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THESIS

EFFECT OF ACCELERATED AGING ON FUNCTIONAL PROPERTIES OF RICE GRAIN AND FLOUR

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A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
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The influences of aging process on functional properties of polished Jasmine rice grain and paddy of three cultivars of different amylose content were investigated. For polished Jasmine rice, the accelerated aging was carried out at 60°C, 75% relative humidity for 5 days, in the absence and presence of 25 mM cysteine, a known GRAS reducing agent for food proteins. The aged polished Jasmine rice grains were more opaque and yellowish than the new one ($p<0.05$). The sensory hardness of the cooked aged grains were higher than that of the cooked new ones ($N=30$, $p<0.05$). Confocal Laser Scanning Microscopy revealed the protein network, orderly encased the starch fraction within its honeycomb structure in the cooked grains. The rapid visco analyser (RVA) showed that the aging of rice grain lowered peak viscosity but increased the holding strength, final viscosity and setback of rice flour slurries compared with the new one ($p<0.05$). The presence of cysteine during aging process did not have significant effect on the RVA pasting profile of the aged polished rice ($p<0.05$). Nevertheless, further addition of 25 mM cysteine during cooking in the RVA lowered the holding strength, final viscosity and setback of aged rice flour ($p<0.05$). For paddy, the accelerated aging was carried out at 60°C, 70% relative humidity for 5 days. Aging process decreased peak viscosity for non-waxy rice flours but increased that of waxy rice flour ($p<0.05$). However, holding strength, final viscosity and setback of all rice flours were increased after aging ($p<0.05$). The lowering of protein content by alkali de-protenization showed that when protein was removed, accelerated aging did not have significant effect on pasting properties ($p<0.05$). Nevertheless, removal of protein caused markedly increase of peak viscosity and breakdown viscosity ($p<0.05$). The presence of 25 mM cysteine slightly lowered holding strength, final viscosity and setback of both new rice flour and aged rice flour, regardless of cultivar. SDS-PAGE of glutelin fraction, the main rice storage protein, with β-mercaptoethanol indicated the protein existed in subunits linked by disulfide bond in both new and aged paddy. This study suggested the possibility in the manipulation of rice grain and flour properties by accelerated aging through protein modification to have desirable characteristics in food formulations and processing.

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Teerarat Likitwattanasade

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INTRODUCTION

Rice is one of the staple foods recognized as the most important crop in Asia. The functional properties of rice grains, such as cooking characteristics, pasting profiles, gelation and water holding capacity, are important factors in determining the end use and market of rice grains and flours. Although eating qualities of table rice and processing qualities of rice flours are mainly attributed to the starch component in the grain, rice storage proteins appear to play some roles in determining the rice qualities (Ramesh *et al.,* 2000). Ramesh *et al*. showed that the protein fractions in rice affected the texture to some degree, particularly tenderness and cohesiveness of cooked rice.

Milling of paddy results in head rice around 40-55%, broken rice 10-22%, rice bran 10% and husk 20% (Barter *et al*, 1981). Broken rice is usually further milled to rice flour. The high-amylose flour and waxy rice flour find their uses in food industries such as rice noodle and snack foods, respectively; or can be further processed to starches and modified starches. Domestically, Jasmine rice is considerated to be the premium rice grains grown in the north-eastern and the central region of Thailand. Nonetheless, its low amylose content (approximately 18 g/100 g) is not preferential in either noodle or snack industry. It is too low to generate elastic texture for noodle and too high for the formation of solid foam with good textural properties for snack. Extensive attempts have been made to further utilize the Jasmine rice flour since Thailand's export market of fragrance head rice expands each year.

Protein content of rice for approximately 8% are mainly located in the endosperm (Teo *et al*., 2000). The functional properties of rice grain and flour are determined by glutelin (oryzenin) the major rice protein (Ju *et al*., 2001). The roles of protein on functional properties of rice grain and flour related to disulfide bond have been investigated in recent years (Martin and Fitzgerald, 2002; Teo *et al*., 2000;

Tulyathan and Leeharatanaluk, 2007; Zhou *et al.*, 2002). The formation of disulfide bond could restrict the expansion of starch fraction during gelatinization. Increasing or decreasing the disulfide bond content was reported to alter the pasting properties and textural structure of rice grain and flour (Primo *et al*, 1965).

During storage of rice grain after harvest, the average molecular weight of oryzenin, the major rice storage protein, increased while oryzenin-starch binding decreased (Charastil, 1990; 1992). These changes lead to the alterations in cooking qualities and stickiness of rice grain. The texture of cooked aged rice was harder and less sticky than the cooked newly harvested rice (Teo *et al*., 2000). Apparently, the protein fractions in rice grain are involved in the qualities and functional properties of rice grains and flours. Tulyathan and Leeharatanaluk (2007) have recently reported that naturally aged Jasmine rice grain showed significant changes in pasting profile from newly-harvested one after being stored for 5 months at 30-35°C.

It is apparent that the roles of rice storage proteins on cooking and pasting properties need further investigation. The study on mechanism of accelerated aging, to elucidate the fate of storage proteins and their influences on functional properties of rice grain and flour could be useful for food industry. Moreover, the insights obtained may be used to manipulate the desirable characteristics of rice grains and flours in downstream food processing.

OBJECTIVES

1. To study the effect of accelerated aging on the functional properties of rice grain and flour,

2. to understand the influence of rice proteins on functional properties of rice flour before and after accelerated aging process,

3. to elucidate the fate of storage proteins from different rice cultivars after accelerated aging and

4. to modify functional properties of rice grain and flour by accelerated aging.

LITERATURE REVIEW

Rice

Rice is one of the leading food crops in the world and the staple food for more than half of the world's population. South Asia accounts for about 30% of global rice production. Considering from the major export, Thailand is the largest rice exporter, shipping more than 7 million tons a year, which accounted for 25-30% of the global rice trade. Moreover, Thailand's exports rose for almost 50% during the 1990s, and the country has potential to expand rice export even more (Champagne *et al*., 2004).

1. Structure and composition of rice grain

Rice (*Oryza sativa* L.) is a member of the family *Poaceae* (formerly Gramineae or grass). It is generally considered a semi aquatic, annual grass plant. It can grow in a wide range of water-soil regimes, from deeply flooded land to dry, hilly slope (Luh and Mickus, 1991). Rice is harvested as paddy rice that varies in compositions following different cultivars, but has the same basic structure as shown in Figure 1.

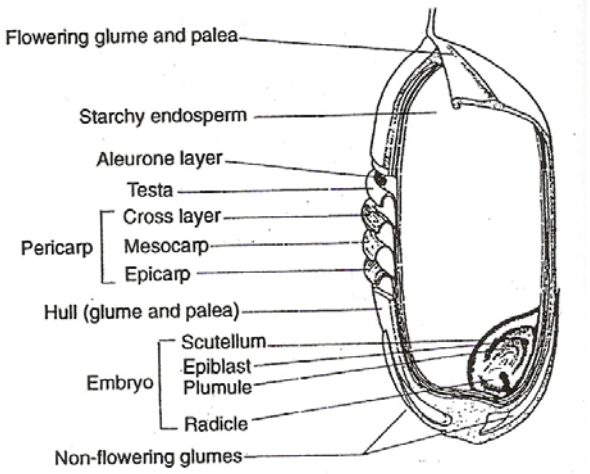

Figure 1 Structure of the mature rice grain.

Source: Henry and Kettlewell (1996)

The structure of the mature paddy grain is shown in Figure 1. The principal parts of the grain are the hull, pericarp, aleurone layer, embryo and starchy endosperm. The hull is outer covering for the caryopsis (brown rice). It presents for 20% of the rough rice. The hull serves as a protection against insect infestation and against rapid changes in moisture content of the grain. Hull is low in protein, fat and starch but high in crude fiber, crude ash (mostly silica) and dietary fiber (Table1). Next to hull is pericarp, which contains several thin layers of differentiated tissues. Aleurone layer, the outermost layer of the endosperm, is bound tightly to the starchy endosperm. However, it would be removed as part of the bran fraction during milling. Most of the protein and oil are stored in this structure. The embryo or germ is roughly 1-3% by weight of the total grain and is located at the basal end of ventral side of the grain. The starchy endosperm is the major storage tissue of the cereal grain, containing most of the starch and storage protein. Starchy endosperm can be divided into an outer endosperm, just below the subaleurone layer and inner endosperm or central core. The regions differ primarily in the number of starch granules and protein bodies contained within the cell. The protein content is the highest in the outer layer and starch content is the highest in the central core. In the central core, the starch granules are hexagonal in shape and highly compact (Marshall and Wadsworth, 1993).

Table 1 Proximate composition of paddy, brown rice, milled rice, rice hull, rice bran and rice embryo (% d.b.).

Constituent	Paddy	Brown rice	Milled rice	Hulls	Bran	Embryo
Protein	6.7-8.3	8.3-9.6	7.3-8.3	2.3-3.2	13.2-17.3	17.7-23.9
Crude fat	2.1-2.7	2.1-3.3	0.4-0.6	0.4-0.7	17.0-22.9	19.3-23.8
Crude fiber	8.4-12.1	0.7-1.2	0.3-0.6	40.1-53.4	9.5-11.5	2.8-4.1
Crude ash	3.4-6.0	1.2-1.8	0.4-0.9	15.3-24.4	9.2-11.5	6.8-10.1
Starch	62.1	77.2	90.2	1.8	16.1	2.4
Dietary fiber	19.1	4.5	2.7	77.3	27.6-33.3	-

Source: Pomeranz and Ory (1982)

2. Classification of rice

Rice can be classified into many categories based on their prominent characteristics, i.e. kernel length, shape, variety, amylose content, cooking properties and industrial use. Based on amylose content, rice is classified as waxy (1-2%), very low amylose (2-12%), low amylose (12-20%), intermediate amylose (20-25%) and high amylose (25-33%) (Juliano, 1979). Another characteristic widely used to classify rice is cooked rice properties. According to these properties, rice can be categorized into two types as follow:

2.1 Glutinous rice or waxy rice

Waxy rice is essentially composed of amylopectin, resulting in the white and opaque kernel. When cooked, this rice provides very chewy texture and the cooked grains stick to one another.

2.2 Non-Glutinous rice or non-waxy rice

Non-waxy rice contains 70-80% amylopectin and 10-30% amylose and provids firm, dry texture and fluffy when cooked.

Rice Protein

The protein content of rice varies from 6-9% depending on the cultivars, climate and agronomic conditions. Protein content is usually calculated from Kjeldahl nitrogen multiplied by the factor of 5.95 based on the nitrogen content (16.8%) of the major rice protein, glutelin. The milling process of rice results in milled rice (40-55%) and three major by-products: hull (20%), bran (10%) and broken (10-22%) (Barter ***et. al***, 1981). Protein contents of these fractions can be categorized as follow: 8% in milled rice, 3% in hull, 17% in bran, and 8.5% in broken rice, (Lasztity, 1995). Due to the removal of protein-rich bran layer during milling, milled rice contains lower

content of protein than that of brown rice (Table 2). Nevertheless, the greatest part of the total protein is located in the endosperm.

Table 2 Proximate protein contents of rough rice and its milling fractions.

Rice fraction	Crude protein (g of Nx5.95)
Rough rice	5.6-7.7
Brown rice	7.1-8.3
Milled rice	6.3-7.1
Rice bran	11.3-14.9
Rice hull	2.0-2.8

Source: Juliano (1993)

1. Classification and form of rice protein

1.1 Classification of rice protein

Proteins in rice can be classified by many characteristics such as solubility, biological function, morphology or their position in the grain like protein in endosperm, in aleurone layer or in the embryo or germ. Each position contains different protein fractions.

Classification of protein based on solubility or Osborne method has been widely used in rice protein chemistry. Protein extracted from rice flour with 8.75% of protein following this method were composed of 79.74% alkali- and acid-soluble glutelins, 13.11% salt-soluble globulins, 4.45% water-soluble albumins and 2.46% alcohol-soluble prolamins (Ju *et al*. 2001).

1.1.1 Albumins

Albumin is generally known as water soluble protein. However, Vallareal and Juliano (1981) reported that separation of the water-soluble albumins and salt-soluble globulins cannot be achieved by simple sequential extraction because of minerals present in the rice grain dissolved in the water during extraction. Houston and Mohammad (1970) pointed out that albumins are obtained by precipitating off the globulins from 0.7-0.9 M NaCl extract of milled rice, either by dialysis against water or by addition of ammonium sulfate to a 1.3 M concentration. Studies on molecular weight of albumin showed that rice albumins have a wide range of molecular weights (Juliano, 1972), with major components with apparent molecular weights of 18-20 kDa (Houston and Mohammed, 1970).

1.1.2 Globulins

Globulin is protein soluble in NaCl solution. A major globulin was obtained by ammonium sulfate precipitation from a 2.5-5% NaCl solution of milled rice (Houston and Mohammad, 1970). Rice globulins consist of α-,β-, γ-, and δ-globulins with apparent molecular weights of 25.5, 15, 200 kDa and higher, respectively (Morita and Yoshida, 1968). Chromatographic studies of purified γ-globulin showed three components, designated as γ_1, γ_2 and γ_3 in order of increasing basicity and decreasing migration rate on electrophoresis at pH 9.8 (Sawai and Morita, 1970).

1.1.3 Glutelins

Glutelin is the major storage protein of rice accounting for 70-80 % of the total protein of rice grain. Glutelins in rice have a specific name as oryzenin. Generally, glutelin is largely soluble in acidic (pH below 3.0) or alkali solutions (pH above 10.0) (Shih, 2004). However, extraction of glutelin also uses a detergent such as sodium dodecyl sulfate (SDS) with or without the addition of a reducing agent. Molecular weight of glutelins have been identified. It is composed of two major polypeptide subunits classified as α, or acidic and β, or basic subunits with apparent molecular weights of 30-39 and 19-25 kDa, respectively (Juliano, 1985, Kawaga *et al*.,

1988, Kishimoto *et al*., 1999, Shih, 2004). These two groups of polypeptides, formed by the cleavage of a 57 kDa polypeptide precursor (Sarker *et al*., 1986), are believed to cross-link by disulfide bonding, resulting in glutelin molecules with molecular weight ranging from 64-500 kDa (Sugimoto *et al*., 1986). Hamada (1996) reported that native rice glutelin is extremely insoluble in water because of hydrophobic and disulfide bonding. In addition, an increase in disulfide bond content and a decrease of sulfhydyl groups during storage were observed in the glutelin fraction (Chrastil and Zarins, 1992).

1.1.4 Prolamins

The prolamin is another rice storage protein that soluble in alcohol/water mixture (usually is extracted with 70% ethanol). It has high content of proline and glutamine which led Osborne called the name prolamin. Prolamins consist of three polypeptide subunits with apparent molecular weights of 10, 13 and 16 kDa. The dominating 13 kDa polypeptide is readily solubulised in alcohol, while the 10 and 16 kDa polypeptides with a high level of sulfur-containing amino acids require a reducing agent for solubilization in alcohol (Ogawa *et al*., 1987).

The molecular weights of rice proteins were investigated extensively by Agboola *et al*. (2005). Australian rice protein isolates contained many protein subunits as characterized by different methods as shown in Table 3.

Table 3 Molecular weight of proteins in Australian rice protein isolates prepared by the Osborne method and characterized by capillary electrophoresis (CE) and SDS-PAGE.

Fraction from CE	Molecular weight (kDa)							
	Albumin		Globulin		Glutelin		Prolamin	
Peak	CE	PAGE	CE	PAGE	CE	PAGE	CE	PAGE
1	7.6	14.9	22.9	13.9	5.9	15.8	12.8	15.4
2	39.8	19.8	26.9	17.1	12.7	19.7	37.7	21.9
3	55.0	26.5	37.9	21.5	21.4	21.4	43.7	25.8
4		38.8	53.7	32.1	29.5	38.5	45.3	41.1
5		49.6	97.7	39.9	39.8		49.0	
6		56.3	104.7	54.8			105.2	

Source: Agboola *et al*. (2005)

Rice proteins were also classified by their biological functions as cytoplasmic proteins and storage proteins (Lasztity, 1995). Cytoplasmic proteins are the proteins that contain a number of biologically important components. They are low molecular weight proteins, globular form and water- or salt-soluble proteins, mainly albumins and globulins. Storage proteins were divided into high molecular weight proteins glutelins and low molecular weight proteins prolamins.

Prolamin protein is a single peptide chain, it can form disulfide bond in the chain that called intra-molecular disulfide bond. While glutelin or oryzenin is composed of many polypeptide chains so it can form disulfide bond both of inner and among the chains that called inter-molecular disulfide bond (Figure 2) (Chrastil and Zarin, 1994).

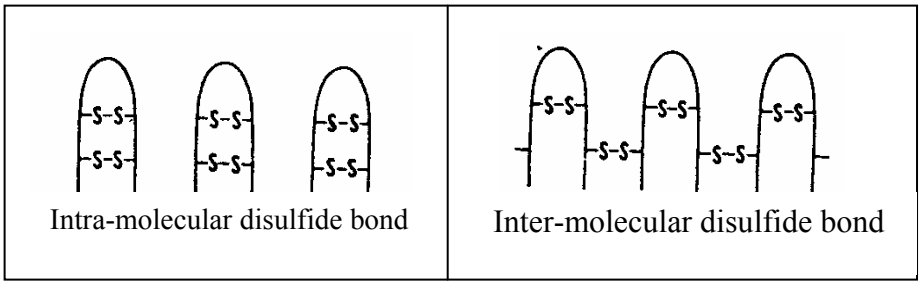

Figure 2 Intra-molecular disulfide bond and inter-molecular disulfide bond of prolamin and glutelin.

Source: Modified from Wall (1971)

1.2 Form of rice protein

In general, two forms of protein are found in rice storage proteins. One is protein bodies and another is protein matrix. In rice, up to 95% of the endosperm proteins were observed in the form of protein bodies and there was little or no matrix protein (Lasztity, 1995). This differentiates rice from other cereals which contain large amounts of protein existing as an inter-granular matrix. Three types of protein bodies have been identified; i.e. crystalline, small spherical and large spherical (Figure 3). Bechtel and Pomeranz (1978) reported that the central region of rice grain contains the only large spherical protein bodies. However, the small spherical bodies were most numerous and large bodies the least numerous. The storage protein bodies can be divided into type I protein body or PB-I which is rich in prolamin. The other is type II protein body or PB-II which was rich of glutelin. In low-glutelin rice, type I protein body is widely distributed over the endosperm tissues; while in the other cultivars mainly consisted of type II protein, the protein was distributed predominantly in the center part of the endosperm tissues (Furukawa *et al*., 2003).

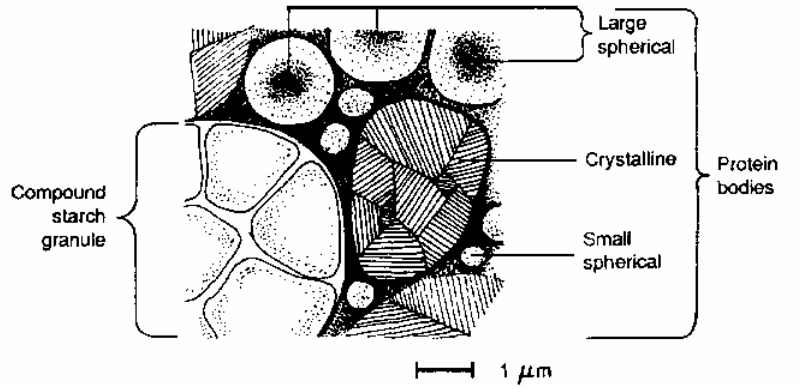

Figure 3 Schematic diagram of various protein bodies and compound starch granule.

Source: Juliano (1993)

2. Influence of rice protein on qualities and functional properties of rice

Although cooking, eating and processing qualities of rice are mainly attributed to the starch component of the grain, Champagne *et al*. (1999) demonstrated that many cultivars with similar amylose contents showed rather different pasting and textural properties. Rice proteins, the second most abundant component in rice next to starch, seem to play an important role on rice quality. Thus, recent researches have been conducted on rice proteins and their impacts.

Table 4 shows the thermal properties of proteins, rice starch and rice flour that can provide information on their cooking properties, physical and chemical effects. The denaturation temperatures of albumin, globulin and glutelin were 73.3, 78.9 and 82.2^{o}C, respectively which were slightly lower than the gelatinization temperature of the starch (Ju *et al*., 2001). The phase transition temperature of rice starch is the gelatinization temperature. Gelatinization temperature of rice flour which contained 8.8% protein was lower than that of the rice starch. These results indicated that heating at 73^{o}C to 85^{o}C is critical in rice cooking and the formation of textural structure. Enthalpy values of albumin and globulin were lower than that of glutelin, suggesting that these two fractions were more easily denaturated (Ju *et al*. 2001).

Table 4 Thermal property of rice proteins, starch and flour.

Sample	Phase transition Temperature (^{o}C)	Enthalpy value of Denaturation (J/g)
Albumin	73.3[a]	2.88[a]
Globulin	78.9[b]	3.14[b]
Glutelin	82.2[c]	3.79[c]
Prolamin	NA	NA
Rice starch	84.7[e]	10.53[e]
Rice flour	80.5[d]	8.49[d]

Means within a column followed by the same superscript letter are not significantly different ($p<0.05$).

Source: Ju *et al.* (2001)

Xie *et al.* (2008) compared the changes of pasting and cooking properties of non-waxy and waxy rice after protein removal and disulfide bond disruption in rice flour. The result showed that peak, breakdown and consistency viscosity of waxy rice flour treated with protease or dithiotreitol (DTT) significantly decreased ($p<0.05$). For non-waxy cultivars, pasting temperatures of flour treated with protease were increased and there were decreases in viscosity along all the point of the curve. For cooking properties, adhesiveness of waxy rice was decreased while that of non-waxy rice was increased, but hardness of cooked waxy and non-waxy rice both decreased after adding DTT to cooked rice water.

In 2004, Baxter *et al*. studied the effect of prolamin removal by 100% propanan-2-ol on the pasting and textural properties of rice flour. The result showed that removal of prolamin resulting in significant ($p<0.05$) reduction in peak viscosity

and final viscosity. In addition, significant increasing in hardness, adhesiveness and gumminess of the rice flour gels can be observed ($p<0.05$).

Martin and Fitzgerald (2002) determined how proteins affected the viscosity curve using RVA analysis and concluded that protein influence viscosity curve, first , through binding water, which causes the concentration of the dispersed and viscous phases of gelatinized starch to increased; and second, through the network linked by disulfide bonds. Hamaker and Griffin (1993) reported that protein with disulfide bonds in rice flour restricted starch granule swelling during gelatinization and made the swollen granule less susceptible to disruption by shear.

Protein not only determined the functionality of rice grains and flours naturally existed but also the functionality of the flour added with extracted storage proteins. Baxter *et al*. (2004) demonstrated that addition of prolamin to rice flour caused an increase in RVA breakdown viscosity but decrease in hardness, adhesiveness and gumminess of the composite gel. However, the opposite effect was observed when prolamin was removed.

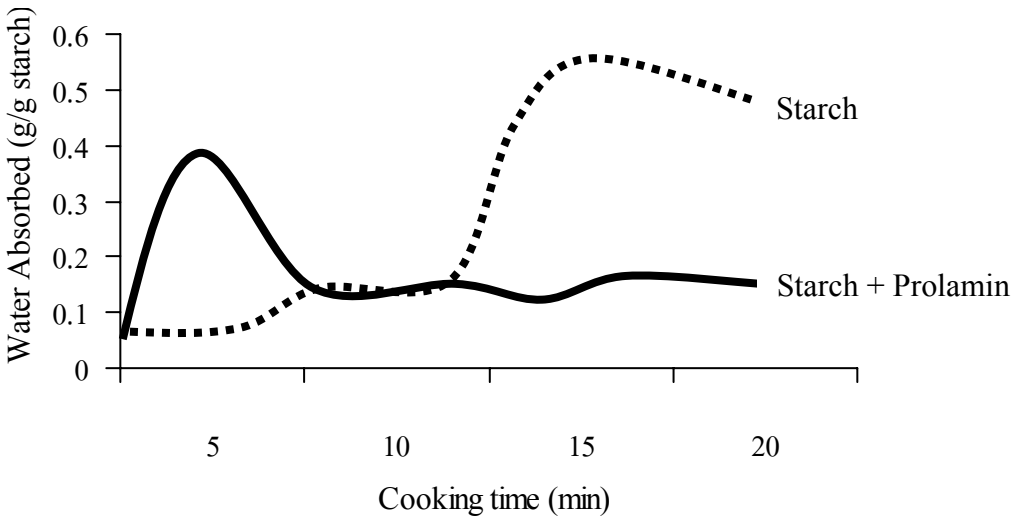

Figure 4 Water absorption of rice starch with the addition of prolamin.

Source: Baxter *et al*. (2004)

Moreover, Baxter *et al*. determined the water absorption of rice starch and they found that protein adding caused increasing the rate of water absorption in the early stage of cooking and this is a reason for increasing rupturing of starch granule during RVA processing (Figure 4).

Rice aging

1. Definition of rice aging and its effect on the properties of rice grain and flour

Aging is natural and spontaneous phenomenon that commences after harvesting and continues as a time-, temperature- and moisture-dependent index (Nakakete, 2000). During the aging or storage process, although the overall starch, protein and lipid content in the rice grain remain essentially unchanged, there are physicochemical interaction among these components and enzymatic reactions (Table 5).

Table 5 Mechanisms in rice aging process.

Substrate	Storage change	Cooking effect	Sensory effect
Starch →	Increase of strength of micelle binding →	Inhibit swelling of starch granule →	Texture
		↓	
		Fatty acid-amylose complex →	Texture
		↑	
Lipid →	1. Hydrolysis →	Complex FFA oxidation	
	2. Oxidation →	Hydroperoxides, carbonyl compounds →	Aroma
Protein →	Oxidation →	-SH → S-S →	Aroma
		↓	
		Inhibit swelling of starch granule →	Texture

Source: Modified from Zhou *et al*. (2002)

Starch forms micelle with protein, thus increasing the strength and inhibiting swelling of starch granule. These change the texture of cooked rice. During aging, fat is hydrolyzed to free fatty acids with then complexed with amylose and affected the texture. Fat and free fatty acids could undergo autoxidation to carbonyl compounds which caused aroma changes. Although total protein content does not change during aging, chemical and physicochemical properties of proteins, especially that of the storage protein oryzenin, changed significantly during storage. This was by increasing of the molecular weight and the number of disulfides bond. Protein oxidation leads to formation of disulfide linkages from sulfhydryl groups that causes the decrease of volatile sulphur compounds. Disulfide linkages inhibited swelling of starch granules and finally affected cooked rice texture (Ramesh, *et al*., 2000).

Aging is one of the factors responsible for textural changes in cooked rice and the properties, especially pasting properties of rice flour. Cooked aged rice becomes harder and less sticky than that of new or fresh rice (Ohno and Ohisa, 2005, Toyoshima *et al*., 1998, Tulyathan and Leeharatanaluk, 2007 and Watanabe *et al*., 1991). The stickiness/hardness (S/H) ratio of aged rice became lower than that of new rice (Ohno and Ohisa, 2005). Aged cooked rice had increased firmness but lower stickiness as compared with new rice (Perdon *et al*., 1999). Perez and Juliano (1981) reported that 3 months was the minimum storage period for major changes to occur in the hardness of cooked rice.

In 2001, Sowbhagya and Bhattacharya stored the paddy at 26°C for over 4 years. The Brabender viscogram showed that paste breakdown steadily decreased with time of storage, while there was a steady increase in setback. It implied that aging rendered the rice substances progressively more organized and resistant to swelling and disintegration. Besides, they found that the changes were relatively rapid at first, gradually slowed down, but not showed signs of being halted even after 4 years.

Zhou, *et al*. (2002) stored milled rice at 37°C up to 16 months. The most significant change in the pasting curve was the decrease in breakdown over time and the gradual disappearance of a clearly defined peak in aged samples. The decrease in

breakdown indicated the capacity of the starch granules to rupture after cooking was increased significantly and the decrease in peak viscosity showed that the starch granule of stored rice were more resistant to swelling than those of fresh rice. In addition, they also studied the effect of rice storage on pasting properties of rice flour and found that aged rice flour treated with protease showed a peak and holding strength as normally seen in fresh rice. Furthermore, addition of β-mercaptoethanol to aged rice flour increased peak viscosity significantly. These changes can be attributed to disruption of the protein disulfide bonds, allowing greater hydration and swelling of the starch granules.

Changes in protein properties contribute to the effect of aging on the pasting properties of rice (Zhou *et al*, 2002). Chrastil (1990) reported that the number of disulfide bonds and the average molecular weight of oryzenin, which is a major protein in rice, increased during storage of rice grain. In addition, the interactions between oryzenin and starch decreased during storage and were related to a decrease in rice stickiness during storage. Chrastil and Zarins (1992) reported a decrease of low molecular weight peptide subunits and an increase of high molecular weight peptide subunits in both medium and long rice grains during storage.

In 2005, Ohno and Ohisa revealed the textual changes in aged rice were inferred to be due to oxidation of protein in the external layer of grains. From their analysis by SDS-PAGE, the proteins of the external layer in aged rice grains were oxidized to greater extent than those of new rice grains. Addition of a reducing agent to cooking water to cleave the disulfide linkages increased the stickiness/hardness ratio of aged rice to approximately that of new rice.

2. Accelerated aging and its effect on the properties of rice grain and flour

The natural aging of rice takes approximately 4-6 months and also requires much more space for storage of paddy. This increases operating cost. Moreover, paddy is susceptible to insect damages, as well as microorganisms and rodents during

storage (Soponronnarit *et al*., 2008). From the above reasons, accelerated aging used could be more practical commercially.

Aging may be accelerated by treating the grain with dry or wet heat (Juliano, 1979). However, dry treatment is preferred since it is easier and cheaper. Soponronnarit *et al*. (2008) investigated the characteristics of accelerated and naturally aged rice using fluidized bed drying followed by tempering and ventilation. The result showed that rice properties; namely elongation ratio, whiteness, volume expansion, water uptake, solid loss and pasting properties changed in a similar way to those of the naturally aged paddy. Nevertheless, the head rice yield of rice undergone accelerated aging process was significant lower than that of the naturally aged paddy ($p<0.05$).

Parnsakhorn (2001) studied the effect of accelerated aging by using heat treatment at equilibrium moisture content of 60°C, 81% RH for 4 days on the quality of Kao Dok Mali (KDML-105) polished rice. After incubating for 4 days, the color, water absorption of rice grain and hardness of cooked rice changed to those of naturally aged rice stored at 30°C for 5 months (Figure 5).

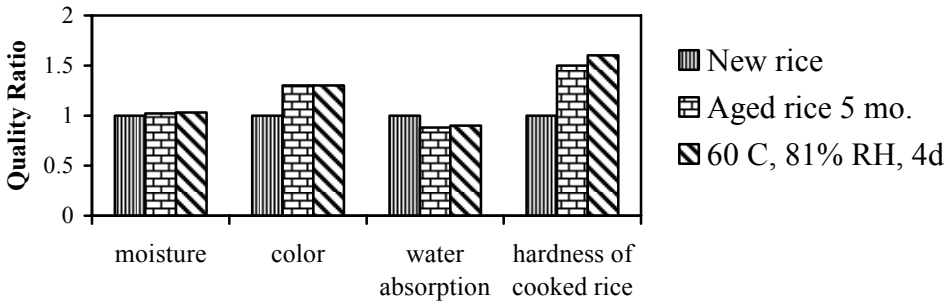

Figure 5 Comparison of rice characteristics of new rice, naturally aged rice and accelerated aged rice.

Source: Parnsakhorn (2001)

Gujral and Kumar (2003) studied the effect of steaming on the physicochemical and textural properties of brown and milled rice by steaming paddy

(14, 18 and 22% moisture content) for 30 min in a close vessel at atmospheric pressure and drying to 9-11% moisture content in shade. They found that water absorption, swelling capacity and cooking time increased markedly after steaming whereas solids loss decreased. The cohesiveness, springiness and hardness increased. Nevertheless, adhesiveness of aged rice decreased significantly ($p<0.05$). Moreover, the result showed that the more content of moisture, the more severity of the aging treatment.

It is apparent that accelerated aging of paddy could be used as a means to alter the rice characteristics. However, the investigation of accelerated aging on the second most constituent-protein, is quite limited. This study thus elucidated the alteration of rice storage proteins and their influences on the characteristics of rice flour.

MATERIALS AND METHODS

In this study, the experiments were set in the laboratory and were divided into 2 parts consisted of Part I: the effect of accelerated aging and cysteine on functional properties of polished jasmine rice grain and flour and Part II: the effect of accelerated aging and cysteine but focus on functional properties of paddy and their flours.

Materials

1. Rice samples

For Part I, commercially available polished Jasmine rice, long grain fragrant rice, (Hongtong brand, Chiameng Marketing Ltd., Thailand) was bought from a local supermarket. It had 13.44% moisture content (AACC, 1995), 7.51% protein detected by the Kjeldahl method (AOAC, 2000).

For part II, the freshly harvested paddy samples of three cultivars; Sanpah-Tawng1, Pathum Tani1 and Leuang Pratew123, grown in Chiang Mai, Chachoengsao and Ratchaburi farms of Rice Research Institute (RRI) respectively, were obtained from the Department of Rice, Ministry of Agriculture and Cooperatives, Thailand. All cultivars were harvested in 2007.

Sanpah-Tawng1 is a waxy; 1.30% amylose content determined using the method of Chrastil (1987) contained 10.94% moisture content, 8.32% protein, 2.34% fat (AACC, 1995) and 1.23% ash content (AACC, 1995).

Pathum Tani1 is a non-waxy; 14.09% amylose content, long grain fragrant rice contained 11.12% moisture content, 6.73% protein, 1.19% fat and 0.75% ash.

Leuang Pratew123 is a non-waxy; 28.57% amylose content, long grain rice contained 11.43% moisture content, 9.64% protein, 0.64% fat and 0.57% ash.

2. Chemicals

2.1 Reagents for chemical analysis

2.1.1 Amylose content determination

1) Amylose (Amylose Type III: From Potato, lot. 084K3808, SIGMA, Germany)

2) Iodine (I_2, analytical grade, lot. AF505180, Asia Pacific Specially Chemicals Limited, Australia)

3) Methanol (CH_3OH, analytical grade, lot. K36801509703, MERCK, Merck KGaA, Germany)

4) Potassium iodide (KI, analytical grade, lot. AF506181, UNIVAR, Ajax Finechem, Australia)

5) Sodium hydroxide (NaOH, analytical grade, lot. B888598 630, MERCK, Merck KGaA, Germany)

6) Trichloroacetic acid (CCl_3COOH, analytical grade, Merck KGaA, Germany)

2.1.2 Protein content determination

1) Sulfuric acid (H_2SO_4, analytical grade, lot. E41W62, Mallinckrodt Chemicals, USA)

2) Boric acid (H_3BO_3, analytical grade, lot. A884465 750, MERCK, Merck KGaA, Germany)

3) Copper (II) sulfate ($CuSO_4.5H_2O$, analytical grade, lot. F3G115, UNIVAR, Ajax Finechem, Australia)

4) Potassium sulfate (K_2SO_4, analytical grade, lot. 0801267, UNIVAR, Ajax Finechem, New Zealand)

5) Methyl red ($C_{15}H_{15}N_3O_2$, analytical grade, lot. 10278KP, Panreac, Panreac Quimica SK, Spain)

6) Bromocresol green ($C_{21}H_{14}BR_4O_5S$, analytical grade, lot. AF404011, LABCHEM, Ajax Finechem, New Zealand)

7) Sodium hydroxide (NaOH, commercial grade, Thasco Chemical Co., Ltd., Thailand)

8) Ethanol (C_2H_5OH, analytical grade, lot. K364776983634, MERCK, Merck KGaA, Germany)

2.1.3 Fat content determination

1) Petroleum ether (analytical grade, lot. B10756, Maillickrodt Chemicals, USA)

2.2 Reagents for determination of disulfide linkage

2.2.1 L-Cysteine ($C_3H_7NO_2S$, analytical grade, lot. 30090, Fluka Bio Chemika, Japan)

2.3 Reagent for low protein rice flour preparation

2.3.1 Sodium hydroxide (NaOH, analytical grade, lot. B250098 249, MERCK, Merck KGaA, Germany)

2.3.2 Hydrochloric acid (HCl, analytical grade, lot. K28575417 049, MERCK, Merck KGaA, Germany)

2.4 Reagents for protein extraction

2.4.1 Hexane ($CH_3(CH_2)_4CH_3$, analytical grade, lot. 0895413, Fisher scientific, UK Limited, UK)

2.4.2 Sodium chloride (NaCl, analytical grade, lot. AF612180, UNIVAR, Ajax Finechem, Australia)

2.4.3 Sodium hydroxide (NaOH, analytical grade, lot. B250098 249, MERCK, Merck KGaA, Germany)

2.4.4 Ethanol (C_2H_5OH, analytical grade, lot. K364776983634, MERCK, Merck KGaA, Germany)

2.4.5 Hydrochloric acid (HCl, analytical grade, lot. K28575417 049, MERCK, Merck KGaA, Germany)

2.5 Reagents for Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

2.5.1 Protein standard (Amersham Full-Range Rainbow Recombinant Protein Molecular Weight Markers, lot. 3/28925341, Amersham Biosciences AB, Sweden)

2.5.2 Acrylamide-PAGE ($CH_2CHCONH_2$, lot. K32714603 429, PlusOne, Amersham Biosciences AB, Sweden)

2.5.3 Metylenebisacrylamide ($(CH_2CHCONH_2)_2CH_2$, lot. K36962617718, PlusOne, Amersham Biosciences AB, Sweden)

2.5.4 Tris (Hydroxylmethyl) aminomethane ($NH_2C(CH_2OH)_3$, Ultrapure, lot. 119617, usb, USB Corporation, USA)

2.5.5 Tetradimethylethylenediamine (TEMED:$C_6H_{16}N_2$, Ultrapure, lot. 114219, usb, USB Corporation, USA)

2.5.6 2-Mercaptoethanol ($HSCH_2CH_2OH$, Ultrapure, lot. S3901589 403, PlusOne, Amersham Biosciences AB, Sweden)

2.5.7 Ammonium Persulfate ($(NH_4)_2S_2O_8$, lot. 210004026, BIO-RAD, Bio-Rad Laboratories, USA)

2.5.8 Hydrochloric acid (HCl, analytical grade, lot. K28575417 049, MERCK, Merck KGaA, Germany)

2.5.9 Glycerol ($CH_2OHCHOHCH_2OH$, analytical grade, lot. 3M018064B, CARLO ERBA Reagents, Italy)

2.5.10 Coomassie brilliant blue R-250 ($C_{45}H_{44}N_3O_7S_2Na$, lot. 21003683, BIO-RAD, Bio-Rad Laboratories, USA)

2.5.11 Bromophenol Blue Sodium Salt ($C_{19}H_9BR_4NaO_5S$, Ultrapure, lot. 110583, usb, USB Corporation, USA)

2.5.12 Sodium dodecyl sulfate (SDS: $C_{12}H_{25}OSO_3Na$, lot.L56038550 749, Amersham Biosciences AB, Sweden)

2.5.13 Glycine (NH_2CH_2COOH, electrophoresis purity grade, lot. 210003974, BIO-RAD, Bio-Rad Laboratories, USA)

2.5.14 Acetic acid (CH_3COOH, analytical grade, lot. E15W63, J.T.Barker, Thailand)

2.5.15 Methanol (CH_3OH, analytical grade, lot. K36801509703, Merck KGaA, Germany)

3. Instruments and apparatus

3.1 Instruments and apparatus for sample preparation

3.1.1 Incubator (WTC binder, model KBF240, Tuttlingen, Germany)

3.1.2 Hammer mill (model SK1, Retsch, Germany)

3.1.3 Hot air oven (model 400, Memmert GmbH&Co. KG, Germany)

3.1.4 Refrigerated centrifuge (model RC-5C, Sorvall, DoPont Company, USA)

3.1.5 Free dryer (model HetoFD2.5, Heto Lab Equipment Manufactured, Denmark)

3.1.6 pH meter (model Microcomputer pH-VISION 6071, Jenco Electronics, LTD., China)

3.1.7 Magnetic stirrer (model SLR, SCHOTT Intruments GmbH&Co. KG, Germany)

3.1.8 Hot plate (model HS-101, GEM, Thailand)

3.1.9 Test sieve (Aperture size 90 Mic., Endecotts Limited, England)

3.1.10 Test sieve (Aperture size 150 Mic., Endecotts Limited, England)

3.1.11 Refrigerator (Sanyo, Japan)

3.1.12 Ultrasonic bath (model Brasonic 32, Sunvalley, USA)

3.2 Instruments and Apparatus for sample analysis

3.2.1 Water bath (model OB14, Memmert GmbH&CO. KG, Germany)

3.2.2 Muffle furnace (model Tactical 308, Gallenkamp, U.K.)

3.2.3 Minolta Spectrophotometer (model CM-3500d, Minolta Co.LTD, Japan)

3.2.4 Rapid Visco analyzer (model RVA3D, Newport Scientific Instrument & Engineering, Australia)

3.2.5 Balance (model SPB31, Scale Tech, Germany)

3.2.6 Electrophoresis Cell (model Mini-PROTEAN 3 Cell, Bio-RAD Laboratories, Inc., USA)

3.2.7 Kjeldahl apparatus (BUCHI, B-324, LabortechnikAG, Switzerland)

3.2.8 Soxtec system (model HT 1043, Tecator, Sweden)

3.2.9 Confocal Laser Scanning Microscope (CLSM, model LSM5 PASCAL, ZEISS, Germany)

3.2.10 UV-Visible spectrophotometer (model Genesys 10, Thermo Fisher Scientific, USA)

3.2.11 Vortex (model G-560E, Scientific Industries, Inc., USA)

3.2.12 Microcentrifuge (model Labnet Spectrafuge 16M, Labnet International, Inc., USA)

Methods

Part I: Effect of accelerated aging and cysteine on functional properties of polished rice grain and flour

1. Accelerated aging process

The polished Jasmine rice grain samples were incubated at 60°C, 75% relative humidity (RH) for 5 days [modified from method described by Parnsakhorn (2001)] and stored at -18°C prior to the analyses.

2. Cysteine treatment of rice grain and flour

The effect of cysteine on rice grain and flour was determined on colorimetry and pasting characteristic. For the cysteine-treated aged grain, a solution of 25 mM cysteine was sprayed onto the grain for 6.5% by weight of rice grain before being subjected to aging process. For rice flour, 0, 25 and 50 mM cysteine solution were added directly to RVA canister instead of water.

3. Preparation of rice flour

Polished rice afterwards kept at -18°C was ground to flour with a Hammer mill (ZM1, Retsch, Germany) and sifted through 100 mesh sieve.

4. Characterization of rice grain and flour

4.1 Sensory evaluation of cooked rice

Unwashed 280 g sample of polished rice, both of new and aged rice samples were cooked in an electric rice cooker for about 15 min with 440 mL of water. The difference in sensory hardness of cooked rice was determined using triangle test (AACC, 1995) by 30 panelists. In each set, one or two of the three samples were cooked new rice or cooked aged rice selected randomly, and the position of the spiked sample was randomized across each series. All samples were served in white plastic cups to the panelists at warm temperature. The cysteine-treated aged rice was not included in this sensory evaluation due to the distinct smell of H_2S after cooking.

4.2 Pasting characteristics

The dry-milled flours from new and aged rice were suspended in 0, 25 and 50 mM cysteine solution and analyzed by the Rapid Visco Analyser (RVA) (Newport Scientific Warriwood, Australia) using the AACC Approved Method 61-02 (1995). The sample was held at 50°C for 1 min, heated to 95°C in 3.75 min, held at 95°C for 2.5 min, cooled to 50°C in 3.75 min and finally held at 50°C for 1.40 min. Apparent viscosity was recorded in Pa.s. Pasting characteristics were described using

amylograms including peak viscosity (the maximum viscosity developed soon after the heating cycle ended), holding strength (the viscosity after holding at 95°C for 2.5 min), breakdown viscosity (the viscosity difference between peak viscosity and holding strength), final viscosity (viscosity after cooling at 50°C for 1.40 min), setback viscosity (the viscosity difference between final viscosity and peak viscosity) and pasting temperature (temperature of initial viscosity increased).

4.3 Color measurements

The color of the samples (uncooked and cooked rice grain) were determined using CIE color system (Minolta Spectrophotometer CM-3500d, Japan) with the measurement condition 10°field of vision/standard illuminant D. Color values (L*, a* and b*) were measured using a white standard tile to calibrate the color colorimeter before measurement. The colorimetric measurements were performed three times for each sample.

4.4 Microstructure of protein in cooked rice

The microstructure of cooked rice grain was observed under the confocal laser scanning microscope (CLSM, model LSM5 PASCAL, ZEISS, Germany) to locate the protein fraction in the grain using Rhodamine B as fluorescent-labelling dye and method described by Hongsprabhas *et al*. (2007). A solution of Rhodamine B (0.01% in 95% ethanol) was added to the cooked new rice and cooked aged rice on the sample similar to those used for sensory analysis. After incubation for 2 min, each sample was rinsed by distilled water and loaded into a slide well and observed for a location of fluorescent-labeled protein. A HeNe laser with an excitation wavelength of 543 nm was used. CLSM digital image were acquired using the LSM 5 PASCAL program.

5. Statistical analysis

The experiments were carried out in three separated trials. Each trial was run in triplicates. The data were analyzed by Analysis of Variance (ANOVA) with significance at $p<0.05$. All statistical analyses were performed using the SPSS Software Version 12.

Part II: Effect of accelerated aging on functional properties of paddy and their flour

1. Accelerated aging process

The paddy of three cultivars were incubated at 60°C, 70% relative humidity (RH) for 5 days [modified from method described by Parnsakhorn (2001)] and stored at -18 °C prior to the analyses.

2. Preparation of rice flour

The new and aged paddies were dehulled and polished at the Department of Rice, Ministry of Agriculture and Cooperatives, Thailand. Polished rice was milled in a Hammer mill (ZM1, Retsch, Germany) and sifted through 100 mesh sieve.

3. Preparation of low protein rice flour

Low protein rice flour was produced from new and aged rice flour using the method described by Yamamoto *et al*., (1973) with minor modification. Rice flour (200 g) was soaked in 0.05N sodium hydroxide (500 mL) at ambient temperature for 3 h and then centrifuged at 3000xg for 20 min. The supernatant was discarded. The residue was again soaked in 0.05 N sodium hydroxide for 3 h, then passed through a 170 mesh sieve and only filtrate was centrifuge at 3000xg for 20 min. The supernatant was again discarded. The residue was added of water and adjusted to pH 6.5-7.0 with 1 M HCl. The suspension was centrifuged. The residue was washed by distilled water and centrifuged. This step was repeated for 3 times. At the end of each centrifugation, the top brownish portion of protein was scraped out. The low protein rice residue was

dried in a hot air oven at 40°C for 48 h to give moisture content closed to that of the rice flour. The dried low protein rice flour was ground into flour and passed trough a 100 mesh sieve.

4. Extraction of rice protein

The straight extraction method using the NaOH described by Agboola *et al.* (2005) was used. Flour (25 g) was mixed with 300 ml of 0.1 M NaOH and stirred at ambient temperature for 1 h. The suspension was then centrifuged at 3000xg for 30 min. The supernatant was removed and adjusted to pH 4.8 with 1 M HCl. The precipitated proteins were collected by centrifugation at 3000xg for 30 min. The precipitated proteins were then washed twice with distilled water, freeze-dried and stored at -18°C.

For Osborne method, proteins were extracted from rice flour based on their solubility in specific solutions using the procedure described by Ju *et al.* (2001) with minor modifications. Rice flour (100 g) was defatted with hexane (400 mL) with continuous stirring for 1 h. Residual solvent in flour was evaporated in a ventilation hood at ambient temperature for at least 24 h. The flour was then extracted by stirring with distilled water (400 mL) at ambient temperature for 4 h (albumin extract) and centrifuged at 3000xg for 30 min. After water extraction, the flour was extracted with 5% NaCl (400 mL) at ambient temperature for 4 h (globulin extract) and centrifuged at 3000xg for 30 min. The flour was then extracted for glutelin with 0.1M NaOH (400 mL) at ambient temperature for 1 h, and followed by prolamin extraction with 70% ethanol (400 mL) at ambient temperature for 4 h. The supernatants of albumin, globulin, glutelin and prolamin fractions were removed and adjusted to their isoelectric pH at pH 4.1, 4.3, 4.8 and 5.0 respectively to precipitate the proteins. The precipitated proteins were collected by centrifugation at 3000xg for 30 min, then washed twice with distilled water, freeze-dried and stored at -18°C.

5. Characterization of rice grain and flour

5.1 Pasting characteristics

The dry-milled flours and low protein flours from new and aged rice were determined for pasting characteristics and analyzed by the Rapid Visco Analyser (RVA) (Newport Scientific Warriwood, Australia) using the AACC Approved Method 61-02 (1995).

5.2 Electrophoresis of proteins extracted from the flour

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was run according to the method by described by Laemmli (1970) using electrophoresis model Mini PROTEAN 3 Cell (BIORAD, USA). A 4% stacking gel and 15% running gel were used. Protein (10 μg) was loaded on the gel. The electrophoresis was run under non-reducing and reducing condition at a constant voltage of 150 volts for 1 h. Protein was stained in 0.1% coomassie brilliant blue R-250 and destained in 40% methanol and 10% acetic acid. Molecular weight markers were also run on the same gel and used to estimate the molecular weight profile of the protein bands in the stained gel.

6. Statistical analysis

The experiments were carried out in three separated trials. Each trial was run in triplicates. The data were analyzed by Analysis of Variance (ANOVA) with significance at $p<0.05$. Significant differences among mean values were determined by the Student *t* test or Duncan's multiple range test. All statistical analyses were performed using the SPSS Software Version 12.

Place and Duration

Place

At the Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University (Bangkhen campus), Thailand

Duration

From June 2006 to December 2008

RESULTS AND DISCUSSION

Part I: Effect of accelerated aging and cysteine on functional properties of polished rice grain and flour

The texture of cooked aged rice was different from the new one as determined by 30 panelists ($p<0.05$). The cooked aged rice was moderately harder than the cooked new one in sensory term although they contained similar protein content (Table 6). It is possible that disulfide bond formed during accelerating aging process induced the alteration of protein network with thicker barrier around the starch granules. This might retard water uptake by granules and contributed to harder cooked rice grain.

Table 6 Moisture and protein content of new rice, aged rice and cysteine-treated aged rice.

Sample	Moisture content (% d.b.)	Protein content (% d.b.)
New rice	13.44[a]	7.51[a]
Aged rice	11.70[b]	7.53[a]
Cysteine-treated aged rice	11.65[b]	8.08[a]

Means in the same column followed by different superscript are significantly different ($p<0.05$).

The aging process applied in this study altered the RVA pasting profiles of polished rice grain as reported in Table 7. The accelerated aging process slightly lowered peak viscosity of polished rice flour, increased peak time, pasting temperature, holding strength, drastically decreased breakdown and raised both final viscosity and setback ($p<0.05$). The lower breakdown and an increase in both setback and hardness values of the aged Khao Dawk Mali 105 rice were also reported by Tulyathan and Leeharatanaluk (2007). The decrease in peak viscosity, the increase in peak time and pasting temperature showed that starch granules of aged rice were more

resistant to swelling than those of new rice. These result supported the above hypothesis that thicker barrier protein after aging might retard water uptake and effect of cooked rice texture.

Table 7 RVA pasting profiles of new rice flour, aged rice flour and cysteine-treated rice flour.

Treatment	Peak viscosity (Pa.s)	Peak time (min)	Holding strength (Pa.s)	Breakdown viscosity (Pa.s)	Final viscosity (Pa.s)	Setback (Pa.s)	Pasting temperature (°C)
New rice flour	3.52[a]	5.65[b]	2.04[b]	1.47[a]	3.33[b]	1.29[b]	74.32[b]
Aged rice flour	3.11[b]	5.95[a]	2.30[a]	0.81[b]	3.83[a]	1.52[a]	75.84[a]
Cysteine-treated aged rice flour	2.86[b]	6.04[a]	2.19[a]	0.67[b]	3.74[a]	1.55[a]	75.56[a]

Means in the same column followed by different superscript are significantly different ($p<0.05$).

Results on sensory differentiation of hardness and the RVA pasting profiles suggested the alterations in functional properties of polished rice grain and its flour after accelerated aging. Nevertheless, the presence of cysteine during accelerated aging process in this study did not retard the change in pasting profiles induced by aging process ($p<0.05$). This was probably due to the oxidation of cysteine and its degradation to H_2S at high aging temperature and prolonged aging time using the accelerated process.

Apart from the changes in RVA pasting profile, the accelerated aging process altered the appearance of both uncooked and cooked rice. The aged rice grains were more opaque (higher L*-value) and more yellowish (higher b*-value) than the new ones ($p<0.05$) (Table 8). The changes in b*-values could be due to the Maillard reactions of protein and starch in rice endosperm during the accelerated aging process at high temperature.

Table 8 Effect of aging and cysteine on CIE color of uncooked and cooked rice grain.

	Treatment	L*	a*	b*
Uncooked rice	New rice	75.56^{c}	-0.43^{b}	16.42^{c}
	Aged rice	77.16^{b}	-0.02^{a}	18.63^{a}
	Cyteine-treated aged rice	78.09^{a}	-0.10^{a}	18.18^{b}
Cooked rice	New rice	75.11^{b}	-1.82^{b}	8.44^{b}
	Aged rice	76.82^{a}	-1.62^{a}	9.16^{b}
	Cyteine-treated aged rice	77.53^{a}	-1.52^{a}	9.63^{a}

Means in the same column within the same category (uncooked or cooked) followed by different superscript are significantly different ($p<0.05$).

The CLSM shown in Figure 6 illustrates the distribution of protein fractions in rice endosperm after cooking. The proteins, which fluoresced in red under CLSM, formed a continuous network with honeycomb structure. Although proteins in rice endosperm exist in the form of protein bodies, they underwent heat denaturation under

thermal process and formed three dimensional networks. In aged rice, there was the periodic structure of dark area (protein-free phase) present and the honeycomb structure of the protein network was less ordered than that of the new one. The dark area was likely to be the starch fraction, which concentrated randomly within the geometry of the cooked rice grain due to the thermodynamic incompatibility of starch and protein molecular structures. Although present for only 8% (d.b.), the fact that the proteins forms continuous phase after cooking suggests their significance in determining the sensory hardness, which correlated with the mechanical properties of cooked rice grains. Nevertheless, the changes in physicochemical properties of proteins during accelerated aging needs further clarification. In addition, the mechanisms involved in the phase separation of starch and protein fractions in the formation of such composite structure required more investigation whether they are filled structure or a bi-continuous geometry in nature.

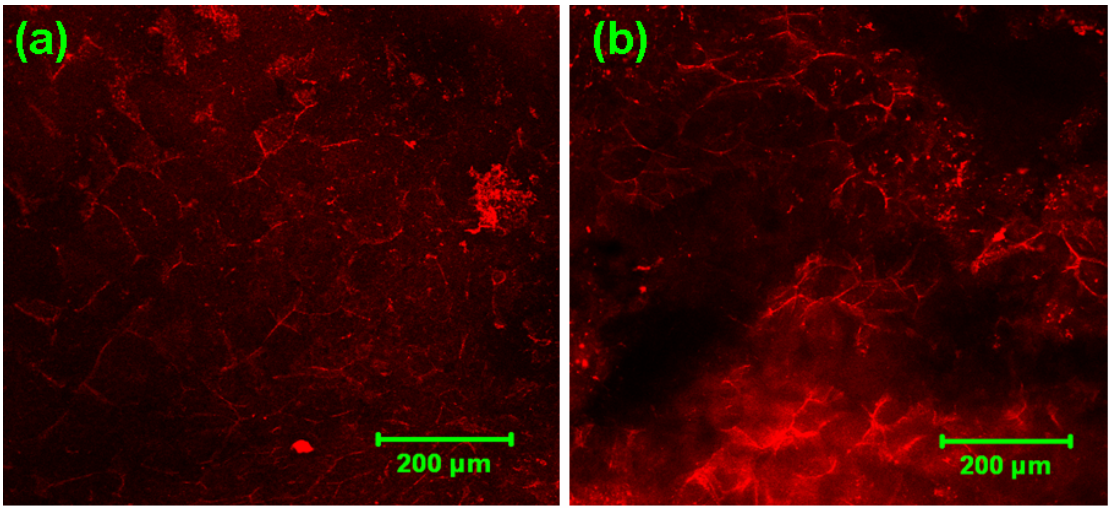

Figure 6 Confocal laser scanning micrographs of cooked rice grain (a) new rice, (b) aged rice.

The direct addition of cysteine to the flour suspensions lowered holding strength, increased breakdown, decreased final viscosity and setback of both new and aged rice flours (Table 9). The addition of cysteine was to reduce the disulfide bond of the rice storage proteins in flour. In the presence of cysteine, the gelatinized rice flour was less

resistant to shear at high temperature, observed as lower holding strength and increased breakdown viscosity. These weaker gelatinized rice flour paste further lowered final viscosity and setback during cooling stage in the RVA. These results are in good agreement with those reported by Hamaker and Griffin (1993) on the effect of dithiotreitol (DTT), the reducing agent, on the increase of breakdown. Moreover, the presence of cysteine lowered pasting temperature, this result supported the hypothesis that protein network could restrict swelling of starch granule.

Table 9 Effect of cysteine concentration on RVA pasting properties of new rice flour and aged rice flour.

Treatment	Peak viscosity (Pa.s)	Peak time (min)	Holding strength (Pa.s)	Breakdown viscosity (Pa.s)	Final viscosity (Pa.s)	Setback (Pa.s)	Pasting temperature (°C)
New rice flour							
Distilled water	3.67^{a}	5.88^{ab}	2.24^{a}	1.42^{c}	3.55^{b}	1.30^{b}	74.52^{bc}
25 mM cysteine	3.84^{a}	5.83^{ab}	1.92^{cd}	1.92^{a}	3.00^{d}	1.08^{d}	73.88^{cd}
50 mM cysteine	3.78^{a}	5.81^{b}	1.82^{d}	1.96^{a}	2.83^{e}	1.02^{d}	72.68^{d}
Aged rice flour							
Distilled water	3.05^{b}	6.03^{a}	2.28^{a}	0.77^{d}	3.84^{a}	1.55^{a}	76.77^{a}
25 mM cysteine	3.67^{a}	5.83^{ab}	2.11^{b}	1.56^{bc}	3.33^{c}	1.22^{c}	76.12^{a}
50 mM cysteine	3.73^{a}	5.72^{b}	1.99^{bc}	1.74^{ab}	3.19^{c}	1.20^{c}	75.83^{ab}

Means in the same column followed by different superscript are significantly different ($p<0.05$).

Part II: Effect of accelerated aging on functional properties of paddy and their flour

Previous results suggested the significant effect of accelerated aging on functional properties of polished rice. The accelerated aging applied to paddy, which is more complex because of the presence of hull, bran and endosperm, was investigated.

After aging, protein content of Sanpah-Tawng1, Pathum Tani1 and Leuang Pratew123 rice flour were not significantly different from the new ones (Table 10). The result indicated that accelerated aging had no significant effect on protein content regardless of cultivar. However, the high amylose rice (Leuang Pratew123) had the highest protein content among the cultivars investigated.

Table 10 Amylose and protein content of Sanpah-Tawng1, Pathum Tani1 and Leuang Pratew123 new and aged rice flour.

Cultivar	Amylose content (% d.b.)	Protein content (% d.b.)	
		New rice flour	Aged rice flour
Sanpah-Tawng1	1.30	8.32^{aB}	8.11^{aB}
Pathum Tani1	14.09	6.73^{aC}	6.56^{aC}
Leuang Pratew123	28.57	9.64^{aA}	9.56^{aA}

Means within a row followed by different lowercase superscript are significantly different and means within a column followed by different uppercase superscript are significantly different ($p<0.05$).

Accelerated aging of paddy significantly altered the pasting properties of all three cultivars rice flour (Table 11). Peak viscosity of Sanpah-Tawng1, the waxy rice, increased after aging. But in non-waxy rice, peak viscosity of Pathum Tani1 and Leuang Pratew123 decreased. The decrease in peak viscosity showed that the cooked granules of aged non-waxy rice had less friction than those of cooked new non-waxy rice. For waxy rice containing little amount of amylose, the starch granule generally

swelled more readily during heating as compared with that of high amylose rice (Lii *et al*., 1996). It was possible that, at temperature that peak viscosity occurred, the aged rice protein network involved in the flow-induced structure altered bulk viscosity by changing the flow behavior of the paste, through restriction of swelling. A significant ($p<0.05$) increasing in peak time also supported this assertion. For breakdown viscosity, there had no significant effect of aging on waxy rice, but a notable decrease was observed on both non-waxy rice cultivars; Pathum Tani1 and Leuang Pratew123. The decrease in breakdown after aging indicated that the capacity of the swollen starch granule to rupture after cooking was reduced significantly by aging (Noomhorm *et al*., 1997). The difference in peak viscosity and breakdown of waxy and non-waxy rice flour could be due to the differences in the properties of their starch, protein and interactions. Such difference is responsible for the disperse phase or continuous phase of rice starch and protein fractions undergoing phase transition during heating between waxy and non-waxy rice. Although the changes of peak viscosity and breakdown in waxy rice were not similar to those of non-waxy rice, the holding strength, final viscosity and setback were dramatically increased after aging. The results indicated that the aged cooked paste was more resistant to shear at high temperature regardless of cultivars.

Table 11 The effect of cultivars and accelerated aging on RVA pasting profile of Sanpah-Tawng1, Pathum Tani1 and Leuang Pratew123 rice flour.

Cultivar	Treatment	Peak viscosity (Pa.s)	Peak time (min)	Holding strength (Pa.s)	Breakdown viscosity (Pa.s)	Final Viscosity (Pa.s)	Setback (Pa.s)	Pasting temperature (°C)
Sanpah-Tawng1	New rice	2.22^{b}	3.88^{b}	1.12^{b}	1.09^{a}	1.37^{b}	0.24^{b}	68.84^{a}
(amylose=1.30%)	Aged rice	2.64^{a}	4.10^{a}	1.53^{a}	1.12^{a}	1.88^{a}	0.36^{a}	68.62^{a}
Pathum Tani1	New rice	3.61^{a}	5.50^{b}	1.72^{b}	1.90^{a}	2.78^{b}	1.07^{b}	73.96^{b}
(amylose=14.09%)	Aged rice	3.20^{b}	5.70^{a}	2.05^{a}	1.16^{b}	3.38^{a}	1.34^{a}	76.35^{a}
Leuang Pratew123	New rice	2.68^{a}	6.16^{a}	2.01^{b}	0.68^{a}	3.35^{b}	1.34^{b}	77.74^{b}
(amylose=28.57%)	Aged rice	2.60^{b}	6.01^{b}	2.21^{a}	0.38^{b}	3.99^{a}	1.78^{a}	79.80^{a}

Means in the same column in each cultivar followed by different superscript are significantly different ($p<0.05$).

The influence of protein on pasting properties was studied by removing the rice protein by alkali solution. Protein content of low protein new rice flour and low protein aged rice flour of all the three rice cultivars is shown in Table 12. Comparing with the native flour, alkali de-protinization reduced more than 60% of protein content. Interestingly, proteins in aged rice flours were more difficult to remove than those of the new ones (compared with protein content shown in Table 10). This was probably due to interactions between rice components, especially starch and protein, which are the main components in rice that may occur during aging process.

Table 12 Protein content of Sanpah-Tawng1, Pathum Tani1 and Leuang Pratew123 low protein rice flour.

Cultivar	Protein content (% d.b.)	
	Low protein new rice flour	Low protein aged rice flour
Sanpah-Tawng1	1.42[b]	2.47[a]
Pathum Tani1	1.17[b]	1.45[a]
Leuang Pratew123	1.53[b]	2.01[a]

Means in the same row followed by different superscript are significantly different ($p<0.05$).

Pasting properties of low protein rice flour are shown in Figure 7. The similarity of RVA pasting curves between low protein new rice flour and low protein aged rice flour suggested that accelerated aging did not influence pasting properties of rice flour at low protein concentration ($p<0.05$).

Further details of the influences of proteins on pasting properties of native rice flour and low protein rice flour of three rice cultivars were summarized in Table 13. Removal of protein up to 60% caused an increase in peak viscosity and breakdown viscosity but shortened peak time in both new rice flour and aged rice flour, compared with their native rice flour. This suggested that in low protein rice flour, the granule swelled to a greater degree and produced more viscous paste due to friction. With shear

in RVA, the greater swollen starch granule broke down more easily. The result implied that rice protein played a significant role on pasting by possibly encasing the starch granules and regulating their swelling. Chakrabarthy *et al.* (1972) also reported that cooking time was positively correlated to protein content, more protein formed a thicker barrier around the starch granules, thus slowing water uptake by the granule.

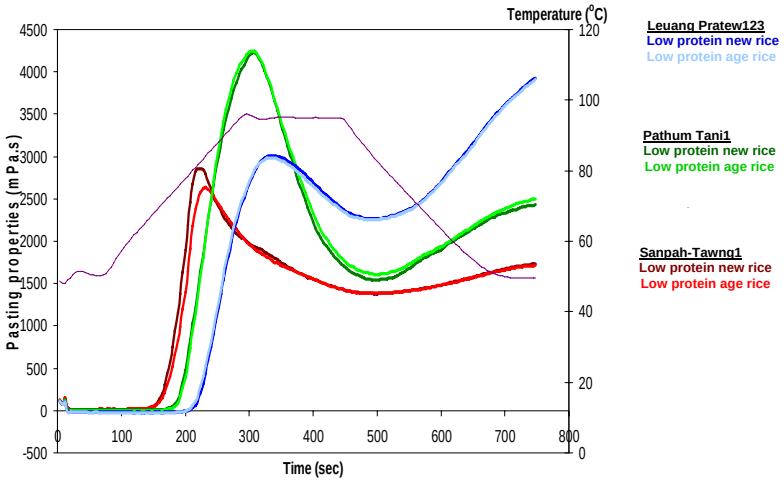

Figure 7 RVA pasting profiles of low protein new rice flour and low protein aged rice flour of Sanpah-Tawng1, Pathum Tani1 and Leuang Pratew123 cultivar.

Table 13 Effect of removal of rice protein from new rice flour and aged rice flour of Sanpah-Tawng1, Pathum Tani1 and Leuang Pratew123 rice cultivar on RVA pasting profile.

Cultivar	Treatment	Peak viscosity (Pa.s)	Peak time (min)	Holding strength (Pa.s)	Breakdown viscosity (Pa.s)	Final Viscosity (Pa.s)	Setback (Pa.s)	Pasting temperature (°C)
Sanpah-Tawng1 (amylose=1.30%)	New rice	2.22^{c}	3.88^{b}	1.12^{c}	1.09^{b}	1.37^{c}	0.24^{b}	68.84^{a}
	Low protein new rice	2.89^{a}	3.72^{c}	1.38^{b}	1.42^{a}	1.73^{b}	0.35^{a}	66.39^{b}
	Aged rice	2.64^{b}	4.10^{a}	1.53^{a}	1.12^{b}	1.88^{a}	0.36^{a}	68.62^{a}
	Low protein aged rice	2.83^{a}	3.78^{c}	1.35^{b}	1.49^{a}	1.67^{b}	0.32^{b}	66.19^{b}
Pathum Tani1 (amylose=14.09%)	New rice	3.61^{b}	5.50^{b}	1.72^{b}	1.90^{b}	2.78^{b}	1.07^{b}	73.77^{b}
	Low protein new rice	4.24^{a}	5.01^{c}	1.54^{c}	2.70^{a}	2.43^{c}	0.89^{c}	73.03^{b}
	Aged rice	3.20^{c}	5.70^{a}	2.05^{a}	1.15^{c}	3.38^{a}	1.34^{a}	76.27^{a}
	Low protein aged rice	4.33^{a}	5.33^{c}	1.61^{bc}	2.72^{a}	2.50^{c}	0.89^{c}	73.26^{b}
Leuang Pratew123 (amylose=28.57%)	New rice	2.68^{b}	6.16^{a}	2.01^{b}	0.68^{b}	3.35^{b}	1.34^{b}	77.74^{b}
	Low protein new rice	3.01^{a}	5.58^{c}	2.26^{a}	0.75^{a}	3.93^{a}	1.67^{a}	77.65^{b}
	Aged rice	2.60^{c}	6.01^{b}	2.21^{a}	0.38^{c}	3.99^{a}	1.78^{a}	79.80^{a}
	Low protein aged rice	2.98^{a}	5.57^{c}	2.25^{a}	0.74^{ab}	3.80^{a}	1.66^{a}	77.32^{b}

Means in the same column in each cultivar followed by different superscript are significantly different ($p<0.05$).

We further investigated the influence of cysteine, a reducing agent, on the pasting properties of new rice and aged rice flour of three rice cultivars and the results are shown in Table 14. The presence of 25mM cysteine significantly lowered peak viscosity of both new rice and aged rice of Sanpa-Tawng1 ($p<0.05$). Nearly 50% reduction of peak viscosity in the presence of reducing agent indicated the influences of disulfide bond on the swelling of waxy starch granule. Unlike the waxy rice, cysteine had no effect on peak viscosity of Pathum Tani1 new rice flour but caused higher in aged rice flour. For Leuang Pratew123, the decreased peak viscosity was observed in new rice, in contrast, the increased peak viscosity was observed in aged rice. The increase in peak viscosity in aged rice sample of non-waxy rice can be attributed to the disruption of the protein disulfide bonds by cysteine, allowing greater hydration and swelling of the starch granule. Moreover, the presence of cysteine slightly lowered holding strength, final viscosity and setback of both new rice flour and aged rice flour, regardless of cultivar. The decreasing of those RVA parameters indicated that, in the absence of the disulfide-bound protein network associated with the granule, the gelatinized paste was less resistant to shear during holding stage and cooling stage. Although the disulfide bonds existed in both new rice and aged rice, the impact of cysteine on the aged rice was greater than on the new rice. This implicated that the number of disulfide bridges increased during rice accelerated aging process.

Table 14 Effect of cysteine on RVA pasting profiles of Sanpah-Tawng1, Pathum Tani1 and Leuang Pratew123 new rice and aged rice flour.

Cultivar and Treatment	Solution	Peak viscosity (Pa.s)	Peak time (min)	Holding strength (Pa.s)	Breakdown viscosity (Pa.s)	Final viscosity (Pa.s)	Setback (Pa.s)	Pasting temperature (°C)
Sanpah-Tawng1								
New rice flour	Distilled water	2.16^{b}	3.89^{b}	1.12^{b}	1.03^{a}	1.38^{b}	0.26^{b}	66.48^{b}
	25 mM cysteine	1.05^{d}	4.10^{a}	0.39^{d}	1.07^{a}	0.53^{d}	0.14^{d}	66.46^{b}
Aged rice flour	Distilled water	2.67^{a}	3.71^{c}	1.60^{a}	0.65^{c}	1.96^{a}	0.36^{a}	69.75^{a}
	25 mM cysteine	1.50^{c}	3.85^{b}	0.73^{c}	0.78^{b}	0.92^{c}	0.18^{c}	69.30^{a}
Pathum Tani1								
New rice flour	Distilled water	3.48^{ab}	5.87^{ab}	1.92^{b}	1.56^{b}	3.04^{b}	1.12^{b}	75.47^{b}
	25 mM cysteine	3.38^{ab}	5.98^{a}	1.60^{c}	1.01^{c}	2.50^{d}	0.90^{d}	74.94^{a}
Aged rice flour	Distilled water	3.22^{b}	5.88^{ab}	2.21^{a}	1.79^{a}	3.58^{a}	1.37^{a}	77.23^{a}
	25 mM cysteine	3.59^{a}	5.79^{b}	1.80^{b}	1.79^{a}	2.86^{c}	1.06^{c}	76.64^{a}
Leuang Pratew123								
New rice flour	Distilled water	2.72^{b}	6.24^{a}	2.16^{b}	0.55^{c}	3.36^{c}	1.19^{b}	77.23^{b}
	25 mM cysteine	2.51^{c}	6.05^{b}	1.86^{c}	0.30^{d}	2.81^{d}	0.95^{c}	77.32^{b}
Aged rice flour	Distilled water	2.59^{c}	6.18^{a}	2.28^{a}	0.65^{b}	3.93^{a}	1.64^{a}	78.72^{a}
	25 mM cysteine	2.96^{a}	5.82^{c}	2.11^{b}	0.85^{a}	3.65^{b}	1.54^{a}	78.36^{a}

Means in the same column in each cultivar followed by different superscript are significantly different ($p<0.05$).

Rice proteins seemed to play a significant role on pasting properties of rice. Accelerated aging process used in this study could both induce disulfide bond formation and glycation of proteins via non-enzymatic browning or Maillard reactions. Figure 8 showed the effects of cultivar and aging on protein patterns. Rice proteins were consisted of three major protein bands with molecular weight between 10-15, 15-25 and 30 kDa in all cultivars. Accelerated aging had no significant effect on protein pattern regardless of cultivars. However, Pathum Tani1 was lack of polypeptide with molecular weight of less than 13 kDa compared to the others.

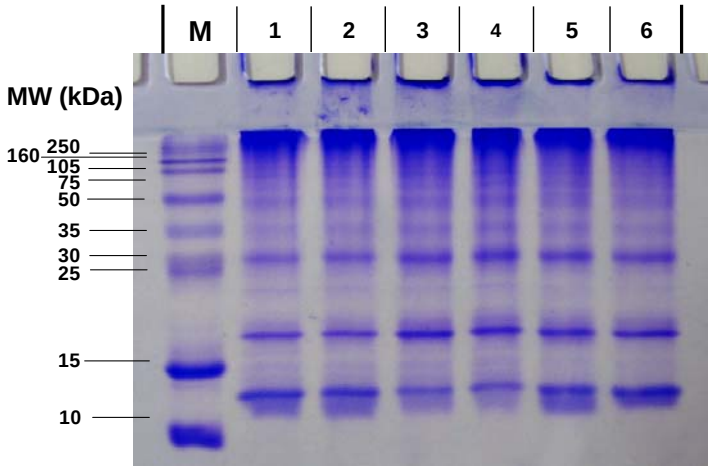

Figure 8 Effect of rice cultivars and accelerated aging on rice protein pattern (Extraction of rice proteins by using 0.1 M NaOH).

M=Standard marker,

Lane No.1=Sanpah-Tawng1 new rice, Lane No.2=Sanpah-Tawng1 aged rice,

Lane No.3=Pathum Tani1 new rice, Lane No.4=Pathum Tani1 aged rice,

Lane No.5=Leuang Pratew123 new rice, Lane No.6=Leuang Pratew123 aged rice

Figure 9 shows the effect of cultivar and aging on pattern of proteins extracted from rice flour using Osborne method. The result showed that proteins in the same fraction in each cultivar were different. In globulin fraction (lanes3 and 4), the intensity of polypeptide with molecular weigh between 15-25 kDa in Sanpah-Tawng1 was more than that of Pathum Tani1 and Leuang Pratew123. However, polypeptide in globulin fraction of Pathum Tani1 with molecular weigh of 250 kDa was less than the others.

Considering the effect of aging, globulin fraction from aged rice of Sanpah-Tawng1 (lanes3 and 4) had less intensed band at 15-25 kDa than the protein globulin from new rice. Besides, there was an extra band with molecular weight between 50-75 kDa after aging Pathum Tani1 and the disappearance of protein of molecular weight around 75 kDa of Leuang Pratew123. For glutelin fraction (lanes5 and 6), protein bands with molecular weigh between 15-250 kDa were slightly detected compared to the others. After aging, there were a few extra bands in glutelin fractions observed at molecular weight between 15-25, 30 and 50 kDa in Sanpah-Tawng1. The differences in protein patterns shown in Figure 9, owing to cultivar and aging, might attribute to the differences in pasting properties of rice grains and flours.

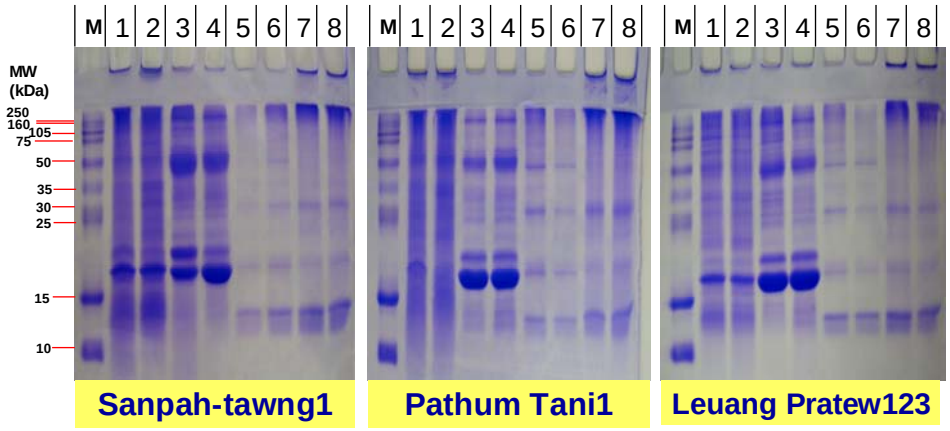

Figure 9 Effect of rice cultivars and accelerated aging on protein pattern (Extraction of rice proteins by Osborne method).

M=Standard marker,

Lane No.1=New rice albumin, Lane No.2=Aged rice albumin,

Lane No.3=New rice globulin, Lane No.4=Aged rice globulin,

Lane No.5=New rice glutelin, Lane No.6=Aged rice glutelin,

Lane No.7=New rice prolamin, Lane No.8=Aged rice prolamin

Glutelin fraction which is the major storage protein was examined in presence β-mercapto ethanol (β-ME). In the presence of β-ME suggested that the glutelin protein exists in subunit linking by disulfide bond (Figure 10). However, no difference in the

patterns of protein from new rice and aged rice, indicating that accelerated aging process did not alter the molecular weigh proteins and the intensity of the bonds in glutelin fraction.

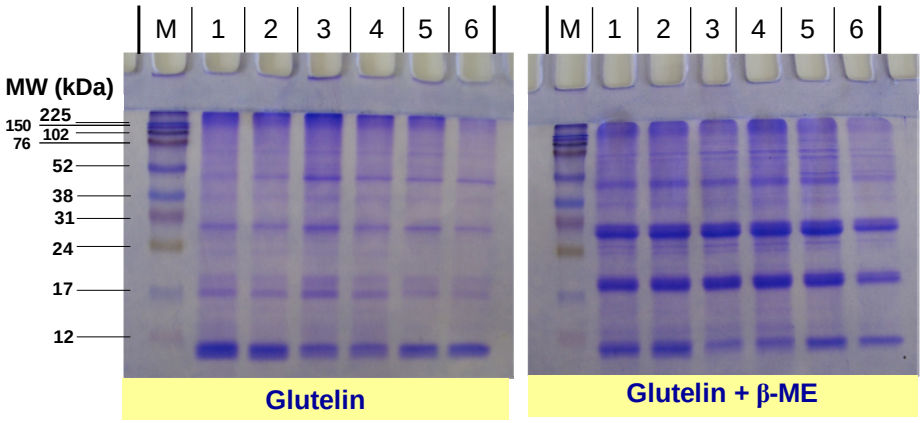

Figure 10 Effect of reducing agent on protein pattern.
M=Standard marker,
Lane No.1=Sanpah-Tawng1 new rice, Lane No.2=Sanpah-Tawng1 aged rice,
Lane No.3=Pathum Tani1 new rice, Lane No.4=Pathum Tani1 aged rice,
Lane No.5=Leuang Pratew123 new rice, Lane No.6=Leuang Pratew123 aged rice

Effect of aging on CIE color of Sanpah-Tawng1, Pathum Tani1 and Leuang Pratew123 rice flour is shown in Table 15. The accelerated aging process had no significant effect on L*-value of Sanpah-Tawng1 and Pathum Tani1, but a decrease in L*-value was observed in Leuang Pratew123 rice flour. The result indicated that aged rice flour of Leuang Pratew123 was less opaque and darker than that of new rice. For b*-value which positive values indicate yellow color, the result showed that the aged rice flour of non-waxy rice; Pathum Tani1 and Leuang Pratew123 were more yellowish (higher b*-value) than the new ones. However, b*-value of Sanpah-Tawng1 waxy rice flour did not change after aging. As explained above in polished rice, the changes in b*-values could be due to the Maillard reactions of protein and starch in rice endosperm during the accelerated aging process at high temperature. It is noticeable that significant changes in CIE color both L* and b* occurred particular in non-waxy rice.

Table 15 Effect of aging on CIE color of Sanpah-Tawng1, Pathum Tani1 and Leuang Pratew123 rice flour.

Cultivar	L*		a*		b*	
	New	Aged	New	Aged	New	Aged
Sanpah-Tawng1	90.08[a]	90.20[a]	-0.27[b]	-0.09[a]	6.17[a]	6.67[a]
Pathum Tani1	90.34[a]	89.72[a]	-0.34[b]	-0.27[a]	6.11[b]	6.95[a]
Leuang Pratew123	91.39[a]	90.23[b]	-0.35[b]	-0.18[a]	4.98[b]	6.21[a]

Means in the same row in each cultivar under the same color category followed by different superscript are significantly different ($p<0.05$).

This study clearly showed that the storage proteins played an important role in determining functional properties of rice grains and flours after accelerated aging. Alterations of those properties such as pasting properties strongly related to protein interactions during aging which also affected the eating quality in term of hardness of cooked rice. Apart from the disulfide bonding, proteins may involve in other reactions during aging, particularly the non-enzymatic browning or Maillard reaction. Although the determination of extracted rice protein pattern by SDS-PAGE was not clearly identified the alteration of protein subunits after aging, it showed that the rice proteins existed in subunits linked by disulfide bond.

Obviously, some of the alterations on functional properties of rice grains and flours were not in similar trend for all cultivars. The different changes in properties of rice between waxy and non-waxy rice flour may be contributed to the difference in functions of rice proteins. Xie *et al*., 2008 reported that the protein network of waxy rice linked by disulfide bonds increased the gelatinized paste rigidity, the hardness and adhesiveness of cooked rice. While in non-waxy rice, both the network and the increase of the gelatinized paste concentrations resulting from protein hydration contributed to the enhancement of the paste rigidity and the hardness of cooked rice.

CONCLUSION AND RECOMMENDATIONS

1. Alteration of functional properties of rice grain and flour can be observed after accelerated aging process at 60°C, 70-75% RH for 5 days. After aging process of polish rice, the hardness in sensory term of cooked rice was increased. Peak viscosity of non-waxy cultivars decreased while that of waxy cultivar increased after aging. However, holding strength, final viscosity and setback of aged rice flour increased regardless of cultivar. In addition, the rice grain and flour were more yellowish after aging. These alterations could be due to other interactions apart from disulfide bond formation. Thus, for complete elucidate the mechanism of rice aging influencing by rice proteins further investigation would be necessary.

2. The removal of rice proteins and the presence of 25 mM cysteine resulted in the alterations of RVA pasting profile, particularly in non-waxy rice flour with shorter peak time and higher peak viscosity. This suggested that the proteins played an important role in the regulation of rice starch granule swelling and resistance to shear at constant temperature. However, more work is needed to clarify the roles of protein at inter-phase of starch-rich phase and protein-rich phase.

3. The alterations in proteins profiles of extracted globulin fraction while there was no detectable changes in the extracted glutelin fraction after aging suggested the physico-chemical changes of storage proteins during aging. Nevertheless, more efficient extraction methods are required if the insights on unextracted proteins, which could be low in solubility due to the polymerization caused by aging, needs further elucidation.

4. The knowledge from this research suggested the possibility in manipulation of rice grain and flour properties through protein modification to have desirable characteristics in the food formulations and processing. Nevertheless, specific usage of such knowledge in rich-based products such as noodle or rice snack need further

investigation since chemical interactions among food ingredients in each product might be involved.

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APPENDICES

Appendix A

Chemical analysis

Appendix A1

Determination of amylose content (Chrastil, 1987)

1. Instruments and apparatus

    1.1 Water bath
    1.2 Vortex
    1.3 Refrigerated centrifuge
    1.4 Spectrophotometer

2. Chemicals

    2.1 Methanol, 85%
    2.2 NaOH, 0.4 N
    2.3 NaOH, 0.13 N
    2.3 Trichloroacetic acid, 0.5%
    2.4 Iodine solution (0.127% I_2 and 0.3% KI in distilled water)
    2.5 Potato amylose

3. Procedure

    3.1 Preparation of test sample

        3.1.1 Weigh 10-20 mg of sample and transfer to test tube
        3.1.2 Add 5 mL 85% methanol in the test tube, cap the tube and vortex thoroughly
        3.1.3 Heat for 15 min in a water bath at 60 ^{0}C
        3.1.4 Centrifuge at 3000xg for 15 min and discard the supernatant
        3.1.5 Repeat step 3.1.2-3.1.4
        3.1.6 Add 2 mL 0.4 N NaOH and then vortex
        3.1.7 Add 4 mL distilled water and vortex
        3.1.8 Cap the tube and heat for 30 min in a water bath at 95°C

3.1.9 Cool and use it as a test sample (Solution A)

3.2 Solution for standard curve

3.2.1 Weight 50 mg of potato amylose and transfer to test tube

3.2.2 Add 8.3 mL 0.4 N NaOH in the test tube and vortex thoroughly

3.2.3 Adjust volume to 25 mL by adding distilled water and cap the tube

3.2.4 Heat for 30 min in a water bath at 60°C

3.2.5 Cool and use it as a stock (100% concentration)

3.2.6 Use the stock to prepare working solutions at 20, 40, 60 80 and 100% (dilute stock solution to 5 mL by 0.13 NaOH) (Solution B)

3.3 Iodine color measurement for test sample (Solution A) and standard curve (Solution B)

3.3.1. Pipette 5 mL of 0.5% Trichloroacetic acid in test tube (for blank, prepare test tube using 5 mL 0.13 N NaOH)

3.3.2. Add 0.1 mL Solution A or Solution B

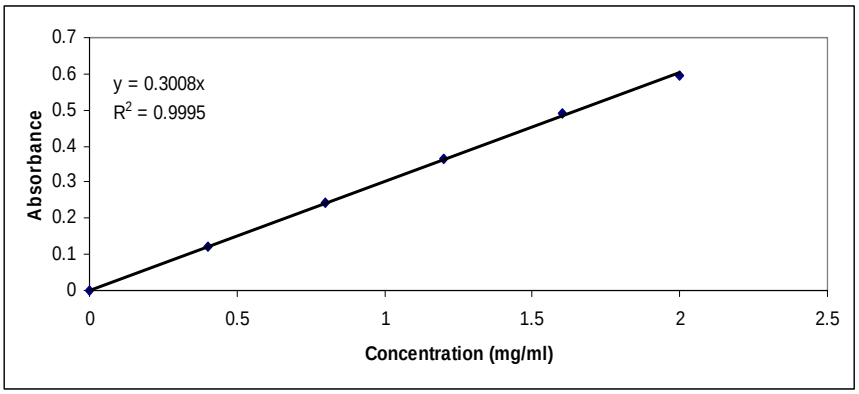
3.3.3. Add 0.05 mL iodine solution

3.3.4. Read color absorbance at 620 nm, using the blank to zero the spectrometer

4. Calculation

4.1 Plot absorbance at 620 nm against amylose concentration of working solutions for a standard curve

4.2 Use absorbance values obtained from the test sample to calculate the apparent amylose content of the sample from the equation obtained from the standard curve (Appendix Figure A1)



Appendix Figure A1 Standard curve for amylose content determination.

Appendix A2

Moisture content determination (AACC, 1995)

1. Instruments and apparatus

    1.1 Moisture can and cover
    1.2 Hot air oven
    1.3 Dessicator
    1.4 Microbalance

2. Procedure

    2.1 Dry the moisture can by heating in hot air oven at 130°C for 1 h
    2.2 Cool in dessicator and weight
    2.3 Weigh the sample (2-3 g) and place into the moisture can
    2.4 Heat uncover moisture can for 1 h
    2.5 Remove the can from oven, cool in dessicator and weigh
    2.6 Repeat step 2.4-2.5 to constant weight

3. Calculation

$$\%\,Moisture = \frac{A \times 100}{B}$$

Where,

A=g of moisture loss

B=g of original sample

Appendix A3

Determination of protein content (AOAC, 2000)

1. Instruments and apparatus

1.1 Microbalance
1.2 Kjeldahl digestion flask
1.3 Digestion unit
1.4 Scrubber
1.5 Distillation unit
1.6 Flasks

2. Chemicals

2.1 Concentrated Sulfuric acid
2.2 Boric acid, 2%
2.3 Copper (II) sulfate
2.4 Potassium sulfate
2.5 Sodium hydroxide, 40%
2.6 Mix indicator (0.1 g bromocresol green and 0.1 g of methyl red in 100 mL of ethanol)

3. Procedure

3.1 Place weighted sample (0.5-1 g) in a Kjeldahl digestion flask
3.2 Add 5 g of catalyst consisted of copper (II) sulfate and potassium sulfate (1:9 w/w) in the flask with a few glass beads
3.3 Add 20 mL H_2SO_4 in the flask
3.4 Connect the flask with digestion unit and heat (At first heating was provided gently until all the water was removed and charring is completed. The heat is then gradually increased so that the solution is brought to constant boiling with slight bubbling.) until solution clears and then for at least 15-20 min longer

3.5 Cool and remove the flask to distillation unit

3.6 Add 20 mL distilled water and 60 mL of 40% NaOH

3.7 Place the receiving flask containing 60 mL of 2% boric and acid 2-3 drops of mixed indicator in the distillation unit

3.8 Distill for 3 min

3.9 Titrate the solution in receiving flask with standard 0.1 N H_2SO_4 until the color changes from green to colorless

3.10 Correct for blank determinations on reagents

4. Calculation

$$\%\,Nitrogen = \frac{(S-B)\times N\times 1.401\times 100}{W}$$

Where,

S=mL sulfuric acid titration of sample titer

B=mL sulfuric acid titration of blank titer (mL of standard)

N=Normality of sulfuric acid

W=g of sample weight

$$\%\,\mathrm{Pr}otein = \%\,Nitrogen\times 5.95$$

Where,

5.95 is conversion factor for rice protein

Appendix A4

Determination of fat content (AOAC, 2000)

1. Instruments and apparatus

1.1 Soxtec System HT2; Extraction unit
1.2 Cooling generator
1.3 Extraction cup
1.4 Extraction thimble
1.5 Filter paper
1.6 Dessicator

2. Chemicals

2.1 Petroleum ether (boiling point 40-60°C)

3. Procedure

3.1 Weigh 2 g of sample, place on a filter paper and fold the filter paper to cover the sample
3.2 Transfer sample to extraction unit
3.3 Add 60 mL petroleum ether into an extraction cup and connected it to extraction unit
3.4 Dip the extraction thimble containing the sample into petroleum ether that was constantly heated to boiling for 20 min
3.5 Lift the extraction thimble up and rinse by petroleum ether for 45 min
3.6 Evaporate petroleum ether for 10 min
3.7 Remove extraction cup and place I hot air oven at 100°C for 1 h
3.8 Cool in dessicator and weigh

4. Calculation

$$\%\,Fat \quad = \quad \frac{(W_3 - W_2)\times 100}{W_1}$$

Where,

W_1=g of sample

W_2=g of extraction cup

W_3=g of extraction cup and fat

Appendix A5

Ash content determination (AACC, 1995)

1. Instruments and apparatus

1.1 Porcelain crucible

1.2 Muffle furnace, capable of operating at temperatures up to 600 ± 15°C

1.3 Hot plate

1.4 Dessicator

1.5 Microbalance

2. Procedure

2.1 heat a porcelain crucible at 525°C for 2 h, cool, and weigh

2.2 Weigh accurately 1 g rice flour into porcelain crucible

2.3 Heat gently on hot plate until sample is thoroughly carbonized

2.4 Place in muffle furnace at and heat for 2 h or until ash is free from carbon, white color

3. Calculation

$$\%\,Ash \quad = \quad \frac{(W_2 - W_0)\times 100}{(W_1 - W_0)}$$

Where,

W_0=g of porcelain crucible

W_1=g of porcelain crucible and sample before heating

W_2=g of porcelain crucible and sample after heating

Appendix B

Characterization of rice grains, rice flours and rice proteins

Appendix B1

Determination of the pasting properties of rice flour (AACC, 1995)

1. Instruments and apparatus

1.1 Rapid Visco Analyser

1.2 Computer, loaded with control software provided with instrument

2. Procedure

2.1 Instrument preparation

2.1.1 Switch on instrument and associated computer

2.1.2 Select the test profile for rice that consists of the following time and temperature cycle in table B1

Appendix Table B1 The test RVA profile for rice**.**

Temperature (°C)	Time (min:sec)
50.0 (Idle temperature)	
50.0	1:00
95.0	4:45
95.0	7:15
50.0	11:06
Total time	12:30

2.1.3 Enter paddle rotation speed , initial speed is 960 rpm for first 10 sec of test, followed by 160 rpm for remainder of test

2.1.4 Allow instrument at least 30 min to warm up before use

2.2 Sample determination

2.2.1 Weigh 3.00 g rice flour (12% moisture basis) into weighing vessel before transfer into test canister

2.2.2 Dispense 25 mL (±0.1 mL) water (12% moisture basis) into test canister. Equivalent sample and water mass can be calculated using formulas:

$$S = \frac{88 \times 3.0}{100 - M} \qquad \text{For flour}$$

$$W = 25 + (3.0 - S) \qquad \text{For water}$$

Where,

S=corrected sample weight

W=corrected water weight

M=actual moisture content of sample

2.2.3 Transfer flour onto water surface in canister

2.2.4 Place paddle into canister and vigorously jog blade through sample up and down 10 times

2.2.5 Place paddle into canister, and insert paddle and canister assembly firmly into paddle coupling so that paddle is properly centered

2.2.6 Initiate measurement cycle by depressing motor tower of instrument

2.2.7 Note peak viscosity, minimum viscosity after peak, and final viscosity at 50°C (viscosity is measured in rapid visco units cP)

2.2.8 Typically measured pasting properties that characterize rice are as follows:

Pasting temperature=temperature of the initial viscosity increase

Peak=maximum viscosity recorded during the heating and holding cycles

Peak time=time required to reach peak

Holding strength=minimum viscosity after peak

Final viscosity=viscosity achieved at the end of the test

Breakdown viscosity=the difference between peak and trough

Setback from peak=the difference between final viscosity and peak

Appendix B2

Determination of molecular weight of protein by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970)

1. Instruments and apparatus

    1.1 Electrophoresis Cell
    1.2 Hotplate
    1.3 Microcentrifuge

2. Chemicals

    2.1 Protein standard marker
    2.2 Acrylamide
    2.3 Metylenebisacrylamide
    2.4 Tris (Hydroxylmethyl) aminomethane
    2.5 Tetradimethylethylenediamine
    2.6 2-Mercaptoethanol
    2.7 Ammonium persulfate
    2.8 Hydrochloric acid
    2.9 Glycerol
    2.10 Coomassie brilliant blue R-250
    2.11 Bromophenol Blue Sodium Salt
    2.12 Sodium dodecyl sulfate
    2.13 Glycine
    2.14 Acetic acid
    2.15 Methanol

3. Procedure

    3.1 Assembling the glass plate on clean surface

3.1.1 Assemble the glass plates on the clean surface (the bottom of both glass plates must be aligned)

3.1.2 Loosen the clam assembly and gently slide the glass plate sandwich in to it

3.1.3 Tighten the clam assembly and place it into casting stand

3.2. Casting the gels (preparation of 15% separating gel and 4% stacking gel)

3.2.1 Prepare the separating gel monomer solution (following Appendix Table B2) by combining all reagents except ammonium persulfate (APS) and TEMED

Appendix Table B2 Recipe for preparation of separating gel and stacking gel**.**

Chemicals	15% separating gel (0.375 M Tris, pH 8.8)	4% stacking gel (0.125 M Tris, pH 6.8)
1. Distilled water	2.35 mL	6.1 mL
2. 1.5 M Tris-HCl/SDS, pH 8.8	2.5 mL	-
3. 0.5 M Tris-HCl/SDS, pH 6.8	-	2.5 mL
4. 10% (W/V) SDS	100 µL	100 µL
5. 30% Acrylamide + 2.67% Bis-acrylamide	5 mL	1.33 mL
6. 10% Ammonium persulfate (APS)	50 µL	50 µL
7. TEMED	5 µL	5 µL
Total monomer	10 mL	10 mL

3.2.2 Deaerate and mix the solution after adding each reagent by swirling the container gently

3.2.3 Add APS and TEMED to the monomer solution and mix well by swirling gently

3.2.4 Pipette the solution to the assembled gel sandwich

3.2.5 Immediately overlay the monomer solution with distilled water

3.2.6 Allow the gel to polymerize for 45 min to 1 hour

3.2.7 Pour the water overlaying the gel

3.2.8 Prepare the stacking gel monomer solution by combine all reagents except APS and TEMED

3.2.9 Deaerate and mix the solution by swirling gently

3.2.10 Add APS and TEMED to the solution and pipette the solution down until the sandwich is filled completely

3.2.11 Place a comb in the gel sandwich

3.2.12 Allow the gel to polymerize for 15 min

3.2.13 With a marker pen, place a mark on the glass plate 1 cm below the teeth of the comb

3.2.14 Remove the comb

3.2.15 Place the gel in the buffer chamber and diluted running gel buffer (following Appendix Table B3) is added into the chamber (dilute the electrode running gel buffer by adding 4 time distilled water)

Appendix Table B3 Recipe for preparation of electrode running gel buffer**.**

Chemicals	Weight or volume
1. Tris base	9.0 g
2. Glycine	43.2 g
3. SDS	3.0 g
4. Distilled water	600 mL

3.3 Preparation of samples

3.3.1 Determine the volume of protein samples that contains appropriated concentration of protein

3.3.2 Place the volume of sample into a labeled microfuge tube

3.3.3 Add 1 mL sample buffer (following Appendix Table B4) to the samples

3.3.4 Place the tubes in a boiling water bath and boil the samples for 4 min

3.3.5 Cool and centrifuge at 5000 rpm for 5 min

3.3.6 Load the boiled protein samples to the bottom of a well

Appendix Table B4 Recipe for preparation of sample buffer**.**

Chemicals	With reducing agent	Without reducing agent
1. Distilled water	3.8 mL	4.2 mL
2. 0.5 M Tris-HCl/SDS, pH 6.8	1.0 mL	1.0 mL
3. Glyceral	0.8 mL	0.8 mL
4. 2-Mercaptoethanol	0.4 mL	-
5. 1% (w/v) Bromophenol blue	0.4 mL	0.4 mL
Total	8.0 mL	8.0 mL

4. Loading the samples

4.1 Load the first well with Standard protein marker

4.2 Load the second and other wells with protein samples (do not pipette the pellet at the bottom of the microfuge tube)

5. Running the gel

5.1 Check that the electrode running gel buffer in chamber is full

5.2 Place the lid on top of the lower buffer chamber (make sure that the connection is correct, black to black and red to red)

5.3 Attach the electrical leads to a suitable power pack with the proper polarity (black to black and red to red) and run the gel at a constant current of 150 volts

5.4 Stop the electrophoresis when the tracker dye is approximately 1 cm above the end of the glass plates (about 1 h)

6. Removing and staining the gel

6.1 After electrophoresis, carefully remove the gel from between the glass plates

6.2 Submerge the gel in staining solution following Appendix Table B5 (make sure that the gel is fully submerged in the staining) for 30 min

6.3 Destain the gel in a destaining solution (following Appendix Table B5) a few times until protein bands are visualized and the background is clear

6.4 Approximately determine the molecular weight of the visualized protein bands by comparing them with the molecular weight markers

Appendix Table B5 Recipe for preparation of staining solution and destaining solution**.**

Chemicals	Staining solution	Destaining solution
1. Distilled water	500 mL	825 mL
2. Methanol	400 mL	100 mL
3. Glacial acetic acid	100 mL	75 mL
4. Coomassie brilliant blue	1.0 g	-
Total	1000 mL	1000 mL

Appendix C

Sensory analysis

Appendix C1

Questionnaire for sensory analysis

Triangle Test: Difference analysis of cooked rice quality

Date: _____ Time: _____ Product: Cooked rice

Instructions:

Here are three samples for evaluation, two are the same and one is different; starting from the left evaluate samples and circle the code that different in the eating quality of cooked rice in term of hardness and circle that is different from the other two. Indicate the degree of the hardness and decide which evaluated sample is harder by make a mark in the box. Please rinse your mouth with water before tasting each sample.

1. Separate the different sample in hardness quality

Sample code	631	749	285

2. Indicate the degree of the hardness between the same samples and the different samples

- □ Very slightly different
- □ Slightly different
- □ Moderately different
- □ Much different
- □ Extremely different

3. The harder sample is

- □ The different sample
- □ The same samples

Comments _____

Appendix D

Statistical analysis

Appendix Table D1 Analysis of variance of moisture and protein content of polished rice flour.

Treatment	Source	Degree of Freedom	Sum of Squares	Mean Square	F-Value	Sig.
Moisture content	Treatment	2	18.632	9.316	63.589	0.000
	Error	24	3.516	0.147		
	Total	26	22.148			
Protein content	Treatment	2	1.857	0.928	2.010	0.157
	Error	23	10.621	0.462		
	Total	25	12.477			

Appendix Table D2 Analysis of variance of RVA pasting profile of new rice flour, aged rice flour and cysteine-treated rice flour.

Treatment	Source	Degree of Freedom	Sum of Squares	Mean Square	F-Value	Sig.
Peak viscosity	Treatment	2	1.972	0.986	12.862	0.000
	Error	24	1.840	0.077		
	Total	26	3.812			
Holding strength	Treatment	2	0.312	0.156	8.409	0.002
	Error	24	0.445	0.018		
	Total	26	0.757			
Breakdown viscosity	Treatment	2	3.348	1.674	46.766	0.000
	Error	24	0.820	0.034		
	Total	26	2.107			
Setback	Treatment	2	0.360	0.180	24.767	0.000
	Error	24	0.174	0.007		
	Total	26	0.534			
Peak time	Treatment	2	0.758	0.379	9.186	0.001
	Error	24	0.990	0.041		
	Total	26	1.747			
Pasting temperature	Treatment	2	11.829	5.914	6.725	0.005
	Error	24	21.108	0.879		
	Total	26	32.937			

Appendix Table D3 Analysis of variance of CIE color of polished uncooked new rice, aged rice and cysteine-treated aged rice grain.

Treatment	Source	Degree of Freedom	Sum of Squares	Mean Square	F-Value	Sig.
L*	Treatment	2	29.514	14.757	81.935	0.000
	Error	24	4.322	0.180		
	Total	26	33.836			
a*	Treatment	2	0.840	0.420	23.640	0.000
	Error	24	0.427	0.018		
	Total	26	1.267			
b*	Treatment	2	24.544	12.272	92.764	0.000
	Error	24	3.175	0.132		
	Total	26	27.719			

Appendix table D4 Analysis of variance of protein content of CIE color of polished cooked new rice, aged rice and cysteine-treated aged rice grain.

Treatment	Source	Degree of Freedom	Sum of Squares	Mean Square	F-Value	Sig.
L*	Treatment	2	27.715	13.858	6.399	0.006
	Error	24	51.977	2.166		
	Total	26	79.693			
a*	Treatment	2	0.411	0.205	6.735	0.005
	Error	24	0.732	0.030		
	Total	26	1.143			
b*	Treatment	2	6.450	3.225	13.113	0.000
	Error	24	5.903	0.246		
	Total	26	12.353			

Appendix Table D5 Analysis of variance of RVA pasting profile of new rice flour and aged rice flour added with 25 and 50 mM Cysteine solution.

Treatment	Source	Degree of Freedom	Sum of Squares	Mean Square	F-Value	Sig.
Peak viscosity	Treatment	5	2.450	0.490	6.295	0.000
	Error	30	2.336	0.078		
	Total	35	4.786			
Holding strength	Treatment	5	1.016	0.203	18.740	0.000
	Error	30	0.325	0.011		
	Total	35	1.341			
Breakdown viscosity	Treatment	5	5.759	1.152	18.338	0.000
	Error	30	1.884	0.063		
	Total	35	7.643			
Final viscosity	Treatment	5	4.010	0.802	54.840	0.000
	Error	30	0.439	0.015		
	Total	35	4.448			
Setback	Treatment	5	1.071	0.214	70.385	0.000
	Error	30	0.091	0.003		
	Total	35	1.162			
Peak time	Treatment	5	0.316	0.063	2.357	0.064
	Error	30	0.805	0.027		
	Total	35	1.121			
Pasting temperature	Treatment	5	71.375	14.275	9.207	0.000
	Error	30	46.515	1.551		
	Total	35	117.891			

Appendix Table D6 Statistical analysis from independent samples test of protein content of Sanpah-Tawng1, Pathum Tani1 and Leuang Pratew123 new rice and aged rice flour.

Cultivar	Source	Levene's Test for Equality of Variances		T-test for equality of means					
		F-value	Sig.	Degree of Freedom	Mean Difference	t	Sig. (2-tailed)	95% Confidence Interval of the difference	
								Lower	Upper
Sanpah-Tawng1	Equal variances assumed	22.169	0.00	15	0.207	0.207	0.043	0.007	0.406
	Equal variances not assumed			8.02	0.207	0.207	0.070	-0.021	0.434
Pathum Tani1	Equal variances assumed	1.953	0.18	15	0.165	1.689	0.112	-0.043	0.373
	Equal variances not assumed			13.72	0.165	1.734	0.105	-0.039	0.369
Leuang Pratew123	Equal variances assumed	0.853	0.37	14	0.075	0.333	0.744	0.409	0.559
	Equal variances not assumed			13.65	0.075	0.333	0.744	0.410	0.560

Appendix Table D7 Analysis of variance of protein content of Sanpah-Tawng1, Pathum Tani1 and Leuang Pratew123 new rice and aged rice flour.

Treatment	Source	Degree of Freedom	Sum of Squares	Mean Square	F-Value	Sig.
New rice	Treatment	2	36.211	18.106	185.07	0.000
	Error	22	2.152	0.098		
	Total	24	38.364			
Aged rice	Treatment	2	36.084	18.042	213.08	0.000
	Error	22	1.863	0.085		
	Total	24	37.946			

Appendix Table D8 Statistical analysis from independent samples test of RVA pasting profile of Sanpah-Tawng1 new rice and aged rice flour.

Treatment	Source	Levene's Test for Equality of Variances		T-test for equality of means					
		F-value	Sig.	Degree of Freedom	Mean Difference	t	Sig. (2-tailed)	95% Confidence Interval of the Difference	
								Lower	Upper
Peak viscosity	Equal variances assumed	49.085	0.00	16	-0.427	-7.440	0.000	-0.548	-0.305
	Equal variances not assumed			8.17	-0.427	-7.440	0.000	-0.558	0.294
Holding strength	Equal variances assumed	8.558	0.01	16	-0.405	-15.041	0.000	-0.462	-0.348
	Equal variances not assumed			11.65	-0.405	-15.041	0.000	-0.464	0.346
Breakdown viscosity	Equal variances assumed	16.517	0.00	16	-0.022	-0.597	0.559	-0.099	0.055
	Equal variances not assumed			10.00	-0.022	-0.597	0.564	-0.102	0.059
Final viscosity	Equal variances assumed	24.849	0.00	16	-0.517	-14.588	0.000	-0.593	-0.442
	Equal variances not assumed			9.40	-0.517	-14.588	0.000	-0.597	0.437
Setback	Equal variances assumed	11.103	0.00	16	-0.112	-8.834	0.000	-0.139	-0.085
	Equal variances not assumed			9.34	-0.112	-8.834	0.000	-0.141	0.084

Appendix Table D8 (Continued)

Treatment	Source	Levene's Test for Equality of Variances		T-test for equality of means					
		F-value	Sig.	Degree of Freedom	Mean Difference	t	Sig. (2-tailed)	95% Confidence Interval of the Difference	
								Lower	Upper
Peak time	Equal variances assumed	0.607	0.44	16	-0.215	-6.160	0.000	-0.289	-0.141
	Equal variances not assumed			14.13	-0.215	-6.160	0.000	-0.290	-0.140
Pasting temperature	Equal variances assumed	7.201	0.02	16	0.222	0.306	0.763	-1.316	1.761
	Equal variances not assumed			9.83	0.222	0.306	0.766	-1.398	1.843

Appendix Table D9 Statistical analysis from independent samples test of RVA pasting profile of Pathum Tani1 new rice and aged rice flour.

Treatment	Source	Levene's Test for Equality of Variances		T-test for equality of means					
		F-value	Sig.	Degree of Freedom	Mean Difference	t	Sig. (2-tailed)	95% Confidence Interval of the Difference	
								Lower	Upper
Peak viscosity	Equal variances assumed	18.869	0.00	16	0.411	3.650	0.002	0.172	0.650
	Equal variances not assumed			8.89	0.411	3.650	0.005	0.156	0.666
Holding strength	Equal variances assumed	1.265	0.27	16	-0.329	-6.049	0.000	-0.445	-0.214
	Equal variances not assumed			14.99	-0.329	-6.049	0.105	-0.446	-0.213
Breakdown viscosity	Equal variances assumed	19.819	0.00	16	0.740	9.287	0.000	0.571	0.909
	Equal variances not assumed			9.32	0.740	9.287	0.070	0.5610	0.919
Final viscosity	Equal variances assumed	5.860	0.02	16	-0.598	-9.180	0.000	-0.737	-0.460
	Equal variances not assumed			12.40	-0.598	-9.180	0.105	-0.740	-0.456
Setback	Equal variances assumed	2.689	0.12	16	-0.269	-11.740	0.000	-0.318	-0.220
	Equal variances not assumed			13.69	-0.269	-11.740	0.105	-0.318	-0.219

Appendix Table D9 (Continued)

Treatment	Source	Levene's Test for Equality of Variances		T-test for equality of means					
		F-value	Sig.	Degree of Freedom	Mean Difference	t	Sig. (2-tailed)	95% Confidence Interval of the Difference	
								Lower	Upper
Peak time	Equal variances assumed	5.149	0.03	16	-0.193	-3.521	0.003	-0.309	-0.077
	Equal variances not assumed			12.84	-0.193	-3.521	0.004	-0.311	-0.074
Pasting temperature	Equal variances assumed	0.005	0.94	16	-2.494	-4.924	0.000	-3.568	-1.421
	Equal variances not assumed			15.11	-2.494	-4.924	0.000	-3.574	-1.415

Appendix Table D10 Statistical analysis from independent samples test of RVA pasting profile of Leuang Pratew123 new rice and aged rice flour.

Treatment	Source	Levene's Test for Equality of Variances		T-test for equality of means					
		F-value	Sig.	Degree of Freedom	Mean Difference	t	Sig. (2-tailed)	95% Confidence Interval of the Difference	
								Lower	Upper
Peak viscosity	Equal variances assumed	0.067	0.79	16	0.087	2.375	0.030	0.009	0.165
	Equal variances not assumed			15.88	0.087	2.375	0.031	0.009	0.164
Holding strength	Equal variances assumed	0.148	0.70	16	-0.204	-6.302	0.000	-0.273	-0.136
	Equal variances not assumed			15.99	-0.204	-6.302	0.000	-0.273	0.135
Breakdown viscosity	Equal variances assumed	24.013	0.00	16	0.292	12.772	0.000	0.243	0.340
	Equal variances not assumed			9.17	0.292	12.772	0.000	-0.240	0.343
Final viscosity	Equal variances assumed	0.000	0.98	16	-0.642	-12.162	0.000	-0.754	-0.530
	Equal variances not assumed			15.99	-0.642	-12.162	0.000	-0.754	-0.530
Setback	Equal variances assumed	5.441	0.03	16	-0.437	-10.259	0.000	-0.528	-0.347
	Equal variances not assumed			13.41	-0.437	-10.259	0.000	-0.529	-0.345

Appendix Table D10 (Continued)

Treatment	Source	Levene's Test for Equality of Variances		T-test for equality of means					
		F-value	Sig.	Degree of Freedom	Mean Difference	t	Sig. (2-tailed)	95% Confidence Interval of the Difference	
								Lower	Upper
Peak time	Equal variances assumed	0.315	0.58	16	0.148	4.047	0.001	0.070	0.226
	Equal variances not assumed			15.77	0.148	4.047	0.001	0.070	0.226
Pasting temperature	Equal variances assumed	0.162	0.69	16	-2.067	-6.854	0.000	-2.706	-1.428
	Equal variances not assumed			15.93	-2.067	-6.854	0.000	-2.706	-1.427

Appendix Table D11 Statistical analysis from independent samples test of protein content of Sanpah-Tawng1, Pathum Tani1, Leuang Pratew123 low protein new rice and low protein aged rice flour.

Treatment	Source	Levene's Test for Equality of Variances		T-test for equality of means					
		F-value	Sig.	Degree of Freedom	Mean Difference	t	Sig. (2-tailed)	95% Confidence Interval of the Difference	
								Lower	Upper
Sanpah-Tawng1	Equal variances assumed	0.602	0.45	15	-1.046	-16.663	0.000	-1.180	-0.912
	Equal variances not assumed			14.78	-1.046	-16.922	0.000	-1.178	-0.914
Pathum Tani1	Equal variances assumed	0.025	0.88	16	-0.2824	-2.490	0.024	-0.523	-0.042
	Equal variances not assumed			15.99	-0.2824	-2.490	0.024	-0.523	-0.042
Leuang Pratew123	Equal variances assumed	3.169	0.09	16	-0.485	-4.246	0.001	-0.727	-0.243
	Equal variances not assumed			13.99	-0.485	-4.246	0.001	-0.730	-0.239

Appendix Table D12 Analysis of variance of RVA pasting profile of Sanpah-Tawng1 new rice, aged rice, low protein new rice and low protein aged rice flour.

Treatment	Source	Degree of Freedom	Sum of Squares	Mean Square	F-Value	Sig.
Peak viscosity	Treatment	3	2.543	0.848	65.589	0.000
	Error	32	0.414	0.013		
	Total	35	2.956			
Holding strength	Treatment	3	0.755	0.252	79.174	0.000
	Error	32	0.102	0.003		
	Total	35	0.857			
Breakdown viscosity	Treatment	3	1.430	0.477	93.486	0.000
	Error	32	0.163	0.005		
	Total	35	1.593			
Final viscosity	Treatment	3	1.273	0.424	74.911	0.000
	Error	32	0.181	0.006		
	Total	35	1.454			
Setback	Treatment	3	0.074	0.024	23.971	0.000
	Error	32	0.033	0.001		
	Total	35	0.107			
Peak time	Treatment	3	0.746	0.249	61.023	0.000
	Error	32	0.130	0.004		
	Total	35	0.876			
Pasting temperature	Treatment	3	53.804	17.935	12.808	0.000
	Error	32	44.810	1.400		
	Total	35	98.614			

Appendix Table D13 Analysis of variance of RVA pasting profiles of Pathum Tani1 new rice, aged rice, low protein new rice and low protein aged rice flour.

Treatment	Source	Degree of Freedom	Sum of Squares	Mean Square	F-Value	Sig.
Peak viscosity	Treatment	3	7.650	2.550	41.126	0.000
	Error	32	1.984	0.062		
	Total	35	9.634			
Holding strength	Treatment	3	1.366	0.455	33.990	0.000
	Error	32	0.429	0.013		
	Total	35	1.795			
Breakdown viscosity	Treatment	3	15.028	5.009	164.308	0.000
	Error	32	0.976	0.030		
	Total	35	16.004			
Final viscosity	Treatment	3	5.053	1.684	87.706	0.000
	Error	32	0.615	0.019		
	Total	35	5.667			
Setback	Treatment	3	1.179	0.393	175.495	0.000
	Error	32	0.072	0.002		
	Total	35	1.251			
Peak time	Treatment	3	2.776	0.925	52.179	0.000
	Error	32	0.567	0.018		
	Total	35	3.344			
Pasting temperature	Treatment	3	59.886	19.962	25.129	0.000
	Error	32	25.420	0.794		
	Total	35	85.306			

Appendix Table D14 Analysis of variance of RVA pasting profiles of Leuang Pratew123 new rice, aged rice, low protein new rice and low protein aged rice flour.

Treatment	Source	Degree of Freedom	Sum of Squares	Mean Square	F-Value	Sig.
Peak viscosity	Treatment	3	1.206	0.402	75.860	0.000
	Error	32	0.170	0.005		
	Total	35	1.375			
Holding strength	Treatment	3	0.381	0.127	24.774	0.000
	Error	32	0.164	0.005		
	Total	35	0.544			
Breakdown viscosity	Treatment	3	0.797	0.266	58.238	0.000
	Error	32	0.146	0.004		
	Total	35	0.943			
Final viscosity	Treatment	3	2.415	0.805	77.841	0.000
	Error	32	0.331	0.010		
	Total	35	2.746			
Setback	Treatment	3	0.958	0.319	20.983	0.000
	Error	32	0.487	0.015		
	Total	35	1.444			
Peak time	Treatment	3	2.483	0.828	124.334	0.000
	Error	32	0.213	0.007		
	Total	35	2.696			
Pasting temperature	Treatment	3	34.590	11.530	31.343	0.000
	Error	32	11.772	0.368		
	Total	35	46.362			

Appendix Table D15 Analysis of variance of RVA pasting profiles of Sanpah-Tawng1 new rice flour and aged rice flour added with 25 mM Cysteine solution.

Treatment	Source	Degree of Freedom	Sum of Squares	Mean Square	F-Value	Sig.
Peak viscosity	Treatment	3	13.675	4.558	368.973	0.000
	Error	31	0.383	0.012		
	Total	34	14.058			
Holding strength	Treatment	3	7.241	2.414	653.853	0.000
	Error	31	0.114	0.004		
	Total	34	7.355			
Breakdown viscosity	Treatment	3	1.082	0.361	106.118	0.000
	Error	31	0.105	0.003		
	Total	34	1.187			
Final viscosity	Treatment	3	10.295	3.432	689.058	0.000
	Error	31	0.154	0.005		
	Total	34	10.450			
Setback	Treatment	3	0.271	0.090	328.180	0.000
	Error	31	0.008	0.000		
	Total	34	0.280			
Peak time	Treatment	3	0.713	0.238	52.246	0.000
	Error	31	0.141	0.004		
	Total	34	0.854			
Pasting temperature	Treatment	3	82.763	27.588	15.998	0.000
	Error	31	53.459	1.724		
	Total	34	136.222			

Appendix Table D16 Analysis of variance of RVA pasting profiles of Pathum Tani1 new rice flour and aged rice flour added with 25 mM Cysteine solution.

Treatment	Source	Degree of Freedom	Sum of Squares	Mean Square	F-Value	Sig.
Peak viscosity	Treatment	3	0.660	0.220	3.232	0.035
	Error	32	2.179	0.068		
	Total	35	2.839			
Holding strength	Treatment	3	1.770	0.590	22.519	0.000
	Error	32	0.839	0.026		
	Total	35	2.609			
Breakdown viscosity	Treatment	3	3.633	1.211	33.982	0.000
	Error	32	1.140	0.036		
	Total	35	4.773			
Final viscosity	Treatment	3	5.478	1.826	57.576	0.000
	Error	32	1.015	0.032		
	Total	35	6.493			
Setback	Treatment	3	1.026	0.342	223.116	0.000
	Error	32	0.049	0.002		
	Total	35	1.075			
Peak time	Treatment	3	0.169	0.056	1.935	0.144
	Error	32	0.932	0.029		
	Total	35	1.101			
Pasting temp.	Treatment	3	29.655	9.885	10.014	0.000
	Error	32	31.587	0.987		
	Total	35	61.241			

Appendix Table D17 Analysis of variance of RVA pasting profiles of Leuang Pratew123 new rice flour and aged rice flour added with 25 mM Cysteine solution.

Treatment	Source	Degree of Freedom	Sum of Squares	Mean Square	F-Value	Sig.
Peak viscosity	Treatment	3	1.042	0.347	34.771	0.000
	Error	32	0.320	1.010		
	Total	35	1.361			
Holding strength	Treatment	3	0.846	0.282	38.984	0.000
	Error	32	0.232	0.007		
	Total	35	1.078			
Breakdown viscosity	Treatment	3	1.388	0.463	66.650	0.000
	Error	32	0.222	0.007		
	Total	35	1.610			
Final viscosity	Treatment	3	6.192	2.064	149.998	0.000
	Error	32	0.440	0.014		
	Total	35	6.633			
Setback	Treatment	3	2.778	0.926	47.500	0.000
	Error	32	0.624	0.020		
	Total	35	3.402			
Peak time	Treatment	3	0.947	0.316	17.561	0.000
	Error	32	0.575	0.018		
	Total	35	1.521			
Pasting temperature	Treatment	3	14.918	4.973	6.723	0.001
	Error	32	23.669	0.740		
	Total	35	38.588			

Appendix Table D18 Analysis of variance of CIE color of Sanpah-Tawng1 new rice and aged rice flour.

Treatment	Source	Levene's Test for Equality of Variances		T-test for equality of means					
		F-value	Sig.	Degree of Freedom	Mean Difference	t	Sig. (2-tailed)	95% Confidence Interval of the Difference	
								Lower	Upper
L*	Equal variances assumed	3.207	0.09	16	-0.203	-0.381	0.708	-1.335	0.928
	Equal variances not assumed			13.20	-0.203	-0.381	0.709	-1.355	0.948
a*	Equal variances assumed	2.088	0.17	16	-0.179	-4.479	0.000	-0.264	-0.094
	Equal variances not assumed			14.89	-0.179	-4.479	0.000	-0.264	-0.094
b*	Equal variances assumed	8.761	0.01	16	-0.500	-1.471	0.161	-1.221	0.221
	Equal variances not assumed			12.45	-0.500	-1.471	0.166	-1.238	0.238

Appendix Table D19 Analysis of variance of CIE color of Pathum Tani1 new rice and aged rice flour.

Treatment	Source	Levene's Test for Equality of Variances		T-test for equality of means					
		F-value	Sig.	Degree of Freedom	Mean Difference	t	Sig. (2-tailed)	95% Confidence Interval of the Difference	
								Lower	Upper
L*	Equal variances assumed	1.966	0.18	16	0.617	1.367	0.190	-0.339	1.573
	Equal variances not assumed			13.49	0.617	1.367	0.194	-0.354	1.587
a*	Equal variances assumed	14.551	0.00	16	-0.073	-2.728	0.015	-0.130	-0.016
	Equal variances not assumed			9.35	-0.073	-2.728	0.023	-0.134	-0.013
b*	Equal variances assumed	5.819	0.03	16	-0.846	-2.721	0.015	-1.504	-0.187
	Equal variances not assumed			12.44	-0.846	-2.721	0.018	-1.520	-0.171

Appendix Table D20 Analysis of variance of CIE color of Leuang Pratew123 new rice and aged rice flour.

Treatment	Source	Levene's Test for Equality of Variances		T-test for equality of means					
		F-value	Sig.	Degree of Freedom	Mean Difference	t	Sig. (2-tailed)	95% Confidence Interval of the Difference	
								Lower	Upper
L*	Equal variances assumed	0.087	0.77	16	0.470	3.272	0.005	0.166	0.774
	Equal variances not assumed			16	0.470	3.272	0.005	0.165	0.775
a*	Equal variances assumed	2.561	0.13	16	-0.170	-8.143	0.000	-0.214	-0.126
	Equal variances not assumed			16	-0.170	-8.143	0.000	-0.215	-0.125
b*	Equal variances assumed	27.321	0.00	16	-1.233	-13.629	0.000	-1.425	-1.042
	Equal variances not assumed			16	-1.233	-13.629	0.000	-1.439	-1.028

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