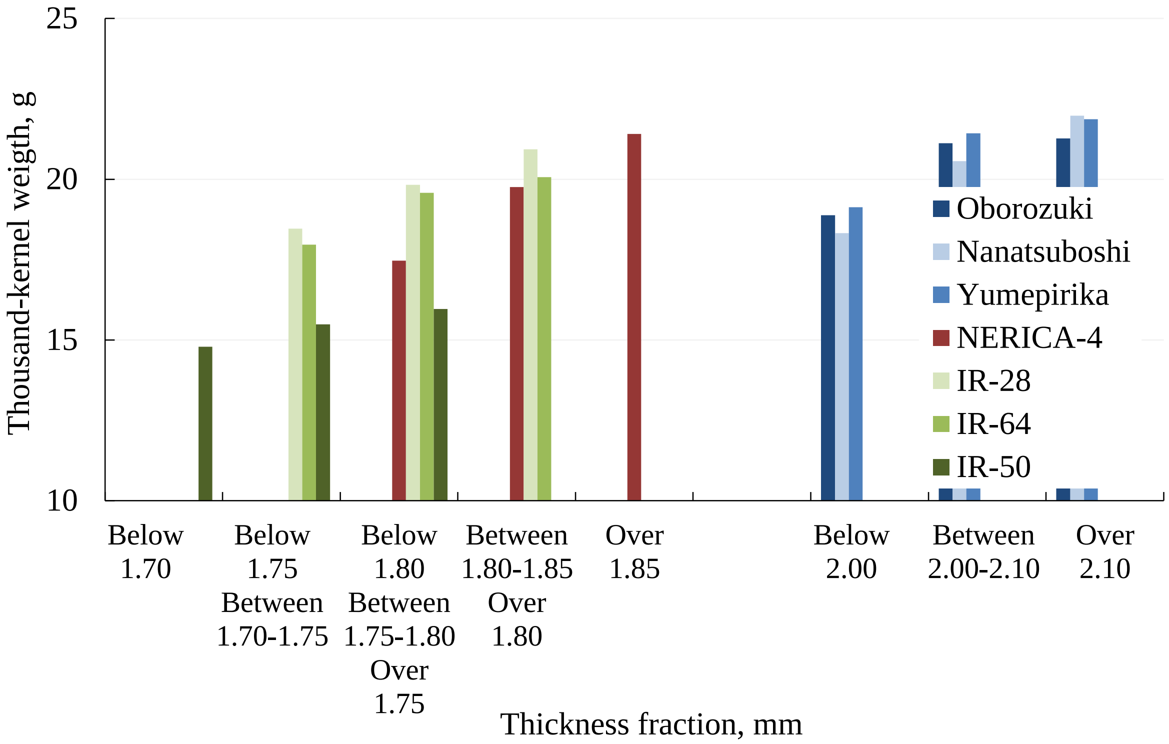

Figure 2.7 Thousand-kernel weight of milled rice by thickness fraction

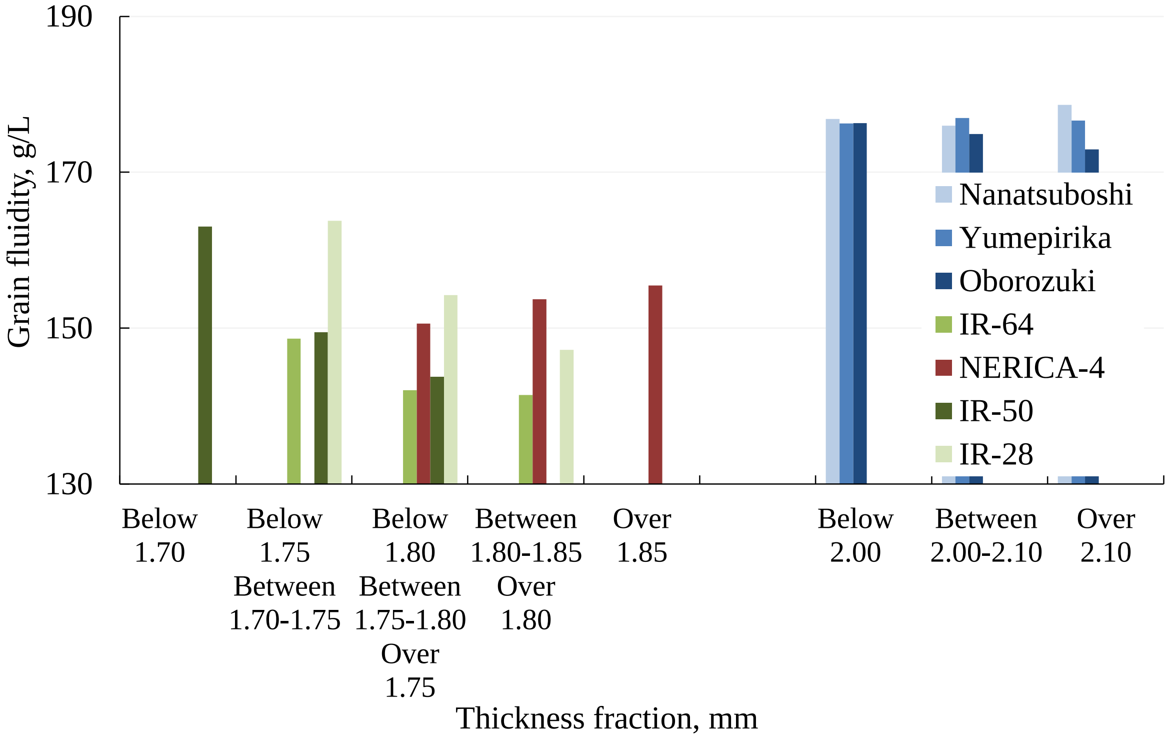

Figure 2.8 Fluidity of milled rice by thickness fraction

Additionally, one-way ANOVA reported a significant difference in thousand-kernel weight among thickness fractions within each variety (Figure 2.7). Meanwhile, in grain fluidity, significant differences were reported among thickness fractions within each Indica variety (Figure 2.8).

Frictional characteristics of milled rice

In the angle of repose and coefficient of friction, the NERICA variety *NERICA-4* was closer to Indica varieties. However, Japonica varieties indicated the lower angle of repose and higher coefficient of friction (Figure 2.9, Figure 2.10).

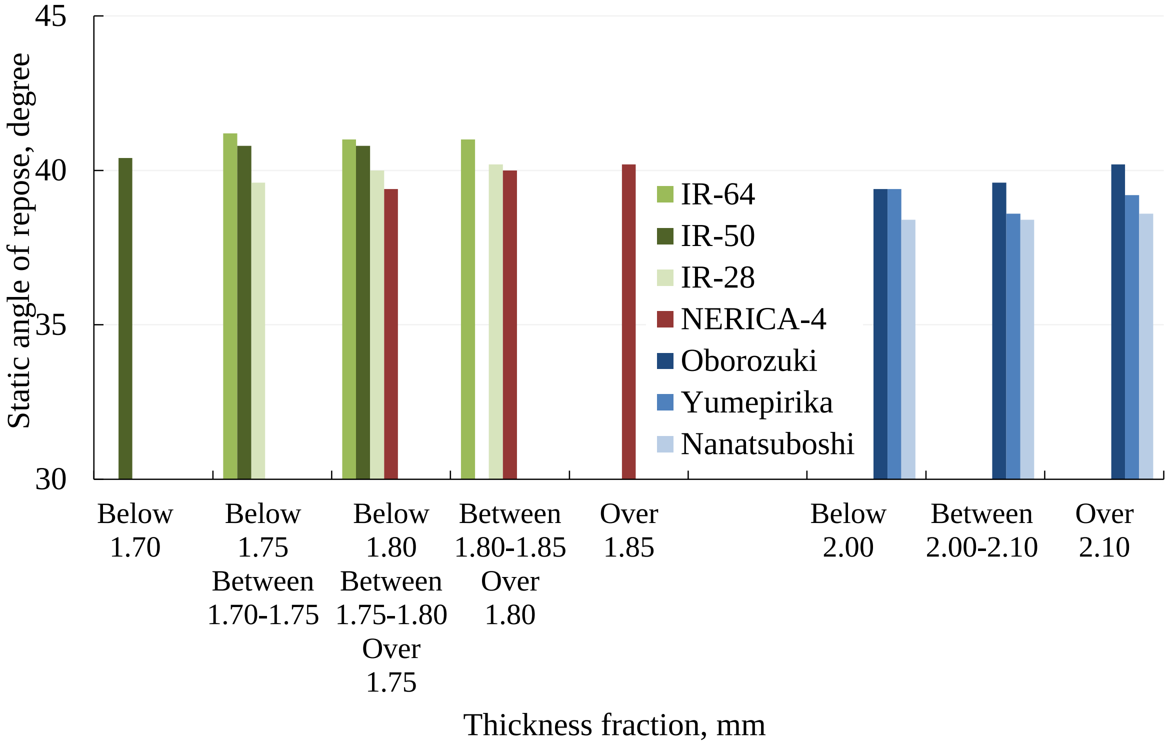

Figure 2.9 Static angle of repose of milled rice by thickness fraction

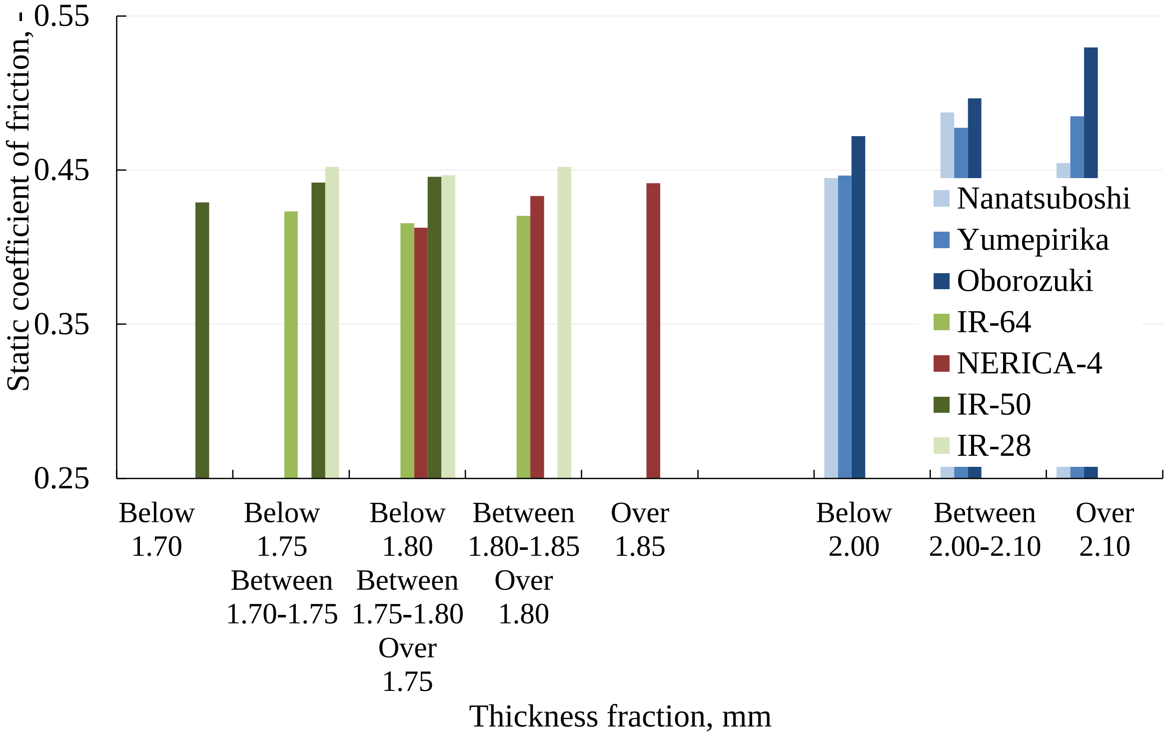

Figure 2.10 Static coefficient of friction of milled rice by thickness fraction

Moreover, one-way ANOVA did not report a significant difference in static angle of repose among thickness fractions within each variety (Figure 2.9). Meanwhile, in static coefficient of friction, significant differences were reported among thickness fractions within each Japonica variety (Figure 2.10).

Rough rice kernel of NERICA varieties apparently had a lower void space between the outer husk and the inner caryopsis compared to other varieties, and hence was heavier in mass despite not being higher in kernel volume. Moreover, rough rice of NERICA varieties indicated higher bulk density because the proportion of empty space in a bulk was lower due to their slenderness. However, the behavior of the slenderness of NERICA varieties during drying affected the degree of packing of the kernel in bulk; they thus stood higher when piled and needed a greater force to initiate movement on a rubber surface.

Milled rice of the NERICA variety *NERICA-4* showed similar physical properties to those of the Indica varieties. Consequently, both types flowed in volume, stood when piled, and needed force to initiate movement on a rubber surface in the same way.

Information obtained in this study could be used for technology development. Dimensional properties can be used for designing cleaning process, pneumatic conveying systems, fluidized bed dryers, and aeration systems in the drying process (Sablani and Ramaswamy, 2003). Mass characteristics can be useful for determining the diameter of pneumatic and chute tube conveyors (Bucklin et al., 2007). Frictional characteristics can be useful for determining the horsepower required to drive belt conveyors (Wimberly, 1983), and the design of the hopper at the bottom of a bin (Kunze et al., 2004).

Data obtained from the assessment of physical properties of NERICA, Indica and Japonica types of rice provided useful information for improving deficiency of technology for postharvest processing. Furthermore, because the NERICA and Indica types indicated similarity in kernel dimensions of rough rice and in dimensional, mass and frictional characteristics of milled rice, the technology used in the postharvest processing of Indica varieties could be transferred and inserted in those countries where NERICA production has been expanding. Consequently, postharvest losses could be reduced and rice quality enhanced.

2.5 Conclusions

Physical properties of rice were highly affected by moisture content and thickness of the kernel. Information obtained in this study could be useful in designing more efficient equipment for postharvest processes. Furthermore, the similarity between NERICA and Indica varieties in the kernel dimensions of rough rice and dimensional, mass and frictional characteristics of milled rice could help in developing a technology-transfer strategy to reduce postharvest losses and mitigate constraints to NERICA expansion, increasing production and the improving grain quality in developing countries.

2.6 Recommendation for further studies

Based on the similarity between NERICA and Indica types in kernel dimensions of rough rice and in dimensional, mass and frictional characteristics of milled rice, further research should be done into how technology used in the postharvest processing of Indica varieties can be applied to processing NERICA types. Particular attention should be paid to the technology used in processes such as cleaning, drying, and milling, where higher percentages of postharvest losses have been reported. Such research would support the development of a technology-transfer strategy.

Since the number of NERICA varieties used in this study was low, a study should be done with a greater number of NERICA varieties to analyze the physical properties of rice considering different levels of moisture content of rough rice and different thickness fractions of milled rice.

3. CHAPTER III: DIVERSITY OF PHYSICAL AND CHEMICAL PROPERTIES OF RICE VARIETIES PRODUCED IN REGIONS OF HOKKAIDO, JAPAN

3.1 Summary

This chapter is the first of three chapters focused on the enhancement of rice quality to meet Japanese consumer requirements. Thus, information on the diversity of physical and chemical properties of multiple varieties of rice produced in various regions of Hokkaido will be analyzed to reduce dimensionality and increase interpretability among properties.

3.2 Introduction

During the grain filling of rice, ambient air temperature and location in the panicle have indicated the most negative impact on agronomic yield of rice and thus quality of the rice grain (Champagne et al., 2004; Siebenmorgen et al., 2013; Chun et al., 2015a; Patindol et al., 2015; Kinoshita et al., 2017; Ma et al., 2017).

Elevated day/night temperature caused premature termination of grain filling in some cultivars, reduced amylose content, thousand-kernel weight, and kernel thickness, and increasing protein content and the number of chalky kernels (Siebenmorgen et al., 2013; Patindol et al., 2015). Cool temperatures increased amylose content and protein content (Kinoshita et al., 2017a). On the other hand, since the flowering period of spikelets within the rice panicle is long and the flowering date of an individual spikelet is dependent on its location on the panicle, and since the grain-fill happens from the top of the panicle downward (Ma et al., 2017) there are differences in levels of protein and amylose, as well as in growth, weight, maturity and quality, between kernels located in the upper and lower part, as well as between kernels located in primary and secondary rachis branches (Matsue et al., 1994; Myers McClung, 2004; Ma et al., 2017).

Both ambient air temperature and location in the panicle during grain filling have an enormous impact on agronomic yield of rice. They affect rice milling quality, appearance, viscosity, and gelatinization temperatures, and thus determine rice grain quality (Siebenmorgen et al., 2013; Patindol et al., 2015; Kinoshita et al., 2017).

Information on the diversity of physical and chemical properties of multiple varieties of rice produced in various regions of Hokkaido was analyzed. Also, the effect of ambient temperature during grain development on those physical and chemical properties was analyzed by varieties and by production regions. The hypothesis was that by analyzing information on the diversity of physical and chemical properties of multiple varieties of rice produced in various regions of Hokkaido, the large dimensionality of the data would be reduced, increasing the interpretability of relationship among properties, which will be used for further analysis in this research.

3.3 Materials and methods

3.3.1 Rice sample

This study was conducted using brown rice of non-waxy Japonica varieties (718 samples) from various varieties such as *Oborozuki*, *Kirara-397*, *Yumepirika*, *Fukkurinko*, *Nanatsuboshi* among others, produced in different areas of Hokkaido, Japan, namely Hidaka, Hiyama, Iburi, Ishikari, Kamikawa, Oshima, Rumoi, Shiribeshi, and Sorachi, between 2010 and 2017.

3.3.2 Devices and methods of measurement

Physical properties information

Physical properties such as estimated percentages of mature and immature kernels and length and width of brown rice kernel were obtained using a visible light segregator (VIS) grain segregator (Shizuoka Seiki, ES-1000, Fukuroi, Shizuoka, Japan).

Chemical properties information

Principal rice constituents such as amylose content and protein content were measured.

Amylose content (*AC*) in milled rice was determined based on Iodine colorimetry using an auto-analyzer (Bran-Luebbe, Solid Prep III, Tokyo, Japan) following the protocol of Williams (Williams et al., 1958) with modifications by Inatsu (Inatsu, 1988). The absorption of the amylose-iodine complex was measured at 620 nm with a spectrophotometer, and the apparent amylose content was quantified against a calibration curve. In this study, the *Hoshinoyume* variety of rice grown in Hokkaido (moisture content: 13.09%, amylose content: 21.12%) and glutinous rice (amylose content: 0%) were used as standard amylose content to calculate the apparent amylose content (*AC*) of each sample. Apparent amylose content was expressed as a percentage (%).

Protein content (*PC*), was predicted by chemometric analysis. Partial least squares regression (PLS) was carried out using reference protein content and NIR spectra information of milled rice.

Temperature information

Average day (24 hrs) temperatures, as well as average maximum and minimum temperatures within a day in July, August, and September, were used in this study. July, August, and September represent the growing period of rice grain on Hokkaido Island. Average temperatures were collected from the Japan Meteorological Agency homepage: (http://www.jma.go.jp/jma/index.html).

Statistical analysis

The chemometric technique principal component analysis (PCA) within the statistical software The Unscrambler (Version 10.3 Upgrade 10.3.0r4) was used for processing the data.

Principal Component Analysis is one of the most powerful multivariate exploratory data analysis methods (Bro and Smilde, 2014; Jolliffe and Cadima, 2016). It takes information from the original variables (multidimensional and/or large data) and projects such information onto a smaller set of latent variables called principal components (PC). Each PC describes a definite amount of the total information within the original data. However, the first PC is a linear combination of the original variables and explains as much variation as possible in the original data, thus it is the component that has the maximum variance. Each subsequent PC explains, in order, less information than the previous one and it is uncorrelated with the previous components. Consequently, we can conclude that all the information of the data is included among the first principal components (Hatcher and O'Rourke, 2013).

By plotting principal components (mostly PC1 vs PC2), important sample and variable interrelationships can be revealed, leading to the interpretation of certain sample groupings, similarities or differences (Bro and Smilde, 2014; CAMO, 2014; Jolliffe and Cadima, 2016).

Data were standardized before being processed. Weighting or scaling and centering the data, which is the common way of standardizing the data, was carried out by multiplying each X-variable with the inverse of the standard deviation of the corresponding variable (1/SD). In this way, each scaled or weighted variable gets the same variance. No X-variable can dominate over another and thus influence the model (Bro and Smilde, 2014; CAMO, 2014).

3.4 Results and discussions

The properties analyzed in this study were processed with principal component analysis (PCA) to interpret the main components of variation. PCA was carried out to reduce the original data (15 characteristics of 718 samples) to two principal components, PC-1 and PC-2, which explained 54 % of the total variance.

The relationship between the principal components (PC-1 and PC-2) and physicochemical properties is shown in Table 3.1.

PC-1, which explained 38% of the total variance, was positively correlated to amylose, the percentage of immature kernels, and the average day and the average maximum temperatures within a day in July. PC-1 was negatively correlated to protein, percentages of mature kernels, and kernel length as well as average daily temperature within a day, average maximum and minimum temperatures within a day in August and September. However, PC-1 correlated significantly high with average day temperature (-0.92) and average minimum temperature (-0.92) in September. Average day temperature in August (-0.87) also indicated highly significant correlation with PC-1. Based on this correlation, PC-1 is primarily a measure of September temperatures including average day and average maximum and minimum temperature within a day (Table 3.1).

On the other hand, PC-2, which explained 16% of the total variance, was positively correlated to protein, the percentage of immature kernels, and average temperatures in July, August, and September. PC-2 was highly correlated to the percentage of mature kernels (-0.64) and immature kernels (0.69). Based on this correlation, PC-2 is mainly a measure of maturity (Table 3.1).

Table 3.1 Correlation of principal components PC-1 and PC-2 with physicochemical properties

	PC-1 (38%)		PC-2 (16%)	
July Day	0.54	**	0.44	**
July Maximum	0.66	**	0.19	**
July Minimum	0.01		0.66	**
August Day	-0.87	**	0.31	**
August Maximum	-0.69	**	0.24	**
August Minimum	-0.83	**	0.34	**
September Day	-0.92	**	0.01	
September Maximum	-0.81	**	0.07	*
September Minimum	-0.92	**	-0.08	**
Amylose	0.50	**	-0.30	**
Protein	-0.16	**	0.46	**
Mature	-0.48	**	-0.64	**
Immature	0.33	**	0.69	**
Length	-0.15	**	-0.02	
Width	-0.03		-0.58	**

For each test, the mean followed by ** and * do differ statistically at 1% and 5 % probability respectively

Relationship among physicochemical properties of varieties of rice produced in Hokkaido

Principal components PC-1 and PC-2 were plotted to analyze the relationship among properties. The cosine of the angle between the properties approximates the relationship between the variables. Average temperatures in August and September were positively correlated to each other and negatively correlated to amylose content. This reflects the fact that as temperatures in August and September increased, amylose content of Hokkaido varieties tended to decrease. Amylose was also negatively correlated to protein and kernel length. Protein content and average minimum temperature in July were positively correlated to each other. Protein content and average minimum temperature in July were also negatively correlated to kernel width and percentage of mature kernels. Moreover, the percentage of mature kernels was negatively correlated to the percentage of immature kernels (Figure 3.1).

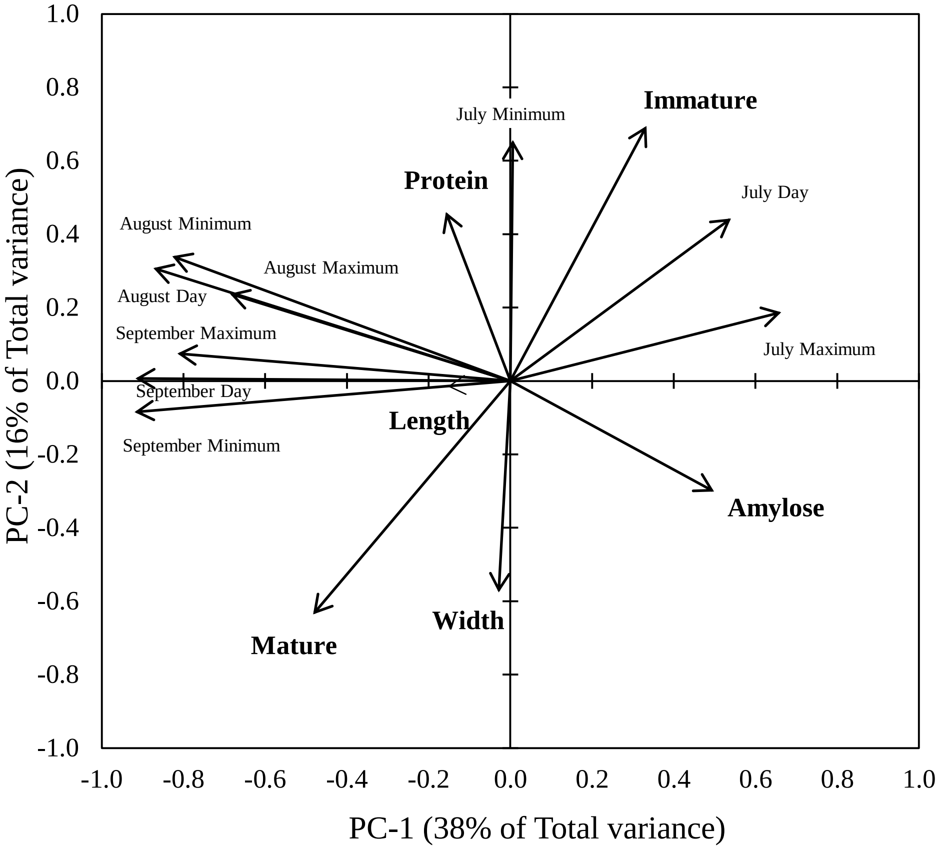

Figure 3.1 Bivariate plot of properties for PC-1 and PC-2 from PCA analysis

Similar results have been reported by Siebenmorgen et al. (2013); Patindol et al. (2015); and Kinoshita et al. (2017b). They suggest a relationship between maturity and physicochemical properties.

Diversity of physicochemical properties of varieties of rice produced in Hokkaido

Principal components PC-1 and PC-2 of samples (scores) and properties (loadings) were plotted with a bi-plot to analyze the diversity of physicochemical properties considering the type of variety, production area and year of production.

Diversity of physicochemical properties considering the type of variety

Bi-plot of scores (samples) and loadings (properties) from principal components PC-1 and PC-2 from PCA analysis considering varieties produced in Hokkaido is shown in Figure 3.2. In the plot, each marker represents a sample (boldface) and each arrow represents a property.

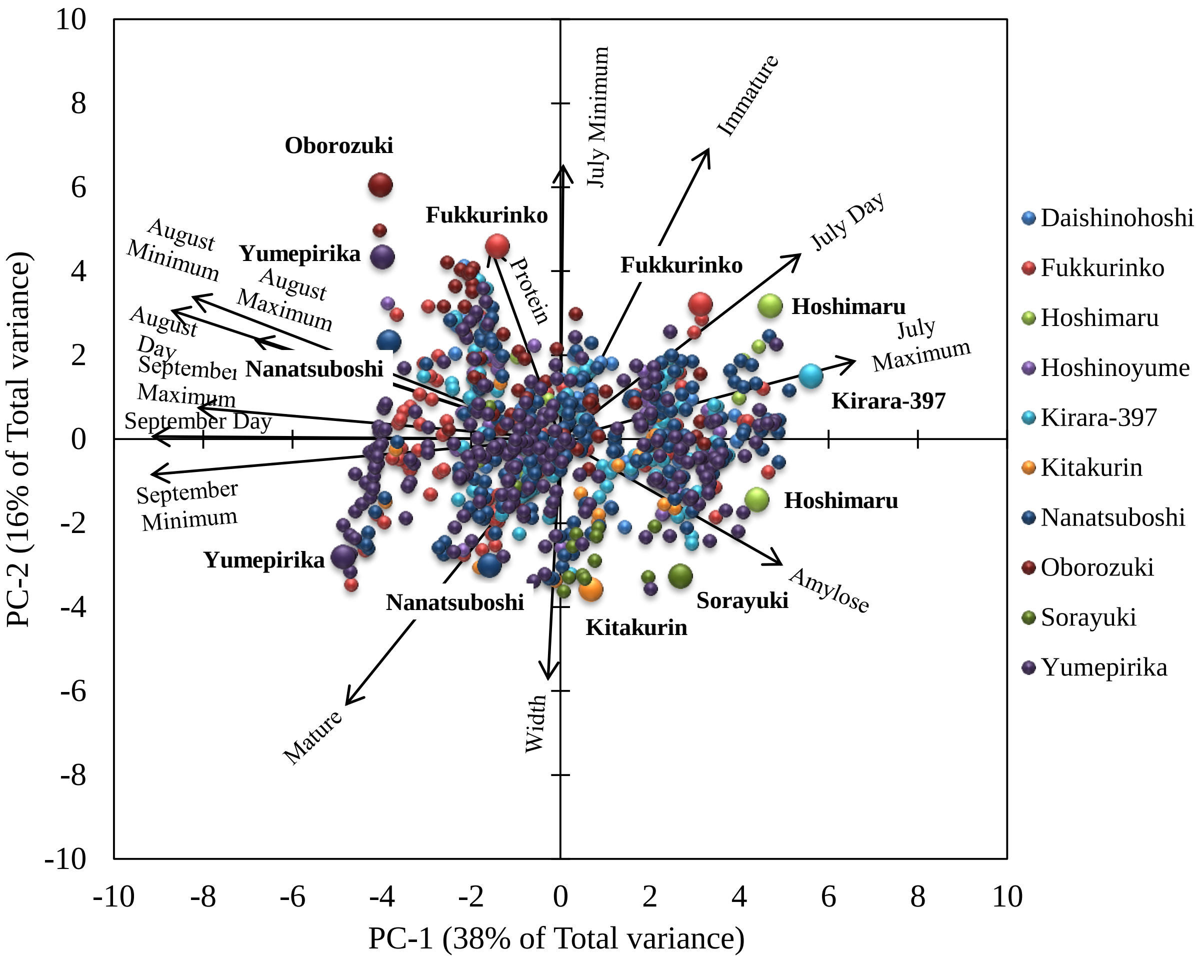

Figure 3.2 Bi-plot of samples and properties from PC-1 and PC-2 from PCA analysis considering varieties of rice

Some rice varieties such as *Oborozuki* and *Yumepirika* were positively correlated with average temperatures in August and September and thus negatively correlated with amylose content. In addition, some varieties of *Sorayuki* and *Yumepoirika* indicated higher percentages of amylose content. Also, some varieties of *Oborozuki* and *Fukkurinko* reported high percentages of immature kernels as well as protein. Meanwhile, some *Nanatsuboshi* and *Yumepirika* varieties reported higher percentages of mature kernels and were found to be comprised of wider kernels (Figure 3.2).

Diversity of physicochemical properties considering the production area

Bi-plot of scores (samples) and loadings (properties) from principal components PC-1 and PC-2 from PCA analysis considering production area in Hokkaido is shown in Figure 3.3. In the plot, each marker represents a production area (boldface) and each arrow represents a property.

Rice produced in some areas of Hiyama, Oshima, and Sorachi were positively correlated to temperatures in August and September, and thus negatively correlated to amylose content. By contrast, rice produced in some areas of Kamikawa and Iburi and Sorachi reported higher levels of amylose content. In addition, some rice produced in some areas of Ishikari and Shiribeshi reported higher percentages of mature kernels and wider kernels. Meanwhile, rice produced in some areas of Ishikari and Sorachi indicated higher percentages of protein content (Figure 3.3).

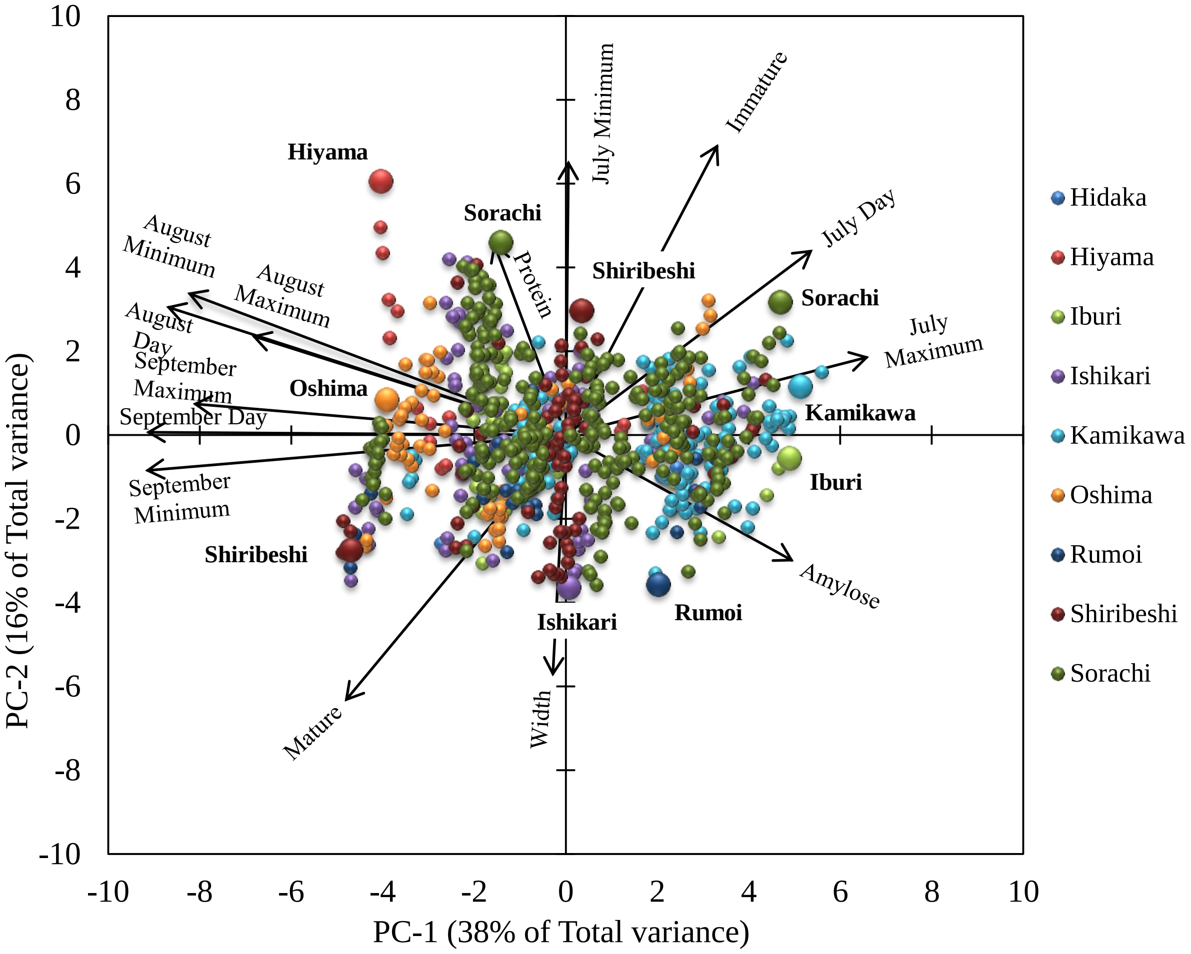

Figure 3.3 Bi-plot of samples and properties from PC-1 and PC-2 from PCA analysis considering production area

Diversity of physicochemical properties considering the production year

Bi-plot of scores (samples) and loadings (properties) from principal components PC-1 and PC-2 from PCA analysis considering production year in Hokkaido is shown in Figure 3.4. In the plot, each marker represents a production year (boldface) and each arrow represents a property.

Rice varieties produced in some areas in 2010, 2012, 2013 and 2016 were correlated to temperatures in August and September, and thus negatively correlated to amylose content. By contrast, samples produced in some areas of 2014, 2015 and 2017 were positively correlated to amylose content. Furthermore, samples produced in some areas in 2010, 2011 and 2013 reported higher levels of protein content and immature kernels. Meanwhile, samples produced in some areas in 2011, 2015 and 2016 reported higher levels of maturity and wider kernels (Figure 3.4).

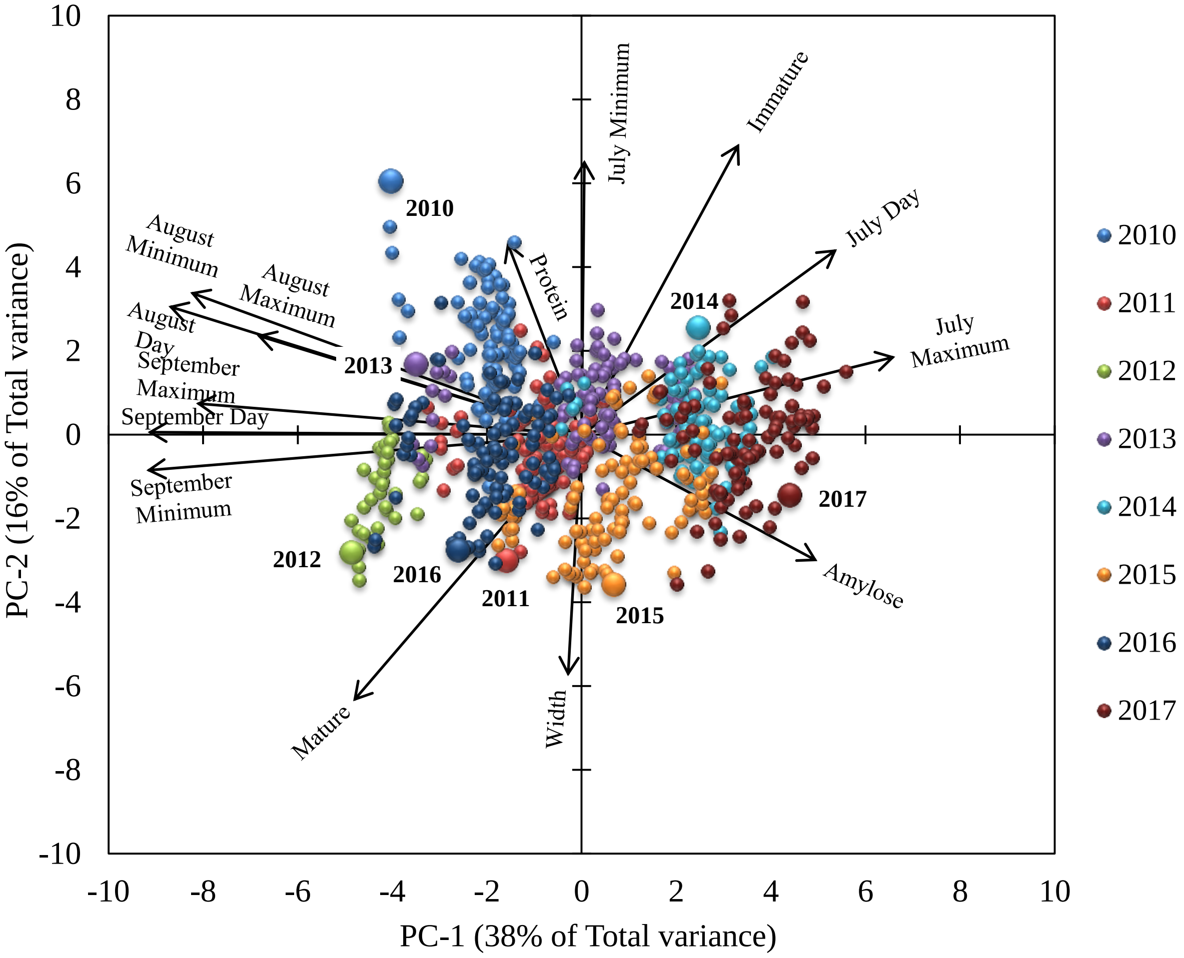

Figure 3.4 Bi-plot of samples and properties from PC-1 and PC-2 from PCA analysis considering production year

Principal component analysis (PCA) allowed extensive data on physicochemical information to be reduced to principal component PC-1 and PC-2. By mean of PC-1 and PC-2 the diversity of physicochemical properties by variety, production area and year of production were obtained.

3.5 Conclusions

Principal component analysis (PCA) revealed the diversity of physicochemical properties by variety, production area and year of production in Hokkaido from 2010 to 2017. It also showed the effect of temperature during grain development on those physicochemical properties of rice by variety, production area and year of production as well as the relationship among those properties.

As average temperatures in August and September increased, amylose content of Hokkaido varieties tended to decrease. In addition, as the average minimum temperatures in July increased, there was also an increase in protein content, the percentage of immature kernels, and kernel length.

Moreover, as the percentage of immature kernels increased, the level of protein content also increased, and the percentage of mature kernels and kernel width decreased. This result suggested a relationship between maturity and physicochemical properties.

3.6 Recommendation for further studies

The explorative and interpretative analysis of the physicochemical information of rice produced in various regions in Hokkaido from 2010 to 2017 indicated a relationship between kernel maturity and physicochemical properties. It is therefore recommended to analyze the relationship between physicochemical properties and kernel maturity, with a focus on amylose and protein content, the most important constituents for assessing rice quality.

4. CHAPTER IV: RELATIONSHIP BETWEEN PHYSICOCHEMICAL PROPERTIES AND KERNEL MATURITY FOR ASSESSING QUALITY CONSTITUENTS OF RICE VARIETIES PRODUCED IN HOKKAIDO, JAPAN

4.1 Summary

This chapter is also focused on enhancing rice quality to meet Japanese consumer requirements.

As stated in previous chapters, during the growing period of rice there are differences in growth, weight, maturity, and quality among the kernels located in the upper and lower part, as well as the kernels located in primary and secondary rachis branches, of a rice panicle. The results presented in chapter three indicated a relationship between physicochemical properties and kernel maturity. This chapter will, therefore, examine the relationship between physicochemical properties and kernel maturity for assessing quality constituents during postharvest processes of rice produced in Hokkaido.

4.2 Introduction

Due to the fact that 1) the flowering period of spikelets within the rice panicle is long and the flowering date of an individual spikelet is dependent on its location on the panicle, and 2) the grain-fill happens from the top of the panicle downward and thickness is the last of the principal rice dimensions to reach its maximum level when the caryopsis is developing, there are differences in growth, weight, thickness as well as in levels of protein and amylose and viscosity among the kernels located in the upper and lower part, and kernels located in primary and secondary rachis branches, of a rice panicle during rice grain development (Matsue et al., 1994a; Myers McClung, 2004; Ma et al., 2017). This leads to a difference in maturity, quality, and palatability for Japanese consumers between grains located in the upper and lower parts of the panicle (Matsue, et al, 2001; Ma et al., 2017).

Since kernel thickness is highly related to an increase in storage materials in the rice caryopsis during grain filling, as kernel thickness increases, a corresponding increase has been observed in kernel maturity, weight and in the percentage of amylose content, along with a decrease in the percentage of protein content (Matsue et al., 2001; Siebenmorgen et al., 2006; Siebenmorgen et al., 2013). The increase in amylose content is caused by the higher activity of GBSSI enzymes and hence a higher accumulation of amylose in starch per kernel (Umemoto et al., 1994; Umemoto et al., 2002; Biselli et al., 2014). The decrease in protein content is caused by the fact that glutelin molecular weight decreases with grain-fill (Shih, 2004). Likewise, high protein content has been significantly correlated with light kernels and early heading (Hillerislambers et al., 1973; Tanaka, 2012).

On the other hand, as Japanese studies have shown, amylose content and protein content are essential constituents for determining the high palatability and good taste demanded by rice consumers. Also, both constituents have been found to influence the physicochemical properties of Japonica varieties produced in the Kyushu and Honshu areas of Japan during the growing period (Matsue et al., 2001; Umemoto et al., 1994). Furthermore, in Japan brown rice sample with an average moisture content of 15% is required to have more than 70% of the sound whole kernel to be classified as high-quality rice (Mizuho National Foundation, 2011). Meanwhile, in Hokkaido, the northern island in Japan, a high-quality sample of rice is required to be comprised of more than 80% sound whole kernel. Similarly, for the *Yumepirika* variety produced in Hokkaido, the best combination for high palatability and good taste for people in Hokkaido has been reported for milled rice with amylose content of < 19 % and protein content of < 7.5 % and/or amylose content of ≥ 19 % and protein content of ≤ 6.8 % (Kawamura et al., 2013).

At harvest time, the bulk of harvested rice is considered mature. However, as was explained above, since the grain-fill happens from the top of the panicle downward and thickness is the last of the principal rice dimensions to reach its maximum level when the caryopsis is developing, there are differences in growth within the bulk of harvested rice. Also, given that rice kernels are passed through various sorting systems during processing, including a thickness grader and color sorter (Yoshizaki, 1995; Kondo and Kawamura, 2013), I examined the relationship between physicochemical properties and kernel maturity of rice produced in Hokkaido. The analysis was carried out while the rice was being processed by a color sorter machine and during processing in a grain elevator. The hypothesis was that an analysis of the relationship between physicochemical properties and kernel maturity could lead to a better understanding of the behavior of quality constituents such as amylose and protein content in Hokkaido varieties during postharvest processes. This information could be useful for developing a protein content sorter, which could contribute to more accurate quality assessment and help to secure the high palatability and good taste demanded by Japanese rice consumers.

4.3 Materials and methods

4.3.1 Rice sample

Brown rice of Japonica-type varieties *Kirara-397* and *Nanatsuboshi*, produced in Hokkaido, Japan in 2014 and 2015 respectively were used to examine the relationship during processing by a color sorter machine.

Brown rice of Japonica type variety *Nanatsuboshi*, produced in Fukagawa, Hokkaido, Japan in 2014 was used to examine the relationship during processing in a grain elevator.

4.3.2 Rice sample preparation

Samples for analysis during processing by a color sorter machine

A total of seventy-two samples of brown rice were created with different sound whole kernel rates. Since sound whole kernel has been found to be well-developed kernel (Japan Rice Millers Association, 1997), the level of maturity was varied by creating different rates of the sound whole kernel.

Thirty samples of *Kirara-397* with sound whole kernel rates ranging from approximately 12% to 88% were made using one model of color sorter machine (ANZAI, model LHQ-1100, Chiba, Japan) (Figure 4.1).

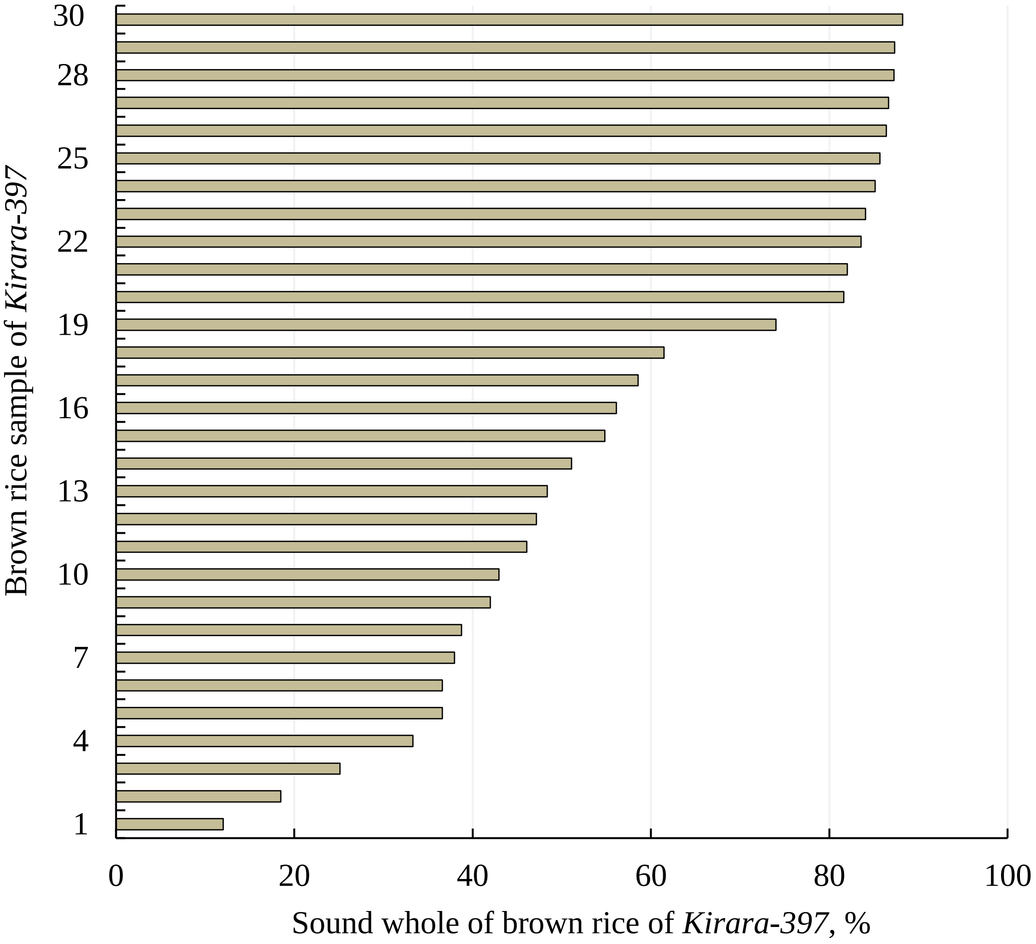

Figure 4.1 Percentage of sound whole kernels per sample of brown rice of *Kirara-397*

The remaining forty-two samples of *Nanatsuboshi* with sound whole kernel rates ranging from approximately 11% to 93% were made using another model of color sorter machine (SATAKE, model GRAND ES-03 AMS, Hiroshima, Japan) (Figure 4.2).

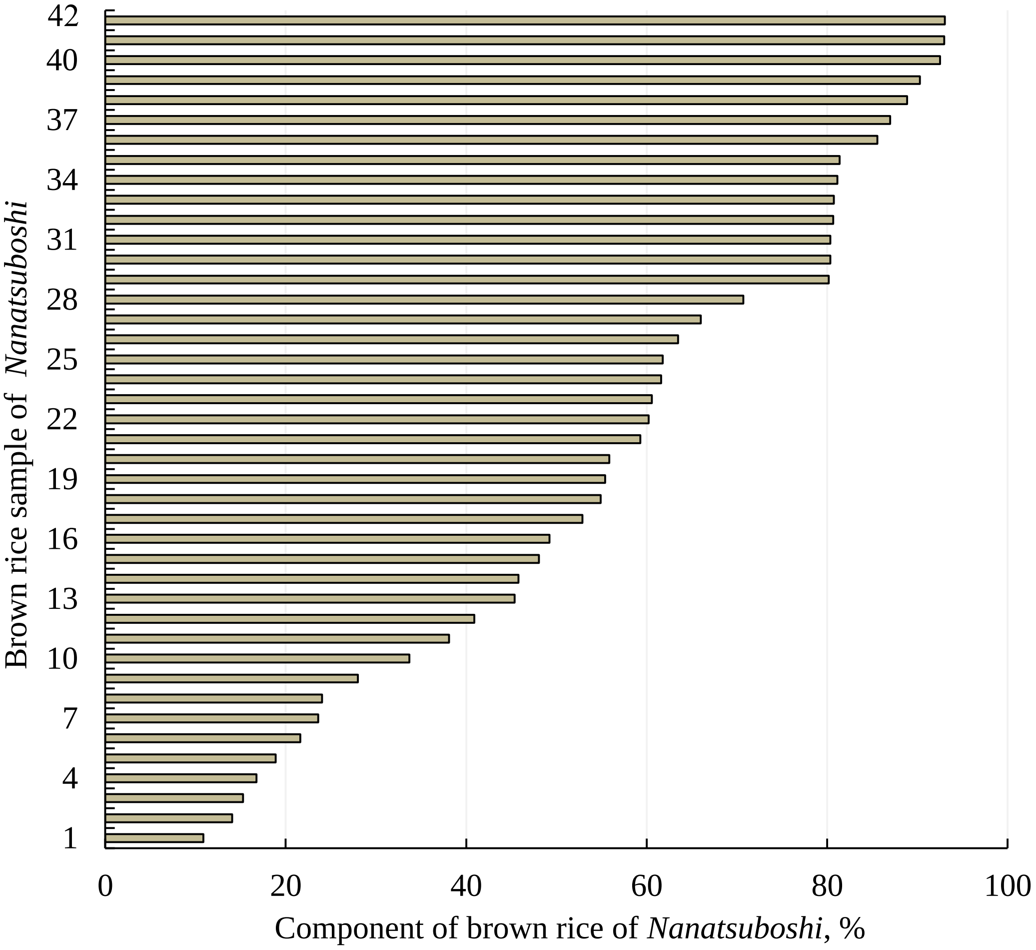

Figure 4.2 Percentage of sound whole kernels per sample of brown rice of *Nanatsuboshi*

Samples for analysis during processing in a grain elevator

Samples were collected at the rice grain elevator at Fukagawa Mainary grain elevator in Fukagawa, Hokkaido, Japan (Figure 4.3) over two hours at different stages of processing: after the huller, after the thickness grader, in the waste of the thickness grader, after the color sorter and in the waste of the color sorter.

Over the two hours, 15 samples were collected after the huller, after the thickness grader and in the waste of the thickness grader and 21 samples were collected after the color sorter and in the waste of the color sorter. The difference in the number of samples collected before and after the color sorter was due to the difference in the volume of rice processed in the huller and thickness grader (higher volume) and in color sorter machine (lower volume).

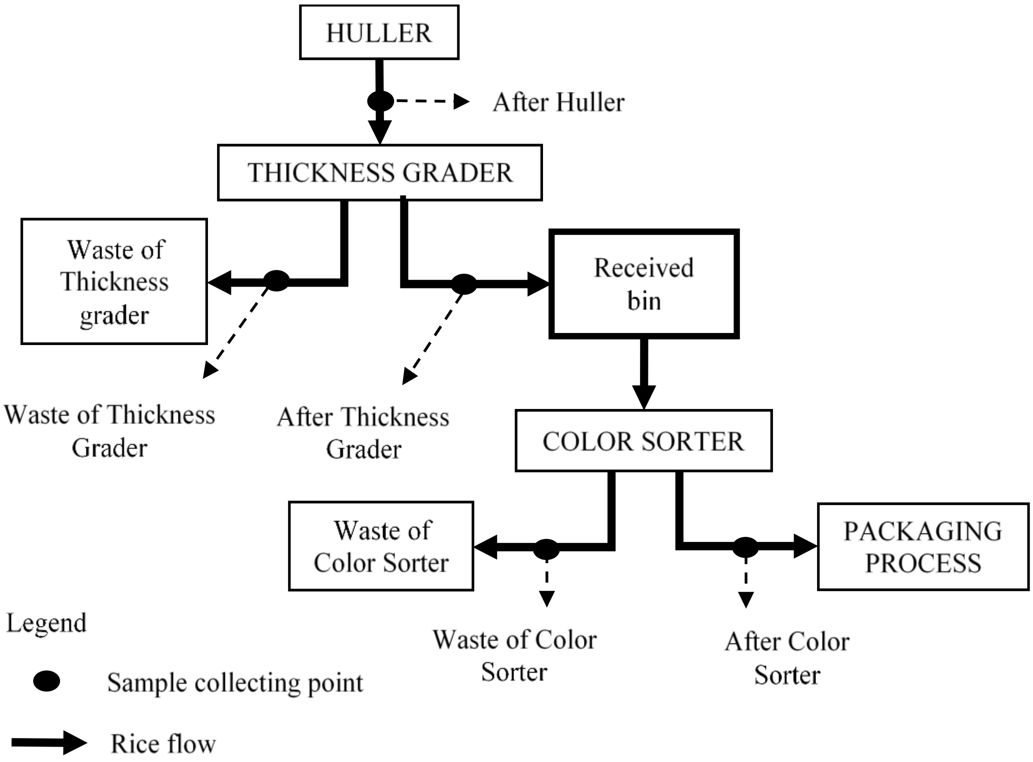

Figure 4.3 Flow chart of sample collection in Fukagawa Mainary grain elevator

4.3.3 Devices and methods of measurement

Moisture content

Moisture content was determined by the Japanese Society of Agricultural Machinery and Food Engineers (JSAM) standard method: about 10 g of whole grain rice was placed in a forced-air oven at 135°C for 24 h and computed on a wet basis.

Rice component analysis

Components of both brown and milled rice were divided by human observation and were expressed as a percentage of the weight of approximately 20 g of sample. Components of brown rice were divided into sound whole (*SWK*), immature (*IK*), chalky (*CK*), broken (*BK*), and discolored kernels (*DK*); meanwhile, components of milled rice were classified into sound whole, broken, chalky, damaged, discoloured kernels, and foreign material (*FK*) (less than one quarter of the sound whole kernel of milled rice) (Ohtsubo, 1995; Japan Rice Millers Association, 1997).

Physical properties

Thickness distribution was measured with a sieve shaker. A volume of 100 grams was separated into 8 thickness categories at intervals of 0.1 mm, from less than 1.6 mm to more than 2.2 mm, which were expressed as a percentage of weight.

Meanwhile, the mean thickness (*MT*) of each sample was calculated by the application of Equation 4.1, and expressed in millimeters (mm),

$$MT = \frac{\begin{gathered}((2.25 \cdot WP_{o2.2}) + (2.15 \cdot WP_{2.1-2.2}) + (2.05 \cdot WP_{2.0-2.1}) + (1.95 \cdot WP_{1.9-2.0}) + \\ (1.85 \cdot WP_{1.8-1.9}) + (1.75 \cdot WP_{1.7-1.8}) + (1.65 \cdot WP_{1.6-1.7}) + (1.55 \cdot WP_{B1.6})\end{gathered}}{100} \qquad \text{Equation 4.1}$$

where *WP* is the weight percentage in each thickness fraction, the subscript $_{o2.2}$ represents kernels more than 2.2 mm thick, and the subscript $_{b1.6}$ represents kernels less than 1.6 mm thick.

Thousand-kernel weight *TKW* was determined by weighing 1,000 randomly drawn regular rice kernels in an electronic balance (Sartorius Lab Holding GmbH, Germany) and expressed in g (Bhattacharya, 2011b).

Chemical properties

Principal rice constituents such as amylose content and protein content were measured.

Amylose content (*AC*) in milled rice was determined based on Iodine colorimetry using an auto-analyzer (Bran-Luebbe, Solid Prep III, Tokyo, Japan) following the protocol of Williams (Williams et al., 1958) with modifications by Inatsu (Inatsu, 1988). The absorption of the amylose-iodine complex was measured at 620 nm with a spectrophotometer, and the apparent amylose content was quantified against a calibration curve. In this study, the Hoshinoyume variety of rice grown in Hokkaido (moisture content: 13.09%, amylose content: 21.12%) and glutinous rice (amylose content: 0%) were used as standard amylose content to calculate the apparent amylose content (AC) of each sample. Apparent amylose content was expressed as a percentage of dry starch of milled rice (%, MR, Auto-analyzer).

Crude protein content (PC) was determined using the Kjeldahl and Dumas methods as well as by using a near-infrared (NIR) spectrometer (Shizuoka Seiki, BR-5000, Fukuroi, Shizuoka, Japan). The protein content of *Kirara-397* samples was determined using the Kjeldahl method, while protein content of *Nanatsuboshi* samples was determined using the Dumas method. In both methods, the Nitrogen form in the samples is transformed to Nitrogen Oxides and then converted into protein using a conversion factor (5.95). Protein content determined by Kjeldahl was expressed as a percentage of dry matter of milled rice (%, DM, MR, Kjeldahl). Protein content determined by the Dumas method was expressed as a percentage of dry matter of brown rice (%, DM, BR, Dumas). In the other hand, protein content predicted by NIR spectrometer was expressed as a percentage of dry matter of milled rice (%, DM, MR, NIR BR-5000). In plot were the protein content of *Kirara-397* and *Nanatsuboshi* is combined the result is expressed only in percentage (%).

Statistical analysis

The statistical analysis in this chapter included linear regression analysis to determine the relationship among physicochemical properties with 99 % of confidence during processing by a color sorter machine.

One-way ANOVA and Tukey's test with 99% of confidence were carried out to determine any significant differences among the means of physicochemical properties among processes during processing in a grain elevator.

4.4 Results and Discussion

4.4.1 Relationship between physicochemical properties and kernel maturity during processing by a color sorter machine

Rice component analysis

Component analysis of both brown and milled rice samples indicated that the percentage of undesirable kernels, such as immature, chalky, broken, damaged, and discolored kernels, increased as the percentage of sound whole kernels decreased within each sample. Components of brown rice of *Kirara-397* and *Nanatsuboshi* are shown in Figure 4.4 and Figure 4.5.

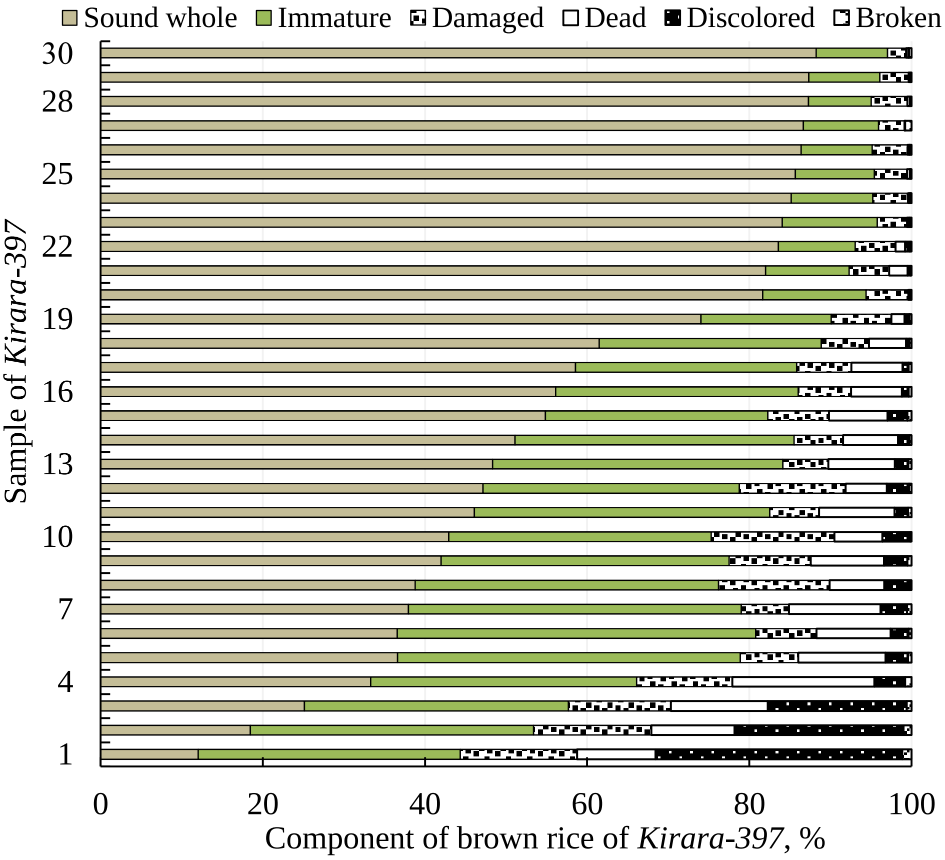

Figure 4.4 Component per sample of brown rice of *Kirara-397*

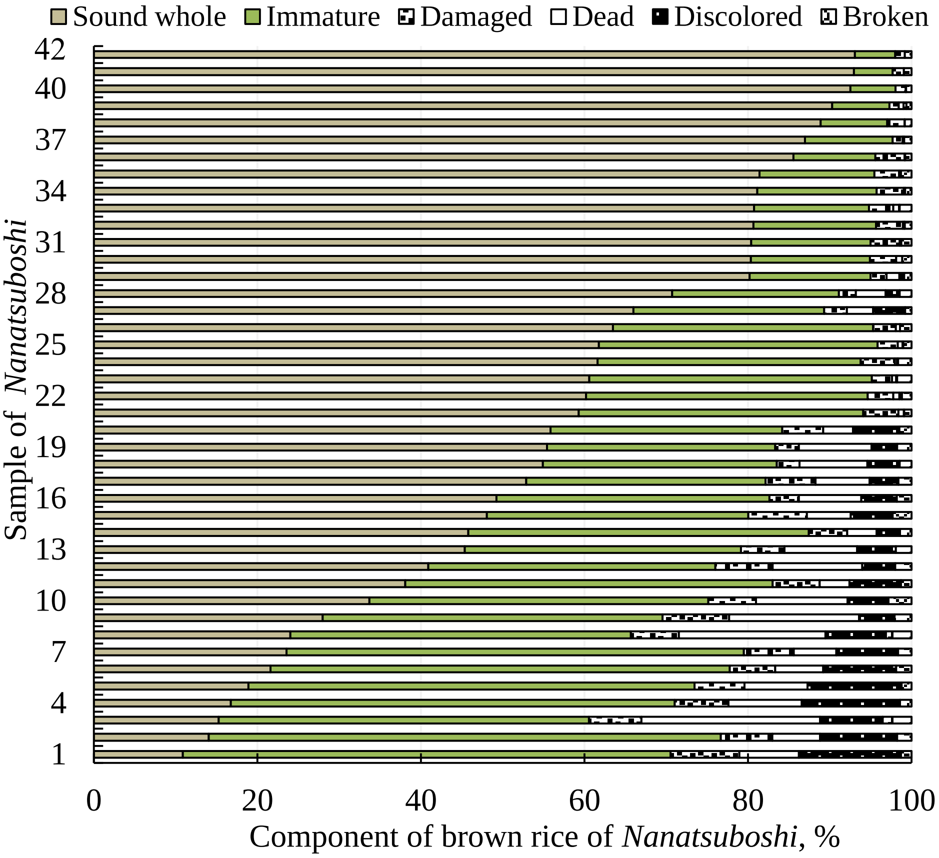

Figure 4.5 Component per sample of brown rice of *Nanatsuboshi*

This result was expected because the samples were created based on sound whole kernel rate and because higher percentages of undesirable kernels are usually found in samples with lower percentages of sound whole kernel

Physical properties

In general, the values of physical properties in both brown and milled rice increased as sound whole kernel rate increased.

Regression analysis reported a highly significant relationship between mean thickness and mean sound whole kernel of both brown (left-hand plot) and milled rice (right-hand plot) within each variety (Figure 4.6). There was also a highly significant relationship between mean thousand-kernel weight and mean sound whole kernel of both brown (left-hand plot) and milled rice (right-hand plot) within each variety (Figure 4.7).

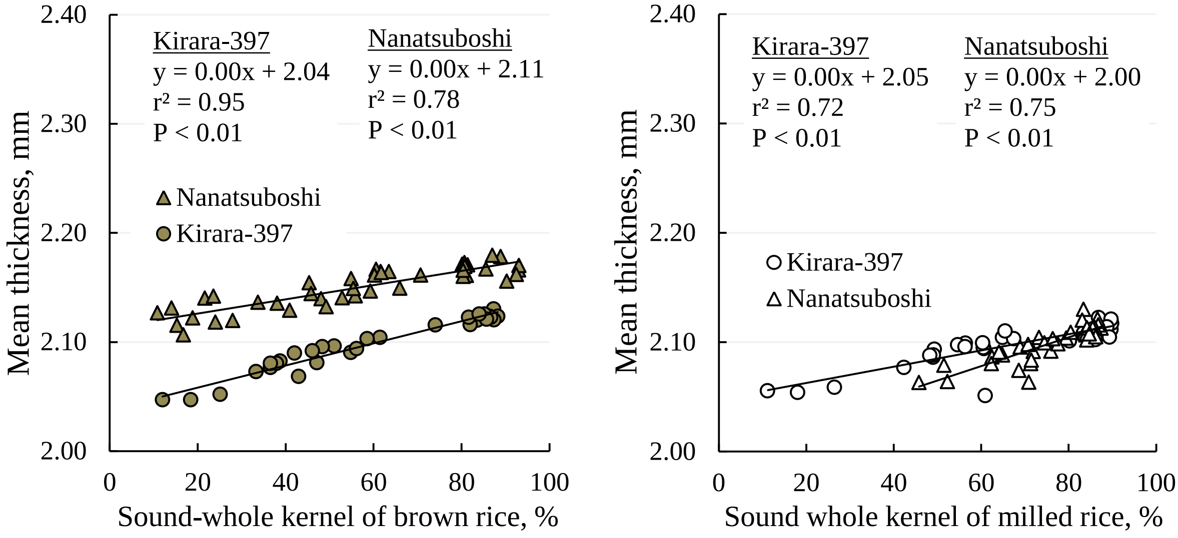

Figure 4.6 Relationship between thickness and maturity for brown and milled rice

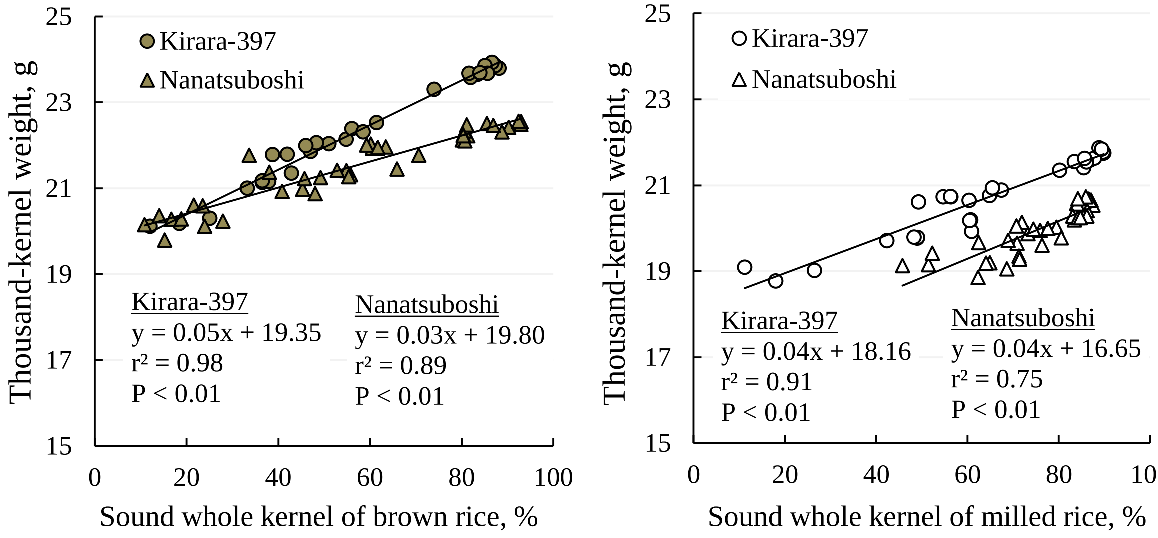

Figure 4.7 Relationship between thousand-kernel weight and maturity for brown and milled rice

In both brown and milled rice, samples with higher percentages of sound whole kernels were found to contain thicker and heavier kernels. In other words, samples comprised mostly of mature kernels tended to have thicker and heavier kernels.

Chemical properties

The values of amylose content in both brown and milled rice increased as sound whole kernel rate increased.

Regression analysis reported a highly significant relationship between mean amylose content and mean sound whole kernel of both brown and milled rice within each variety. Thus, samples comprised mostly of matured kernels tended to have higher amylose content within each variety. However, at higher percentages of the sound whole kernel (over approximately 70%), the level of amylose content remained similar for both varieties, mostly in milled rice (Figure 4.8).

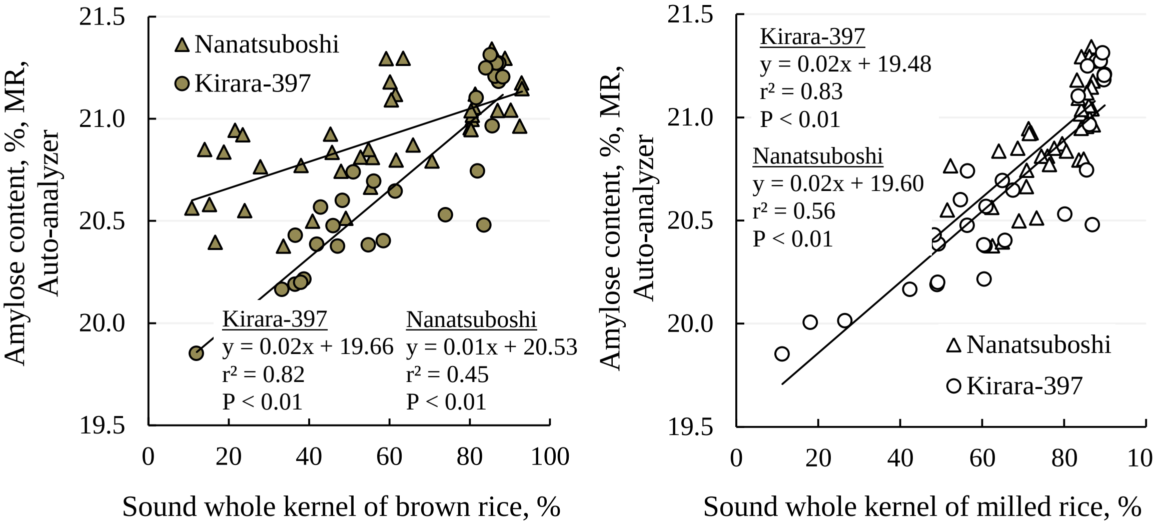

Figure 4.8 Relationship between amylose content and maturity for brown and milled rice

Samples with higher percentages of sound whole kernel had higher percentages of amylose content compared to samples with lower percentages of the sound whole kernel. This result could be due to the low activity of granule-bound starch synthase 1 (*GBSS-1*), which is responsible for amylose synthesis, in kernels located in the lower part of the rice panicle (Chun et al., 2015; Li et al., 2017).

Values of protein content in both brown and milled rice indicated varied behavior as sound whole kernel rate increased.

Regression analysis reported a highly significant relationship between mean protein content and mean sound whole kernel of both brown and milled rice within each variety. However, in contrast to *Kirara-397*, as sound whole kernel rate increased the protein content of *Nanatsuboshi* increased. This is an unexpected behavior, considering that glutelin molecular weight decreases with grain-fill (Shih, 2004) (Figure 4.9).

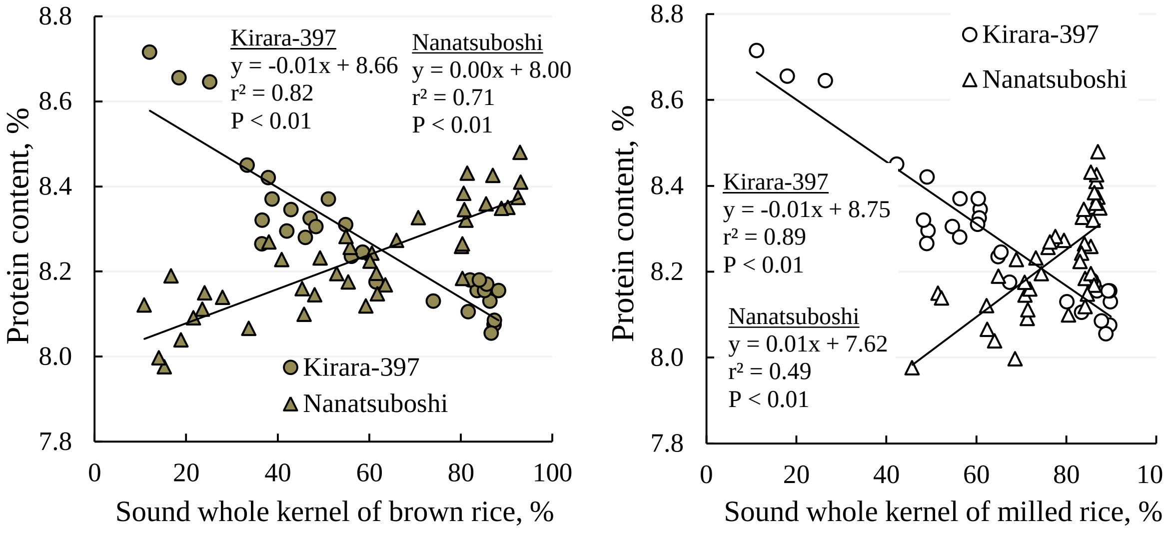

Figure 4.9 Relationship between protein content and maturity for brown and milled rice

This unexpected behavior could be related to the narrow range of thickness in samples of the *Nanatsuboshi* variety. The narrow range in thickness of the *Nanatsuboshi* variety indicated that the immature kernel fraction contains a higher percentage of thicker kernels (Figure 4.10). The immature kernel fraction usually indicated a higher percentage of protein content. In other words, there was a similarity in thickness in samples with different levels of maturity (mature and immature kernels). This suggested a detailed study of the effect of thickness and maturity on protein content.

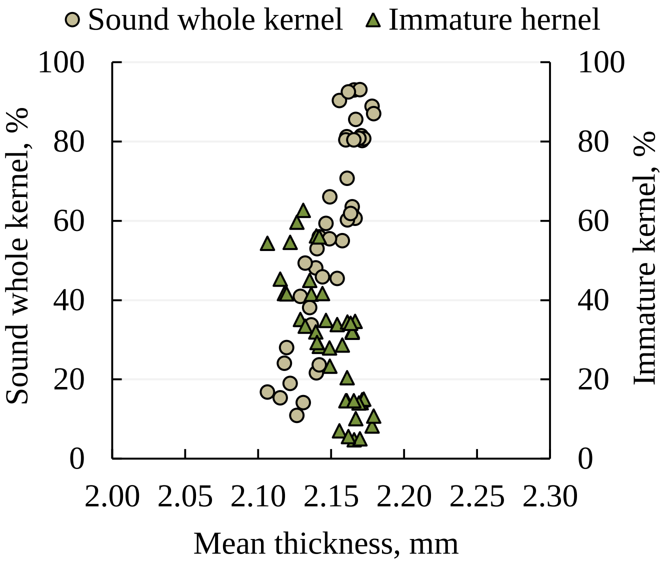

Figure 4.10 Relationship between mean thickness and immature kernel of brown rice of *Nanatsuboshi*

Moreover, the highly significant relationship between amylose content and sound whole kernel (Figure 4.8) suggested a relationship between amylose content and physicochemical properties.

Regression analysis reported a highly significant relationship between mean amylose content and mean thickness of both brown (left-hand plot) and milled rice (right-hand plot) (Figure 4.11).

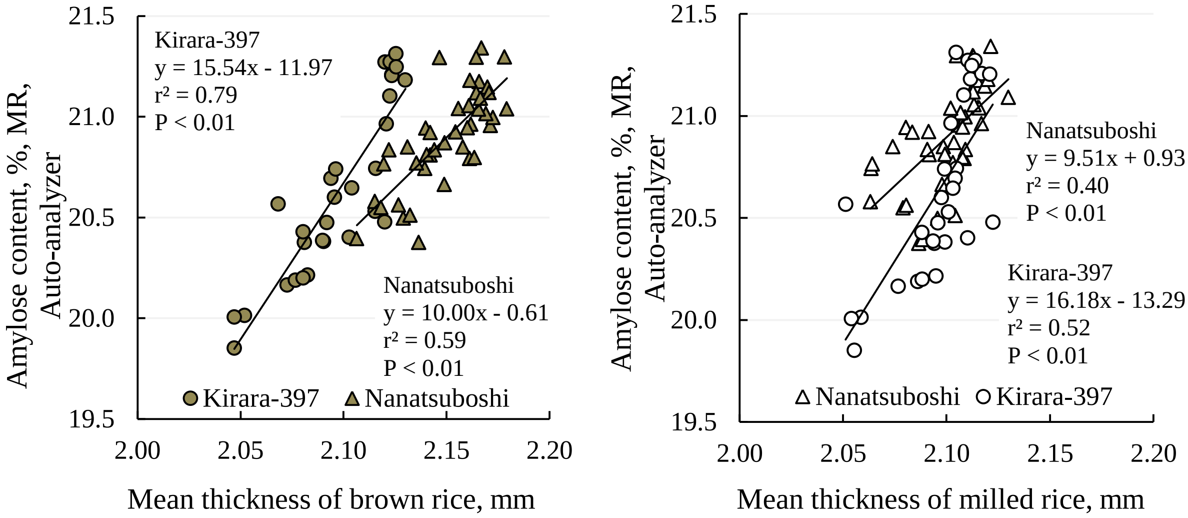

Figure 4.11 Relationship between amylose content and mean thickness of brown and milled rice

Regression analysis also indicated a highly significant relationship of mean amylose content on the mean thousand-kernel weight of both brown (left-hand plot) and milled rice (right-hand plot) (Figure 4.12).

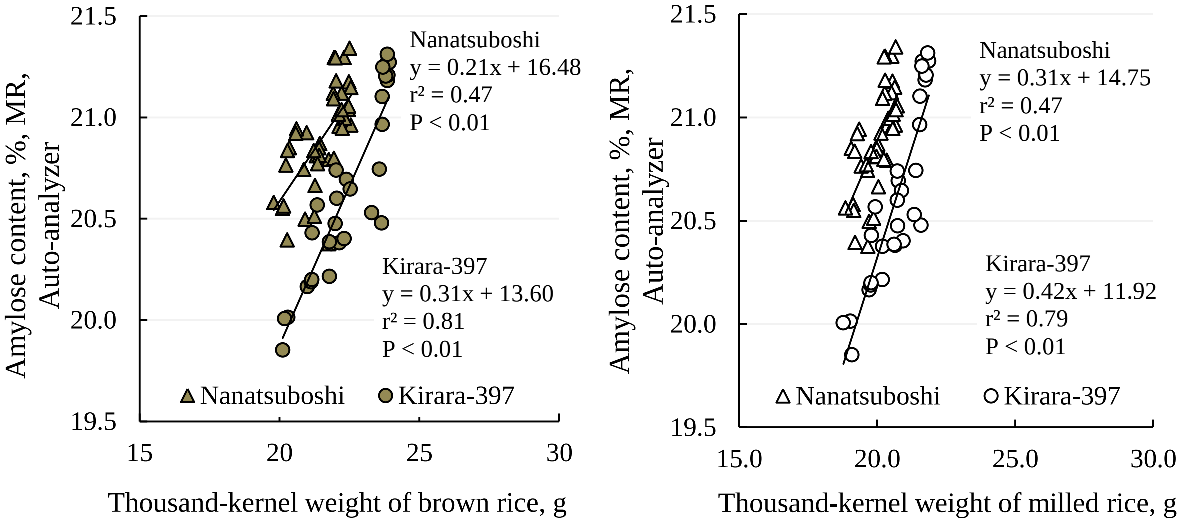

Figure 4.12 Relationship between amylose content and thousand-kernel weight of brown and milled rice

In addition, regression analysis indicated a highly significant negative effect of mean amylose content on both mean percentage of immature kernels of brown rice (left-hand plot) and mean percentage of chalky kernels of milled rice (right-hand plot). This means that milled samples comprised of kernels with higher percentages of amylose content tended to have lower percentages of both immature and chalky kernels (Figure 4.13).

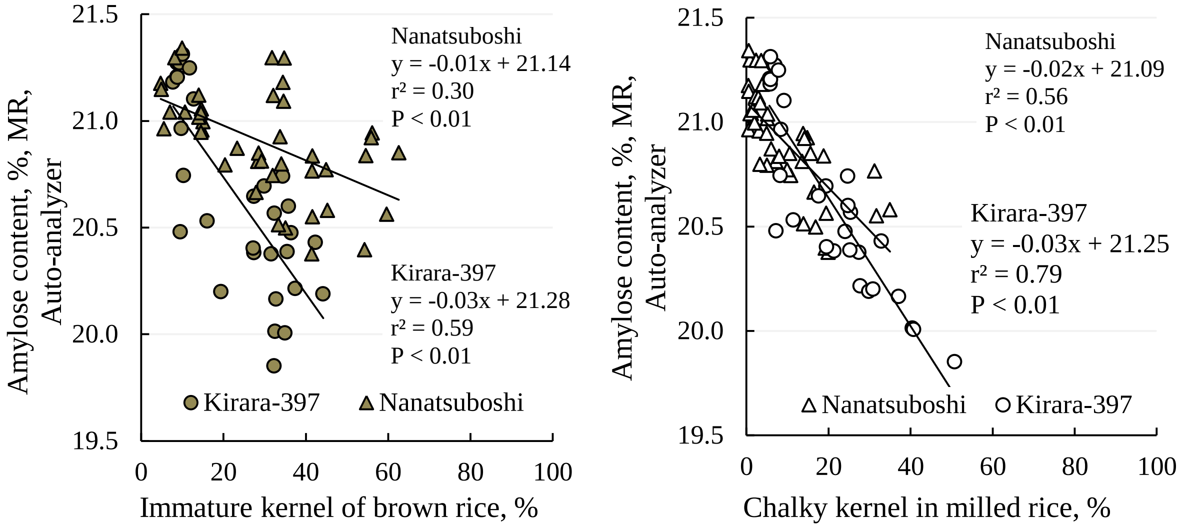

Figure 4.13 Relationship of amylose content with the percentage of immature kernels of brown rice and percentage of chalky kernel of milled rice

This result could be explained by the fact that rice chalkiness, which develops as a white cloudy region that could occupy part of or the total volume of milled kernel as a consequence of genetic and environmental factors (Patindol et al., 2015), has been reported to contain less amylose (Patindol and Wang, 2003) and to increase in kernels located in the lower and middle parts of the rice panicle (Siebenmorgen et al., 2013). Chalky kernels, in contrast to translucent ones, have been found to have loosely packed starch granules with notable air space between them. Those air spaces are responsible for both the refraction of light through the grain as well as the fissure formation in the kernel and its consequent breakage under mechanical stress (Buggenhout et al., 2013; Siebenmorgen et al., 2013; Patindol et al., 2015).

On the other hand, the lower percentage of immature kernels in samples with higher percentages of amylose content could be related to the lower activity of GBSSI enzymes, which is responsible for amylose synthesis, and hence a lower accumulation of amylose in starch per kernel (Umemoto et al., 1994; Umemoto et al., 2002; Biselli et al., 2014).

This result indicated that samples comprised mostly of thicker and heavier kernels tended to have higher percentages of amylose content, which is due to the fact that samples comprised of thicker and heavier kernels tended to have higher activity of GBSSI enzymes, and hence a higher accumulation of amylose in total starch per kernel. This leads to an increase in storage materials in the developing caryopsis. This corresponds to the findings of (Matsue et al., 1994b; Umemoto et al., 1994) in their study on Japanese varieties produced in the Honshu and Kyushu regions when the rice caryopsis was developing, as well as with the findings of (Matsue et al., 2001) in their study of Japanese varieties, and the findings of (Siebenmorgen et al., 2006) in their study of long-grain Indica varieties.

Furthermore, regression analysis reported a highly significant relationship between mean amylose content and mean protein content. However, the behavior in the relationship was varied. Samples of *Kirara-397* comprised of kernels with higher percentages of amylose content were found to have lower percentages of protein content. Meanwhile, samples of *Nanatsuboshi* comprised of a higher percentage of amylose content indicated a higher percentage of protein content. This is an unexpected result caused by the similarity in thickness in samples with different levels of maturity (mature and immature kernels). This suggested a detailed study of the effect of thickness and maturity on protein content, as was reported above (Figure 4.14).

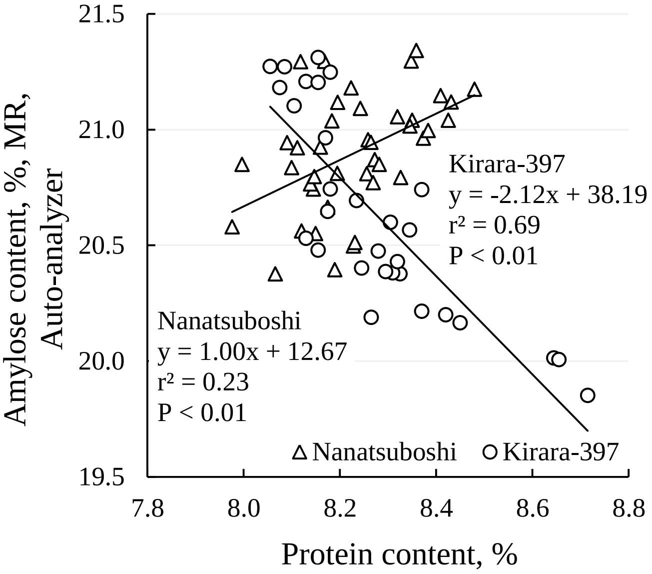

Figure 4.14 Relationship between amylose content and protein content of milled rice

In general, during processing by a color sorter machine, those samples of Japonica varieties produced in Hokkaido, Japan which had higher percentages of amylose content were found to be comprised mostly of thicker and heavier and to have lower percentages of both immature kernels of brown rice and chalky kernels of milled rice. This result was due to the fact that, because grain-fill happens from the top of the panicle downward at the stage of maturation, samples with higher percentages of the sound whole kernel were comprised mostly of matured kernels. That is, at harvesting time, these kernels could be located at both primary rachis branches and the upper part of the rice panicle and be exposed to a longer growing period, causing the greater development of kernel dimensions and a higher accumulation of starch. This result might also suggest a relationship between amylose content and the physicochemical properties of rice.

However, the behavior of protein content varied among varieties produced in Hokkaido, which suggested a detailed study of the effect of thickness and maturity on protein content.

Furthermore, based on the influence of amylose content on rice whiteness and the degree of translucency through the grain, the combined use of predicted amylose content and color information decreased the standard error of prediction and thus enabled reasonable non-destructive determination of the amylose content of rice at grain elevators on Hokkaido island in Japan (Jo et al., 2015; Kawamura et al., 2017). Here, a question arises. Based on the relationship between amylose content and physicochemical properties of Japonica rice, could the accuracy of the model to predict amylose content by the combined use of predicted amylose content and color information be improved by adding information on physicochemical properties to the model?

4.4.2 Relationship between physicochemical properties and kernel maturity during processing in a grain elevator

Brown rice component analysis

Component analysis of the *Nanatsuboshi* brown rice indicated that the percentage of the sound whole kernel was highest in the samples collected after the color sorter. Meanwhile, undesirable kernel, such as immature, broken, damaged, discolored and dead kernel, was highest in samples collected in the waste of the thickness grader (Figure 4.15).

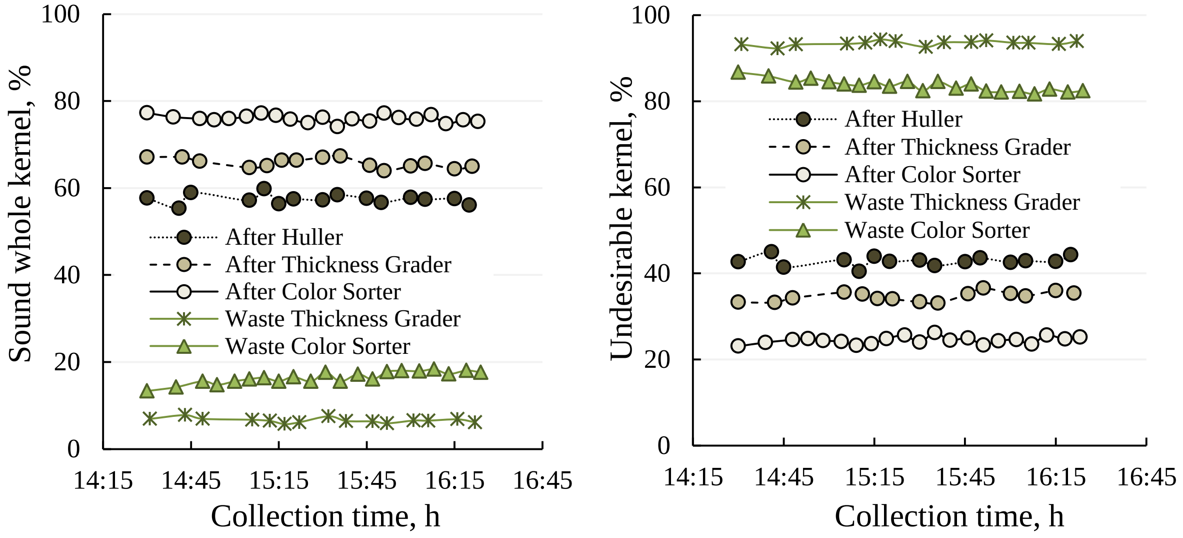

Figure 4.15 Fluctuation of component of brown rice of *Nanatsuboshi* during processing

These results were expected because the samples collected after the color sorter had been sorted by the thickness grader and by the brown rice color sorter machine, and therefore contained a higher percentage of sound whole kernels, indicating higher quality. On the other hand, samples in the waste of the thickness grader had been rejected after being processed by the thickness grader due to the higher percentage of undesirable kernels, indicating lower quality.

Physicochemical properties

Minimum, maximum, and mean (in bold) values of mean thickness, thousand-kernel weight and protein content of each sample collected at different stages of processing at a grain elevator are summarized in Table 4.1.

Table 4.1 Values of physicochemical properties of brown rice of *Nanatsuboshi* during processing

Process	Mean thickness, mm			Thousand-kernel weight, g			Protein content, %, DM, BR, Dumas		
	Min	Max	**Mean**	Min	Max	**Mean**	Min	Max	**Mean**
After Huller	2.12	2.14	**2.13 d**	21.3	21.8	**21.5 c**	7.5	7.8	**7.7 b**
After Thickness Grader	2.14	2.17	**2.16 b**	22.6	22.9	**22.7 b**	7.5	7.7	**7.6 b**
Waste Thickness Grader	1.87	1.89	**1.88 e**	13.8	14.5	**14.1 e**	7.9	8.2	**8.1 a**
After Color Sorter	2.16	2.18	**2.17 a**	23.0	23.2	**23.1 a**	7.5	7.7	**7.6 b**
Waste Color Sorter	2.14	2.15	**2.14 c**	20.7	21.3	**21.0 d**	7.2	7.4	**7.3 c**

For each test, the mean followed by the same letter in the column do not differ statistically at 1% probability through the one-way ANOVA and Tukey's simple main effect.

One-way ANOVA reported significant differences in mean thickness and mean thousand-kernel weight among the samples collected at different stages of processing at a grain elevator (Table 4.1 and **Error! Reference source not found.**).

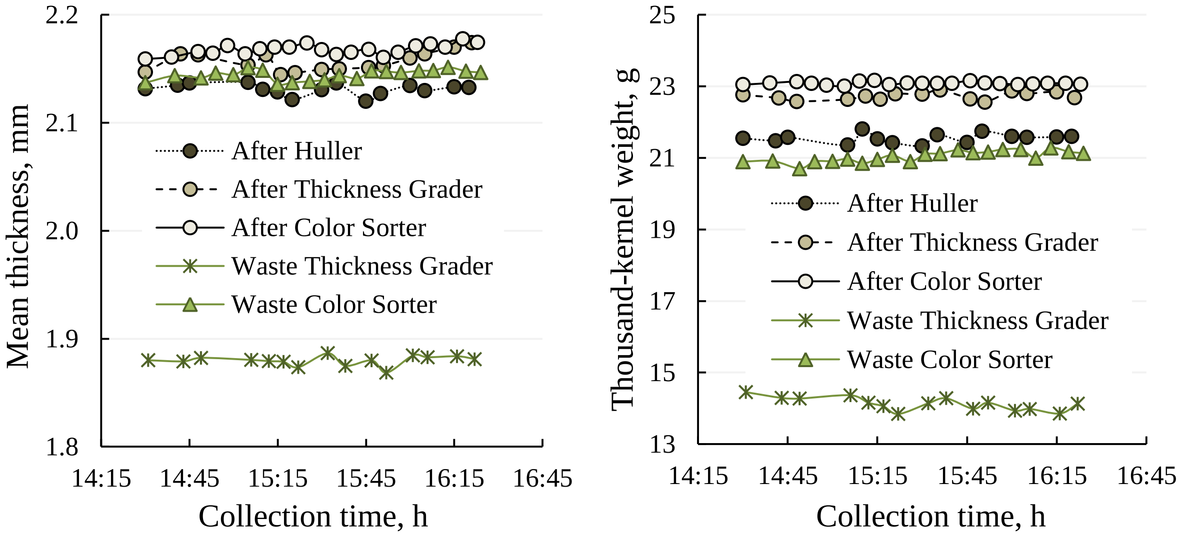

Figure 4.16 Fluctuation of physical properties of brown rice of *Nanatsuboshi* during processing

Moreover, one-way ANOVA also indicated significant differences in mean protein content between samples collected after the huller, after the thickness grader and after the color sorter on the one hand, and samples collected in the waste of thickness grader and color sorter on the other (Table 4.1 and Figure 4.17).

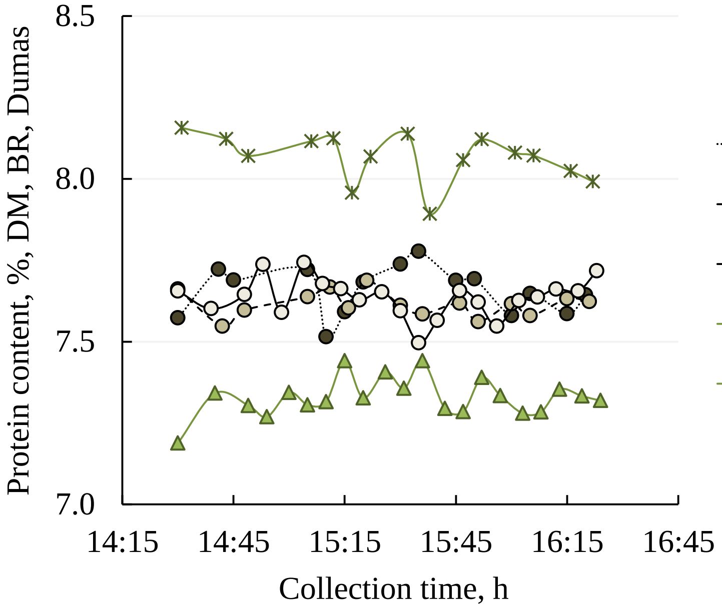

Figure 4.17 Fluctuation of protein content of brown rice of *Nanatsuboshi* during processing

Measurements of physical properties such as component analysis, mean thickness, and kernel thickness also reported expected results. In general, samples with higher percentages of sound whole kernel (after the huller, after the thickness grader, and after the color sorter), which were mostly composed of matured kernels, were found to have thicker and heavier kernels, and therefore tended to have higher quality than the less matured kernels which were found more in the rejected samples (waste of thickness grader and waste of color sorter).

On the other hand, samples collected from the waste of the color sorter showed the lowest protein content, which is an unexpected result considering the lower thousand-kernel weight and the higher percentage of the undesirable kernel. Previous studies have reported that samples with higher percentages of the undesirable kernel, which tend to contain thinner and lighter kernels, tend to have higher percentages of protein (Hillerislamberset al., 1973; Matsue, et al., 1992; Siebenmorgen et al., 2006).

This unexpected behavior could be related to the results for kernel thickness: mean thickness in samples collected in the waste of the color sorter was found to be similar to that in samples taken after the huller, after the thickness grader, and after the color sorter. In other words, there were similarities in thickness in samples with different levels of maturity (**Error!**

Reference source not found.). This suggested a detailed study of the effect of thickness and maturity on protein content.

4.5 Effect of brown rice thickness and maturity on protein content

As described earlier in this chapter, during processing by a color sorter and in a grain elevator in Hokkaido, Japan, rice of the Japonica variety produced in Hokkaido indicated the unexpected behavior of protein content. This unexpected behavior could be related to similarity in thickness in samples with different levels of maturity, which suggested a detailed study of the effect of thickness and maturity on protein content. Consequently, this part of the study was focused on analyzing the effect of brown rice thickness and maturity on protein content during processing.

4.5.1 Materials and methods

Rice sample

This study was conducted using brown rice of Japonica type variety *Yumepirika*, produced in Naie, Hokkaido, Japan in 2016.

Rice sample preparation

Rice main sample was collected after the huller machine during processing at Sunagawa rice terminal. Since the grain-fill happens from the top of the panicle downward and thickness is the last of the principal rice dimensions to reach its maximum level when the caryopsis is developing, the collected sample was fractionated into six different thickness fractions with thickness ranging at 0.1-mm intervals from less than 1.8 to more than 2.2 mm, and each thickness fraction was classified by human observation into levels of maturity, such as mature, immature and chalky kernel. Completely translucent kernels with light brown color were classified as the sound whole kernel. Meanwhile, completely translucent kernels with green color were classified as immature. On the other hand, kernels with an opaque region were classified as chalky.

Additional samples were collected at processes after the huller at the grain elevator such as after the thickness grader, in the waste of thickness grader, after the color sorter and in the waste of the color sorter.

Physical properties

Rice kernel shape, ratio of kernel length to kernel width (*L/W ratio*) was calculated using Equation 2.1 while ratio of kernel length to kernel thickness (*L/T ratio*) and ratio of kernel width to kernel thickness (*W/T ratio*) were calculated by Equation 4.2 and Equation 4.3 (Mohsenin, 1986).

$$L/T\ ratio = \frac{L}{T}$$ Equation 4.2

$$W/T\ ratio = \frac{W}{T}$$ Equation 4.3

Meanwhile, the volume of kernel *Kv* was calculated using Equation 2.2 (Jain and Bal, 1997).

Dimensional characteristics such as length *L*, width *W*, thickness *T*, length-width *L/W* plane area, and length-thickness *L/T* plane area were determined by a rice grain segregator (SATAKE, Hiroshima, Japan). Thousand well-distributed kernels, randomly drawn from the test samples, were analyzed.

Statistical analysis

Two-way analysis of variance (ANOVA) and Tukey's test with 99% of confidence was carried out to determine any significant differences among the means of thickness fractions and among levels of maturity.

4.5.2 Results and discussion

Rice thickness distribution

The thickness distribution of *Yumepirika* brown rice from a sample collected after the huller at a grain elevator is shown in Figure 4.18.

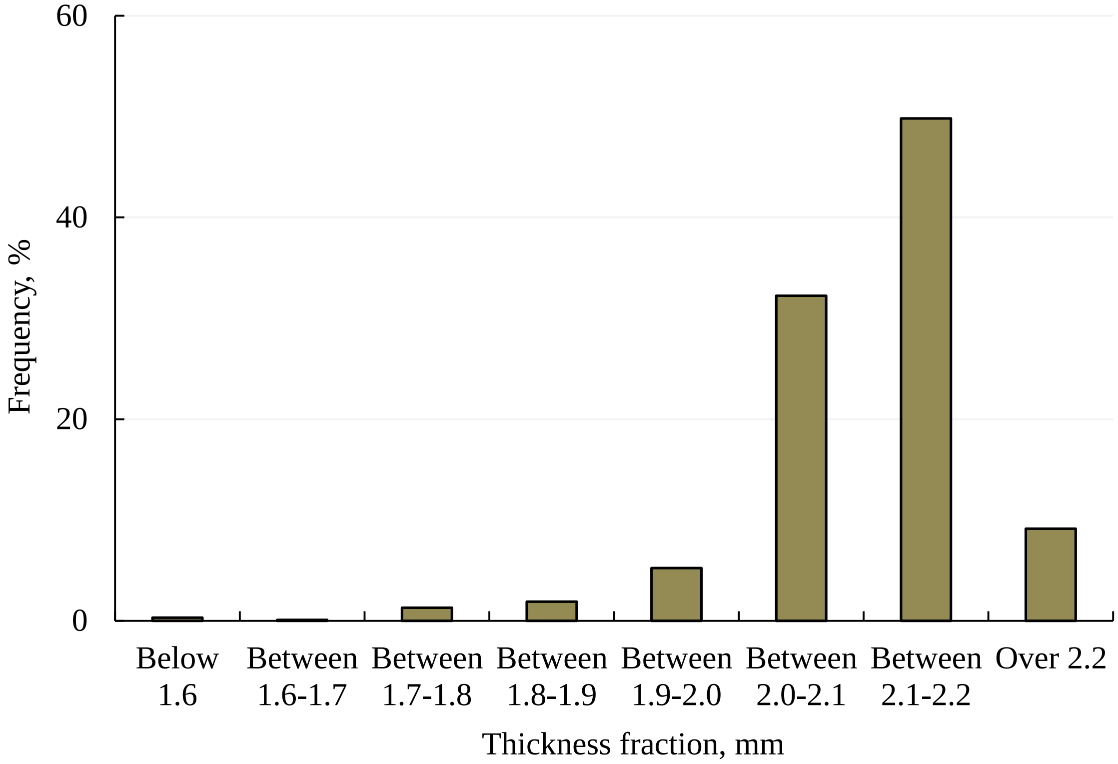

Figure 4.18 Thickness distribution of brown rice of *Yumepirika*

Thickness distribution indicated that the higher volume of *Yumepirika* brown rice was found in thickness fractions higher than between 1.9 and 2.0 (between 1.90.20), with the highest volume in the fraction between 2.1 and 2.2 (between 2.1-2.2). This result indicated the higher quality of the sample collected (Figure 4.18).

Rice component analysis

Component analysis of *Yumepirika* brown rice by thickness fraction collected after the huller at a grain elevator is shown in Figure 4.19.

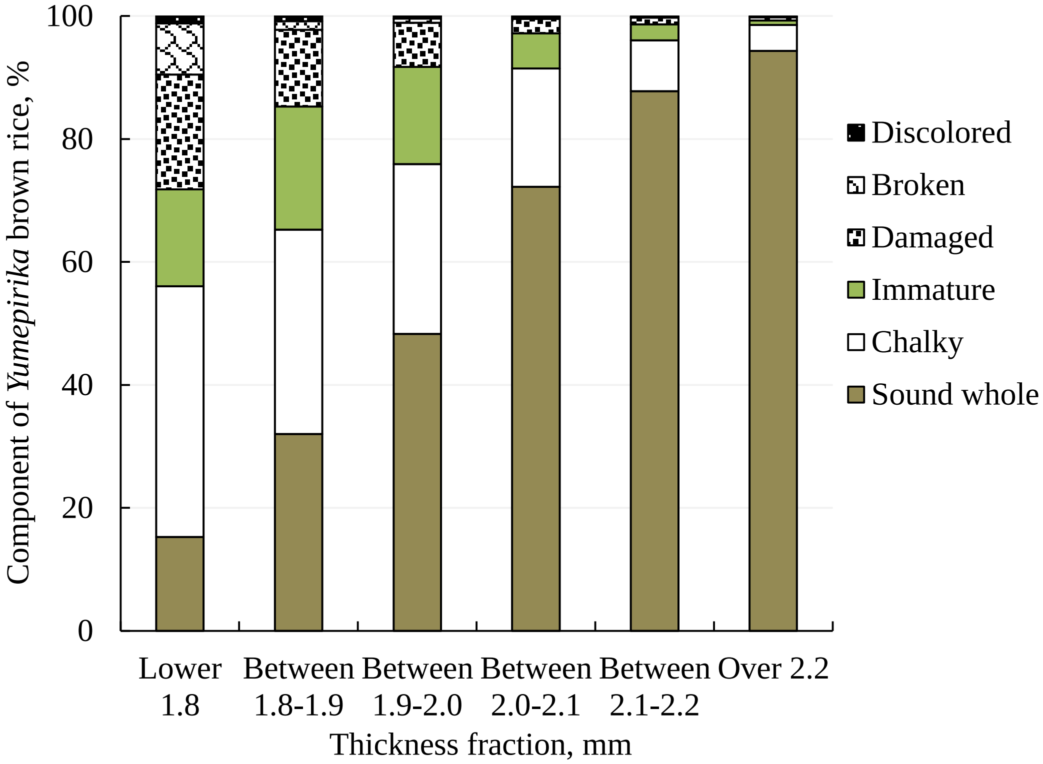

Figure 4.19 Component per thickness fraction of brown rice of *Yumepirika*

Component analysis of *Yumepirika* brown rice by thickness fraction indicated that the mean percentage of the sound whole kernel, by contrast with undesirable kernels, such as chalky, immature, damaged, broken and discolored kernel, increased as thickness fraction increased (Figure 4.19).

Physical properties

In general, kernel principal dimensions of the mature level, such as length, width and thickness, indicated higher values, and the mature level therefore showed the higher kernel volume (Figure 4.20) and thousand-kernel weight (Figure 4.21) in comparison with chalky and immature levels. In addition, these physical properties increased as thickness increased. In other words, the mature level of *Yumepirika* brown rice, which was mostly comprised of fully mature kernels, was found to have longer, wider and thicker, thus higher volume kernel, as well as heavier samples within each thickness fraction.

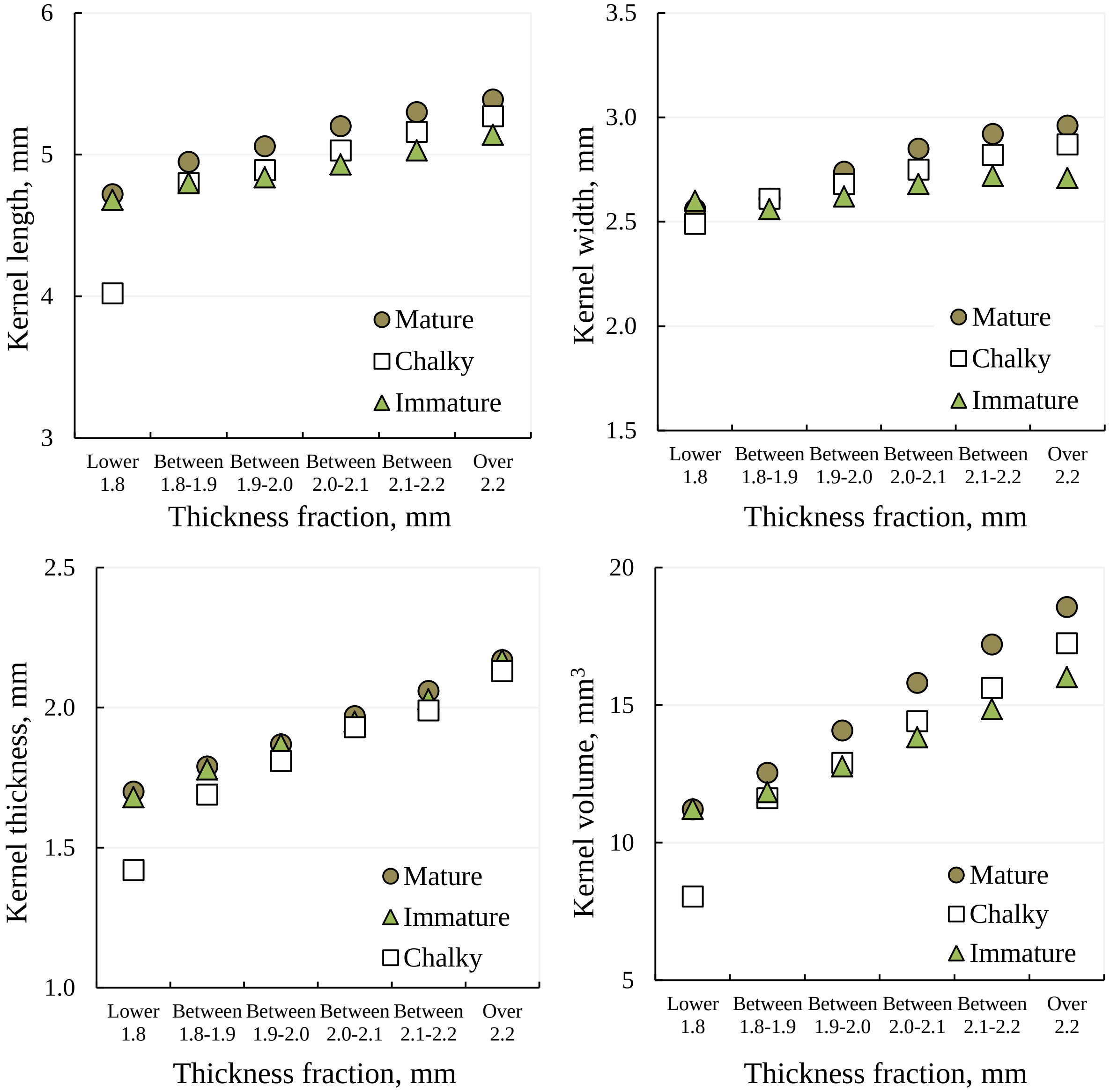

Figure 4.20 Behavior of kernel dimensions and volume of a rice kernel of brown rice of *Yumepirika* according to maturity level per thickness fraction

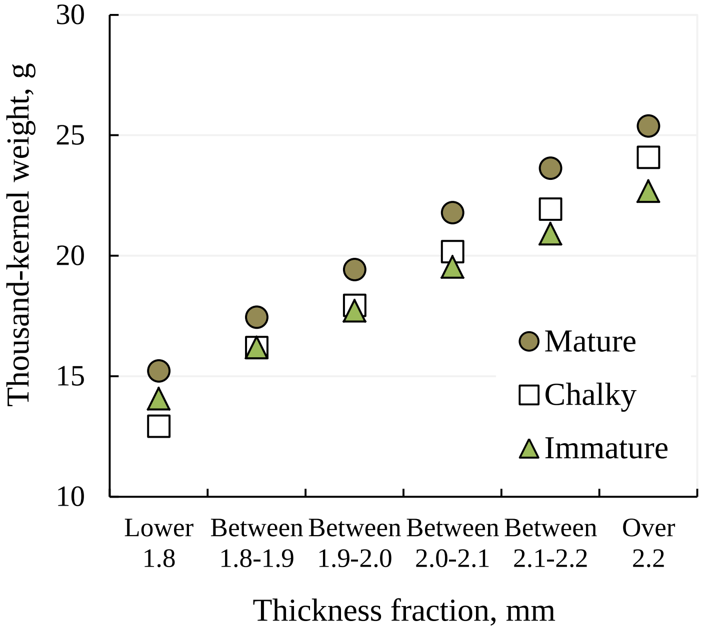

Figure 4.21 Behavior of thousand-kernel weight of brown rice of *Yumepirika* according to maturity level per thickness fraction

Moreover, two-way ANOVA reported highly significant differences among mean of kernel length, width, volume and thousand-kernel weight within each level of maturity as well as among thickness fractions. Significant differences were mostly found among the level of maturity in the higher thickness fractions (Figure 4.21 and Figure 4.21). However, mean kernel thickness did not report significant differences among the levels of maturity or among thickness fractions (Figure 4.20).

In general, this is an expected result due to the fact that kernels in the higher thickness fractions, by contrast with kernels in the lower thickness fractions, were found to be thicker and heavier, because such kernels were exposed to a longer growing period and were, therefore, able to store a higher volume of materials in the caryopsis during grain filling and consequently reach a higher level of maturity (Matsue et al., 2001; Siebenmorgen, Bautista, and Meullenet, 2006; Edenio et al., 2015).

Protein content

In general, the mean protein content of the three levels of maturity of *Yumepirika* brown rice decreased as thickness fraction increased. In addition, the immature level was found to have the highest percentage of protein content among the maturity levels within each thickness fraction (Figure 4.22). This is an expected behavior considering that glutelin molecular weight decreases with grain-fill (Shih, 2004). Likewise, high protein content has been significantly correlated with light kernels and early heading (Hillerislambers et al., 1973; Tanaka, 2012).

Moreover, two-way ANOVA revealed significant differences among levels of maturity within each thickness fraction. This significant difference was mostly found between the immature level and both mature and chalky levels. However, in thickness fractions between 2.0 and 2.1 mm (Between 2.0-2.1) and between 2.1 and 2.2 mm (Between 2.1-2.2), considered samples of high quality, a closer relationship was found between mature and chalky levels (Figure 4.22).

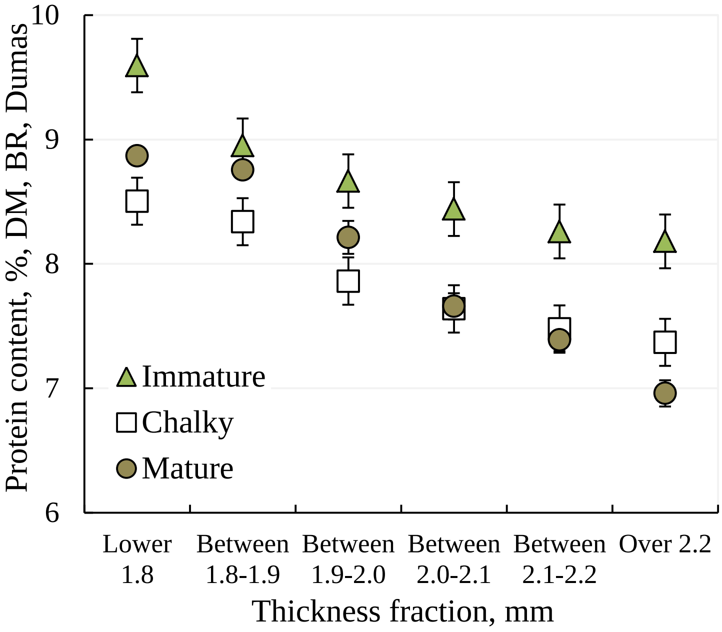

Figure 4.22 Behavior of protein content of brown rice of *Yumepirika* according to level of maturity per thickness fraction

Furthermore, considering that protein content in milled rice is approximately 1% lower than that of brown rice for Japonica varieties, samples in the mature and chalky levels of maturity in the three highest thickness fractions ("between 2.0-2.1", "between 2.1-2.2", and "over 2.2") indicated values of protein content closer to the range (for milled rice: ≤ 6.8 % and < 7.5 %) that has been found to correspond to high palatability and good taste for people in Hokkaido, Japan (Kawamura et al., 2013) (Table 4.2).

In general, samples in the mature level, by contrast with samples in the immature level, indicated the lowest percentage of protein content and were comprised of the thickest and lightest kernel among the levels of maturity within each thickness fraction. The level of protein content also decreased as thickness fraction increased. Consequently, and based also on the fact that higher thickness fractions indicated a range of protein content similar to that associated with high palatability and good taste for Japanese consumers, this suggested that rice could be potentially sorted according to protein content by thickness fraction.

Table 4.2 Mean value of protein content of brown rice of *Yumepirika* according to level of maturity per thickness fraction

Level of maturity	Protein content, %, DM, BR, Dumas					
	Lower 1.8	Between 1.8-1.9	Between 1.9-2.0	Between 2.0-2.1	Between 2.1-2.2	Over 2.2
Mature kernel	8.9 Ab	8.8 Ba	8.2 Cb	7.7 Db	7.4 Eb	7.0 Fc
Chalky kernel	8.5 Ac	8.3 Bb	7.9 Cc	7.6 Db	7.5 Eb	7.4 Fb
Immature kernel	9.6 Aa	9.0 Ba	8.7 Ca	8.4 Da	8.3 Ea	8.2 Fa

For each test, the mean followed by the same capital letter in the row within each level of maturity among thickness fractions and by the same lower letter in the column within each thickness fraction among levels of maturity do not differ statistically at 1% probability through the two-way ANOVA and Tukey's simple main effect.

What is more, the fluctuation of *Yumepirika* brown rice protein content at different processes of the grain elevator is shown in Figure 4.23. Samples collected in the waste of the thickness grader indicated the higher percentage of protein content, followed by the sample collected in the waste of the color sorter. One-way ANOVA also indicated significant differences in mean protein content between samples collected after the huller, after the thickness grader and after the color sorter on the one hand, and samples collected in the waste of thickness grader and color sorter on the other (Figure 4.23).

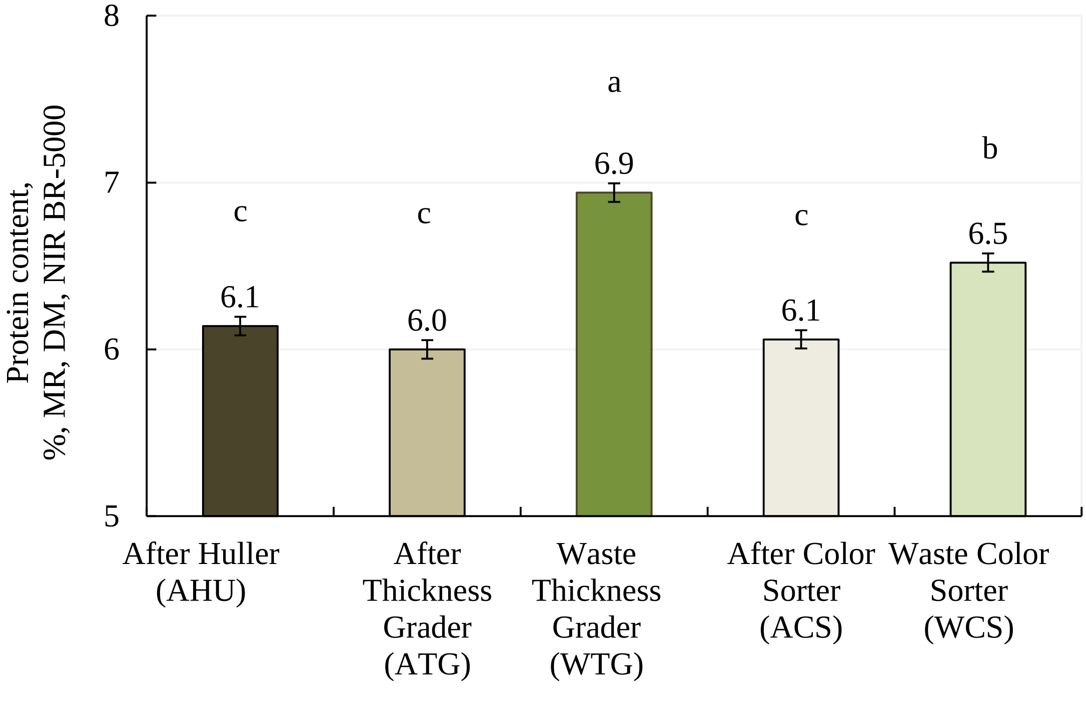

Figure 4.23 Fluctuation of protein content of brown rice of *Yumepirika* during processing

This result is similar to the behavior of the *Nanatsuboshi* protein content at different processes of the grain elevator (Figure 4.17). Thus, based on the fluctuation of protein content after processing by the thickness grader, the process where there was the highest difference in protein content values (after thickness grader indicated lower protein content and in the waste of the thickness grader indicated the highest (Figure 4.17 and Figure 4.23)), this suggested that rice could potentially be sorted according to protein content using the thickness grader.

In other words, the thickness grader could be used to sort rice according to protein content. Thinner and lighter kernels, which indicated higher percentages of protein content, could be found in the bulk of rice rejected in the waste of the thickness grader. Meanwhile, thicker and heavier kernels, which indicated the lower percentage of protein content, which is considered rice of higher palatability, could be obtained after the thickness grader. However, the difference in values of protein content showed in the waste of thickness grader and after the thickness grader was significant (between 0.5 and 0.9%) (Table 4.1 and Figure 4.23). This result indicated that further research is needed into sorting rice according to protein content with narrow values of protein content (approximately 0.1%) obtained in samples collected after the thickness grader.

Relationship between protein content and physical properties

Pearson's correlation analysis indicated not only a close relationship between mean protein content and mean kernel thickness and mean thousand-kernel weight as previous studies have reported (Hillerislamberset al., 1973; Matsue, et al., 1992; Siebenmorgen et al., 2006), but also a highly significant relationship between mean protein content and kernel dimensions (length and width), mean kernel volume, mean whiteness, and mean kernel shape, mostly L/T ratio and W/T ratio, within each level of maturity (Table 4.3).

Table 4.3 Pearson's correlation between protein content and physical properties of brown rice of *Yumepirika* according to level of maturity

Physical Property	Protein content of mature level	Protein content of chalky level	Protein content of immature level
	n = 7	n = 7	n = 7
Protein content	1.00	1.00	1.00
Kernel length	-0.97 **	-0.88**	-0.84 *
Kernel width	-0.99 **	-0.95**	-0.69
Kernel thickness	-0.99 **	-0.95**	-0.87 *
Kernel volume	-0.99 **	-0.94**	-0.83 *
L/W ratio	0.78	-0.67	-0.66
L/T ratio	0.99 **	0.95 **	0.92 **
W/T ratio	0.91 **	0.92 **	0.92 **
Thousand-kernel weight	-0.99 **	-0.95**	-0.92 **
Whiteness	-0.95 **	-0.80 *	-0.86 *

For each test, the mean followed by ** and * do differ statistically at 1% and 5 % probability respectively through Pearson' correlation analysis

Up to now, protein content has been reported to be correlated to kernel dimensions such as length, width, thickness, and volume of the kernel, as well as to weight in mass. These physical properties also showed significant differences in levels of maturity. Nevertheless, in thickness fractions between 2.0 and 2.1 mm and between 2.1 and 2.2 mm, a closer relationship was found between mature and chalky levels. Consequently, another question has arisen. What is the cause of this similarity in the percentage of the protein content of the mature and chalky levels?

The answer to this question could be related to the shape of the kernel. The *L/W ratio* showed a closer similarity among three levels of maturity, and no effect was found on protein content (Table 4.3). On the other hand, kernel shape analyzed by *L/T ratio* (left-hand plot) and *W/T ratio* (right-hand plot) showed a close relationship between the mature and chalky levels of maturity (Figure 4.24). Both ratios were found to highly affect protein content in the higher thickness fractions (Table 4.3).

This result could be caused by the distribution of protein bodies in the aleurone layer. The mature tissue is commonly comprised of radially elongated cells filled up with compound starch granules and protein bodies. Those protein bodies, which are large in number in the aleurone layer, are mostly concentrated in the lateral and dorsal cells (Shih, 2004).

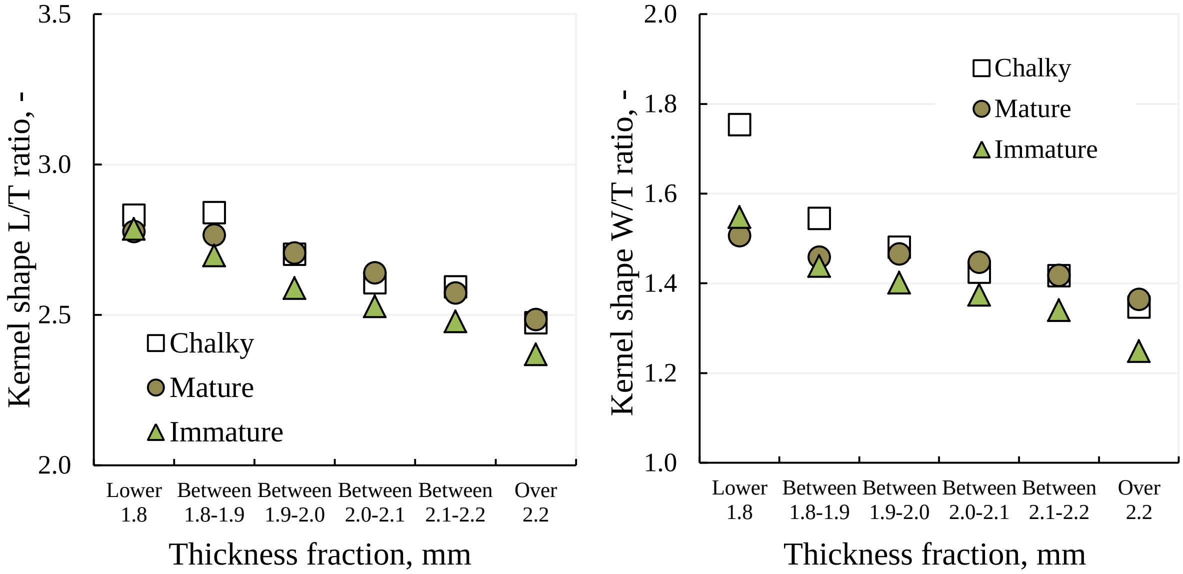

Figure 4.24 Relationship between kernel shape ratios and level of maturity per thickness fraction of brown rice of *Yumepirika*

4.6 Conclusions

From the results achieved in this chapter, it can be concluded that maturity showed an effect on physicochemical properties of Japonica rice produced in Hokkaido during processing by a color sorter and during processing at a grain elevator.

This result was due to the fact that, because grain-fill happens from the top of the panicle downward at the stage of maturation, samples with higher percentages of the sound whole kernel were comprised mostly of matured kernels. That is kernels that, at harvesting time, could be located at both primary rachis branches and the upper part of the rice panicle and be exposed to a longer growing period, causing the greater development of kernel dimensions and a higher accumulation of starch.

This result suggests a relationship between amylose content and the physicochemical properties of rice. Amylose content indicated a highly significant relationship with kernel thickness and thousand-kernel weight, as well as a casual but no correspondence relationship with protein content.

Furthermore, samples in the mature level, by the contrast of samples with samples in the immature level, indicated the lowest percentage of protein content as well as to be comprised by thicker and lighter kernel among the level of maturity within each thickness fraction. The level of protein content also decreased as thickness fraction increased. Consequently, and based also on the fact that higher thickness fractions indicated a range of protein content similar to that associated with high palatability and good taste for Japanese consumers, this suggested that rice could potentially be sorted according to protein content by thickness fraction. As a result, rice of high quality with high palatability and good taste could be obtained.

Moreover, the values of protein content after the rice was processed by the thickness grader suggested that rice could be sorted according to protein content using a thickness grader. However, the difference in values of protein content shown in the waste of the thickness grader and after the thickness grader was significant (between 0.5 and 0.9%). This result indicated that further research is needed into how to sort rice according to protein content with narrow values of protein content (approximately 0.1%) obtained in samples collected after the thickness grader.

In addition, protein content indicated varied behavior when related with maturity because of similarities in thickness in samples with different levels of maturity such as mature and chalky kernels. This similarity was caused by the shape of the kernel. Kernel shape indicators *L/T ratio* and *W/T ratio* showed a closer relationship between the mature and chalky levels of maturity. Both ratios were found to highly affect protein content in the higher thickness fractions. This result could be caused by the distribution of protein bodies in the aleurone layer. These protein bodies are mostly concentrated in the lateral and dorsal cells.

The information obtained in this study could contribute to more accurate quality assessment and help to secure the high palatability and good taste demanded by Japanese rice consumers.

4.7 Recommendation for further studies

Given the influence of amylose content on rice whiteness and the degree of translucency through the grain, the combined use of predicted amylose content and color information decreased the standard error of prediction and thus enabled reasonable non-destructive determination of the amylose content of rice at grain elevators on Hokkaido Island in Japan.

Based on the relationship between amylose content and physicochemical properties of Japonica rice, it is recommended to determine whether the accuracy of calibration model for assessing rice amylose content by near-infrared spectroscopy could be improved by using the information on physicochemical properties.

Moreover, since the difference in values of protein content shown in the waste of the thickness grader and after the thickness grader was significant (between 0.5 and 0.9%). This result indicated that further research is needed into how to sort rice according to protein content with narrow values of protein content (approximately 0.1%) obtained in samples collected after the thickness grader.

5. CHAPTER V: PHYSICAL AND CHEMICAL PROPERTIES FOR ENHANCING ACCURACY OF CALIBRATION MODELS TO DETERMINE AMYLOSE CONTENT OF RICE BY NEAR-INFRARED SPECTROSCOPY

5.1 Summary

This chapter is also focused on enhancing rice quality to meet Japanese consumer requirements. The relationship between physicochemical property of rice and kernel maturity reported in the previous chapter suggested a relationship between amylose content and physicochemical properties of Japonica rice. Consequently, this chapter will investigate whether, by using the information on physicochemical properties, the accuracy of the calibration model for determining amylose content of rice by near-infrared spectroscopy could improve and whether the model could thereby be put to practical use.

5.2 Introduction

Amylose content and protein content are essential constituents for determining the high palatability and good taste demanded by Japanese rice consumers.

Traditional methods, however, for assessing amylose and protein content are labor-intensive, time-consuming, chemicals dependent, and vulnerable to random error (Juliano, 1979; Delwiche et al., 1995; Delwiche et al., 1996; Wang et al., 2011; Duan et al., 2012; Biselli et al., 2014; Xie et al., 2014; Hu et al., 2015; Kaufman et al., 2015). To overcome this shortcoming, near-infrared (NIR) spectroscopy in combination with chemometric techniques, which is rapid (after the calibration model is developed), chemical-free, easy to use, and non-destructive (Manley, 2014), has been used as an alternative non-destructive method for assessing rice amylose and protein content (Satake, 1988; Villareal et al., 1994; Delwiche et al., 1996; Himmelsbach et al., 2000; Sohn et al., 2004; Cozzolino et al., 2014; Xie et al., 2014; Porep et al., 2015; Bagchi et al., 2016; Kawamura et al., 2017).

Several researchers have focused on amylose content as the most important constituent for rice quality. Amylose content has been determined by NIR spectroscopy techniques using milled rice flour (Satake, 1988; Delwiche et al., 1995; Himmelsbach et al., 2000; Sohn et al., 2004; Bao et al., 2007; Wu and Shi, 2007; Sampaio et al., 2017), single-kernel (XIAO et al., 2003; Wu and Shi, 2004, 2007; Bao et al., 2007; Rash and Meullenet, 2010) kernel in bulk (Villareal et al., 1994; Shimizu et al., 2003; Rash and Meullenet, 2010; Bagchi et al., 2016; Kawamura et al., 2017; Ohtsubo and Nakamura, 2017) (brown or milled rice), single-cooked rice kernel (Okadome et al., 2002). It has been determined using transmittance or reflectance modes as well as NIR Fourier-transform Raman (NIR-FT/Raman) spectroscopy

(Himmelsbach et al., 2000). However, almost all the results indicated that the accuracy obtained was only suitable for application in breeding programs.

When rice is received from farmers at grain elevators in Japan, its quality is inspected automatically based on protein content, moisture content and the percentage of the sound whole kernel of brown rice (Kondo and Kawamura, 2013). Ideally, amylose content would be added to the set of properties used to predict rice quality.

Various studies have shown that the measurement of moisture and protein content in rice by NIR is highly accurate and can be put to practical use at rice grain elevators (Kawamura, et al., 1999). However, an accurate calibration model cannot be developed using only a NIR spectrometer (Kawamura, et al., 2014). Based on the influence of amylose content on rice whiteness and the degree of translucency through the grain, combined analysis of NIR spectra and color information has been found to decrease the standard error of prediction (SEP) and thus enables reasonable non-destructive determination of amylose content of rice at grain elevators (Jo, et al., 2015; Kato et al., 2016; Kawamura et al., 2017). However, the desired outcome is to reach higher accuracy in determining amylose content of rice by NIR spectrometer for practical use at grain elevators in Japan.

Based on the suggested relationship between amylose content and physicochemical properties of Japonica rice laid out in chapter four above, I investigated whether, by using the information on physicochemical properties, the accuracy of the calibration model for determining amylose content of rice by near-infrared spectroscopy could be made more accurate and put to practical use. The hypothesis was that if the accuracy of the calibration model for determining amylose content could be improved by using physicochemical information, the amylose content could be added to the set of properties used to predict rice quality. This information could enable grain quality screening at grain elevators, which could help to secure the high palatability and good taste demanded by Japanese rice consumers.

5.3 Materials and methods

5.3.1 Rice sample

This study was conducted using brown rice non-waxy Japonica varieties (738 samples) from various varieties such as *Oborozuki*, *Kirara-397*, *Yumepirika*, *Fukkurinko*, *Nanatsuboshi* among other, produced in different areas of Hokkaido, Japan, namely, Hidaka, Hiyama, Iburi, Ishikari, Kamikawa, Oshima, Rumoi, Shiribeshi, and Sorachi, between 2010 and 2017.

5.3.2 Devices and methods of measurement

Physical properties information

Physical properties, such as estimated percentages of sound whole and immature kernels, length and width of the kernel of brown rice, percentage of sound whole and chalky kernels, and length and width of the kernel of milled rice, were obtained using a visible light segregator (VIS) grain segregator (Shizuoka Seiki, ES-1000, Fukuroi, Shizuoka, Japan).

Color information

Color information of both brown and milled rice, which is reflectance on Red Green and Blue (RGB) detected from the top (R1G1B1) and bottom (R2G2B2) surface of the grain and transmittance detected through the grain (R3G3B3)) were obtained using a VIS grain segregator (Shizuoka Seiki, ES-1000, Fukuroi, Shizuoka, Japan).

Chemical properties information

Amylose content (*AC*) was determined based on Iodine colorimetry using an auto-analyzer (Bran-Luebbe, Solid Prep III, Tokyo, Japan) following the protocol of Williams (Williams et al., 1958) with modifications by Inatsu (Inatsu, 1988). The absorption of the amylose-iodine complex was measured at 620 nm with a spectrophotometer, and the apparent amylose content was quantified against a calibration curve. In this study, the *Hoshinoyume* variety of rice grown in Hokkaido (moisture content: 13.09%, amylose content: 21.12%) and glutinous rice (amylose content: 0%) were used as standard amylose content to calculate the apparent amylose content (*AC*) of each sample. Apparent amylose content was expressed as a percentage (%).

Protein content (*PC*), was predicted by chemometric analysis. Partial least squares regression (PLS) was carried out analyzing reference protein content determined by the Kjeldahl method and NIR spectra information of milled rice.

Near-infrared NIR spectral information

Spectral data of both brown and milled rice were obtained using a NIR spectrometer (Shizuoka Seiki, BR-5000, Fukuroi, Shizuoka, Japan) with a wavelength range of 850 to 1048 nm and with 2-nm intervals.

The Savitzky-Golay derivative order: 2, polynomial order: 2, with left-side and right-side points: 2 as smoothing points, was used for pretreatment.

Chemometric analyses

The chemometric techniques partial least squares (PLS) regression and multiple linear regression (MLR) within the statistical software The Unscrambler (Version 10.3 Upgrade 10.3.0r4) were used for processing the data.

Three analyses were carried out to develop calibration models for non-destructive determination of amylose content of milled rice. These analyses were carried out using physical properties, color information, and spectral information of both brown and milled rice.

The first analysis was named "Only NIR", which is a conventional calibration model. Reference amylose content (AC) and NIR spectra information were analyzed by partial least squares (PLS) regression (Figure 5.1).

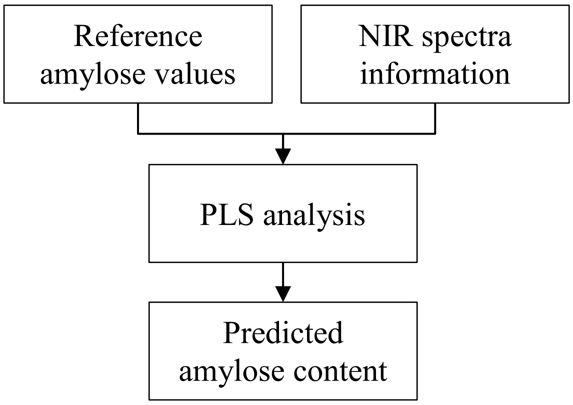

Figure 5.1 Flowchart for "Only NIR" analysis

The second analysis was named "NIR+CI". This is the method which has demonstrated the best accuracy in previous studies. Predicted amylose content (AC_{pred}) obtained from the "Only NIR" analysis and color information (CI) obtained from the VIS grain segregator were analyzed by multiple linear regression (MLR). Because of that, this analysis was classified as dual-step calibration models (Figure 5.2).

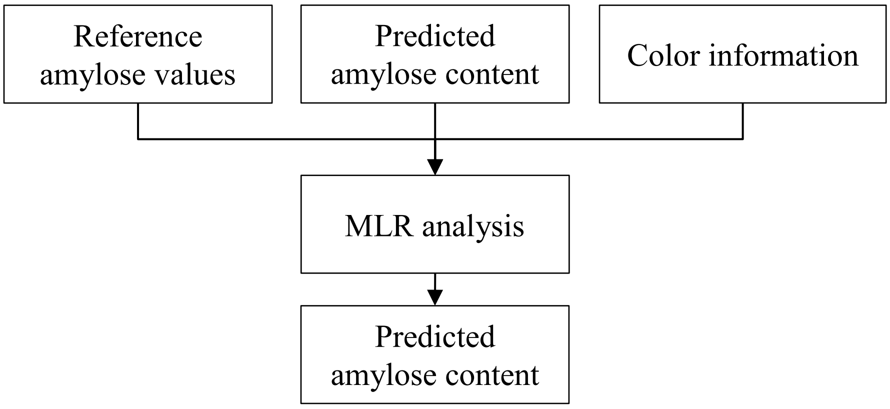

Figure 5.2 Flowchart for "NIR+CI" analysis

The third analysis and final analysis was named "NIR+PC+PP", which represents the exclusive use of physicochemical properties. Predicted amylose content (AC_{pred}) obtained from the "Only NIR" analysis and predicted protein content (PC_{pred}) and physical properties (PP) obtained from the VIS grain segregator were analyzed by multiple linear regression (MLR). This analysis was also classified as dual-step calibration models (Figure 5.3).

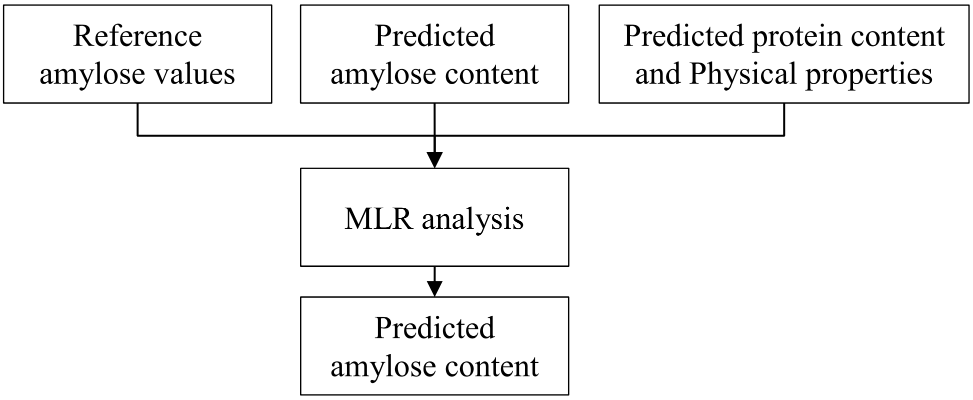

Figure 5.3 Flowchart for “NIR+PC+PP” analysis

The accuracy of the three analyses was compared to determine the most robust calibration model. Validation statistics such as coefficient of determination (r^2), systematic error (difference between reference and prediction values (Bias)), standard error of prediction (SEP), and the ratio of SEP to standard deviation of reference data (RPD) were used to determine the robustness of the calibration model (Williams, 2001; Cao, 2013; Esteve Agelet and Hurburgh, 2014; Porep et al., 2015).

In addition, to determining whether the accuracy of the calibration models for determining amylose content of rice could be of practical use, the analyses were calibrated using 8-production years of calibration set (2009-2016) and validated using 1-production year (2017) as well as using 7-production years of calibration set (2009-2015) and validated using 2-production year (2016-2017). The three analyses were also compared by increasing the years of production.

5.4 Results and discussion

The frequency of the reference amylose content values considering all the samples collected ranged between 11 and 24 % (left-hand plot). However, within all the samples collected, two groups were identified. The first group ranged between 11 and 20% but was largely focused on approximately 15% and was comprised of the low amylose content varieties (only *Oborozuki* and *Yumepirika* varieties). Meanwhile, the second group ranged between 16 and 24%, focused on approximately 20%, and was comprised of the Hokkaido ordinary amylose content varieties (not including *Oborozuki* and *Yumepirika* varieties) (Figure 5.4).

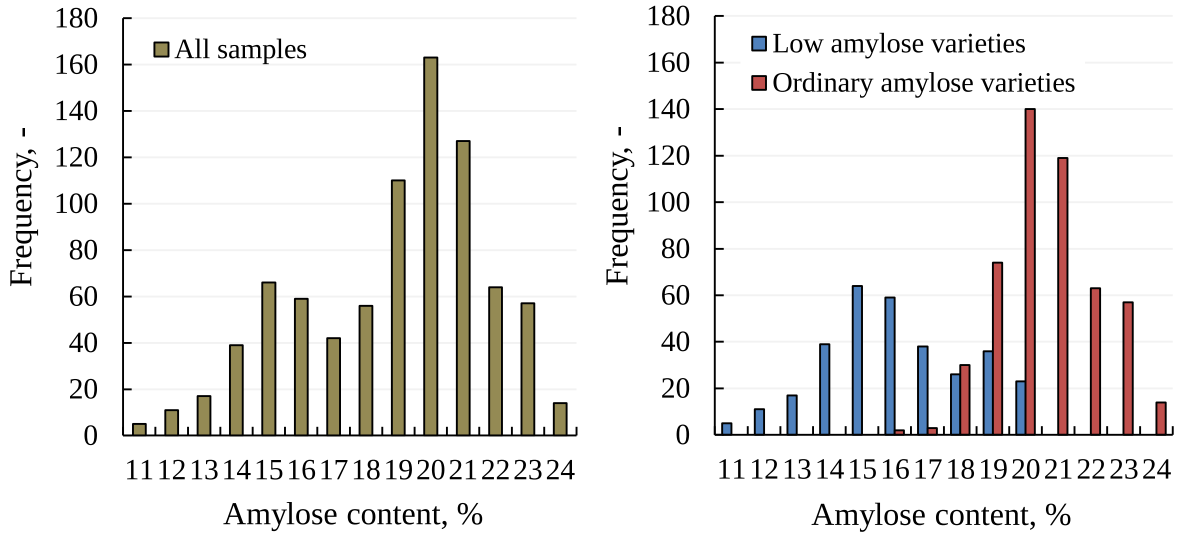

Figure 5.4 Histogram of the reference values of amylose content of Hokkaido varieties

Based on this result, calibration models of both low amylose content varieties and Hokkaido ordinary amylose content varieties were combined and the accuracy of the calibration models among the three analyses was compared.

5.4.1 Calibration models using physical properties, color and spectral information of brown rice validated using one-production year

In this section, the three analyses were carried out using low amylose content varieties and Hokkaido ordinary amylose content varieties within all samples collected using data obtained from brown rice. The analyses were calibrated using 8-production years of calibration set (2009-2016, 738 samples of brown rice) and validated using 1-production year (2017, 94 samples of brown rice). Moreover, the analyses were compared by increasing the years of production.

The frequency of reference amylose content values of the calibration set ranged between 11 and 24 %. Meanwhile, reference amylose content values of the validation set ranged between 18 and 24%. This result indicated that amylose content value of samples collected in 2017 tended to be higher in comparison with previous years (Figure 5.5). This could have been caused by lower temperatures during the rice grain developing period.

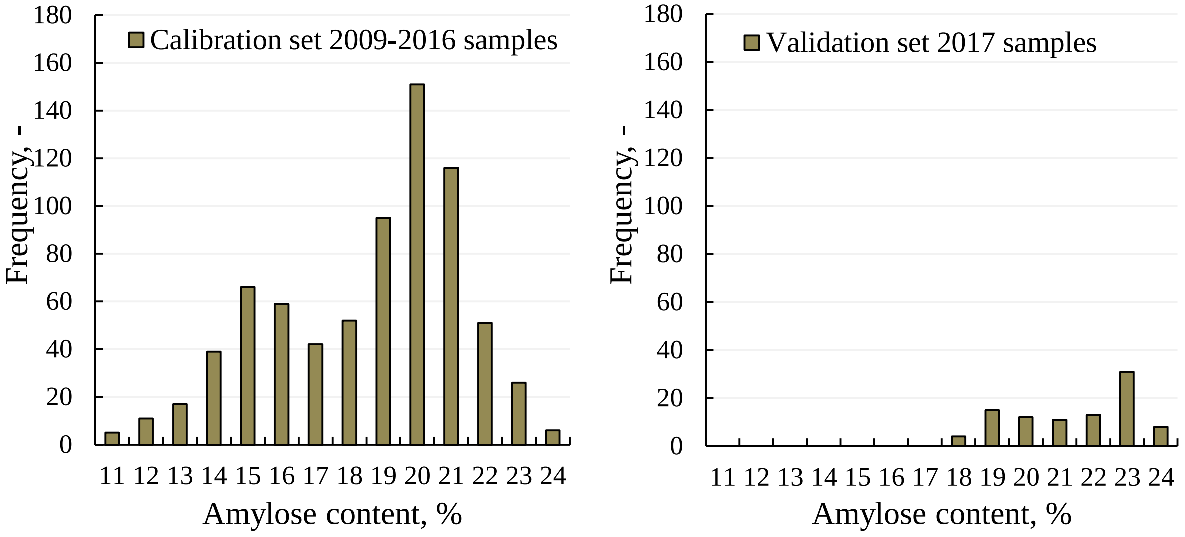

Figure 5.5 Histogram of the reference values of amylose content of the calibration set samples and the validation set samples validated using one-production year

Determination of amylose content by "Only NIR" analysis using brown rice data and validated using one-production year

Calibration models of both low amylose content varieties and Hokkaido ordinary amylose content varieties were determined separately by analyzing reference amylose content (AC) values and NIR spectra information of brown rice (Only NIR analysis). Both calibration models were then combined.

The relationship between reference amylose content and predicted amylose content of the combined calibration models obtained from the "Only NIR" analysis using a validation set of one-year production is shown in Figure 5.6.

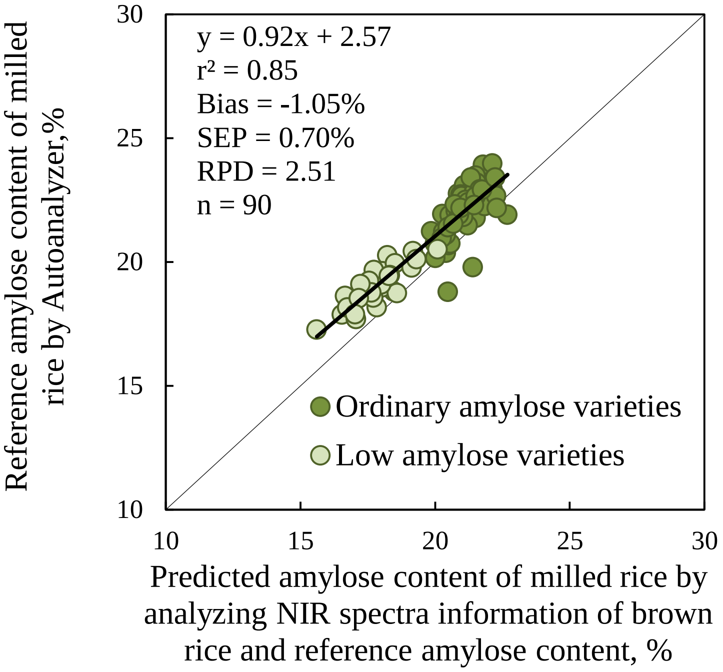

Figure 5.6 Relationship between reference amylose content and predicted amylose content of combined calibration models from "Only NIR" analysis using brown rice data and validated using one-production year

Determination of amylose content by "NIR+CI" analysis using brown rice data and validated using one-production year

Calibration models of both low amylose content varieties and Hokkaido ordinary amylose content varieties were determined separately by analyzing the predicted amylose content (AC_{pred}) obtained from the "Only NIR" analysis and color information (CI) of brown rice obtained from the VIS grain segregator (NIR+CI analysis). Both calibration models were then combined.

The relationship between reference amylose content and predicted amylose content of the combined calibration models obtained from the "NIR+CI" analysis using a validation set of one-year production is shown in Figure 5.7.

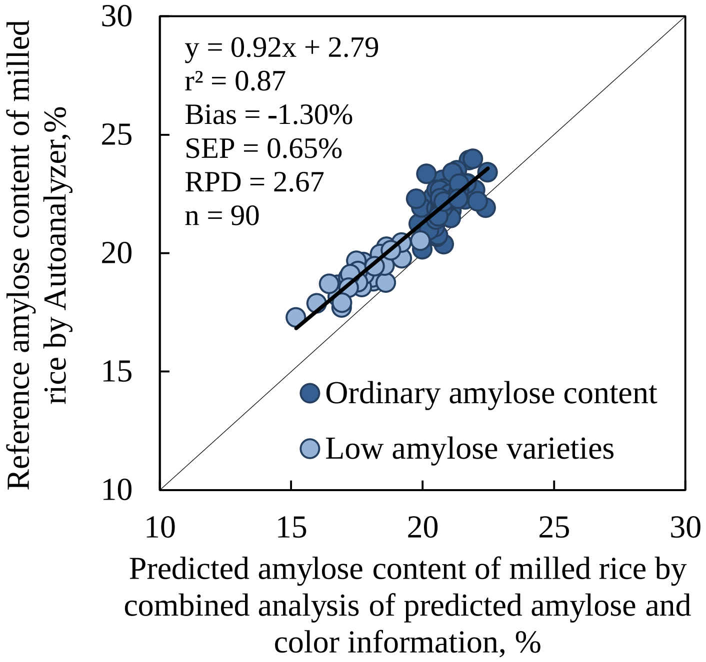

Figure 5.7 Relationship between reference amylose content and predicted amylose content of combined calibration models from "NIR+CI" analysis using brown rice data and validated using one-production year

Validation statistics of "NIR+CI" analysis indicated that $r^2 = 0.87$, SEP = 0.65% and RPD = 2.76. This result was slightly better than the result shown by "Only NIR" analysis, considering that r^2 and RPD values increased and SEP value decreased.

Determination of amylose content by "NIR+PC+PP" analysis using brown rice data and validated using one-production year

Calibration models of both low amylose content varieties and Hokkaido ordinary amylose content varieties were determined separately by analyzing the predicted amylose content (AC_{pred}) obtained from the "Only NIR" analysis, predicted protein content (PC_{pred}) and physical properties (PP) (physicochemical information) of brown rice obtained from the VIS grain segregator (NIR+PC+PP analysis). Both calibration models were then combined.

The relationship between reference amylose content and predicted amylose content of the combined calibration models obtained from the "NIR+PC+PP" analysis using a validation set of one-year production is shown in Figure 5.8.

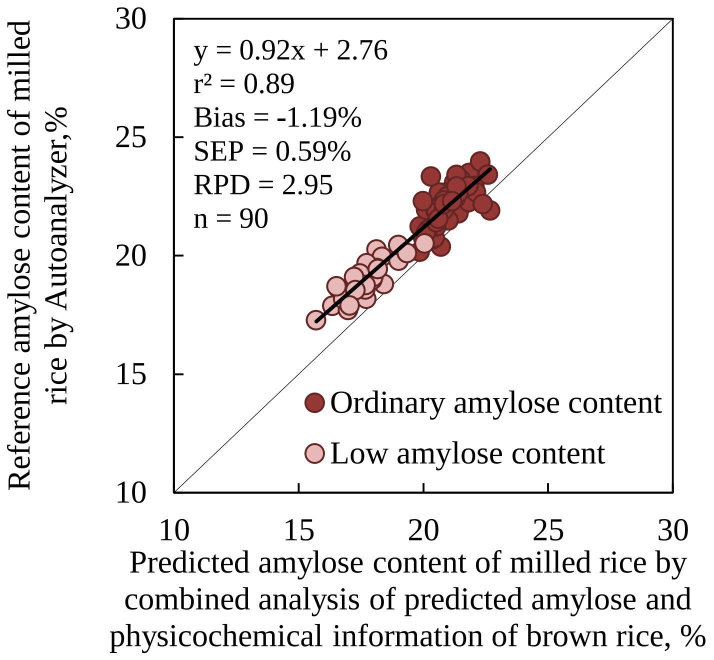

Figure 5.8 Relationship between reference amylose content and predicted amylose content of combined calibration models from "NIR+PC+PP" analysis using brown rice data and validated using one-production year

Validation statistics of "NIR+PC+PP" analysis indicated that $r^2 = 0.89$, SEP = 0.59% and RPD = 2.95. This result was better than the results shown by "Only NIR" and "NIR+CI" analyses, considering that r^2 and RPD values increased and SEP value decreased.

This result of the validation statistics indicated that the calibration models developed by the dual-step "NIR+PC+PP" analysis enable grain quality screening to be done in accordance with rice amylose content at grain elevators.

Comparison among analyses using brown rice data and validated using one-production year

The comparison of the analyses was carried out by increasing the years of production based on the SEP and RPD values (Figure 5.9).

The combined calibration models of "NIR+PC+PP" analysis indicated lower values of SEP and higher values of RPD. In addition, by increasing the years of production, SEP values decreased and RPD values increased. These results reflect that "NIR+PC+PP" analysis was the most robust calibration model among the analyses carried out.

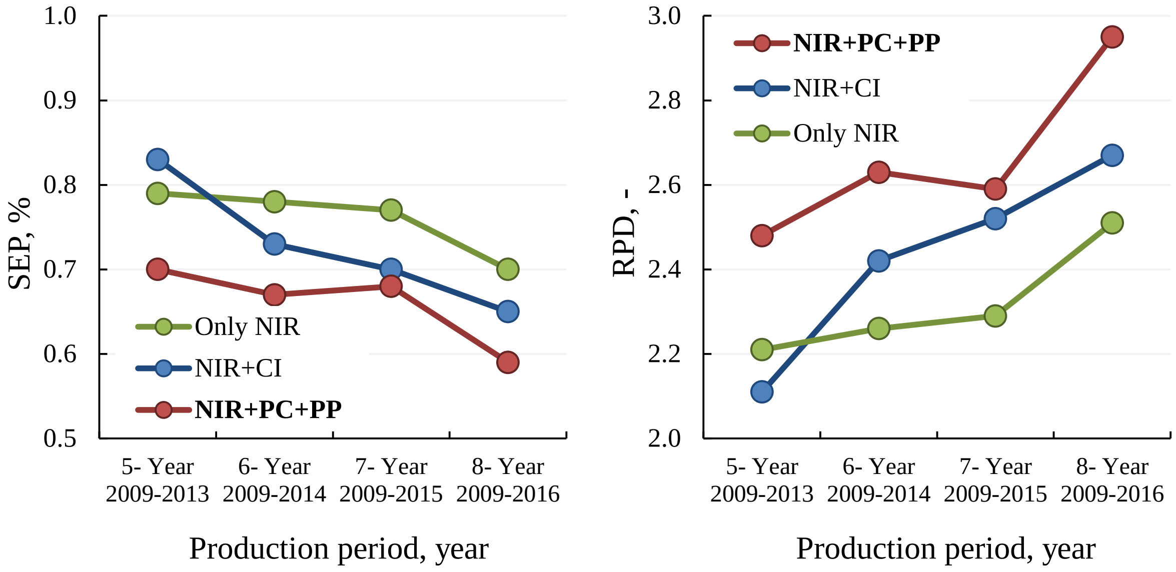

Figure 5.9 Comparison of SEP and RPD values of the analyses using brown rice data and validated using one-production year

5.4.2 Calibration models using physical properties, color and spectral information of brown rice validated using two-production years

In this section, the three analyses were carried out using low amylose varieties and using Hokkaido ordinary amylose content varieties within the all samples collected. The analyses were calibrated using a calibration set of 7-production years (2009-2015, 525 samples of brown rice) and validated using 2-production years (2016-2017, 195 samples of brown rice). Moreover, the analyses were compared by increasing the years of production.

The frequency of reference amylose content values of the calibration set ranged between 11 and 24%. Meanwhile, reference amylose content values of the validation set ranged between 14 and 24%, which is wider than the range shown by the one-year validation set. This result was caused by the higher range of amylose content of samples collected in 2017 (Figure 5.10).

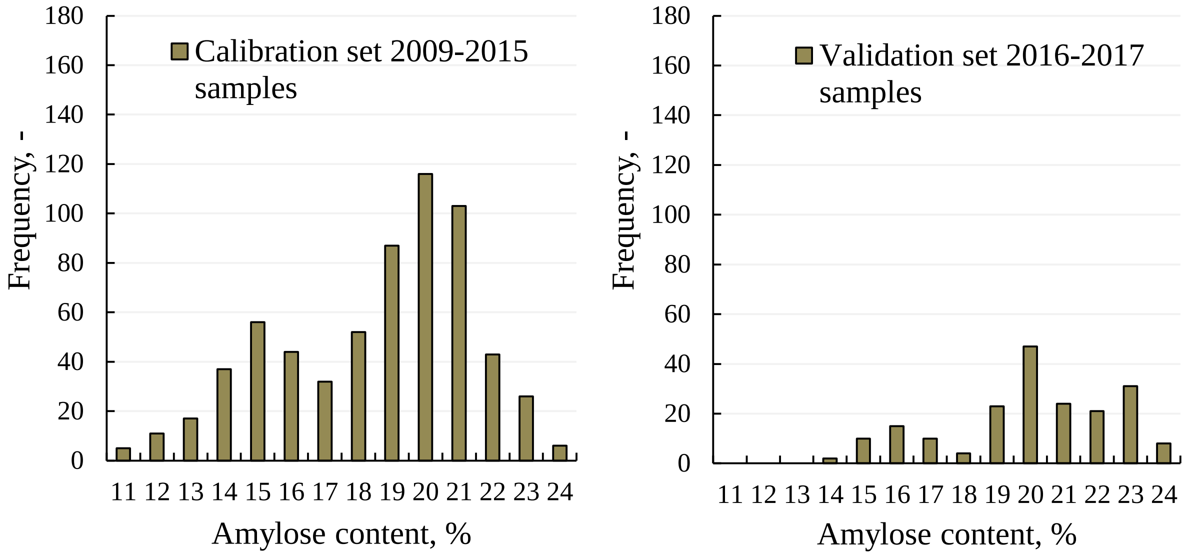

Figure 5.10 Histogram of the reference values of amylose content of the calibration set samples and the validation set samples validated using two-production years

Determination of amylose content by "Only NIR" analysis of brown rice data and validated using two-production years

As in the previous section, Calibration models of both low amylose content varieties and Hokkaido ordinary amylose content varieties were determined separately by analyzing reference amylose content (AC) values and NIR spectra information of brown rice (Only NIR analysis). Both calibration models were then combined.

The relationship between reference amylose content and predicted amylose content of the combined calibration models obtained from the "Only NIR" analysis using a validation set of two-years of production is shown in Figure 5.11.

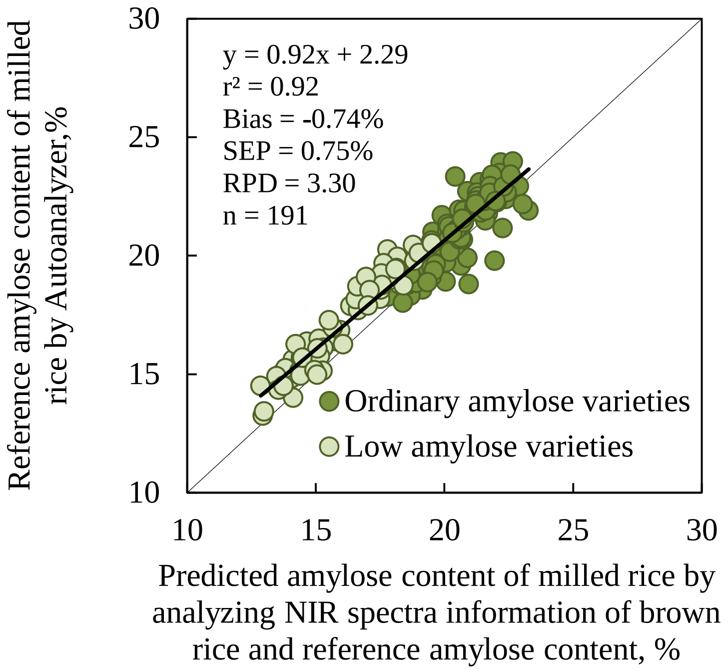

Figure 5.11 Relationship between reference amylose content and predicted amylose content of combined calibration models from "Only NIR" analysis using brown rice data and validated using two-production years

Determination of amylose content by "NIR+CI" analysis using brown rice data and validated using two-production years

Calibration models of both low amylose content varieties and Hokkaido ordinary amylose content varieties were determined separately by analyzing the predicted amylose content (AC_{pred}) obtained from the "Only NIR" analysis and color information (CI) of brown rice obtained from the VIS grain segregator (NIR+CI analysis). Both calibration models were then combined.

The relationship between reference amylose content and predicted amylose content of the combined calibration models obtained from the "NIR+CI" analysis using the two-production years of validation set is shown in Figure 5.12.

Validation statistics of "NIR+CI" analysis indicated that $r^2 = 0.91$, SEP = 0.76% and RPD = 3.25. This result was similar to the result shown by "Only NIR" analysis, considering that r^2, SEP, and RPD values reported closer values.

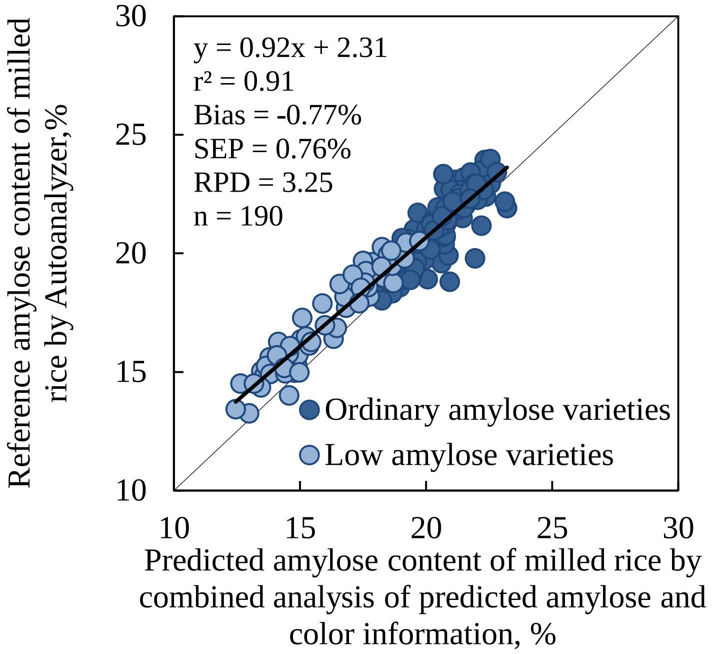

Figure 5.12 Relationship between reference amylose content and predicted amylose content of combined calibration models from "NIR+CI" analysis using brown rice data and validated using two-production years

Determination of amylose content by "NIR+PC+PP" analysis using brown rice data and validated using two-production years

Calibration models of both low amylose content varieties and Hokkaido ordinary amylose content varieties were determined separately by analyzing the predicted amylose content (AC_{pred}) obtained from the "Only NIR" analysis, predicted protein content (PC_{pred}) and physical properties (PP) (physicochemical information) of brown rice obtained from the VIS grain segregator (NIR+PC+PP analysis). Both calibration models were then combined.

The relationship between reference amylose content and predicted amylose content of the combined calibration models obtained from the "NIR+PC+PP" analysis using a validation set of two-years production is shown in Figure 5.13.

Validation statistics of "NIR+PC+PP" analysis indicated that r^2 = 0.93, SEP = 0.70% and RPD = 3.56. This result was better than the results shown by "Only NIR" and "NIR+CI" analyses, considering that r^2 and RPD values increased and SEP values decreased.

This validation result indicated that the calibration models developed by the dual-step "NIR+PC+PP" analysis enable grain quality screening to be done in accordance with rice amylose content at grain elevators.

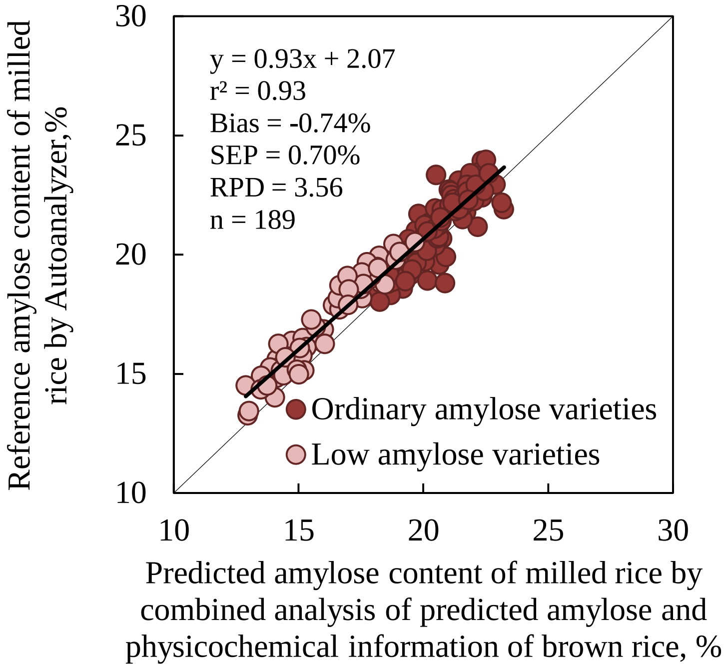

Figure 5.13 Relationship between reference amylose content and predicted amylose content of combined calibration models from "NIR+PC+PP" analysis using brown rice data and validated using two-production years

Comparison among analyses using brown rice data and validated using two-production years

The comparison of the analyses was carried out by increasing the years of production based on the SEP and RPD values (Figure 5.14).

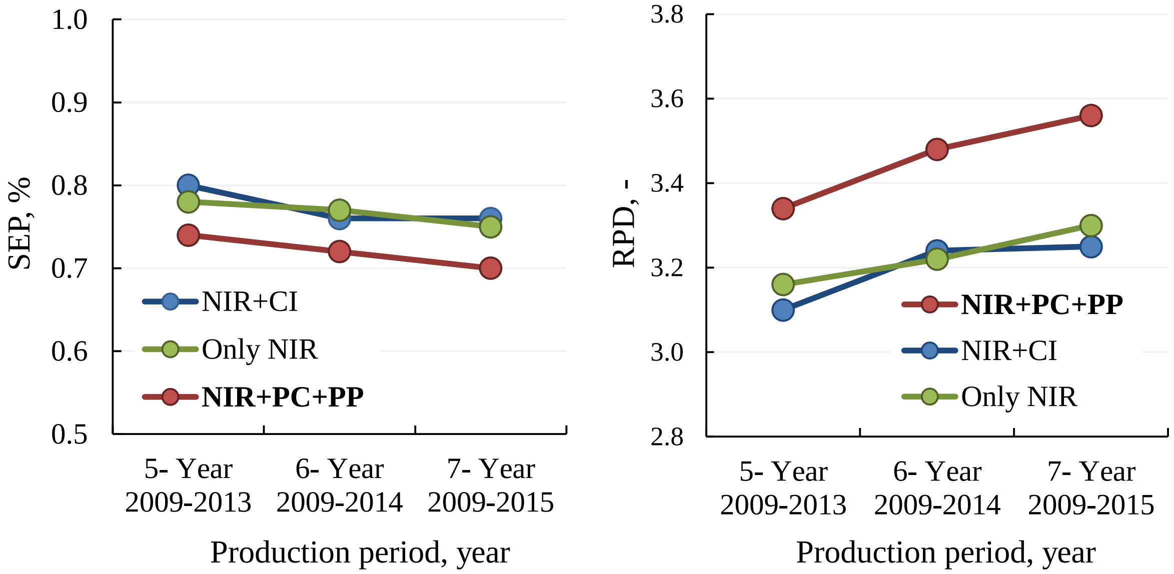

Figure 5.14 Comparison of SEP and RPD values of the analyses using brown rice data and validated using two-production years

5.4.3 Calibration models using physical properties, color and spectral information of milled rice validated by one-production year

In this section, the same three analyses were carried out using low amylose content varieties and using Hokkaido ordinary amylose content varieties within all samples collected using data obtained from milled rice. The analyses were calibrated using 8-production years of calibration set (2009-2016, 687 samples of milled rice) and validated using 1-production year (2017, 94 samples of milled rice). Moreover, the analyses were compared by increasing the years of production.

Determination of amylose content by "Only NIR" analysis using milled rice data and validated using one-production year

Calibration models of both low amylose content varieties and Hokkaido ordinary amylose content varieties were determined separately by analyzing reference amylose content values and NIR spectra information of milled rice (Only NIR analysis). Both calibration models were then combined.

The relationship between reference amylose content and predicted amylose content of the combined calibration models obtained from the "Only NIR" analysis using a validation set of one-year production is shown in Figure 5.15.

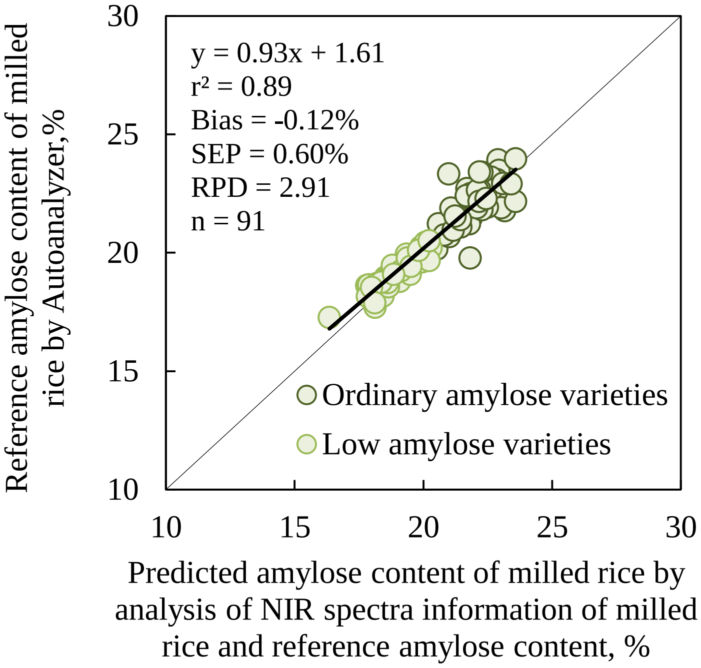

Figure 5.15 Relationship between reference amylose content and predicted amylose content of combined calibration models from "Only NIR" analysis using milled rice data and validated using one-production year

Determination of amylose content by "NIR+CI" analysis using milled rice data and validated using one-production year

Calibration models of both low amylose content varieties and Hokkaido ordinary amylose content varieties were determined separately by analyzing the predicted amylose content (AC_{pred}) obtained from the "Only NIR" analysis and color information (CI) of milled rice obtained from the VIS grain segregator (NIR+CI analysis). Both calibration models were then combined.

The relationship between reference amylose content and predicted amylose content of the combined calibration models obtained from the "NIR+CI" analysis using a validation set of one-year production is shown in Figure 5.16.

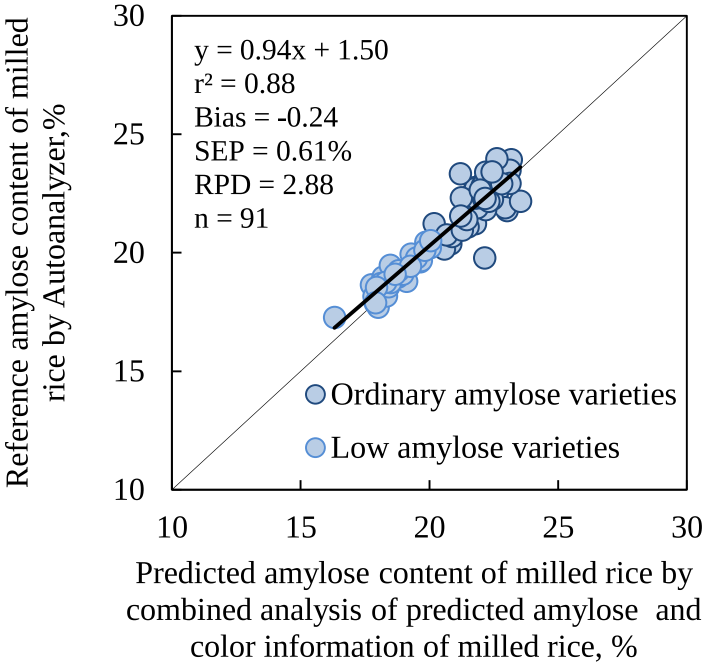

Figure 5.16 Relationship between reference amylose content and predicted amylose content of combined calibration models from "NIR+CI" analysis using milled rice data and validated using one-production year

Validation statistics of "NIR+CI" analysis indicated that $r^2 = 0.88$, SEP = 0.61% and RPD = 2.88. This result was similar to the result shown by "Only NIR" analysis, considering that r^2, RPD, and SEP indicated similar values.

Determination of amylose content by "NIR+PC+PP" analysis using milled rice data and one-production year

Calibration models of both low amylose content varieties and Hokkaido ordinary amylose content varieties were determined separately by analyzing the predicted amylose content

(AC_{pred}) obtained from the "Only NIR" analysis, predicted protein content (PC_{pred}) and physical properties (PP) of milled rice obtained from the VIS grain segregator (NIR+PC+PP analysis). Both calibration models were then combined.

The relationship between reference amylose content and predicted amylose content of the combined calibration models obtained from the "NIR+PC+PP" analysis using a validation set of one-year production is shown in Figure 5.17.

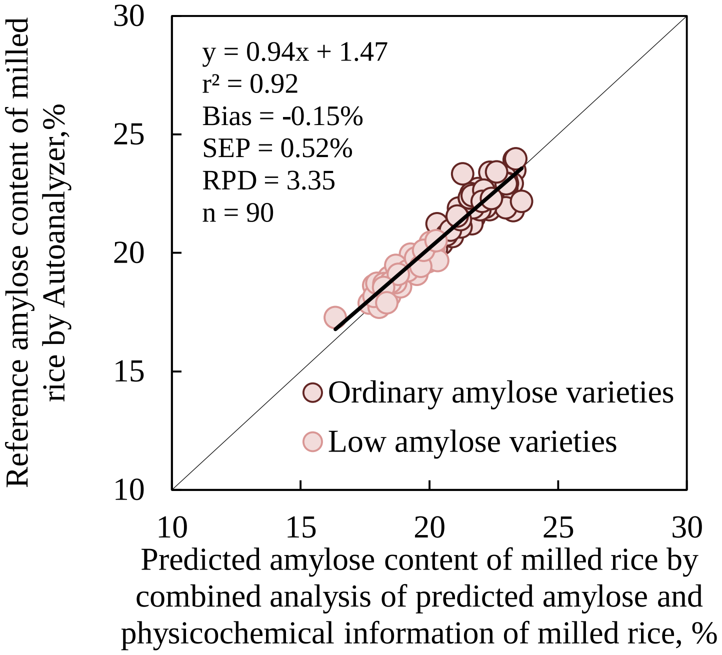

Figure 5.17 Relationship between reference amylose content and predicted amylose content of combined calibration models from "NIR+PC+PP" analysis using milled rice data and validated using one-production year

Validation statistics of "NIR+PC+PP" analysis indicated that $r^2 = 0.92$, SEP = 0.52% and RPD = 3.35. This result was better than the results shown by "Only NIR" and "NIR+CI" analyses, considering that r^2 and RPD values increased and SEP value decreased.

This result of the validation statistics indicated that the calibration models developed by the dual-step "NIR+PC+PP" analysis using validation set of one-year production enable grain quality screening to be done in accordance with rice amylose content at grain elevators.

Comparison among analyses using milled rice data and validated using one-production year

The comparison of the analyses was carried out by increasing the years of production based on the SEP and RPD values (Figure 5.18). By increasing the years of production, SEP values decreased and RPD values increased.

The combined calibration models of "NIR+PC+PP" analysis indicated lower values of SEP and higher values of RPD, which reflects the most robust calibration model for the analyses carried out.

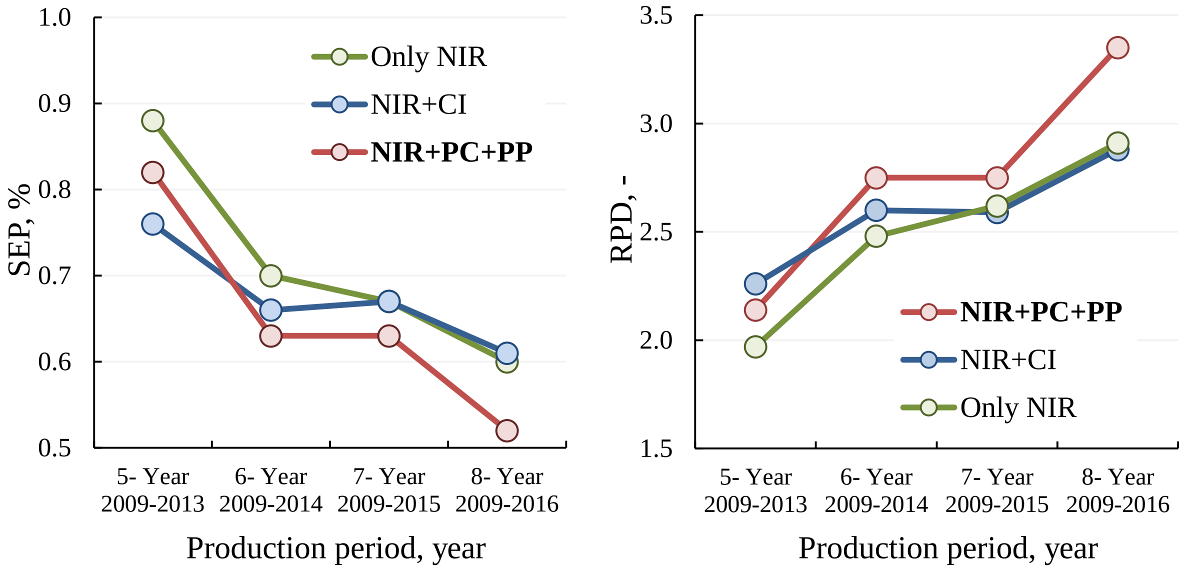

Figure 5.18 Comparison of SEP and RPD values of the analyses using milled rice data and validated using one-production year

5.4.4 Calibration models using physical properties, color and spectral information of milled rice validated by two-production years

In this section, the three analyses were carried out using low amylose content varieties and using Hokkaido ordinary amylose content varieties within the all samples collected. The analyses were calibrated using a calibration set of 7-production years (2009-2015, 585 samples of milled rice) and validated using 2-production years (2016-2017, 195 samples of milled rice). Moreover, the analyses were compared by increasing the years of production.

Determination of amylose content by "Only NIR" analysis using milled rice and validated using two-production years

Calibration models of both low amylose content varieties and using Hokkaido ordinary amylose content varieties were determined separately by analyzing reference amylose content values and NIR spectra information of milled rice (Only NIR analysis). Both calibration models were then combined.

The relationship between reference amylose content and predicted amylose content of the combined calibration models obtained from the "Only NIR" analysis using two-years of production validation set is shown in Figure 5.19.

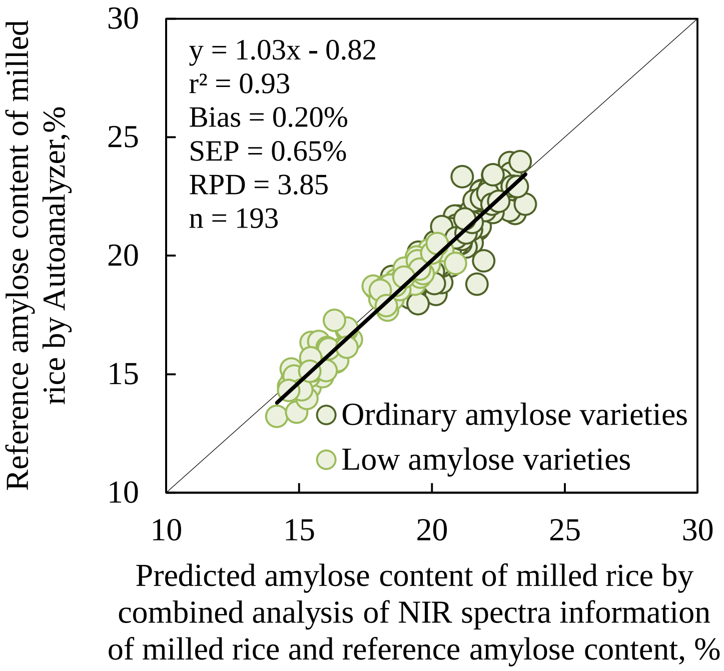

Figure 5.19 Relationship between reference amylose content and predicted amylose content of combined calibration models from “Only NIR” analysis using milled rice data and validated using two-production years

Determination of amylose content by “NIR+CI” analysis using milled rice data and validated using two-production years

Calibration models of both low amylose content varieties and Hokkaido ordinary amylose content varieties were determined separately by analyzing the predicted amylose content (AC_{pred}) obtained from the “Only NIR” analysis and color information (CI) of milled rice obtained from the VIS grain segregator (NIR+CI analysis). Both calibration models were then combined.

The relationship between reference amylose content and predicted amylose content of the combined calibration models obtained from the “NIR+CI” analysis using the two-years production of validation set is shown in Figure 5.20.

Validation statistics of “NIR+CI” analysis indicated that r^2 = 0.93, SEP = 0.69% and RPD = 3.69. This result did not improve the result shown by “Only NIR” analysis, considering that r^2, SEP, and RPD reported worse values.

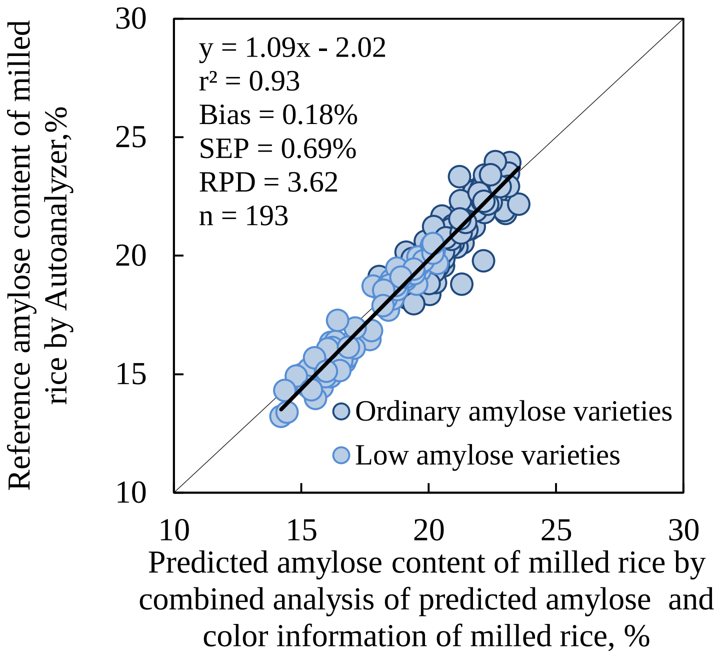

Figure 5.20 Relationship between reference amylose content and predicted amylose content of combined calibration models from “NIR+CI” analysis using milled rice data and validated using two-production years

Determination of amylose content by “NIR+PC+PP” analysis using milled rice data and validated using two-production years

Calibration models of both low amylose content varieties and Hokkaido ordinary amylose content varieties were determined separately by analyzing the predicted amylose content (AC_{pred}) obtained from the “Only NIR” analysis, predicted protein content (PC_{pred}) and physical properties (PP) of milled rice obtained from the VIS grain segregator (NIR+PC+PP analysis). Both calibration models were then combined.

The relationship between reference amylose content and predicted amylose content of the combined calibration models obtained from the “NIR+PC+PP” analysis using a validation set of two-years production is shown in Figure 5.21.

Validation statistics of “NIR+PC+PP” analysis indicated that $r^2 = 0.94$, SEP = 0.61% and RPD = 4.09. This result was better than the results shown by “Only NIR” and “NIR+CI” analyses, considering that r^2 and RPD values increased and SEP values decreased.

This validation result indicated that the calibration models developed by the dual-step “NIR+PC+PP” analysis using the two-years production validation set enable grain quality screening to be done in accordance with rice amylose content at grain elevators.

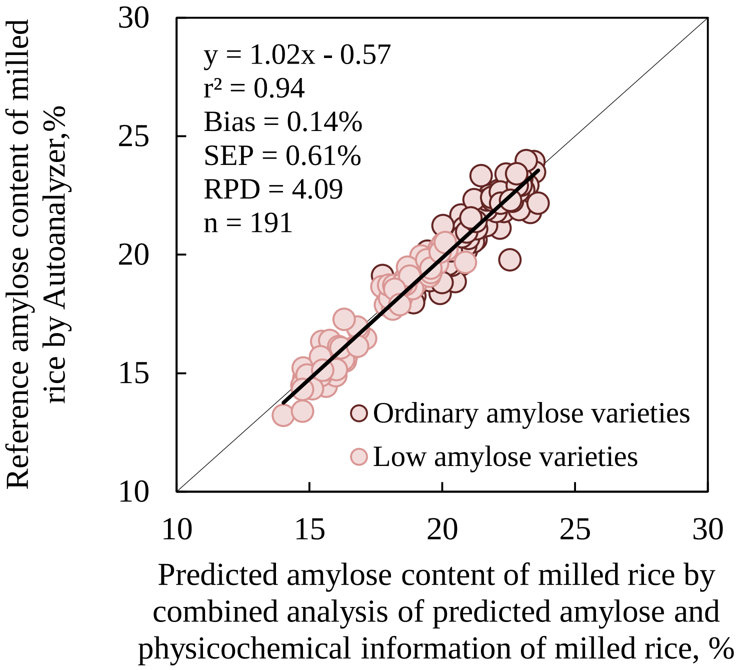

Figure 5.21 Relationship between reference amylose content and predicted amylose content of combined calibration models from "NIR+PC+PP" analysis using milled rice data and validated using two-production years

Comparison among analyses using milled rice data and validated using two-production years

The comparison of the analyses was carried out by increasing the years of production based on the SEP and RPD values (Figure 5.22). By increasing the years of production, SEP values decreased and RPD values increased.

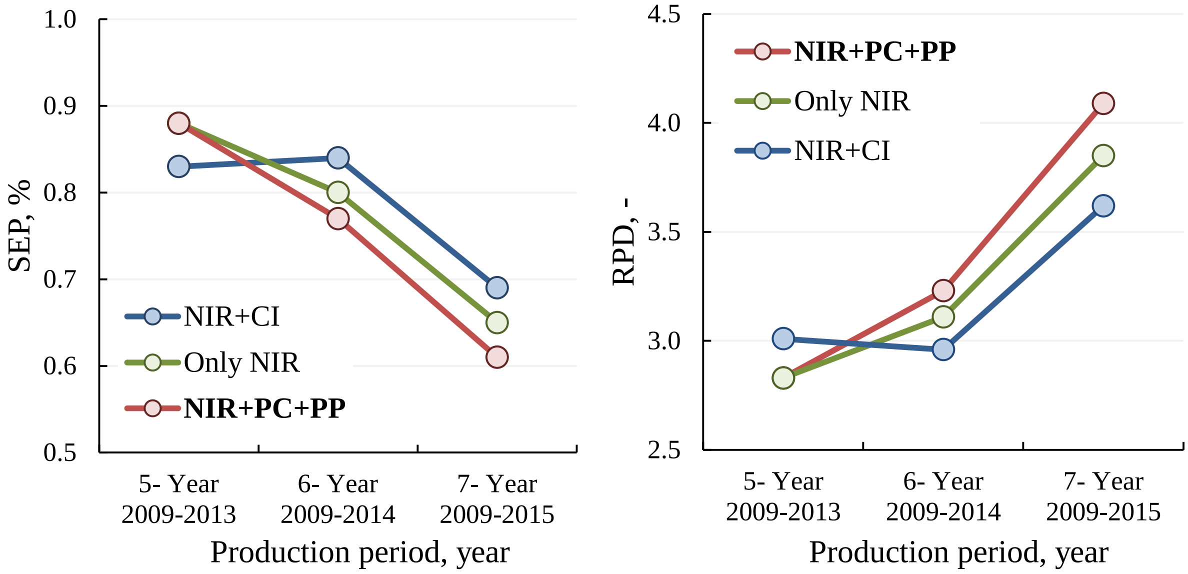

Figure 5.22 Comparison of SEP and RPD values of the analyses using milled rice data and validated using two-production years

The combined calibration models of “NIR+PC+PP” analysis indicated lower values of SEP and higher values of RPD, which reflects the most robust calibration model for the analyses carried out.

In general, the use of physicochemical properties allowed an improvement in the accuracy of the calibration model for assessing amylose content of milled rice using data obtained from both brown and milled rice as well as validation using one-production year and two-production years.

The validation results of the calibration models developed by the dual-step “NIR+PC+PP” analysis indicated that grain quality screening could be done in accordance with rice amylose content at grain elevators.

5.5 Conclusions

From the results achieved in this chapter, it can be concluded that physicochemical properties are a relevant factor in improving the accuracy of multivariate calibration model for determination of rice amylose content by NIR spectroscopy.

In addition, validation statistic results of the calibration models using one-production year and two-production years of validation sets could enable screening of rice amylose content at grain elevators.

Consequently, amylose content could be included in the group of properties that are analyzed to predict grain quality at grain elevators when brown rice is received from farmers. Thus, it could help to secure the high palatability and good taste demanded by Japanese rice consumers.

5.6 Recommendation for further studies

In this study, the accuracy of the multivariate calibration model for determining rice amylose content by NIR spectroscopy was improved by using the physicochemical properties. Physicochemical properties should, therefore, be considered when new calibration models for determining amylose content of rice are being developed for new models of NIR spectrometers that will be used at grain elevators in Japan.

6. CHAPTER VI. GENERAL CONCLUSIONS

6.1 Overall conclusions

The analysis of both physical and physicochemical properties done in this research provided useful information for improving the deficiency of technology for postharvest processing of rice in developing countries and for enhancing the quality and palatability of rice to meet Japanese consumer requirements.

The assessment and comparison of physical properties of NERICA, Indica and Japonica varieties provided information useful for designing more efficient technologies for postharvest processing of rice in developing countries. This information could contribute to the development of a technology-transfer strategy to reduce postharvest losses and mitigate constraints to NERICA expansion. This would help increase production and improve grain quality in developing countries where rice is an essential source of calories and income.

The results of the analysis of the relationship between physicochemical properties and kernel maturity of rice produced in Hokkaido suggested that protein content could potentially be sorted by thickness fraction. Amylose content was also shown to be highly significantly related to protein content and physical properties of rice.

Based on the relationship between amylose and physicochemical properties, I concluded that the use of physicochemical properties information is a relevant factor to improving the accuracy of multivariate calibration models for determining rice amylose content by NIR spectroscopy. The accuracy of the calibration models obtained could enable screening of rice amylose content at grain elevators. This result could thereby contribute to more accurate quality assessment and help to secure the high palatability and good taste demanded by Japanese rice consumers.

6.2 Recommendation for future studies

The results achieved in this research suggested the following ideas for future research.

Based on the similarity between NERICA and Indica types in kernel dimensions of rough rice and in dimensional, mass and frictional characteristics of milled rice, further research should be done into how technology used in the postharvest processing of Indica varieties can be applied to processing NERICA types. Particular attention should be paid to technologies used in processes such as cleaning, drying, and milling, where higher percentages of postharvest losses have been reported. This research would support the development of a technology-transfer strategy.

Since the number of NERICA varieties used in this study was low, a study should be done with a greater number of NERICA varieties to analyze the physical properties of rice considering different levels of moisture content of rough rice and different thickness fractions of milled rice.

Moreover, since the difference in values of protein content shown in the waste of the thickness grader and after the thickness grader was significant (between 0.5 and 0.9%). This result indicated that further research is needed into how to sort rice according to protein content with narrow values of protein content (approximately 0.1%) obtained in samples collected after the thickness grader.

The accuracy of the multivariate calibration model for determining rice amylose content by NIR spectroscopy was improved by using the physicochemical properties. Physicochemical properties should, therefore, be considered when new calibration models for determining amylose content of rice are being developed for new models of NIR spectrometers that will be used at grain elevators in Japan.

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米の品質評価と品質向上のための ネリカ米インディカ米ジャポニカ米の複数の品種の物理化学特性

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