OPTIMUM MICROWAVE HEATING FOR GERMINATED AND NON-GERMINATED PUFFED RICE

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ABTRACT

The optimum microwave heating for germinated and non-germinated puffed rice was investigated. Ten paddy rice varieties, both waxy and non-waxy paddy rice cultivated in the northern and northeastern region were selected to study the effects of some physicochemical properties on the puffing qualities of puffed rice (puffed yield, expansion volume, expansion ratio and bulk density) using microwave at a frequency 2,450 MHz 800 watts. It was found that amylose content (range 5.58-21.24%) was strongly negatively correlated with all qualities of puffed rice; puffed yield (r=-0.95**), expansion volume (r=-0.82**), expansion ratio (r=-0.79**) and bulk density (r=-0.78**). While the tightness of husk (lemma-palea) interlocking, thickness of ventral region layer affected the expansion volume. Paddy rice variety (RD6) was used for studying the effects of salt (0 and 2%), moisture content (10, 13, 16 and 19%) at microwave power (600, 700 and 800 watts) on puffing qualities. The results showed that all the main factors and their interaction significantly ($p<0.05$) affected the puffing qualities included total puffed yield, fully puffed yield and small puffed yield, expansion volume, texture, color and microstructure. Paddy rice soaked with 2% salt solution showed higher puffed yield than paddy rice soaked with water. The higher moisture content and the microwave power produced puffed rice which had higher puffed yield and expansion volume. The highest puffed yield and expansion volume were produced at 13% moisture content soaked with 2% salt solution, puffed at 700 for 110 sec. or 800 watts for 90 sec. Germinated waxy paddy rice improved the bioactive compounds especially GABA and total phenolics contents. The waxy paddy rice was soaked with water to about 33% moisture content and then germinated in the dark cabinet at room temperature for 0, 12, 24, 36, 48 and 60 hrs. At completed time embryos were cut to determine GABA

content, and the germinated paddy rice was dried in the tray dryer at 50°C, dehusked and grounded for analysis. It was found that the germination process significantly affected GABA, dietary fiber and total phenolics and antioxidant capacity increased ($p<0.05$). While fat, protein, starch and amylose contents slightly decreased. There were changes in the early germination stage which markedly increased after 36 hrs. Exception of GABA content increased after soaking and gradually increased from 80 to 220 mg/100 g (fresh weight) from 12 to 60 hrs. Germinated paddy rice at 0, 12, 24, 36, 48 and 60 hrs was dried in the tray dryer 50°C for 4.5-5 hrs. until the moisture content decreased to 8-10%, then rehydrated the moisture with 2% salt solution up to 13% for puffing, puffed at 700 watts for 110 sec. It was found that germinated puffed rice, while germination time increased, puffed yield, expansion volume, hardness, L* value and WAI decreased; on the contrary, WSI and a* value increased. Moreover, drying and puffing caused the GABA decreased 32.66 and 46.89%, respectively by comparing to germinated paddy rice at 36 hrs.

The optimal microwave heating for germinated and non-germinated puffed rice were considered from puffed yield and expansion volume criteria. Because puffed yield and expansion volume are both the represent of quantity and quality of puffed rice. Puffed yield criteria, paddy rice adjusted with 2% salt solution at about 10-13% moisture content, puffed at 700 watts 110 sec. or at 800 watts at 90 sec. Microwave power at 700 or 800 watts, the quantity and quality of puffed rice were not slightly different. This is because puffed paddy rice at 800 watts produced a little higher puffed yield than puffed paddy rice at 700 watts. While puffed paddy rice at 700 watts made brightness (L* value) of puffed rice higher than puffed paddy rice at 800 watts. In the case of expansion volume criteria, with 2% salt solution and the condition of microwave puffing was the same as the criteria of puffed yield. As for the 13% moisture content, it produced an expansion volume higher than the 10% moisture content. The suitable germinated condition for germinated paddy rice for puffing should be the range of 36 hrs.

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CHAPTER 1

INTRODUCTION

Puffed rice (known in Thailand as "kow tok" or "kow Pong") is a whole grain puffed product from paddy rice or milled rice. It is commonly used in snack, cereals drink, ready-to-eat breakfast cereal and infant food. Not only is puffed rice a staple in the diet for carbohydrate and protein, it also contributes beneficial nutrients including dietary fiber, vitamins, minerals and phytochemicals which have been linked to reduce disease risk (FDA, 2006; Seal, 2006). Moreover, many researchers reported that germination of rice caused important changes in the biochemical and the nutritional value of the rice by increasing γ-aminobutyric acid (GABA), oligosaccharides, dietary fiber, certain vitamins and minerals (Saikusa et al., 1994; Shoichi, 2004; Ohtsubo et al., 2005; Choi et al., 2006; Komatsuzaki et al., 2007; Watchraparpaiboon et al., 2007). The most attractive aspect to consumers is the GABA, because many studies have reported that GABA provides benefits to human health such as the inhibitory neurotransmitter in the central nervous system (Jakobs et al., 1993), antihypertensive (Hayakawa et al., 2002) and reducing plasma cholesterol levels (Miura et al., 2006).

Generally, rice is puffed with hot air, hot sand and gun puffing. In Thailand, roasting in hot pan and frying in hot oil are generally used. Roasting has the risk of burning and producing defects, while the oil from frying can be absorbed and easily turns rancid. Thus, a new method has been developed. At the present there is an increasing trend to use microwave for food processing due to the fact that microwave heating is more efficient than the traditional heating process with benefits that include: quicker start-up time, faster heating, energy efficiency, space savings, selective heating, final products with improved nutritive quality (Sumnu, 2001). It is possible to use microwave energy for baking, puffing or popping. One of the most popular applications of microwave heating is the popping of popcorn. However, the quality of popcorn measured by popcorn expansion affected by various factors such as: variety, popping procedure, pericarp thickness and kernel size (Lin and Anantheswaran, 1988; Dofing et al., 1990; Pordesimo et al., 1991; Zhangand and Hoseney, 1998). Previous studies have found that the quality of puffed rice measured by puffing expansion and puffed rice was affected by the maturity, variety, puffing procedure, chemical and physical properties (Srinivas and Desikachar, 1973; Murugesan and Bhattachaya, 1986; 1991). Murugesan and Bhattachaya (1991) reported that paddy rice had a

high degree of husk interlocking that was highly positively correlated with puffing expansion. Srinivas and Desikachar (1973) found that good puffing paddy rice varieties showed a weak point with a thin aleurone layer. Simsrisakul (1991) reported that puffing waxy paddy rice in hot air provided higher expansion and puffed yield than high amylose paddy rice. Contrarily, Murugesan and Bhattachaya (1991) found that amylose and protein had no relationship to puffing expansion. The optimum temperature for maximum volume and kernel puffed percent was difference for the hot sand puffing method (about 200°C) and the hot air puffing method (225°C) (Murugesan and Bhattacharya, 1986). Moraru and Kokini (2003) explained that microwave expansion involves both nucleation and cell growth. Nucleation depends on process parameters and volume of the polymer and cell growth is governed by the rheological properties of the bubble walls. This was consistent with Mariotti et al. (2006) reported that the quality of puffed cereals by gun puffing such as puffed rice strongly was affected by the morphology of starch granules.

Although puffed rice has been produced for a long time in Thailand, there is a very little scientific information, especially puffing paddy rice by microwave heating. This study was investigated because the lack of research explains the effects of microwaving on the physical and chemical properties of puffed rice and its effects on puffing qualities.

The objectives of this study were:

1. To investigate the effects of physicochemical properties of paddy rice varieties on puffing qualities by microwave heating.

2. To study the effects of salt, moisture content and microwave power on the puffing qualities of puffed rice.

3. To study the effects of the germination process on chemical compositions, GABA content and antioxidant capacity of waxy paddy rice.

4. To study the effects of the germination process on puffing qualities and GABA retention of germinated puffed rice.

CHAPTER 2

LITERATURE REVIEWS

The previous studies (Srinivas and Desikachar, 1973; Murugasan and Bhattacharya, 1986; 1991; Simsrisakul, 1991) found that the qualities of puffed rice such as puffing expansion and puffed yield were affected many factors which are described as follows:

2.1 Factors Affecting on the Puffing Qualities

2.1.1 Moisture Content of Paddy Rice Before Puffing

One of the essential factors for a good puffing performance is moisture content creates superheated high pressure to expand the kernel. Thus the proper moisture content is essential for good puffing. Hoseney et al. (1983) explained that moisture creates superheated high pressure or internal steam pressure that will eventually expand the kernel of popcorn. Previous studies have found that the optimum moisture contents for puffing paddy rice by hot air and oil puffing were 13.5-14.5% (Murugesan and Bhattachachaya, 1986) while the microwave puffing was about 15% (Phuaksawat, 2002). Depending on procedure, optimum moisture might differ. In case of popcorn, it was generally observed the maximum moisture content for puffing range from 10 to 15.0% (Pordesimo et al., 1990; 1991; Song et al., 1991; Mohamed et al., 1993; Allred-Coyle et al., 2000a). Ernoult et al. (2002) reported that no expansion for totally dehydrated pellets (half-product from extrude) while maximum expansion was at 10.8% moisture content, and collapse at 11.9% moisture content. Ernoult et al. (2002) and Chen and Yeh (2000) observed the high moisture pellets resulted in expanded products with a coarse structure and thick cell walls, while at a lower moisture content of the pellets the structure was tender.

2.1.2 Physical Properties of Paddy Rice

It might sound puffing requires a delicate balance between proper moisture so that the physical properties of cereal is an important factor. In corn, pericarp as a protective layer plays a major role in puffing. If it is damaged, it ruptures earlier, when the temperature and pressure increase it make less expansion or puffing may not occur at all (Wu, 1991; Hoseney et al., 1983; Hoseney,1986). A kernel of paddy rice consists of the outer covering tissues as the husk (lemma and palea), and the inner kernel that is the caryopsis or brown rice (Figure 2.1). The caryopsis is

coated with the pericarp, or seed coat. An aleurone layer varies from one to five cell layers and is thicker at the dorsal than at the ventral side and is also thicker in short-grain than in long-grain rice (Park et al., 2001). Srinivas and Desikachar (1973) they observed that the good puffing varieties of paddy rice should have a thin aleurone layer and a wider clearance between the husk and kernel or caryopsis. Murugesan and Bhattacharya (1991) found that thickness of the aleurone layer at ventral, dosal or lateral side, had no relation to expansion volume but the degree of husk interlocking or the tightness between lemma and palea showed a very high positive correlation with puffing expansion. Murugesan and Bhattacharya (1991) mentioned that the importance of a tight husk interlocking of paddy rice affected on its puffing act like a pericarp in the popping of popcorn.

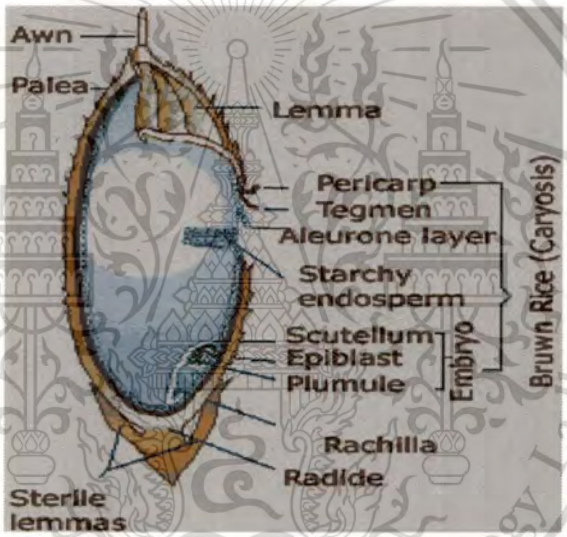


Figure 2.1 The structure of rice kernel.

Source: http://.ikisan.com/links/ap.ricemorp.html.

2.1.3 Chemical Properties

The starch as the dominant polymer in most cereal flours and plays a major role in expansion while protein fat and fiber are the minor component. Maximum expansion has been observed with pure starch extruded (500% increase in product), followed by whole grains (400%), various pet foods with added starch (200 to 300%). Moraru and Kokini (2003) and Schwartzberg et al. (1995) assuming melted starch acts as a pseudoplastic power-law fluid, they elaborated a mathematical model to describe the bubble expansion in molten starch and a visual model of the pore expansion domain for whole cereal kernels. Simsrisakul (1991) reported

puffing waxy paddy rice in hot air provided higher expansion and puffed yield than high amylose paddy rice. Starch is made of linear amylose and branched amylopectin, which impact expansion differently. Mercier and Feillet (1975) mentioned that a high amylopectin content leads to light, elastic, and homogeneous expanded textures, while a high amylose content leads to hard, less expanded extrudates. Amylopectin-rich starch expands more than amylose-based starch because the linear amylose chains align themselves in the shear field and thus are difficult to pull apart during expansion. At the same time, amylopectin starch is not as hard as amylose starch at the same moisture content, which also favors expansion (Kokini et al., 1992; Della Valle et al., 1996).

2.1.4 Salt

Salt is indeed regularly used in the preparation of expanded rice in India. Chinnaswamy and Bhattacharya (1983) reported that the salt addition increasing the expansion of expanded rice from parboiled rice by hot air puffing. While Murugesan and Bhattacharya (1986) found that soaking the paddy rice in salt solution (2% w/w NaCl or $CaCl_2$ or K_2SO_4) substantially increased puffing expansion during hot air puffing. Singh and Singh (1999) studied the effect of different ingredients on popping characteristics and reported that more than 75% kernels can be obtained by using 10% hydrogenated oil, 2% butter and 0.5% sodium chloride. The data obtained by Allred-Coyle et al. (2001b) indicated that increase salt content from 1 to 3% the expansion volume of popcorn was decreased.

2.1.5 Temperature and Time for Puffing

The effect of temperature and time on puffing ability was also studied. Murugesan and Bhattacharya (1986) reported the optimum puffing expanded rice temperature was 190-210°C for a sand puffing and 215-235°C for a hot air puffing, the higher temperature used shorter of puffing time. Allred-Coyle et al. (2000a) also reported that microwave oven wattage significantly affected the popping volume. The higher microwave power produced higher expansion volume. Singh and Singh (1999) found microwave power level showed significantly effects in bulk density, expansion volume and percent of puffed kernels. They suggested that a combination of 10% hydrogenated oil, 2% butter, 0.5 sodium chloride, and 70% microwave power (660 watts) could produce more than 75% popped yield.

2.2 The Mechanism Inducing Puffing or Expansion

Schwartzberg et al. (1995) used corn to be the model to explain puffing by using vapor-induced puffing (VIP), comparing to kernel structure and components of corn with extruder namely; the tough hull or pericarp which surrounds the contents of the kernel provides confinement which in other situations is provided by the shell of a puffing gun or the barrel of an extruder. Other components include the oil-rich germ and two starchy regions: the horny endosperm, which contains tightly packed, protein-coated starch granules; and the floury endosperm which contains loosely packed starch. The starch partially melts when suitably heated, and high internal pressures develop. The pressure progressively increases as temperature, the temperature of the popcorn rises. At 180-190°C the pressure is roughly 800 kPa, and ΔP, the difference between the pressure and the external pressure, causes the pericarp to rupture, and the starch granules in the horny endosperm explosively expand. Corn starch containing 0.08 to 0.28 kg water/kg dry solids starts to melt around 150°C (Wu, 1991) the melting peaks about 180-186°C, close to the temperature where the pericarp ruptures. The extent of melting increases as popcorn's water content increase; the amount of heat absorbed during melting is roughly proportional to the moisture content. Water vapor pressure forces pores at the center of starch granules to expanded converting the endosperm into a bubble-filled matrix, which turns into a foam as showed in Figure 2.3. Other grains that have tough hulls sometimes pop similarly (Schwartzberg et al., 1995). The SEM photographs a bubble-filled matrix, which turns into a foam or structure of puffed rice (Maisont and Narkrugsa, 2009b).

A good understanding of the macromolecular organization and properties of starch during puffing and expansion is cited by Muraru and Kokini (2003). Amylose is a long and linear polymer, while amylopectin has a highly branched molecular structure. Amylopectin is considered responsible for the structural organization of the starch granule and its semicrystalline character (Gallant et al., 1997). Of special relevance to the expansion process is the microscopic pores or hilums, localized near the center of the starch granules (Figure 2.2). The hilums are considered by various researchers to be some of the nuclei at which expansion of vapor bubbles starts (Hoseney, 1986; Hoseney et al., 1992; Schwartzberg et al., 1995; Cisneros, 1999). The starch granule is made up of alternating semicrystalline and crystalline shells which consist of alternating 9 to 10 nm thick amorphous and crystalline lamellae (Jenkins et al., 1993; Gallant et al., 1997; Parker and Ring, 2001). The lamellae are organized into pseudo spherical structures called block lets. The surface pores and interior channels are believed to be naturally occurring

features of the starch granule structure, the pores being the external openings of the interior channels (Fannon et al., 1993; Gallant et al., 1997).

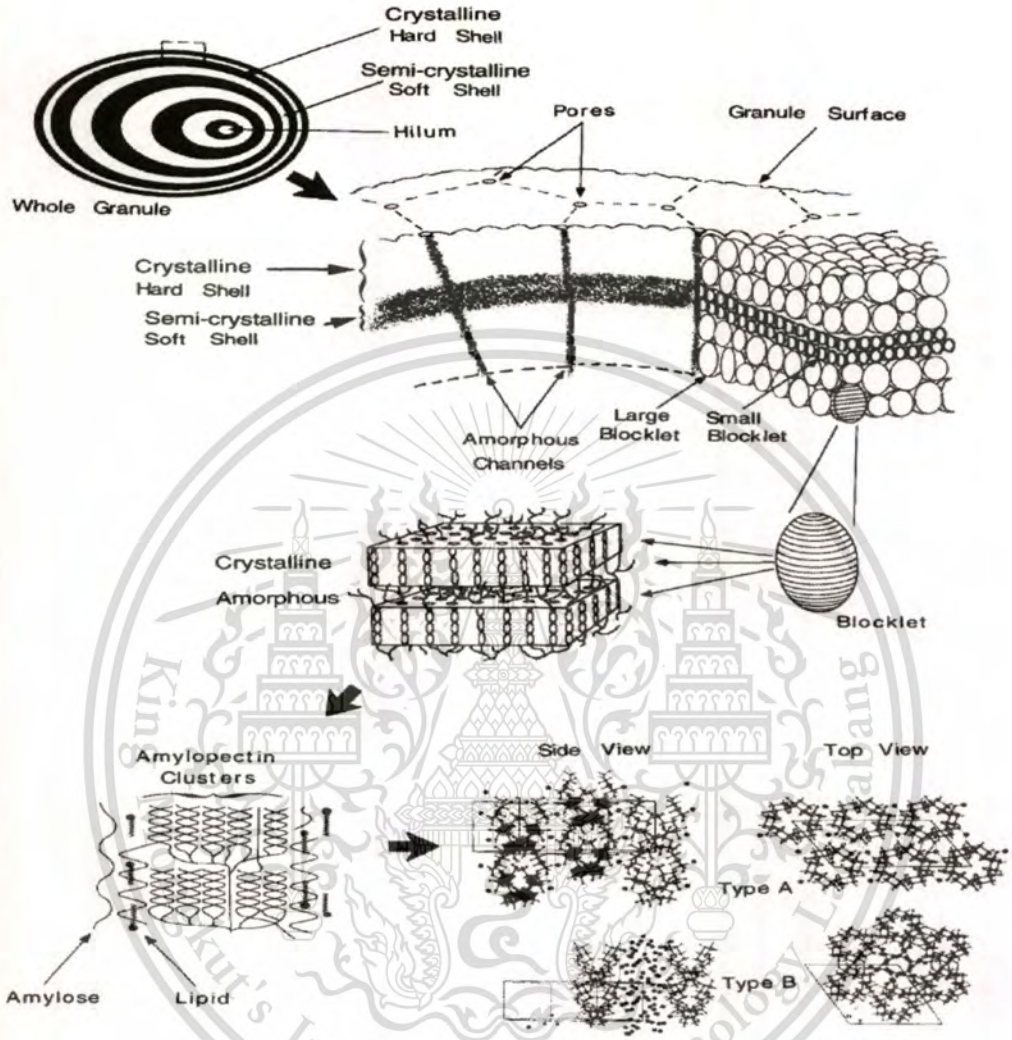
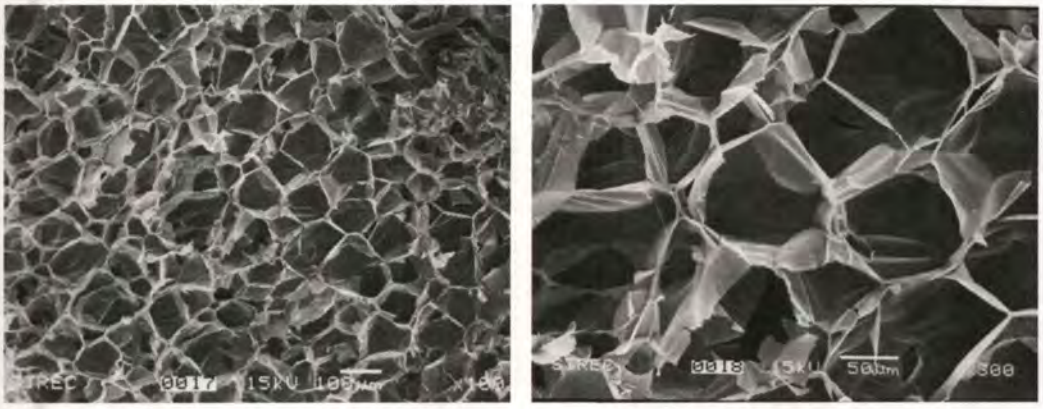


Figure 2.2 Organization of the starch granule structure.

Source: Gallant et al. (1997).



(A) (B)

Figure 2.3 The SEM photograph of puffed rice by microwave heating x 100 (A) and x 300 (B). Source: Maisont and Narkrugsa (2009b).

2.3 Microwave Heating and Puffing

Microwave heating is based on the transformation of electromagnetic field energy into thermal energy by affecting the polar molecules of a material. The most important characteristic of microwave heating is volumetric heating. Volumetric heating means that materials can absorb microwave energy directly and internally and convert it into heat. While conventional heating occur by convection followed by conduction where heat must diffuse in from the surface of the material. In microwave heating, heat is generated through the material, leading to faster heating rates, compared to conventional heating where heat is usually transferred from the surface to the interior (Mudgett, 1982; 1989). Microwave energy is increasingly used for expansion of foods, the typical application being the microwave expansion of popcorn. Third-generation snacks expanded by microwave heating bring tremendous extension to popcorn expansion as it becomes possible to use food polymers to form various biological origin snacks in any desirable shape (Boischot et al., 2003).

Boischot et al. (2003) described the mechanism of glassy amylopectin pellets by microwave expansion as showed in Figure 2.4. Upon microwave heating, when the temperature exceeds the boiling point of water, the water contained by glassy amylopectin pellets is transformed into superheated steam. The vapors accumulate at nuclei in the glassy matrix, creating a locally high pressure. As the cereal matrix undergoes a phase transition from glassy to rubbery state during heating, it starts to yield under the high superheated steam pressure and expansion occurs. As moisture is lost from the matrix, or upon cessation of microwave heating, the matrix reverts to the glassy state, where the mechanical resistance is high enough to maintain the shape of the formed

cells. If the matrix is too soft, which is the case of the high moisture (A_w) samples, collapse occurs. If heating continues after all the moisture is eliminated from the matrix, samples begin to burn.

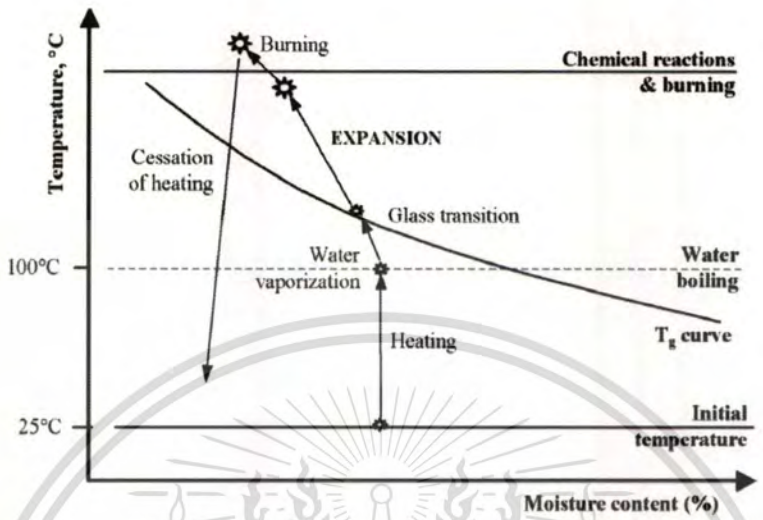


Figure 2.4 The state diagram for microwave expansion of unexpaned amylopectin extrudates. Source: Boischot et al. (2003).

2.4 Germination

Germination is a series of events that begins with soaking, the uptake of water by the dry seed, followed by activated of metabolic processes, elongation of the embryonic axis. Germination can be divided into three phases; phases I and II are characterized by the rapid uptake of water and a plateau phase of water uptake, respectively (Figure 2.5). These phases represent a period of large metabolic change that primes the embryo to commence growth during phase III, when further uptake of water occurs (Bewley, 1997). Once the process of germination has commenced, utilization of stored reserves for energy production is necessary before the plant becomes autotrophic by establishing photosynthesis. The importance of energy metabolism in the early stages of seed germination can be seen in studies that inhibit germination, in phase II, by the use of various bioactive compounds (Carrera et al., 2007; Bassel et al., 2008).

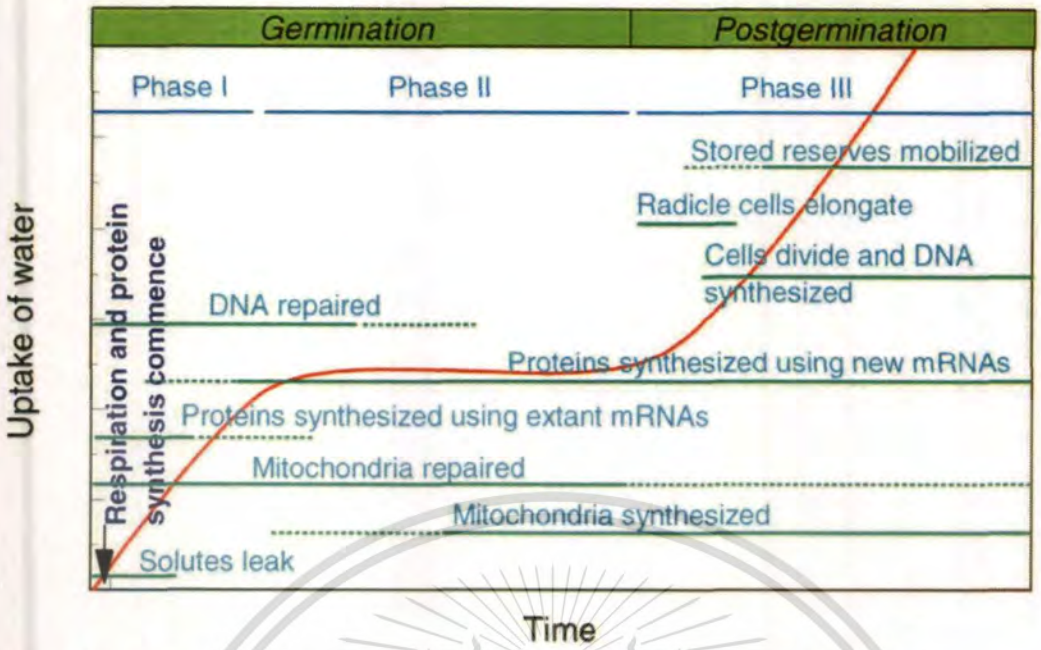


Figure 2.5 Time course of major event associated with germination and subsequent postgerminative growth.

Source: Bewley (1997).

2.5 The Changes of the Components During Rice Germination

Soaking makes the inactive tissues to become living tissues. At this point the grains have an active metabolism in preparation for germination. During germination, the grain reserves nutrients, degraded and used for respiration and synthesis of new cells constituent of the developing embryo, causing changes in the nutritional, biochemical and structure (Bamforth and Barclay, 1993). In case of brown rice, during soaking and germination, γ-aminobutyric acid (GABA) increasing is the most popular (Saikusa et al., 1994; Shoichi, 2004; Ohtsubo et al., 2005; Choi et al., 2006; Komatsuzaki et al., 2007; Watchraparpaiboon et al., 2007). GABA is a gamma amino acid transmitter presents in inhibitory nervous in the central nervous system. Many researches reported that GABA has several physiological functions such as inhibitory neurotransmitter in the central nervous system (Kayahara and Tsukahara, 2000; Jakobs et al., 1993), antihypertensive (Hayakawa et al., 2002), reducing plasma cholesterol levels (Miura et al. 2006), improving of blood glucose levels in diabetic (Ito et al., 2005a; 2005b; Seiki et al., 2005) and preventing chronic alcohol- related diseases (Oh et al., 2003). As reported by Ohtsubo et al. (2004) brown rice was soaked in water at 30°C for 72 hrs, total dietary fiber, total ferulic acid and

γ-aminobutyric acid (GABA), glycine, alanine, aspartic acid and total ferulic acid were higher than those of ordinary brown rice or polished rice.

Saikusa et al. (1994) reported soaking rice in water brought about remarkable changes in the component and content of free amino acid in the kernel of rice cultivar Koshihikari; the most significant of these was an increase of GABA. Kayahara and Tukahara (2000) reported during the process of germinated brown rice GABA, dietary fiber, inositols, ferulic acid, phytic acid, tocotrienols, magnesium, potassium, zinc, and γ-oryzanol, are increasing, that volume of nutrients contained in GBR compared with milled rice, GABA is 10 times, for nearly 4 times for dietary fiber, vitamin E, niacin and lysine, and about 3 times for vitamin B_1 and B_6, and magnesium as showed in Figure 2.6.

Figure 2.6 Ratios of nutritional volumes of pre-germinated brown rice compared with those of polished rice level indicated on the dotted line.

Source: Kayahara and Tukahara (2000).

Wanatabe et al. (2004) prepared pre-germinated rice flour for breadmaking by soaked brown rice (Koshihihikari) in water at 30°C for specified hours. The analytical nutrient components of brown rice (BR) and pre-germinated brown rice flour (PGBR) showed in the Table 2.1

Table 2.1 Analytical data of components in rice flour.

Compositions	BR	PBR at 24 hrs
Energy (kcal/100 g)	367.9	364.2
Carbohydrate (g/100 g)	78.5	78.7
Protein (g/100 g)	6.3	6.8*
Lipid (g/100 g)	3.2	2.5
Fiber (g/100 g)	3.8	3.7
Ash (g/100 g)	1.3	1.5
Vitamin B1 (mg/100 g)	0.54	0.49
Vitamin B2 (mg/100 g)	0.06	0.04
Vitamin E (mg/100 g)	1.6	1.10
Na (mg/100 g)	2.8	2.3*
Ca (mg/100 g)	16.2	37.10
Mg (mg/100 g)	120.6	79.10*
Fe (mg/100 g)	3.1	3.20
Phytic acid (mg/100 g)	675.6	558.0*
Oryzanol (mg/100 g)	46.9	24.9*
Ferulic acid (mg/100 g)	41.4	38.3
GABA (mg/100 g)	3.2	13.0*

Note: BR= brown rice and PBR= pre-germinated brown rice.

* Values significantly different from BR ($p<0.05$).

Source: Wanatabe et al. (2004).

Shoichai et al. (2004) select not only Japanese rice but also California medium grains (Calorose and M401 varieties) and Vietnamese long grains (ordinary grains and jasmine rice) to analyze nutrient contained in germinated brown rice relative to the situation of brown rice before germination. The results indicated that GABA increased from 3.6 to 6.1 mg/100 g for the Vietnamese ordinary long grain despite the extremely low germination rate. GABA in Calrose increased more than times from 4.9 to 10.9 mg/100g and for M401 more than three times from 2.7 to 9.8 mg/100g with germination rates of 90% and 56%, respectively. For the Japananese Koshihikari and Hitomebore varieties, GABA increased from 7.6 to 16.6 mg/100g and from 10.5 to 13.6 mg/100g, respectively.

Sootthiboon (2006) reported brown rice (KDML105 varieties) soaked in 35°C water for 1, 2, 3, 4 and 5 hrs and germinated in gaseous phase processing at the same temperature until the total time of germination process was 24 hrs. The moisture contents of soaked brown rice abount 31-34%. It was found that soaking for 3 hrs and germinated for 21 hrs was the condition that could increase the highest GABA content (14 mg/ 100g) in germinated brown rice.

Komatsuzaki et al. (2007) observed that brown rice was soaked at 35 °C in water for 0.5, 1, 2, 3, 4 and 5 hrs. Gaseous phase sprout processing was then done at the same temperature until total time (soaking and germination process) was 24 hrs. It was found that GABA concentration was the highest in the sample soaked for 3 hrs and germinated for 21 hrs. The highest GABA concentration (42.2 mg/100g) germinated after 22 hrs.

2.6 Mechanism Inducing GABA

γ-aminobutyric acid (GABA) a four-carbon non-protein amino acid, is a significant component of the free amino acid group. GABA has an amino acid group on the γ-carbon rather than on the α-carbon, and exists in unform (Figure 2.7). It is highly soluble in water. It is a structurally flexible molecule that can assume several conformations in solution, including a cyclic structure that is similar to praline. GABA is zwitterionic (carries both a positive and negative charge) at physiological pH values (p*K* values of 4.03 and 10.56) (Shelp et al., 1999).

Figure 2.7 Molecular structure of GABA

Source: http://en.wikipedia.org/wiki/GABAreceptor

Typically GABA levels in plant tissues are low (ranging from 0.03 to 2.00 μmol fresh weight) but it increases several times in corresponding conditions such as anoxia, cytosolic acidification, cold shock, mechanical stimulation, water stress and plant development (Bown and Shelp, 1997; Rhodes et al., 1986). Bouche and Fromm (2004) reported that in plants, the GABA pathway is mainly composed of three enzymes (purple) (Figure 2.8). The first step, glutamate decarboxylase (GAD) is a cytosolic enzyme regulated (green) by the Ca^{2+}/calmodulin (CaM)

complex, which catalyses the irreversible decarboxylation of glutamate to produce GABA. The second, GABA is transported into the mitochondria, where it is converted into succinic semialdehyde by GABA transaminases using either a-ketoglutarate (by GABA-TK) or pyruvate (by GABA-TP) as amino acid acceptors. The last step succinic semialdehyde is then reduced by succinic semialdehyde dehydrogenase (SSADH) to form succinate, which enters the tricarboxylic acid (TCA) cycle. The conversion of glutamate to succinate via the action of glutamate decarboxylase (EC 4.1.1.15), GABA transaminase (EC 2.6.1.19) and succinic semialdehyde dehydrogenase (EC 1.2.1.16) is known as the GABA shunts (Figure 2.8).

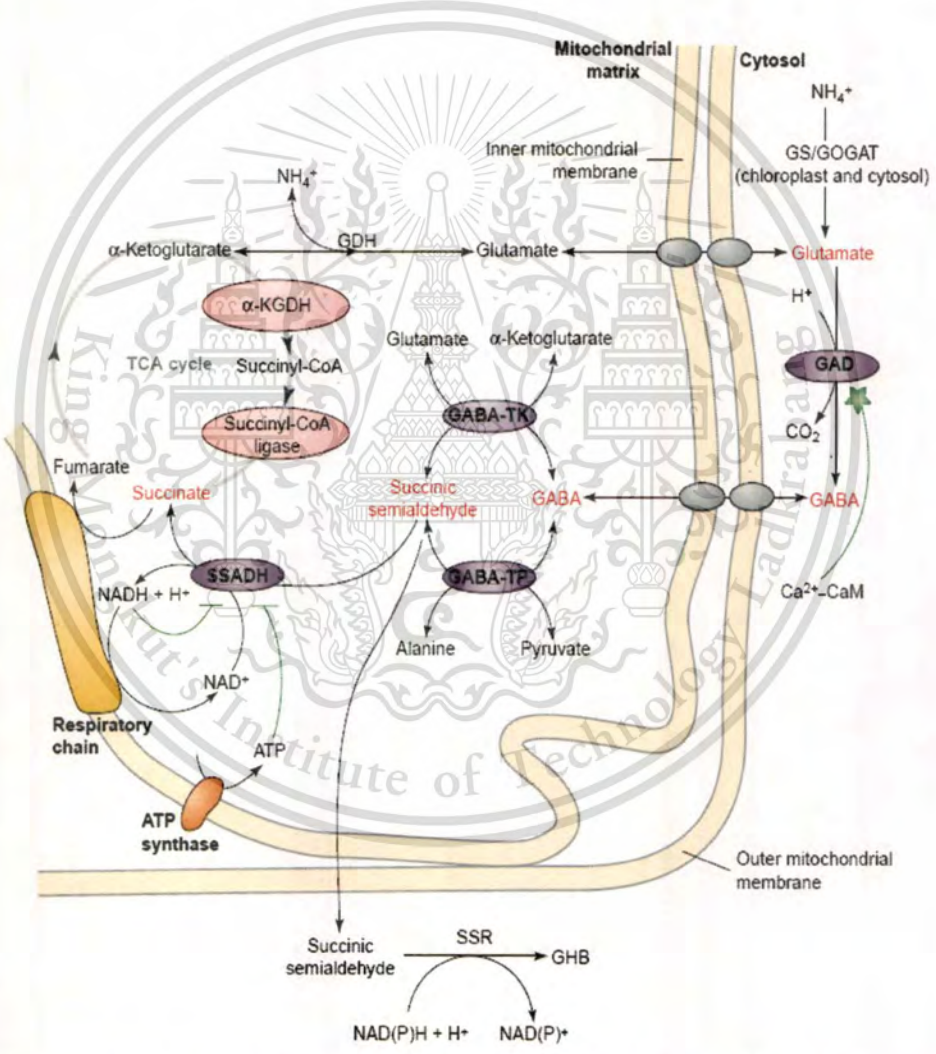
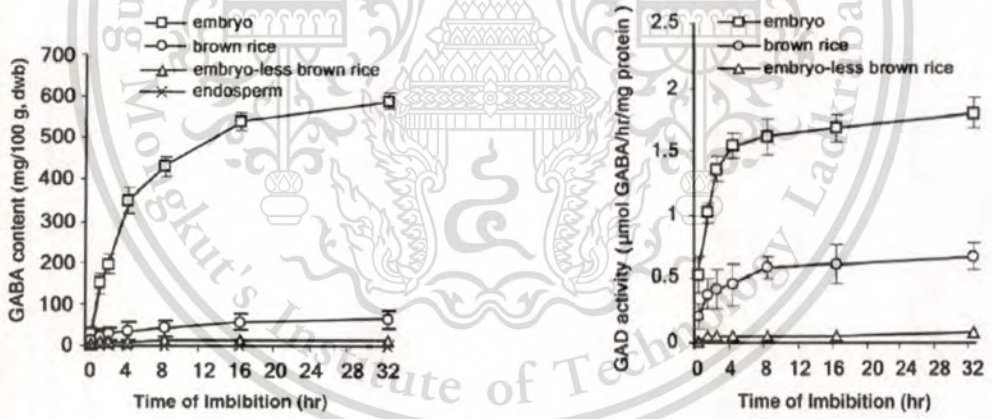


Figure 2.8 The γ-aminobutyric acid (GABA) shunt and its relationship to other metabolic.

Source: Bouche and Fromm (2004).

Among the three enzymes, glutamate decarboxylase (GAD) is the rate-limiting enzyme for GABA synthesis. However, the relationship of GABA accumulation, GAD activity in rice grain during water soaking is unknown yet. Lui et al. (2005) studied the role of GAD activity and its gene expression to GABA in rice grain during water soaking. After soaking Haiminori grain in water for 0, 1, 2, 4, 8, 16, and 32 hrs. The results showed a continuous accumulation of GABA in soaked embryo, brown rice, embryo less brown rice and endosperm respectively (Figure 2.9 A). Among them, embryo of brown rice had the maximal accumulation. The activity of GAD was increased during water soaking, and the enzyme activity was significantly higher in the embryo than in either the embryo less brown rice or whole brown rice (Figure 2.9 B). Bautista et al. (1964) and Chan and Fromm (1994) reported the activity of GAD shows high correlation with percent germination in rice grain and in barley (Limkin et al., 1983). GAD is one of the most abundant soluble proteins with a Ca^{2+}/calmodulin binding domain, it is found in all plant tissues and the level is regulated during development by transcription or posttranscriptional processes (Shelp et al., 1999).



(A) (B)

Figure 2.9 Change of GABA contents (A) and GDA activities (B) in brown rice during soaking in water for 0, 1, 2, 4, 8, 16 and 32 hrs.

Source: Liu et al. (2005).

Varunyanond et al. (2006) studies the relationship between the soaking condition and the amount of GABA in the rice germ of high amylose, low amylose and waxy rice varieties. GABA content in most of rice varieties increased during germination. The GABA accumulation was differed on rice varieties and soaking time with increasing during 4 hrs of incubation at 40°C.

Waxy paddy rice has a trend to provide GABA higher than low-amyose and high-amylose. During rice germination the other compositions have changed (Table 2.2 and 2.3). Watchraparpaiboon et al. (2007) also reported during germination phytic acid and amylose contents in brown rice were decreased.

Choi et al. (2006) reported that giant embryo (GE); and a normal embryo (NE) brown rice were soaked at room temperature 3-4 hrs germinated for 24 and 48 hrs. GABA content, mineral and free sugar contents were showed in Table 2.2 and 2.3.

Table 2.2 GABA contents and Mineral of giant embryo (GE) and normal embryo (NE) rice

Rice	Germination (hr)	Content (mg/100g)					
		GABA	Mg	Fe	Mn	P	K
GE	Raw	1.67±0.05	128.42±16.53	3.03±0.37	2.85±0.37	314.10±50.37	223.34±33.36
	24	29.26±7.20	122.83±20.82	3.26±1.41	2.72±0.70	336.63±54.26	217.45±72.29
	48	35.86±6.40	127.43±10.36	3.57±0.09	2.65±0.32	325.33±1.52	189.70±10.43
NE	Raw	1.58±0.03	123.21±24.12	3.70±1.03	3.38±0.81	302.78±55.40	234.17±33.20
	24	10.95±0.12	124.01±14.82	4.00±1.35	3.43±0.96	312.69±32.56	232.20±39.50
	48	17.65±0.40	134.26±4.80	3.02±0.55	3.27±0.89	327.05±15.94	227.53±47.39

Note: ^{abcd} Different letter in the same column are significantly.

Source: Choi et al. (2007).

Table 2.3 Free sugars contents of giant embryo (GE) and normal embryo (NE) rice.

Rice	Germination (hr)	Free sugars (mg/100g)				Total free sugars
		Fructose	Glucose	Sucrose	Maltose	
GE	0	1.22 ±0.24^{cd}	15.49 ± 0.60^{ab}	22.01 ± 2.48^{a}	33.24 ±3.17^{a}	71.96
	24	4.14 ±0.29^{b}	17.46 ± 0.69^{a}	3.70 ± 0.14^{bc}	19.19 ±2.27^{b}	44.49
	48	5.80 ±0.89^{a}	15.83 ±3.21^{a}	2.24± 0.46^{cd}	11.44 ±1.95^{c}	35.31
NE	0	0.54 ± 0.11^{d}	6.07 ±0.83^{c}	5.33 ± 0.76^{b}	29.23 ±3.35^{a}	41.17
	24	1.82 ±0.38^{c}	11.57 ±1.04^{b}	3.09 ±0.12^{c}	30.31 ±1.70^{a}	46.79
	48	5.73 ±1.09^{a}	15.38 ±3.89^{ab}	0.57 ± 0.07^{d}	18.42 ± 3.47^{b}	40.10

Note: ^{abcd} Different letter in the same column are significantly.

Source: Choi et al. (2007).

2.7 Important Factors for Grain Germination

Seed or grain germination depends on many factors, both internal and external factors. The most important external factors include: water, air (oxygen and carbon dioxide), temperature and light. The conditions depend on the individual seed variety and are closely linked to the ecological conditions in the plants' natural habitat (Copeland and McDonald, 2001).

2.7.1 Water

Water is a basic requirement for germination. It is essential for enzyme activation, breakdown, translocation and use of reserve storage material. In this resting state, seeds are characteristically low moisture and relatively inactive metabolically. Soaking typically take 40 to 50 hours or complete when the moisture content of the grain is raised to 30-35% for brown rice (Komatsuzaki et al., 2007) 18-30% for waxy paddy rice, 35-40% for non-waxy (Puangwerakul, 2005).

2.7.2 Air (oxygen and carbon dioxide)

Air is composed of about 20%, 0.03% carbon dioxide (oat grain). If carbon dioxide concentrations higher than 0.03% this retards germination. Respiration increases sharply during seed germination. Since respiration is essentially an oxidative process, an adequate supply of oxygen must be available. If the oxygen concentration is reduced below that of air, germination of most seed is retarded. Van Toai et al. (1988) demonstrated corn seed germination declined with decreasing oxygen concentration below that of ambient air. Al-Ani et al. (1985) indicated that maximum seed germination foremost crops including wheat, sorghum and corn occurred at oxygen concentration close to those of ambient air. It has been postulated that anoxic conditions lead to the production if increased ethanol in cells which is toxic to normal metabolism (Thomson and Greenway, 1991). However, in case of Komatsuzaki et al. (2007) geminating brown rice under gaseous conditions it was found that GABA content and some amino acids increased.

2.7.3 Temperature

Seeds germinate over a wide range of temperatures, with many optimum temperatures. The optimum temperature may be defined as the temperature giving the greatest percentage of germination within the shorter time. The response to temperature depends on a number of factors, including the species, varieties, cultivars, quality of seed, and duration of time from harvest. The optimum temperature for most seeds is ranged 15 to 30°C. The maximum temperature for most species is between 30-40°C. (Copeland and McDonald, 2001). Puangwerakul (2005) found that at 30°C and relative humidity 90%, most paddy rice had germination 95-98% and at 35°C under gaseous condition for brown rice (Komatsuzaki et al., 2007).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

Ten varieties of paddy rice were used in this experiment. Two non-waxy paddy rice varieties were Suphan-Buri1 and Khao Dawk Mali105 and eight waxy paddy rice. Each variety was purchased from Rice Research Center in each province of Thailand They were Suphan-Buri1(SPBR1): Pathumthani province, Niaw-Phrae1 (NPH1): Phrae province, Niaw-San-Pah-Tawng (NSPT), Gam-Pai15 (GP15) and Sew-Mae-Jan (SMJ): Chiang Mai province, RD6, RD10 and Khao Dawk Mali105 (KDML105): Ubon Ratchathani province, Hahng-Yi71 (HY71): Nong Khai province and Sakon-Nakhon (SKK): Sakon Nakhon province harvested during September 2005 to May 2006 and September to October 2007. All paddy rice were cleaned and stored at 12-15°C until used.

3.2 Chemicals

3.2.1 Gamma-aminobutyric acid (Sigma, USA)

3.2.2 Amylose from potato (Sigma, USA)

3.2.3 Iron (III) chlorides (Sigma, USA)

3.2.4 Ethanol 95% (Italmar, Thailand)

3.2.5 Ferulic acid (Sigma, USA.)

3.2.6 Acetronitrile (Sigma, USA)

3.2.7 Acetic acid (Merck, Germany)

3.2.8 DPPH (2, 2-diphenyl-1-picrylhydrazyl) (Merck, Germany)

3.2.9 Phosphoric acid (Merck, Germany)

3.2.10 Methanol (Merck, Germany)

3.2.11 Folin-Cocialteu reagent (Sigma, USA)

3.2.12 TPTZ (2,4,6-tripyridyl-S-triazine) (Sigma, USA)

3.2.13 Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carbolic acid) (Sigma, USA)

3.2.14 Gallic acid (Sigma, USA)

3.2.15 Sodium chloride (Ajax Finechem, Australia)

3.2.16 Sodium hydroxide (Ajax Finechem, Australia)

3.2.17 Sodium carbonate (Ajax Finechem, Australia)

3.2.18 Hydrochloric acid (Merck, Germany)

3.2.19 Acetic acid (Merck, Germany)

3.2.20 Sulfuric acid (Merck, Germany)

3.2.21 Potassium sulpate (Merck, Germany)

3.2.22 Boric acid (Ajax Finechem, Australia)

3.2.23 Petrolem ether (Ajax Finechem, Australia)

3.2.24 Glucoamylase (Sigma, USA)

3.2.25 D-glucose (Merck, Germany)

3.2.26 Trihydroxymethylaminomethane (Merck, Germany)

3.2.27 Phosphoric acid (Merck, Germany)

3.2.28 α-amylase, heat stable (Sigma, USA)

3.2.29 Protease (Sigma, USA)

3.2.30 Amyloglucosidase (Sigma, USA)

3.2.31 Others

3.3 Equipments

3.3.1 Tray dryer (Path OV663, Thailand)

3.3.2 Microwave oven (SAMSUNG, Model M1712N, Thailand)

3.3.3 Infrared thermometer (Chino, Japan).

3.3.4 Hot-air oven (Path OV663, Thailand)

3.3.5 Hygro meter (RT-102, Chaina)

3.3.6 Analytical balance (Mettler Toledo MP220, Germany)

3.3.7 Digestion block (Buchi, Switzerland)

3.3.8 Muffle furnace(LT40, Germany)

3.3.9 Colorimeter (Minalta CR 300, Japan)

3.3.10 Dehusker (Satake, Germany).

3.3.11 Ultra centrifugal mill (Retsch, ZM100, Germany)

3.3.12 Spectrophotometer (Shimadzu, 1700, Japan)

3.3.13 Scanning Electron Microscopy (SEM) (JEOL, JSM-5800LV, Japan)

3.3.14 Texture analyzer (Stable Micro System Model TA-XT2i, England)

3.3.15 High performance Liquid Chromatography (HPLC) (LDC/Milton Roy, USA.)

3.3.16 Centrifuges (Allegra X-12R, Beckman Coulter, USA)

3.3.17 Water bath (Memmert, Germany)

3.3.18 pH meter (Suntex SP701, Taiwan)

3.3.19 Autoclave (Tomy-SS-325, Japan)

3.3.20 Glass wares for analysis

3.4 Experimental Procedures

3.4.1 To Investigate The Effects of Some Physicochemical Properties of Paddy Rice Varieties on Puffing Qualities of Puffed Rice by Microwave Heating.

3.4.1.1 Chemical Compositions Analysis

The paddy rice samples were dehulled and ground using a Retsch Ultra centrifugal mill with 0.12 mm mesh sieve screen. Moisture and protein content were determined according to AOAC (1990). Amylose content was determined using the method of Julino (1971). The determinations are described in Appendix A.

3.4.1.2 Physical Properties Measurement

The physical properties of the samples were inspected by modifying the method of Murugesan and Bhattacharya (1991).

1) The Degree of Husk Interlocking

The degree of husk interlocking or region overlaping of lemma and palea was measured as follows. Each paddy rice was cut transversely at the center with a sharp razor blade, fixed on a stups with the help of a double-side adhesive tape and sputter-coated with gold. The samples were viewed with a scanning electron microscope (JEOL, JSM-5800LV) under high vacuum conditions at an accelerating voltage of 15 kV. The degree of husk interlocking was scored by the following scores.

	Score
(A) Hooking:	
in both lemma and palea;	3
only in lemma;	2
nil.	1
(B) Length of the overlap of lemma and palea at hooking points:	
high;	2
low.	1

(C) Closeness of the overlapping portions of lemma and palea to each other:

Touching throughout;	4
Partially touching;	3
Not touching but close;	2
Well separated .	1

The average of the score on the two lateral sides was taken as the respective score. The final score was calculated as husk interlocking score = score A* score B* score C and the mean score was calculated. The maximum score was 24 and the minimum score was 1 (Figure 3.1).

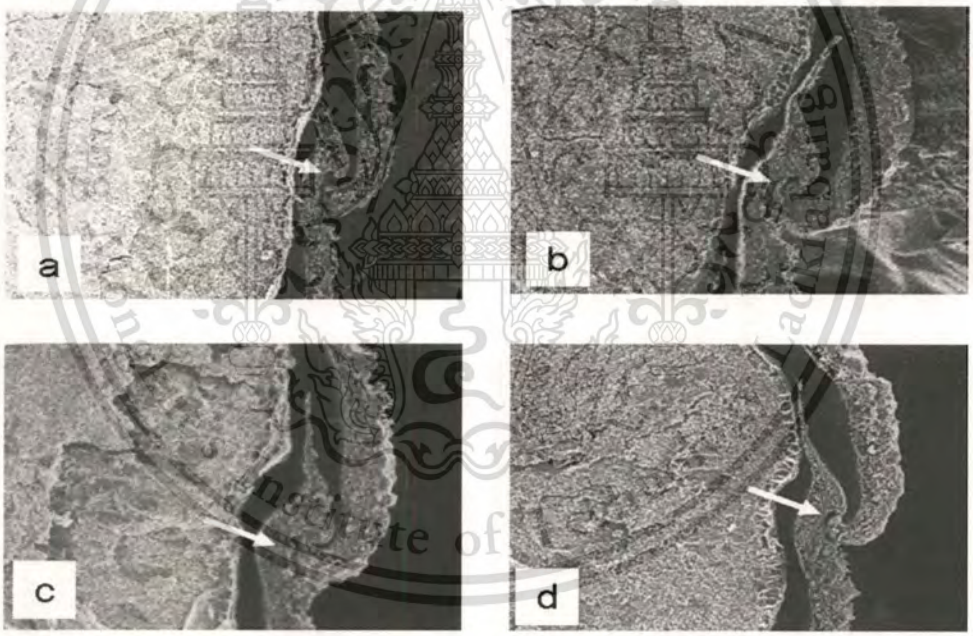


Figure 3.1 SEM macrograph representative some different types of lemma–palea interlocking. The husk interlocking score of the samples are: (a) 1x1x1 SMJ, (b) 3x2x3NSPT, (c) 3x2x4 HY71 and (d) 2x1x2 RD6.

2) The Shellability of Paddy Rice

The shellability of paddy rice (percent by number of paddy rice shelled) It was estimated using a Satake dehusker, with the clearance between the two rubber rolls was fixed at 0.8 mm. One hundred grains were shelled in triplicate.

3) The Husk Content

It was estimated by shelling 30 g of paddy in the Satake dehusker, in triplicate and calculating the mean weight of the husk.

4) The Length and Width

The length and width 20 grains of brown rice were measured using a vernier caliper. The means are reported.

5) The Ventral Region Thickness

It was measured at ventral sides. Brown rice were cut longitudinally, fixed on a stups with the help of a double-side adhesive tape and sputter-coated with gold. The samples were viewed and measured with a scanning electron microscope (JEOL, JSM-5800LV) under high vacuum conditions at an accelerating voltage of 15 kV by modifying the method of Srinivas and Desikachar (1973) (Figure 3.2).

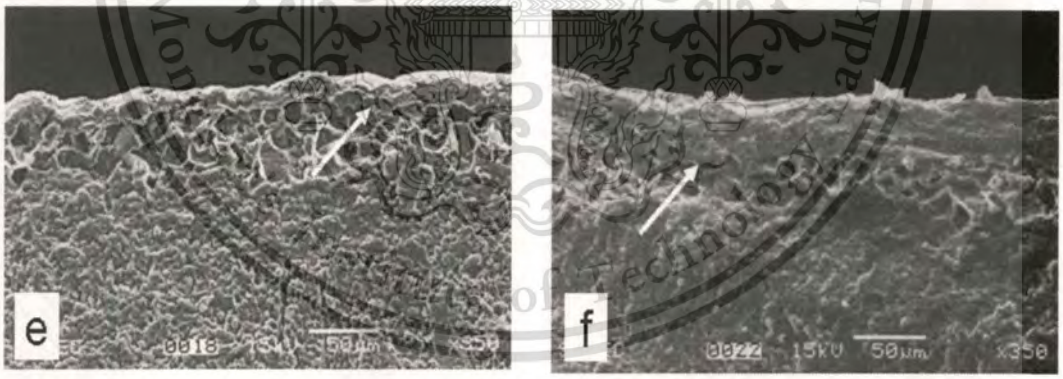


Figure 3.2 SEM micrograph representatives some characteristics of ventral region layer thickness; (e) NSPT and (f) SMJ.

6) White Belly

Grain is the term given to the opaque chalky portions on the ventral side (germ side). It was determined from a random selected from 100 brown rice kernels, cut transversely at the center with a sharp razor blade and viewed in a microbiological colony counter.

7) Morphology Starch Granule

Brown rice was determined by cutting grains transversely at the center with a sharp razor blade. Samples were fixed on a stups with the help of a double-side adhesive tape and sputter-coated with gold. The samples were viewed with a scanning electron microscope (JEOL, JSM 6301F) under high vacuum conditions at an accelerating voltage of 15 kV by modifying the method of Mariotti et al. (2006).

3.4.1.3 Microwave Puffing Operation

After initial moisture of raw materials had been determined, the moisture content of paddy was adjusted to 14±0.3% (wet basis). Paddy rice in the glass bottle was sprayed with distilled water for 3-4 days until it reached equilibrium at room temperature (Srinivas and Desikachar, 1973). A microwave oven (Model M1712N SAMSUNG) was used at 2450MHz 800 watts to puff the paddy rice. A sample of 30 g of paddy rice was put into the paper bag (size 16X30 cm) and placed at the center in the microwave oven and puff for 120 seconds. The time that the grains took to start puffing was recorded and the temperature of puffed rice was measured with an infrared thermometer (Chino, Japan). The data were averaged from triplicate observations.

3.4.1.4 Product Evaluations

Husk and unpuffed paddy rice were separated by hand picking and weighed. The total volume of the puffed samples was measured by the sesame seed displacement. The puffed yield, expansion volume, expansion ratio and bulk density (Simsrisakul, 1991; Murugesan and Bhattacharya, 1991) were calculated using equations as 3.1 to 3.4, respectively.

$$\text{Puffed yield (\%)} = \frac{\text{wt. of puffed rice (g)} * 100}{\text{wt. of paddy rice (g)}} \quad (3.1)$$

$$\text{Expansion volume (mL/g)} = \frac{\text{vol. of puffed rice (mL)}}{\text{wt. of paddy rice (g)}} \quad (3.2)$$

$$\text{Expansion ratio (mL/mL)} = \frac{\text{vol. of puffed rice (mL)}}{\text{vol. of paddy rice (mL)}} \quad (3.3)$$

$$\text{Bulk density (g/mL)} = \frac{\text{wt.of puffed rice (g)}}{\text{vol.of puffed rice (mL)}} \quad (3.4)$$

3.4.1.5 Statistical Analysis

Analysis of Variance (ANOVA) was performed on all tests data. Duncan's multiple range tests was used to compare the means among the paddy rice varieties. Pearson's correlation coefficient was used to compare the physicochemical properties. A simple linear regression was developed using the SPSS software version 11 for Windows to predict puffing qualities.

3.4.2 To Study The Effects of Salt, Moisture Content and Microwave Power on Puffing Qualities of Puffed Rice.

3.4.2.1 Sample Preparation

Paddy rice was soaked in distilled water and 2% salt solution (NaCl) for 24 hrs. The paddy rice dried at an air temperature of 45±3°C with the tray- dryer until moisture content decreased to 19, 16, 13 and 10% (wb). Each sample was stored in the glass bottle for two days before puffing by modifying the method of Srinivas and Desikachar (1973) and Simsrisakul (1991).

3.4.2.2 Microwave Puffing Conditions

A microwave oven used in this experiment was a standard domestic microwave oven (Model M1712N SAMSUNG, THAILAND) of different microwave power setting (600, 700 and 800 watts) at 2,450 MHz. The actual power output of microwave oven testing was determined by weighing 1,000 g of deionized water (19 to 21°C) into a beaker and heating it for 62 sec at high power. When heating was completed, the final temperature of the water was measured with the electronic thermometer. The formula is $P = 70.0*T$ (where P = power in watts and T = change in temperature in °C) was used to estimate output watt power (Allred-Coyle et al., 2000b). A sample of 30 g paddy rice put into a paper bag (size 16 X 30 cm.) and placed in the middle of the microwave oven and puff at 600 watts 130 sec, 700 watts 110 sec and 800 watts 90 sec. The time that the grains took to start puffing was recorded and the temperature of puffed rice was measured with an infrared thermometer (Chino, Japan). After each test, the oven was opened for 3 minutes to cool down the chamber. The data were averaged from triplicate observations. The qualities of puffed rice were evaluated.

3.4.2.3 Product Evaluations

The husk and unpuffed of paddy rice were separated by hand picking. The total puffed rice was weighed. The total of puffed yield was divided into two shapes namely the fully puffed, and the small puffed (Maisont and Narkrugsa, 2009a) (Figure 3.3). The total of puffed yield, fully puffed yield and small puffed yield were calculated using the equations 3.5 to 3.7.

$$\text{Total puffed yield (\%)} = \frac{\text{wt. of total puffed riec (g)} * 100}{\text{wt. of paddy rice (g)}} \quad (3.5)$$

$$\text{Fully puffed yield (\%)} = \frac{\text{wt. of fully puffed riec (g)} * 100}{\text{wt. of paddy rice (g)}} \quad (3.6)$$

$$\text{Small puffed yield (\%)} = \frac{\text{wt. of small puffed riec (g)} * 100}{\text{wt. of paddy rice (g)}} \quad (3.7)$$

3.4.2.4 Expansion Volume

The volumes of puffed rice were measured by the sesame seed displacement and calculated followed calculated using equation 3.2.

Figure 3.3 The characteristics of puffed rice.

3.4.2.5 Hardness

Textural characteristic as a hardness of puffed rice was carried out using Texture Analyzer (model TA-XT2i), (Stable Micro System, 1995). The condition for measured is described in Figure B1 (Appendix B1).

3.4.2.6 Color

The color of the ground puffed rice was measured using colorimeter (Minalta CR 300, Japan) based on CIE system. The color meter was calibrated against a standard calibration plate of a white surface. The color was recorded as L*, a* and b* values, measured six times and averages. The color lightness coordinate L* measures the whiteness value of a color and ranges from black at 0 to white at 100. The coordinate a* measures red when positive and green when negative, and the coordinate b* measures yellow when positive and blue when negative.

3.4.2.7 Microstructure

The microstructure of the puffed rice was examined using SEM model (JEOL, JSM-5800LV) the method according to Mariotti et al. (2006) with some modifications.

3.4.2.8 Statistical Analysis

All statistics were performed by using the SPSS software version 11for Windows. The data was analyzed using descriptive statistics. Dancan's multiple range tests was used for post hoc analyses at the level of significance 0.05.

3.4.3 To Study The Effect of Germination Process on Chemical Compositions, GABA and Antioxidant Activity and Antioxidant Capacity of Waxy Paddy Rice.

3.4.3.1 The Hydration Characteristics of Paddy Rice, Husk and Brown Rice

Paddy rice grain is composed of two major physical components, brown rice and husk. During soaking, moisture content of paddy rice might not be a true moisture of brown rice. Thus, measurement of the moisture content in each section namely paddy rice, brown rice and husk were done according to the method of Abhay et al. (2006) with some modifications. Soak 30 g paddy rice with water (ratio 1:1.5 w/w) at room temperature (28-30°C). The samples were removed at the every 5 hour. The paddy rice was drained for 1 min, blotted with tissue paper 2-3 times to remove the surface water. After blotting, manually separated the husk with a razor blade. Paddy rice, husk and brown rice were determined the moisture content by hot air oven method at 120±2°C (Abhay et al., 2006).

3.4.3.2 Soaking and Germination

Soaking and germination of paddy rice were prepared according to the method of Puangwerakul (2006) with some modifications. Soaking 300 g paddy rice with water (1:1.5 w/w), changed water every 12 hours and drained. The paddy rice separately packed in plastic

boxes on a layer of tissue paper and let it germinate in the cabinet incubator (28-30°C) with a controlling relative humidity at 94-96% for 0, 12, 24, 36, 48 and 60 hrs respectively.

Figure 3.4 Germination process of paddy rice.

3.4.3.3 GABA Content Determination

After soaked and germination, the paddy rice embryos were carefully cut with a razor blade to separate embryo from the endosperm (Liu et al., 2005). Then the GABA content was determined according to the method of Liu et al. (1995) with some modifications. The determination is described in Appendix A3 and the chromatogram of standard GABA shown in Figure A2 (Appendix A).

Figure 3.5 The paddy rice germ or embryo separate from germinated paddy rice at 36 hrs.

3.4.3.4 Chemical Compositions Analysis

Germinated paddy rice were dried at 50±2°C in the tray- dryer until moisture content decreased to 8-10 % (wb.), dehulled and ground using a Retsch Ultra centrifugal mill with a 0.12 mm mesh sieve screen. The content of moisture, protein, fat and ash of samples were determined according to AOAC (1990).

3.4.3.5 Dietary fiber

The dietary fiber content was determined according to AOAC (2000). The determination is described in Appendix A.

3.4.3.6 Starch Content

The starch content was determined by using glucoamylase method according to AACC (1990). The determination is described in Appendix A.

3.4.3.7 Antioxidant Properties

1) Phenolics Extraction

Phenolics of germinated paddy rice flour at 0, 12, 24, 36, 48 and 60 hr, approximately 2 g dry matter were extracted with 20 mL of 80 % ethanol for 30 minutes at room temperature and then centrifuged with Benchtop Centrifuges (Allegra X-12R, Beckman Coulter, Inc. USA) at 6,000 rpm, 4°C for 30 min. After centrifugation, the supernatant was separated from the residue and stored at 4°C for further analysis (Ragaee et al., 2006).

2) Total Phenolic Content Determination

Total Phenolics were determined using the Folin-Ciocalteu method described by Singleton et al. (1999) with some modifications. The determination is described in Figure A3 (Appendix A).

3.4.3.8 Antioxidant Capacities Determination

1) DPPH Radical Scavenging Activity

The radical scavenging activity of the phenolics extracts on DPPH radical was measured according to the method described by Ragaee et al. (2006) and Choi et al. (2006) with some modifications. The determination is described in Figure A4 (Appendix A).

2) Ferric Reducing Ability Power (FRAP)

The FRAP of the phenolics extracts was measured according to the method of Wong et al. (2006) with some modifications. The determination is described in Figure A5 (Appendix A).

3.4.3.9 Statistical Analysis

Three replications of each experiment were performed. All data were analyzed by Statistic Package for Social Science (SPSS). Significant difference ($p<0.05$) among various treatments was detected by Duncan's multiple range tests (DMRT).

3.4.4 To Study the Effect of Germination Process on Puffing Qualities and GABA Retention of Germinated Puffed Rice.

3.4.4.1 Germination Process

The germination process of paddy rice was germinated according to the method of Puangwerakul (2006) with some modifications. Soaked 1,200 g paddy rice with water (1:1.5w/w) 50 hrs, changed water every 12 hour and drained. The paddy rice separately packed in plastic boxes which on a layer of tissue paper and let it germinate in the cabinet incubator (28-30°C) with controlling a relative humidity at 94-96% for 0, 12, 24, 36, 48 and 60 hrs, respectively.

3.4.4.2 Puffing Process

The germinated paddy rice 0, 12, 24, 36, 48 and 60 hrs were dried at 50±2°C in the tray-dryer until moisture content decreased to 8±2% (wb.). 300 g germinated paddy rice was contained in the glass bottle and sprayed with 2% salted for adjusting the moisture content of each germinated paddy rice to 13%±0.3% (wb.) holding at room temperature for 24 hrs before puffing. Then 30 g of germinated paddy rice was put into the paper bag (size 16 X 30 cm) and placed at the center in the microwave oven (Model M1712N, SAMSUNG) at 2450MHz and puffed 700 watts for 110±3 sec. The data were averaged from triplicate observations.

3.4.4.3 Product Evaluations

The husk and unpuffed of paddy rice were separated by hand picking. The total puffed rice was weighed. The total of puffed yield was divided into two shapes namely the fully puffed, and the small puffed (Maisont and Narkrugsa, 2009a) (Figure 3.4). The total of puffed yield, fully puffed yield and small puffed yield were calculated using the equations 3.5 to 3.7. The expansion volumes of puffed rice were measured by the sesame seed displacement and calculated using equation as 3.2.

3.4.4.4 Color

The color of the ground germinated puffed rice was measured using colorimeter (Minalta CR 300, Japan), based on CIE system. The color meter was calibrated

against a standard calibration plate of a white surface. The color was recorded as L*, a* and b* values, measured six times and averages. The color lightness coordinate L* measures the whiteness value of a color and ranges from black at 0 to white at 100. The coordinate a*measures red when positive and green when negative, and the coordinate b* measures yellow when positive and blue when negative.

3.4.4.5 Hardness

Texture of puffed rice as the hardness was carried out on the Texture analyzer (model TA-XT2i), mode: measure force in compression, test speed: 5.0 mm/s, post-test speed 10.0 mm/s with Ottawa Cell with wire plate probe. Six replications of the puffed rice obtained for each puffing condition were used for the texture test. (Stable Micro System, 1995). The condition for measured is described in Appendix B1.

3.4.4.6 Water Absorption Index (WAI) and Water Solubility Index (WSI)

The WAI and WSI of germinated puffed rice were determined by the method of Anderson et al. (1969) with some modifications. Puffed germinated rice flour (80 mesh) 0.20 g was suspended in 9 ml distilled water at room temperature (28±2°C) in a centrifuge tube, stirred intermittent, hold for 30 min and then centrifuged at 3,000 g for 10 min. After pouring the supernatant into moisture can and evaporating until the weight constant, the gel was weighed. WAS and WSI were calculated using equations as 3.8 to 3.9.

$$\text{WAI (g/g)} = \frac{\text{wt. of germinated puffed rice flour gel}}{\text{wt. of puffed germinated rice flour}} \quad (3.8)$$

$$\text{WSI (\%)} = \frac{\text{wt. of total soluble solid}}{\text{wt. of puffed germinated rice flour X 100}} \quad (3.9)$$

3.4.4.7 GABA Content Determination

Germinated paddy rice at 36 hours was selected to investigate the effect of drying and puffing process on GABA content. Fresh embryos were carefully cut with a razor blade to separate from each kernel of paddy rice (Liu et al., 2005). The embryos were dried and puffed with above conditions. The GABA content of samples was determined according to the method of Liu et al. (1995). The determination described in Appendix A.

3.4.4.8 Statistical Analysis

Three replications of each experiment were performed. All data were analyzed by Statistic Package for Social Science (SPSS) version11. Significant difference ($p<0.05$) among various treatments was detected by Duncan's multiple range tests (DMRT).

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Effects of Some Physicochemical Properties of Paddy Rice Varieties on Puffing Qualities by Microwave Heating.

4.1.1 Chemical Properties of Paddy Rice Varieties

The chemical compositions of paddy rice varieties are shown in Table 4.1. The moisture and protein content of paddy rice were in the range 10.52-12.26% and 6.32-8.34%, respectively. The protein content of NPH1 was the lowest 6.32%, whereas the highest was 8.34% for HY71. The protein content in the paddy rice depending on the varieties ($p<0.05$).

Table 4.1 The chemical properties of paddy rice varieties.

Variety	Moisture content (%)	Protein content (%)	Amylose content (%)
SMJ	12.26 ± 0.18^{f}	7.48 ± 0.18^{f}	5.58 ± 0.09^{a}
RD 10	11.66 ± 0.12^{e}	7.03 ± 0.05^{cd}	6.04 ± 0.14^{b}
NPH1	10.72 ± 0.14^{ab}	6.32 ± 0.03^{a}	6.21 ± 0.05^{bc}
RD 6	11.73 ± 0.20^{e}	6.35 ± 0.04^{a}	6.36 ± 0.02^{cd}
NSPT	11.25 ± 0.06^{c}	6.81 ± 0.03^{bc}	6.39 ± 0.16^{cd}
HY71	10.52 ± 0.08^{a}	8.34 ± 0.04^{g}	6.59 ± 0.02^{de}
GP15	11.59 ± 0.12^{de}	6.62 ± 0.18^{b}	6.81 ± 0.14^{e}
SKK	11.36 ± 0.13^{cd}	7.58 ± 0.01^{f}	7.06 ± 0.07^{f}
KDML105	11.81 ± 0.14^{e}	7.37 ± 0.16^{ef}	15.98 ± 0.05^{g}
SPBR1	10.79 ± 0.14^{b}	7.17 ± 0.05^{de}	21.24 ± 0.19^{h}

Note: 1 The data in this table were averaged from triplicate observations.

2 ^{a,b} in the same column with different letters are significantly difference ($p<0.05$)

3 GP15= Gam-Pai15, HY71= Hahng-Yi71, KDML105= Khao Dawk Mali 105, NPH1= Niaw-Phrae1, NSPT=Niaw-San-Pah-Tawng, SKK= Sakon-Nakhon, SMJ= Sew-Mae-Jan, and SPBR1= Suphan-Buri1.

However, Juliano and Hicks (1996) indicated that 2% difference in rice protein content did not affect the quality of cooked rice. In this study, the paddy rice showed less than 2% differences in protein content thus the qualities of rice puffing may not be affected by protein.

Amylose content of paddy rice varieties was different significantly ($p<0.05$) and could be classified into three groups: waxy rice; low-amylose content; and intermediated amylose content with values of 5.96-7.46, 15.98 and 21.24%, respectively. According to Mercier and Feillet (1975) starch is made of linear amylose and branched amylopectin, which impacts on expansion differently. High amylopectin content leads to light, elastic, and homogeneously expanded textures, while a high amylose content leads to hard, less expanded textures. The linear amylose chains align themselves in the shear field and thus are difficult to pull apart during expansion (Moraru and Kokini, 2003).

4.1.2 Physical Properties of Paddy Rice Varieties

The score for the degree of husk interlocking or the overlapping of lemma and palea highlighted the different characteristics of paddy rice. In this study, the score of paddy rice varieties range from 1-24 scores, with the highest score for HY71 and lowest score for SMJ as shown in Table 4.2 and Figure 4.1 a-d. In the contrary, SMJ had a high shallability value (94.33%), but for HY71 had rather low value (81.33%). The much tighter paddy rice husk seemed to be more difficult to dehusk. HY71 had the lowest expansion volume and expansion ratio when compared with the group of waxy paddy rice. This was probably due to the fact that the overlapping of lemma and palea was quite tight, which made the husk harder to explode and resulted in low expansion. The SMJ husks were loose and could not keep the vapor pressure high enough to produce suitable exploding conditions, resulting in rather low values for the expansion volume and expansion ratio. The husk content was lowest for SPBR1 (17.12%) and the highest for GP15 (24.55%). The width and length of brown rice varied from 2.00-2.7 and 7.14-7.68mm, respectively, with the longest for SKK while GP15 was the widest. White belly was the lowest for KMDL105 (2.00%) and the highest for (52% GP15), which difference significantly ($p<0.05$) to other waxy paddy rice varieties. The ventral region thickness was lowest for NSPT (13.54 μg) and the highest for SMJ (28.47 μg) (Figure 4.2 e and f). Morphological starch granule of various brown rice showed the part of endosperm contained polygonal compact starch granules were with a size range 3.44–5.50 μm. SMJ had the smallest sized granules (3.44 μm), whereas the RD10 starch granule were the largest (5.50 μm). GP15 the starch granule tended to be spherical and

much more inter-granular space, which may be a characteristic of white belly kernels, whereas SMJ starch granule was rather compact. RD10, RD6, SKK and NSPT starches granules more uniform size and distribution than starches granules from other paddy rice varieties (Figure 4.3 g-j).

Table 4.2 The physical properties of paddy rice varieties.

Variety	HL (score)	SH (%)	HC (%)	W (mm)	L (mm)	WB (%)	VT (μm)	STG(μm)
SMJ	1.00±0.00^{a}	94.33±2.08^{f}	21.93±1.71^{cd}	2.21±0.04^{bc}	7.29±0.08^{a}	21.67±3.79^{b}	28.47±0.69^{f}	3.44± 0.35^{a}
RD 10	8.00±0.00^{c}	83.67±2.52^{abc}	18.33±2.55^{a}	2.29±0.14^{c}	7.58±0.05^{bc}	3.00±1.00^{a}	15.28±1.30^{ab}	5.50 ±0.17^{e}
NPH1	16.00±3.46^{d}	81.33±3.21^{ab}	18.39±0.71^{a}	2.31±0.10^{c}	7.36±0.14^{ab}	40.00±3.00^{c}	17.71±1.04^{c}	4.61± 0.10^{c}
RD 6	6.67±2.31^{bc}	85.67±2.52^{bcd}	18.47±0.81^{a}	2.18±0.07^{abc}	7.20±0.13^{a}	4.00±1.00^{a}	14.93±1.59^{ab}	4.78± 0.35^{cd}
NSPT	20.00±3.46^{e}	82.00±2.67^{abc}	22.05±0.78^{cd}	2.16±0.13^{abc}	7.24±0.12^{a}	3.00±0.50^{a}	13.54±1.04^{a}	4.39± 0.25^{c}
HY71	24.00±0.00^{f}	80.67±3.06^{a}	21.52±0.95^{bcd}	2.06±0.08^{ab}	7.14±0.12^{a}	24.31±1.50^{b}	27.78±0.67^{f}	4.44± 0.10^{c}
GP15	14.00±2.31^{d}	82.00±3.61^{abc}	22.55±2.79^{d}	2.70±0.10^{d}	7.15±0.05^{a}	52.00±0.67^{d}	18.06±0.57^{cd}	5.11 ±0.25^{de}
SKK	3.33±1.15^{ab}	86.33±1.53^{cde}	20.44±1.71^{b}	2.13±0.12^{abc}	7.68±0.04^{c}	1.67±0.58^{a}	16.67±1.04^{bc}	4.83± 0.17^{cd}
KDML105	5.00±1.37^{bc}	90.67±2.52^{ef}	20.78±1.69^{bc}	2.00±0.14^{a}	7.27±0.23^{a}	1.67±0.58^{a}	19.79±1.04^{de}	3.89± 0.25^{b}
SPBR1	4.67±1.15^{abc}	89.33±1.53^{de}	17.12±3.56^{a}	2.12±0.11^{abc}	7.20±0.24^{a}	2.33±1.53^{a}	20.49±0.65^{e}	4.44± 0.19^{c}

Note: 1 The data in this table were averaged from triplicate observations.

2 ^{a,b} Means in the same column with different letters are significantly difference ($p<0.05$)

3 GP15= Gam-Pai15, HY71= Hahng-Yi71, KDML105=Khao Dawk Mali 105, NPH1= Niaw-Phrae1, NSPT=Niaw-San-Pah-Tawng, SKK=Sakon-Nakhon, SMJ=Sew-Mae-Jan, and SPBR1=Suphan-Buri1 HL=Husk interlocking score, SH=Shallability, HC=Husk content, VT=Ventral region thickness, W=Width of brown rice, L=Length of brown rice, WB=White belly and STG=Starch granules size.

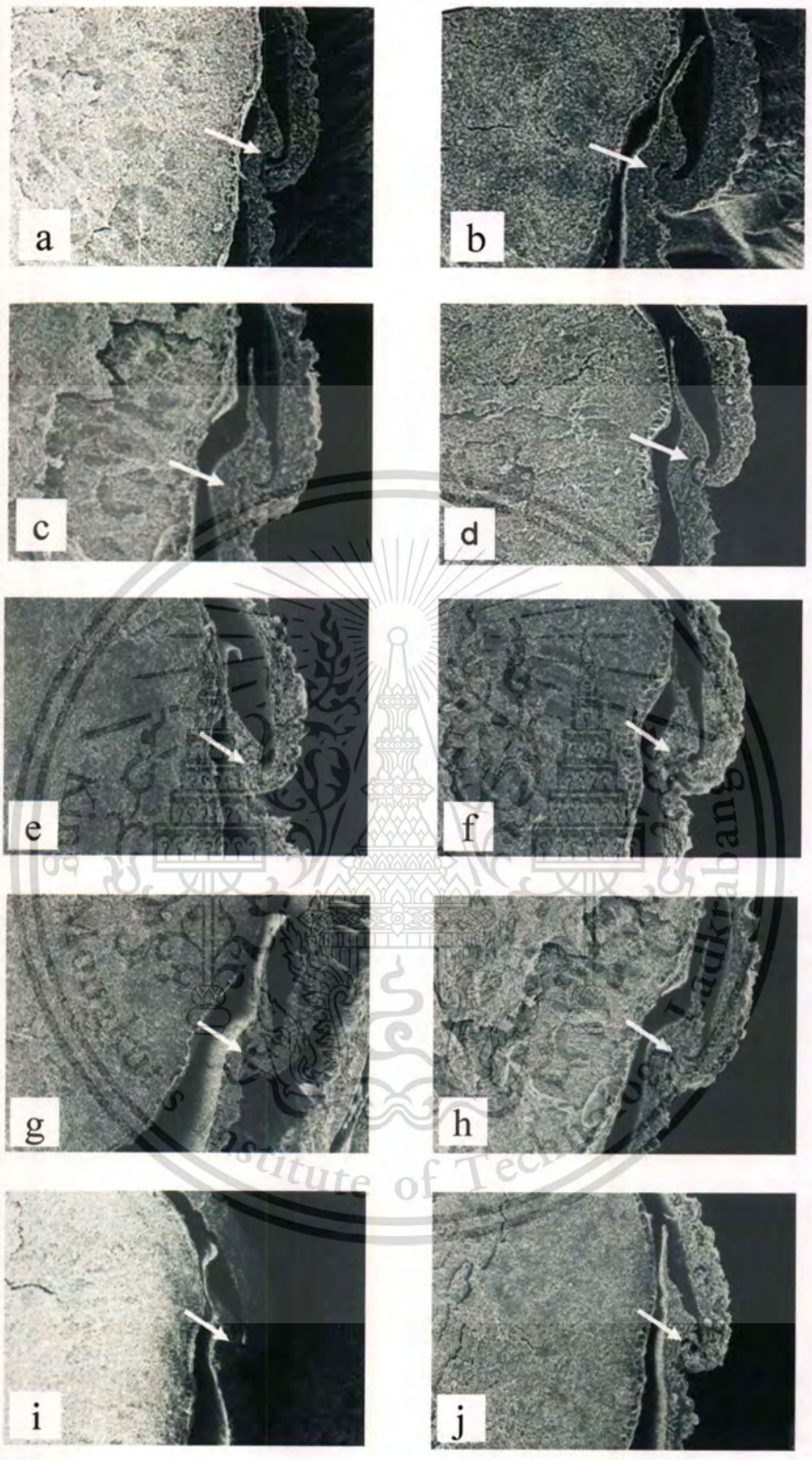


Figure 4.1 SEM micrograpgh lemma –palea interlocking of ten paddy rice varieties (a) Sew-Mae-Jan, (b) Niaw-San-Pah-Tawng, (c) Hahng-Yi71, (d) RD6, (e) RD10, (f) Gam-Pai15, (g) Sakon-Nakhon, (h) Niaw-Phrae1, (i) Khao Dawk Mali 105 and (j) Suphan-Buri1.

4.1.3 Puffing Qualities of Paddy Rice

The data in Table 4.3 presents the characteristics of puffed rice for the paddy rice varieties. Differences were apparent in all qualities of rice puffing, including puffed yield, expansion volume, expansion ratio and bulk density with a range of: 29.79-57.38%, 5.16-10.39 mL/g 2.72-5.97 and 0.21-0.33 g/mL, respectively. Puffed rice from non waxy paddy rice (SPBR1 and KDML105) was lower in value for all quality characteristics than puffed rice from all waxy paddy rice varieties. The main reasons may have been due to the higher amylose content (Table 4.1). There were no relationships among the waxy paddy rice varieties cultivated in the difference areas, but the paddy rice in northeastern area (NPH1, NSPT, GP15 and SMJ) seemed to have greater puffed yield than paddy rice in the north (RD6,RD10, SKK and HY71). The three paddy rice varieties with the highest puffed yield, expansion volume, expansion ratio and bulk density were RD 6, NSPT and RD10, respectively.

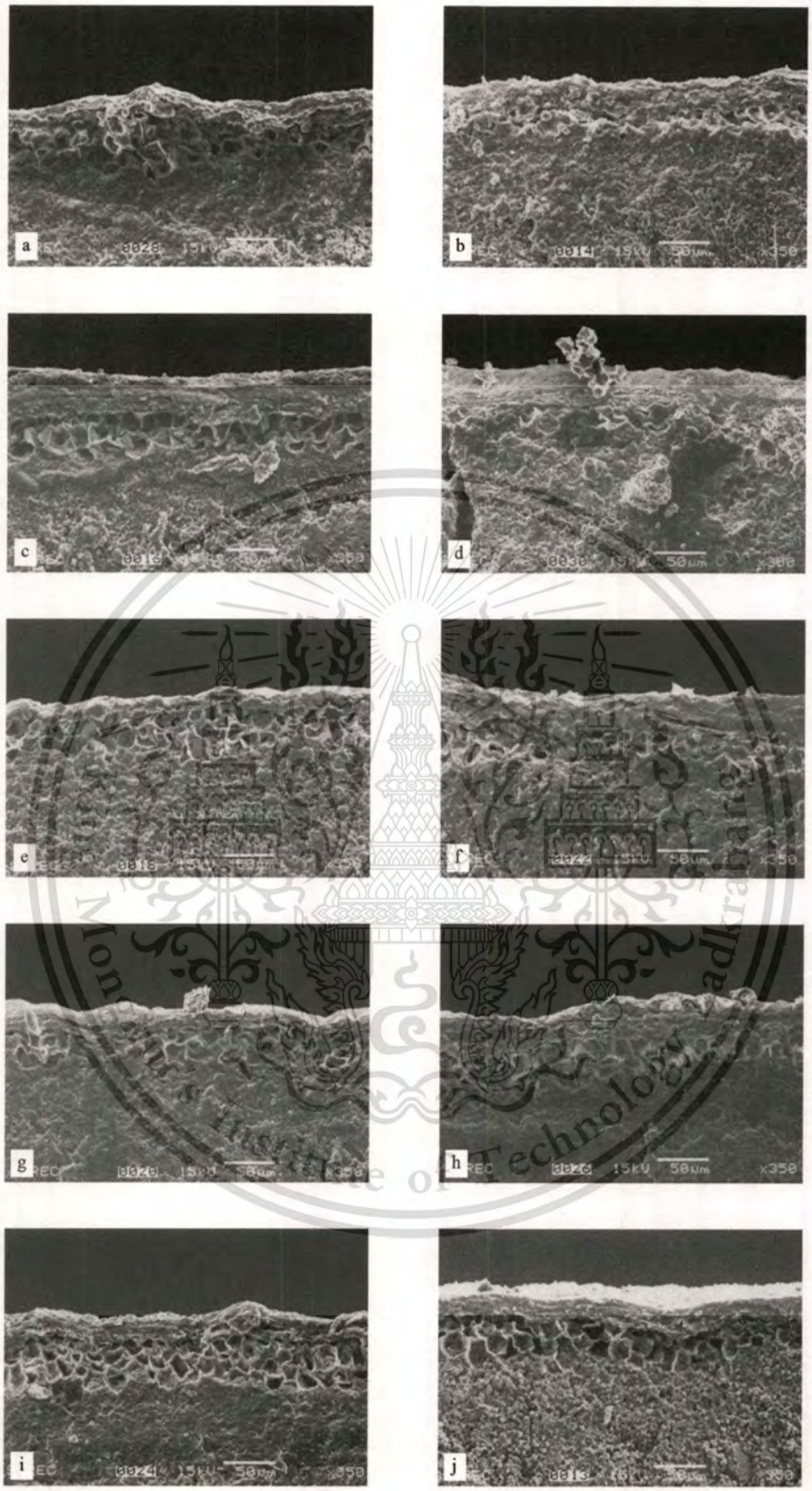


Figure 4.2 SEM micrograph of ventral region layer thickness (a) RD6, (b) Niaw-Phrae1, (c) RD10, (d) Sakon-Nakhon, (e) Niaw-San-Pah-Tawng, (f) Sew-Mae-Jan, (g) Gam-Pai15, (h) Hahng-Yi71, (i) Khao Dawk Mali 105 and (j) Suphan-Buri1.

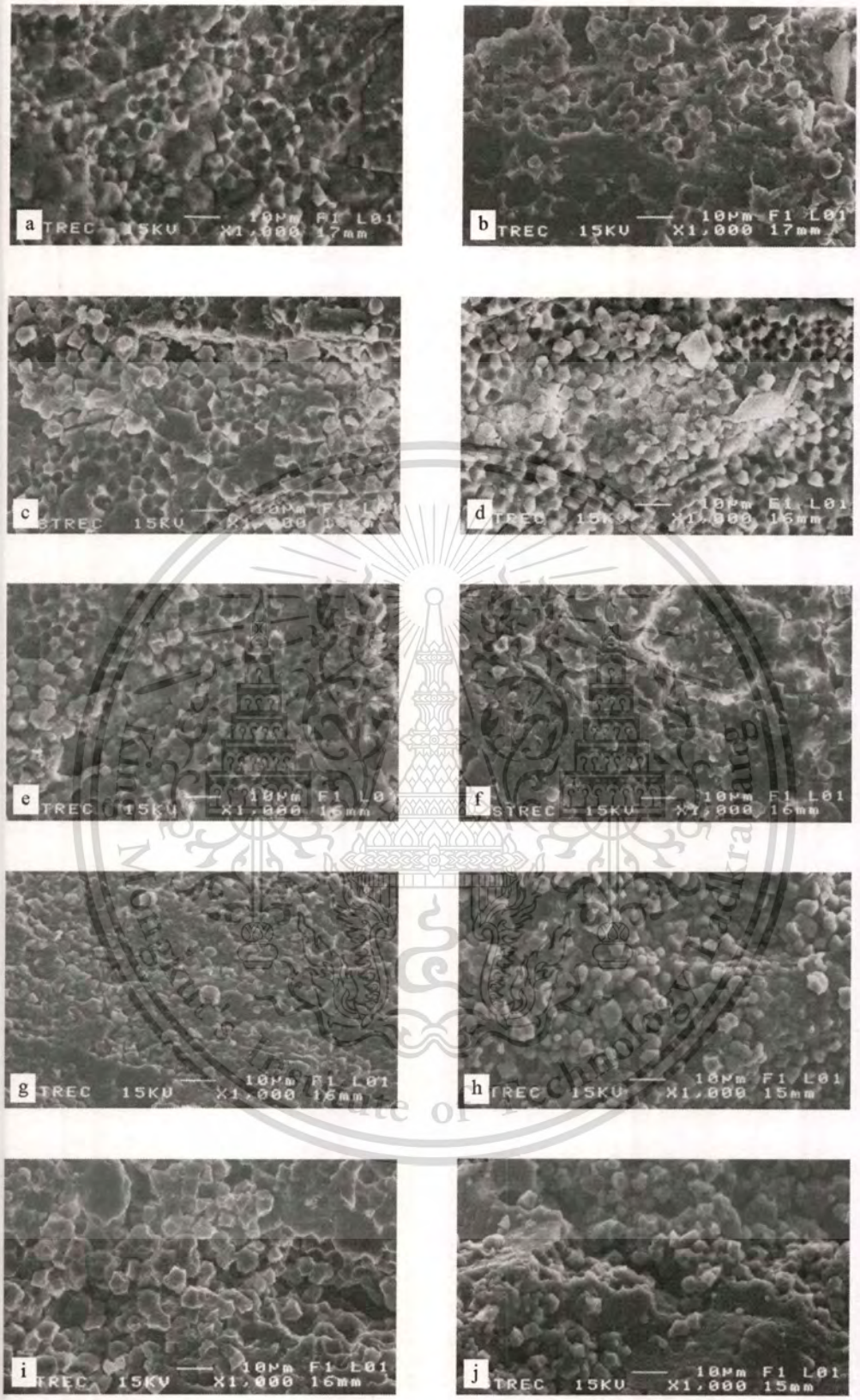


Figure 4.3 SEM micrograph of starch granule: (a) Niaw-San-Pah-Tawng, (b) Hahng-Yi71, (c) Niaw-Phrae1, (d) Sakon-Nakhon, (e) Khao Dawk Mali 105, (f) Suphan-Buri1, (g) Sew-Mae-Jan, (h) Gam-Pai15, (i) RD10 and RD6.

Table 4.3 Puffing qualities of puffed rice.

Variety	Puffed yield (%)	Expansion volume(ml/g)	Expansion ratio(mL/mL)	Bulk density (g/ml)
SMJ	51.54±0.90^{cd}	8.77±0.30^{cd}	4.50±0.11^{c}	0.0588±0.0005^{ab}
RD 10	56.97±1.58^{e}	9.84±0.25^{de}	5.14±0.07^{d}	0.0579±0.0007^{ab}
NSPT	57.09±0.52^{e}	10.27±0.11^{e}	4.95±0.00^{cd}	0.0552±0.0001^{a}
RD 6	57.38±0.86^{e}	10.39±0.76^{e}	5.97±0.51^{e}	0.0553±0.0034^{a}
NPH1	54.66±2.40^{de}	8.61±0.29^{c}	4.49±0.17^{c}	0.0635±0.0030^{b}
HY71	49.58±1.45^{c}	7.00±0.32^{b}	3.71±0.15^{b}	0.0709±0.0032^{c}
GP15	56.24±0.95^{e}	9.42±0.70^{cde}	4.58±0.33^{cd}	0.0598±0.0023^{ab}
SKK	54.00±1.12^{de}	9.99±0.62^{e}	4.74±0.33^{cd}	0.0541±0.0054^{a}
KDMl105	40.86±3.00^{b}	6.66±0.70^{b}	3.48±0.32^{b}	0.0615±0.0020^{ab}
SPBR 1	29.79±1.27^{a}	5.16±0.22^{a}	2.72±0.14^{a}	0.0767±0.0046^{c}

Note: 1 The data in this table were averaged from triplicate observations.

2 ^{a,b} in the same column with different letters are significantly difference ($p<0.05$)

3 GP15= Gam-Pai15, HY71=Hahng-Yi71, KDML105=Khao Dawk Mali 105, NPH=Niaw-Phrae1, NSPT=Niaw-San-Pah-Tawng, SKK= Sakon-Nakhon, SMJ= Sew-Mae-Jan, and SPBR1= Suphan-Buri1.

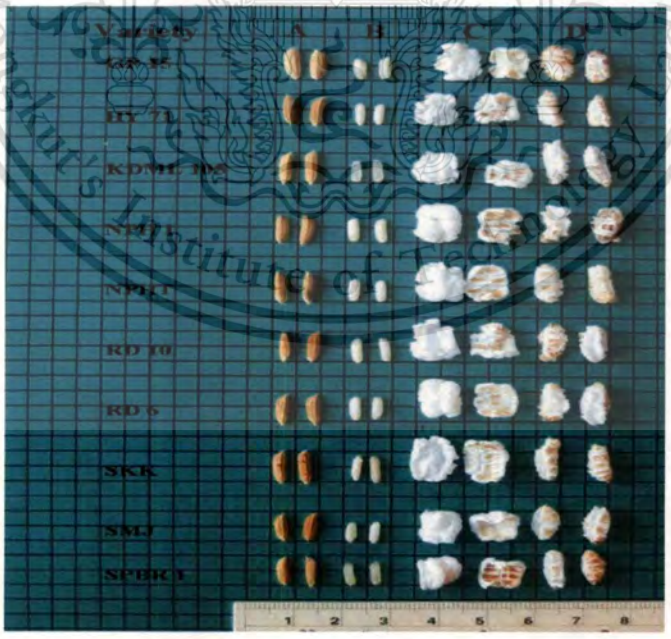


Figure 4.4 Puffed rice from paddy rice varieties; (A) paddy rice, (B) brown rice, (C) fully puffed rice and (D) small puffed rice.

Table 4.4 Pearson's correlation coefficients (r) for the physicochemical properties of paddy rice varieties with the puffing qualities of puffed rice.

Physicochemical properties	Puffing qualities (*n*=13)			
	Puffed yield	Expansion vol.	Expansion ratio	Bulk density
Chemical compositions				
Moisture content	0.26	0.41	0.42	0.18
Protein content	-0.33	-0.44	-0.50	-0.47
Amylose content	- 0.95**	-0.82**	-0.79**	-0.78**
Physical properties				
Husk interlocking	0.30	0.05	0.01	0.18
Husk content	0.35	0.25	0.10	-0.10
Shall ability	-0.24	-0.02	-0.01	-0.09
Width of brown rice	0.44	0.43	0.25	0.15
Length of brown rice	0.01	0.24	-0.22	0.04
Ventral thickness	-0.35	-0.54	-0.51	-0.39
White belly	0.29	0.04	-0.01	0.15
Starch granule size	0.40	0.41	0.40	0.52
Starting time to puffing	-0.67*	-0.56	-0.61	-0.58
Puffing temperature	0.35	0.29	0.26	0.32

Note: 1* means significant: at $p < 0.05$.

2 ** means significant: at $p < 0.01$

4.1.4 Correlations between the Paddy Rice Properties and the Puffing Qualities of Puffed Rice

Table 4.4 presents the correlations between the paddy rice properties and the puffing qualities of puffed rice for: puffed yield, expansion volume, expansion ratio and bulk density. The results showed that none of the physical properties of paddy rice were related to the qualities of rice puffing, which was a similar result to Srinivas and Desikachar (1973). Husk interlocking score, husk content, width, length, white belly and starch granules size showed positive trends, whereas shallability and ventral region thickness layer had negative correlation trends. While chemical composition measured as amylose content was highly negatively correlated with puffed yield (-0.95**), expansion volume (-0.82**), expansion ratio (-0.79**) and bulk density (-0.78**), these results were contrary to Murugesan and Bhattacharya (1991). The starting time of puffing was negatively correlated with puffed yield (-0.67*) and from observations it was found that rice started puffing when is heated from microwave after 39-48 seconds and finished puffing

within 120 seconds at 168-191°C. Paddy rice which varieties that produce a high puffed yield and high expansion volume had an earlier puffing start time and continue puffing longer.

The experiment, produced some interesting results regarding the amylose content and the puffing qualities of the paddy rice varieties SMJ and HY1. These two varieties have low amylose content, but had low puffed yield and expansion volume. When considering physical properties such as the husk interlocking SMJ score is the lowest score (1) in Figure 4.1a while HY1 had the highest score (24) in Figure 4.1c. The Pearson correlation coefficients shown that husk interlocking strongly negative correlated with shallability (r =-0.84 **). Therefore the relationship of husk interlocking with puffing quality might be explained by the fact that the loosely hooked husk affects the capacity to maintain vapor pressure before the kernel explodes, which acts like pericarp in maize. Hoseney et al. (1983) indicated that the pericarp in maize serves as a pressure vapor to hold internal steam pressure until the threshold level required to expand the grain was reached. In addition, Pordesimo et al. (1990) found that the thickness of pericarp affected the popcorn expansion, which corresponds to the experimental result of SMJ where the ventral region layer was thicker than in other varieties (Figure 4.2f). It is probable that the thicker ventral region layer results in the driving force not being enough for the ventral layer to explode. The simple linear regression showed that amylose content (AC) could explain 91 % of puffed yield, 67% expansion volume 62% expansion ratio and 61% bulk density as shown in the equation : Puffed yield = 64.921-1.599AC (R^2 = 0.92),

Expansion volume =11.024-0.273AC (R^2 = 0.67),

Expansion ratio =5.630-0.136AC (R^2 = 0.62),

Bulk density =0.312-0.0053AC (R^2 = 0.61).

As shown in Figure 4.5, the models were in moderated agreement with the observed results of puffed yield in Figure 4.5a, with the predicted data generally clustered around the straight line, which showed the suitability of the established model in describing the puffed yield of rice puffing. The linear relationship of puffed yield can be used to predict the puffing 64.921-1.599AC (R^2 = 0.92) and can be used to predict the puffing quality by resulting from microwave heating. For the other puffing qualities such as expansion volume, expansion ratio and bulk density, the linear relationship with amylose content of paddy rice is not clear, as is shown by the lower R^2 values.

Paddy rice can be puffed by microwave heating, producing puffed rice that has expansion volume, expansion ratio and bulk density similar to cereal grain that has been puffed.

The highest puffed yield was 57.38%. A linear relationship between puffed yield and amylose content could predict the puffed yield up to 90%. While the physical properties were less correlated to the quality of rice puffing. The suitable paddy rice varieties for puffing were RD6, RD10 and NSPT.

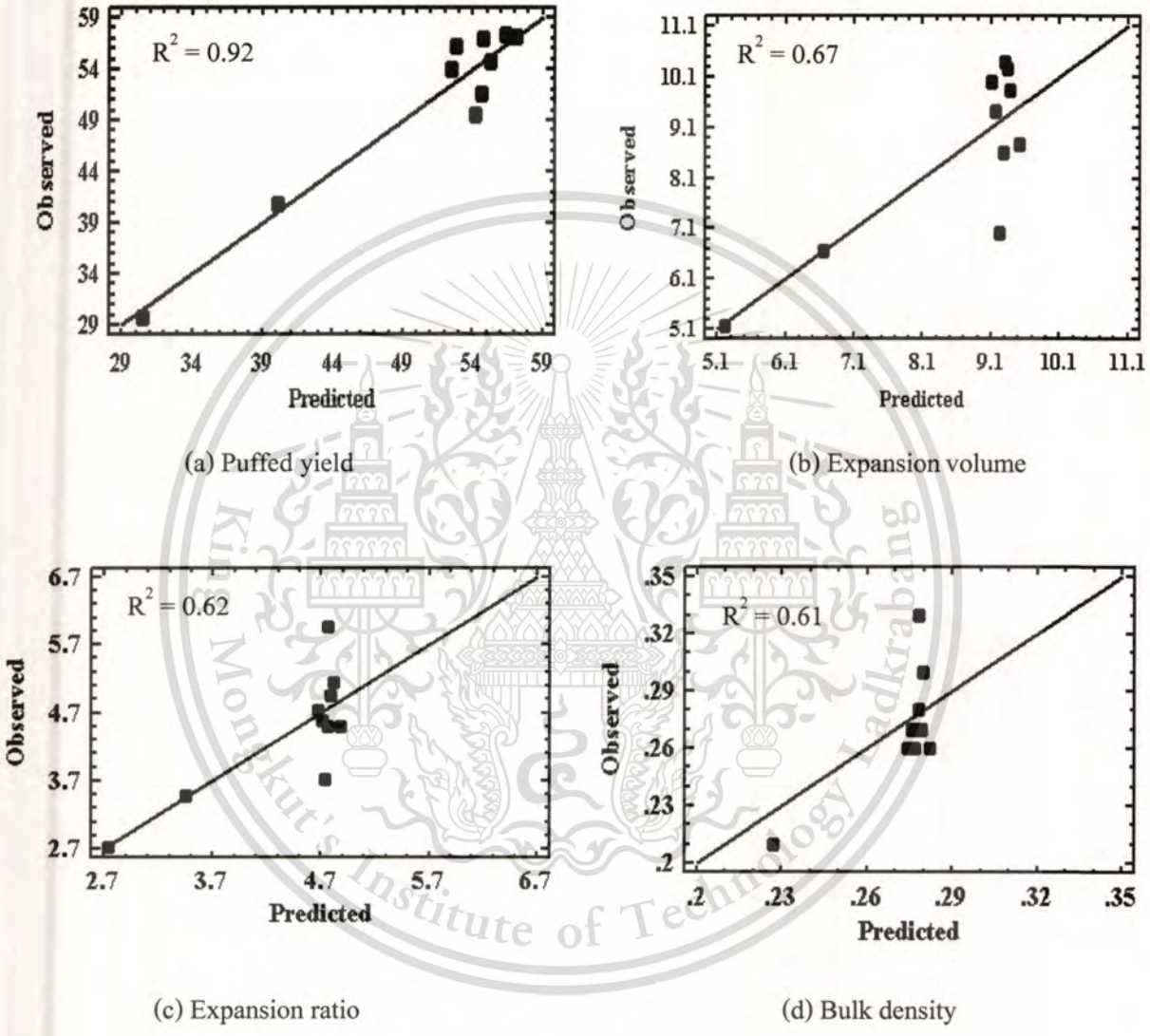


Figure 4.5 The observed and predicted of puffing qualities; (a) Puffed Yield, (b) Expansion volume, (c) Expansion ratio and (d) Bulk density.

4.2 Effects of Salt, Moisture Content and Microwave Power on Puffing Qualities of Puffed Rice by Microwave Heating

4.2.1 Time of Paddy Rice Start Puffing and Temperature of Puffed Rice

Soaking with salt solution, moisture content and microwave power affect the start puffing time and the temperature of puffed rice as shown in Table 4.5. Paddy rice soaked with salt solution at all moisture content levels and puffed with all microwave power levels had a trend to puff faster and also had higher temperature at the end of puffing time than the paddy rice soaked with water. The reason may be caused by high dielectric property of salt that making the rice soaked in salt solution absorb more microwave energy and converting to more heat than those soaked with water. At 10% moisture content, paddy rice required longer time to start puffing than at 13, 16 and 19% moisture content, despite soaking with water and salt solution. Paddy rice with low moisture content requied longer time to produce enough inner vapor pressure in order to be exploded. The results also exhibited that using a higher microwave power especially at 800 watt, the rice started puffing faster and had the temperature higher than those puffed at 600 and 700 watt.

Table 4.5 Effects of salt, moisture content and microwave power to the time of the paddy rice start puffing and the temperature of puffed rice at completed puffing.

Microwave power (watt)	Moisture content (%)	Water		2% Salt solution	
		Time of starting puffing paddy rice (sec.)	Temperature of puffed rice(°C)	Time of starting puffing paddy rice (sec.)	Temperature of puffed rice(°C)
600	10	62-69	162-167	63-68	170-177
	13	59-63	172-176	58-59	174-179
	16	60-66	164-167	55-58	164-169
	19	60-64	162-169	59-63	169-173
700	10	58-59	190-193	43-47	180-189
	13	55-57	176-180	46-50	182-186
	16	56-59	168-174	46-48	173-178
	19	57-59	165-170	53-57	168-174
800	10	48-50	193-194	40-44	196-198
	13	45-48	190-194	39-42	193-197
	16	45-48	182-190	43-46	186-192
	19	45-47	178-184	46-48	180-183

4.2.2 Puffing qualities

The results and F-value of the independent variables namely salt solution, moisture content and microwave power and their interaction affecting qualities of puffed rice are shown in Table 4.6 and 4.7.

Table 4.6 Effects of salted solution, moisture content and microwave power on puffing qualities.

Conditions			Qualities of puffed rice			
Salt solution (%)	Moisture content (%)	Microwave power (watt)	Total puffed yield * (%)	Fully puffed yield * (%)	Small puffed yield* (%)	Expansion volume* (ml/g)
0	10	600	54.33±0.34	28.57±2.08	25.76±2.06	6.87±0.17
		700	55.75±1.27	27.32±0.46	28.42±0.99	7.35±0.71
		800	60.18±1.59	28.80±2.59	31.37±1.37	8.45±0.86
	13	600	58.10±0.71	22.21±1.15	35.90±0.40	9.16±0.67
		700	58.91±1.17	33.15±0.67	25.76±1.50	8.74±0.60
		800	60.80±1.76	21.88±1.20	38.93±2.15	9.66±0.68
	16	600	47.31±2.36	27.00±0.74	20.31±2.76	7.52±0.38
		700	50.61±2.25	35.85±2.69	14.76±2.56	7.50±0.45
		800	56.21±2.86	33.90±0.13	22.31±2.93	7.55±1.81
	19	600	47.64±0.83	33.10±0.12	14.54±0.74	5.73±0.88
		700	48.34±2.53	36.62±0.91	11.72±2.13	6.07±0.28
		800	43.60±1.22	34.02±1.70	9.58±0.48	5.96±0.96
2	10	600	53.82±1.05	34.89±0.83	18.93±0.60	7.72±0.14
		700	57.45±1.03	37.68±2.20	19.71±3.07	8.86±0.55
		800	60.68±0.97	38.05±1.85	22.63±2.32	9.59±0.29
	13	600	60.13±1.16	28.85±0.21	31.28±1.00	8.37±0.46
		700	62.20±0.94	35.09±1.16	27.10±1.88	9.61±0.54
		800	63.53±2.83	42.67±0.93	20.86±1.97	11.23±0.07
	16	600	59.13±1.12	42.05±4.81	17.07±3.82	9.04±1.04
		700	59.31±0.50	45.63±0.86	13.68±1.04	9.01±1.49
		800	58.70±1.25	47.45±1.59	11.25±0.35	9.65±0.63
	19	600	56.20±2.02	45.34±2.77	10.87±1.26	8.22±0.25
		700	57.55±1.94	48.72±1.34	8.83±0.75	6.10±1.50
		800	54.28±1.39	44.57±1.11	9.73±1.37	8.78±0.50

* means significant at $p < 0.05$

Table 4.6 Effects of salted solution, moisture content and microwave power on puffing qualities (cont.).

Conditions			Qualities of puffed rice			
Salt solution (%)	Moisture content (%)	Microwave power (watt)	Hardness (g.f.) *	L*	a*	b*
0	10	600	338.82±5.93	80.10±0.17	3.40±0.20	13.77±0.64
		700	322.29±2.42	80.67±1.46	2.90±0.44	12.97±0.40
		800	310.88±5.48	80.30±0.20	3.07±0.23	12.70±0.78
	13	600	242.89±1.45	80.30±1.79	3.17±0.21	13.03±0.35
		700	241.98±4.75	81.27±1.29	2.83±0.47	12.13±0.92
		800	245.33±10.84	81.77±1.50	2.73±0.31	12.67±0.86
	16	600	224.69±2.69	81.63±1.15	2.70±0.26	12.40±0.52
		700	221.91±3.00	82.20±1.05	2.67±0.37	12.00±0.89
		800	213.58±4.44	82.00±0.30	2.37±0.12	11.73±0.59
	19	600	205.58±2.84	81.80±0.52	2.80±0.26	13.07±0.81
		700	231.46±8.81	82.43±0.55	2.47±0.35	12.30±0.89
		800	207.09±6.95	82.47±0.50	2.47±0.15	12.33±0.22
2	10	600	326.46±2.33	77.60±1.04	3.70±0.52	13.63±0.55
		700	303.44±9.22	76.70±1.51	3.80±0.53	13.57±0.81
		800	264.10±8.19	75.40±1.08	4.27±0.32	14.57±0.37
	13	600	253.43±2.13	78.73±0.46	3.37±0.21	13.63±0.70
		700	246.74±4.43	78.10±0.62	3.63±0.25	13.93±0.71
		800	236.27±5.34	79.73±0.59	3.00±0.20	12.73±0.15
	16	600	220.45±2.13	81.20±0.26	2.83±0.25	12.43±0.81
		700	207.65±8.25	81.10±1.17	3.00±0.17	12.90±0.72
		800	197.37±6.42	81.13±1.21	2.67±0.72	12.63±1.27
	19	600	215.40±6.51	81.20±0.70	2.87±0.06	12.90±0.10
		700	221.29±1.21	82.23±0.35	2.40±0.24	12.23±0.32
		800	18994±7.93	82.50±0.56	2.37±0.28	12.07±0.49

* means significant at $p < 0.05$

Table 4.7 Effects of salt, moisture content and microwave power on the puffing qualities of puffed rice.

Source of variation	df	F-value							
		Puffed Yield (%)			Expansion volume	Hardness	Color		
		Total puffed	Fully puffed	Small puffed			L*	a*	b*
A	1	103.87*	128.29*	362.15*	49.35*	10.16*	72.16*	21.64*	10.18*
B	3	178.87*	667.91*	160.34*	34.67*	203.20*	61.49*	30.53*	11.44*
C	2	16.67*	48.19*	16.70*	12.79*	14.90*	0.96	3.10*	2.73
AB	3	29.95*	5.00*	8.74*	2.37	3.34*	14.32*	4.83*	2.07
AC	2	10.82*	8.39*	9.12*	2.69	4.30*	1.49	1.55	1.87
BC	6	1.76	12.65*	19.84*	2.17	3.44*	2.29*	1.81	0.67
ABC	6	4.26*	13.39*	11.99*	2.37*	0.35	1.00	1.01	1.62

Note: 1. A, salt solution, B, moisture content, C, microwave power.

2. * F-value significant at $p<0.05$.

4.2.3 Puffed Yield

Puffed yield is the puffing ability of the paddy rice under a certain puffing conditions. The effects puffing conditions on puffed yield are reported as Total puffed, fully puffed, and small puffed. It was found that 2% salt solution, moisture content and microwave power and their interaction had significantly effect on the total puffed yield, fully and small puffed yield ($p<0.05$) as shown in Table 4.6. Figure 4.6a shows the effects of salt solution and moisture content on total puffed yield of puffed rice puffing at 800 watts. All the moisture content levels of soaking the paddy rice with salt solution showed higher puffed yield than paddy rice soaked with water. Salt has dielectric properties higher than water, it might change the thermal properties of the grain when absorbing microwave power. When the moisture content increased from 10 to 13%, the total puffed yield of both paddy rice soaked with water and salt solution increased. Soaking paddy rice with salt solution gave the total puffed yield higher than soaking with water. Comparing the puffing of the paddy rice soaked with water and the salt solution, at 16% moisture content, the paddy rice soaked with salt solution gave a higher total puffed yield than the one soaked with water. But in the case of puffing paddy rice soaked with salt solution, at 13% moisture content, it had no significant difference with 16% moisture content. However, at 19% moisture content, total puffed yield from both soaking methods decreased significantly. It might be that when the

moisture of grains were higher and husk (lemma-palea) interlocking lose which made it cannot keep enough internal steam pressure or water vapor pressure to puff (Maisont and Narkrugsa, 2009b). Another reason is the starch granules can produce higher absorption and expansion when the moisture increased, the space between husk and grain smaller and also the expansion lower (Srinivas and Desikachar, 1973). For the effects of salt solution and microwave power, every level of microwave power used in puffing, the puffed rice soaked with salt solution gave higher total puffed yield than the puffed rice soaked with water. When puffing at higher microwave power, the total puffed yield also increased apparently.

For fully and small puffed yield, the effects of salt solution and moisture content show in Figure4.6b and c. Soaking paddy rice with salt solution was higher the fully puffed yield than soaked with water. At 10% moisture content, the fully puffed yield was less than the small puffed yield significantly ($p<0.05$). While the moisture content increased from 10 to13%, 16 and 19%, the fully puffed yield both from the two soaking methods increased more than the small puffed yield. The results agreed to the research of Murugesan and Bhattacharya (1986). They described that at very low moisture content, most of the puffed grains remained cylindrical shape without opening up or popping, probably lack of adequate steam pressure or water vapor pressure needed for bursting. The rice soaked with salt solution and puffed at 600 watts, gave lower fully puffed yield than the one soaking with water. But paddy rice soaked with salt solution puffed at 800 watts, the fully puffed yield was higher than the paddy rice soaked with water. While puffed at 700 watts, the fully puffed yield both from the methods had no difference.

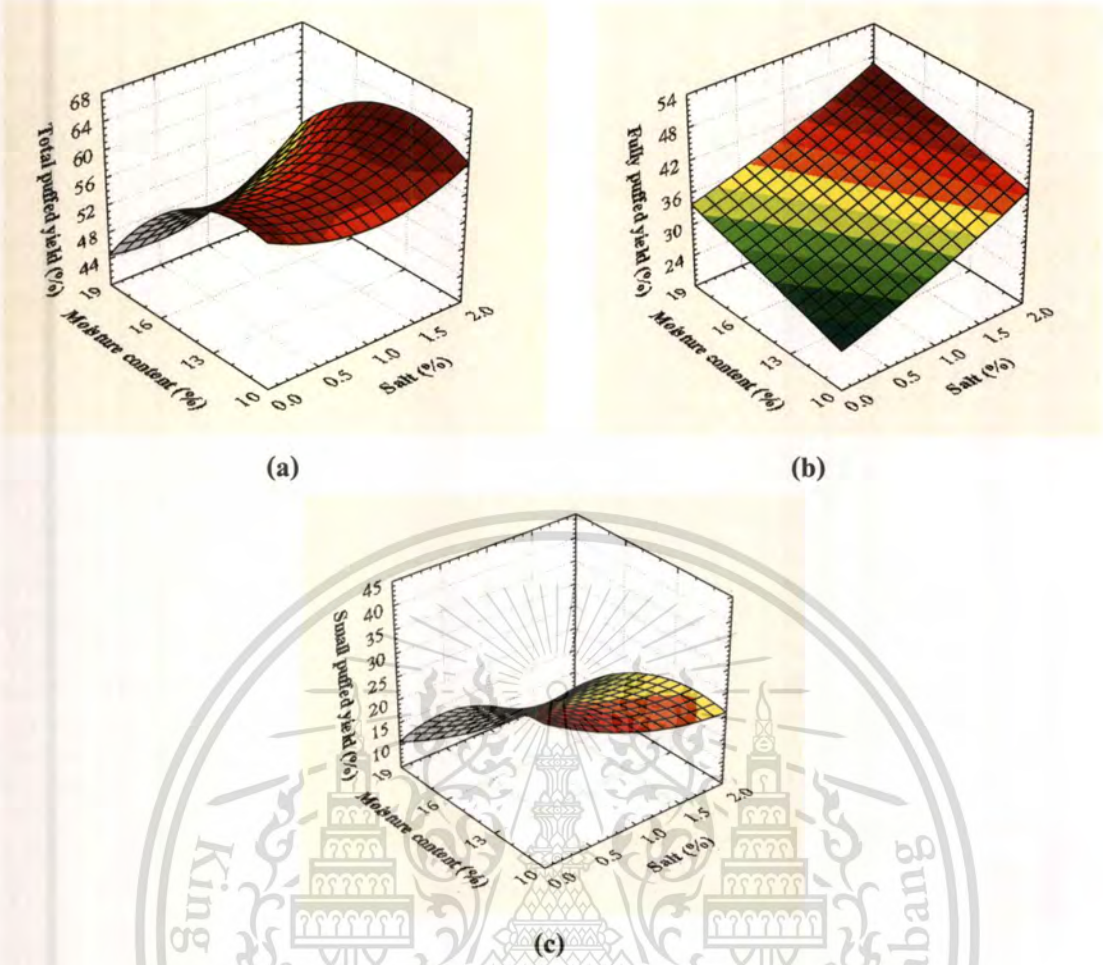
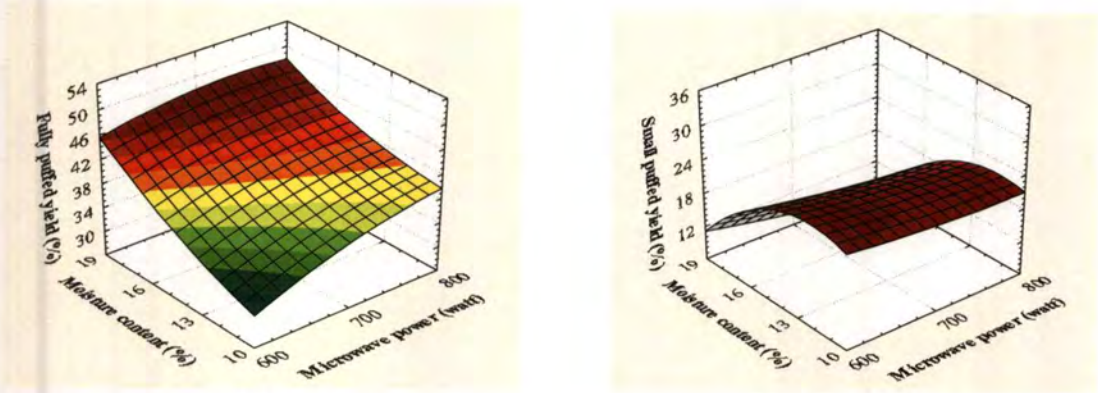


Figure 4.6 Effects of salt solution and moisture content on puffing ability or puffing yield at microwave power 800 watts (a) total puffed yield (b) fully puffed yield and (c) small puffed yield.

The effects of moisture content and microwave power was shown in Figure 4.7a and b. When the moisture increased from 10 to 13, 16, and 19% and puffing at all watt power levels, the fully puffed yield increased and small puffed yield decreased. This is because of at a high moisture level, the starch granules had more water absorption and more volume expansion, resulting in higher internal water vapor pressure. The moisture content range between 13-16%, the paddy rice soaked with water puffed at 600 watts gave higher fully puffed yield than those puffed at 700 and 800 watts. While paddy rice soaked with salt solution and puffed at 800 watts gave higher fully puffed yield than those puffed at 600 and 700 watts. Interestingly, paddy rice soaked with water and salt solution at 19% moisture content puffed at 700 watts, the fully puffed yield from both soaking methods was the highest.

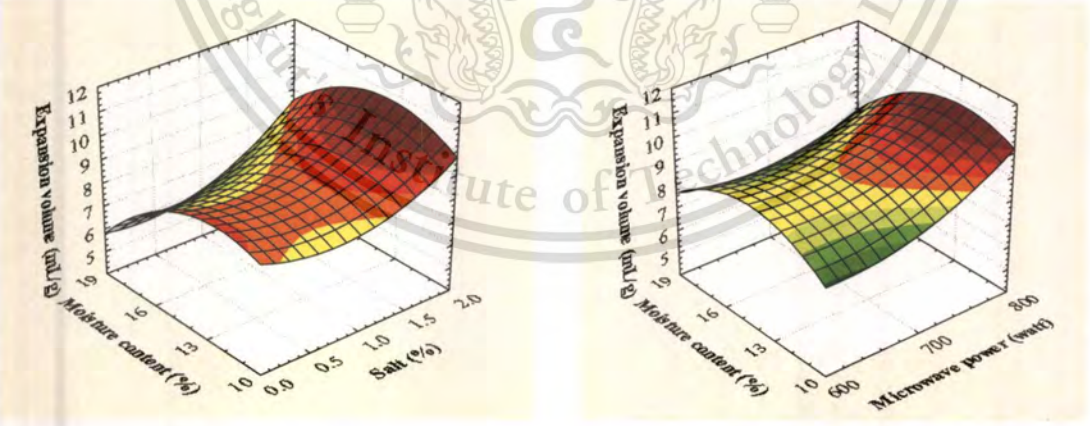


(a) **(b)**

Figure 4.7 Effects of moisture content and microwave power on (a) fully puffed yield and (b) small puffed yield of puffed rice soaked with 2% salt solution.

4.2.4 Expansion volume

The expansion volume of puffed rice is expressed in the degree of expansion when paddy rice was puffed. Salt solution, moisture content, microwave power and interaction between salt solution, moisture content and microwave power significantly affected on the expansion volume ($p<0.05$). Figure 4.8a shows the effects of salt solution and moisture content on expansion volume of puffed rice puffing at 800 watts.



(a) **(b)**

Figure 4.8 Effects of salt solution and moisture content (a) on expansion volume of puffed rice puffing at microwave power for 800 watts and effects of moisture content and microwave power (b) on expansion volume of puffed rice soaked with 2% salt solution.

Comparing paddy rice soaked in salt solution to the one soaked in water, the expansion volume of that soaked in salt solution was more than the one soaked in water. The expansion volume of puffed rice increased when the moisture content increased from 10 to 13%, but decreased when the moisture content increase from 16 to 19%. For the effects of salt solution and microwave power shown in Figure 4.8b, puffing at every level of microwave power, the paddy rice soaked with salt solution gave a higher expansion volume than the rice soaked with water. Puffing at 600 and 700 watts, the expansion volume was not different while puffing at 800 watts, the expansion volume was higher than 600 and 700 watts significantly ($p<0.05$). Comparing when puffing with hot air as reported by Simsrisakul (1991), puffed rice from puffing with hot air at 220°C and 250°C, gave the expansion volume higher than puffing at 280°C. Moisture content plays an important role on the expansion volume of puffed rice. The moisture present in the paddy rice is converted to superheated vapor, providing a driving force for expansion (Chinnaswamy and Bhattacharya, 1983). Thus, in this case, microwave puffing of paddy rice, not only the moisture content but also microwave power and salt solution played the important roles on the expansion volume of puffed rice.

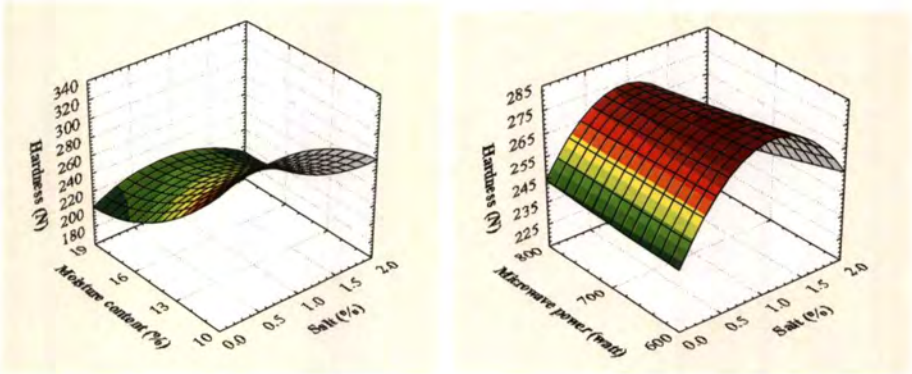
4.2.5 Hardness

The texture of puffed rice is described by hardness, the maximum peak force generated during the breaking test. It was found that salt solution, moisture content, microwave power and their interaction had significant effect on hardness of puffed rice ($p< 0.05$). Figure 4.9a, shows the effects of salt solution and the moisture content on the hardness of puffed rice at a microwave power 800 watts, the paddy rice soaked with salt solution showed less hardness than the paddy rice soaked with water. When the moisture of paddy rice was higher, the hardness of puffed rice trended to be steadily decreased for both soaking with water and salt solution In general, when the moisture of starch increases, the ability in expanding of starch granule is better. Decreasing of hardness found at the higher moisture contents. This was agreed with the report of Phuaksawat, (2002) that the decreasing of the hardness of puffed rice produced at moistures ranging from 10 to 20%.

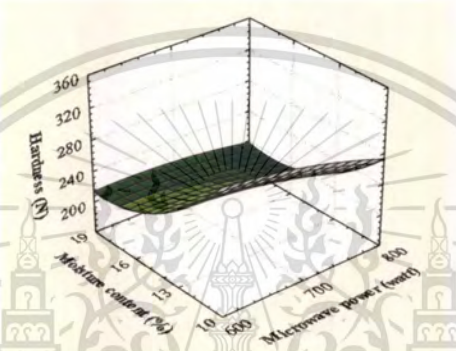
Figure 4.9 shows the effects of salt solution and microwave power. It was found that puffing the paddy rice soaked with salt solution and puffed at 800 watts, the puffed rice was the lowest hardness, while the rice puffed at 700 watts and 600 watts were harder, respectively. In contrast, puffing the paddy rice soaked with water at 600 watts, puffed rice was on the lowest

hardness and at 800 watts and 700 watts were harder respectively. The paddy rice soaked with salt solution and puffing with high microwave power induced the puffed rice to expand well, thus hardness of the puffed rice was low.

Figure 4.9c shows the effects of the moisture content and microwave power, puffing paddy rice soaked with water at moisture content 10%, the puffed rice had a lower hardness. In contrast, the paddy rice soaked with salt solution and puffing at the low microwave power, the puffed rice had higher hardness. Even though the moisture was higher, the hardness trended to change in the same manner. Thus, when puffed paddy rice with the low microwave power had a chance in occurring the case hardening at the aleuron layer, affecting the hardness of puffed rice. At high moisture, salt solution worked as an electrolyte which absorbed microwave power and induced heat transfer better than water. The correlation between the hardness and the expansion volume of the puffed rice was that at high expansion volume, the hardness of puffed rice was lower, the expansion volume was higher. This indicated that there was larger volume of air cells inside the individual puffed rice kernel.



(a) (b)



(c)

Figure 4.9 Effects of puffing parameters on puffed rice hardness:

(a) salt solution and the moisture content (puffing at microwave power 800 watts)

(b) salt solution and microwave power (at moisture content 13 %)

(c) moisture content and microwave power (soaked with 2% salt solution)

4.2.6 Color

It was found that salt solution, moisture content, and the interaction between salt and moisture content, moisture content and microwave power had significant effect on lightness (L*). Salt solution, moisture content, microwave power and the interaction between salt and moisture content had significant effect on redness (a*). While only Salt solution and moisture content had significant effect on yellowness (b*) of the puffed rice as shown in Table 4.6 and 4.7.

Figure 4.10a shows the effects of salt solution and moisture content on lightness. The lightness of the rice soaked with salt solution and puffed at microwave power of 800 watts, was lower than the puffed rice soaked with water. But when the moisture increased from 10 to 13%, lightness trended to be higher in the same direction. At 16 and 19% moisture content, lightness of

puffed rice were not different. Figure 4.10b and 4.10c show the effects of salt solution and moisture content on redness and yellowness of puffed rice puffed at 800 watts of microwave power. Redness and yellowness of puffed rice trended to change as in lightness. The paddy rice soaked with salt solution, the redness and the yellowness value was higher than the puffed rice soaked with water. When the moisture increased, the redness and yellowness decreased. The lowest values were found at 19 % moisture content. The lower moisture content and longer heating time significantly increased the browning reaction, which contributed the lower lightness value and higher the redness and the yellowness value of the puffed rice.

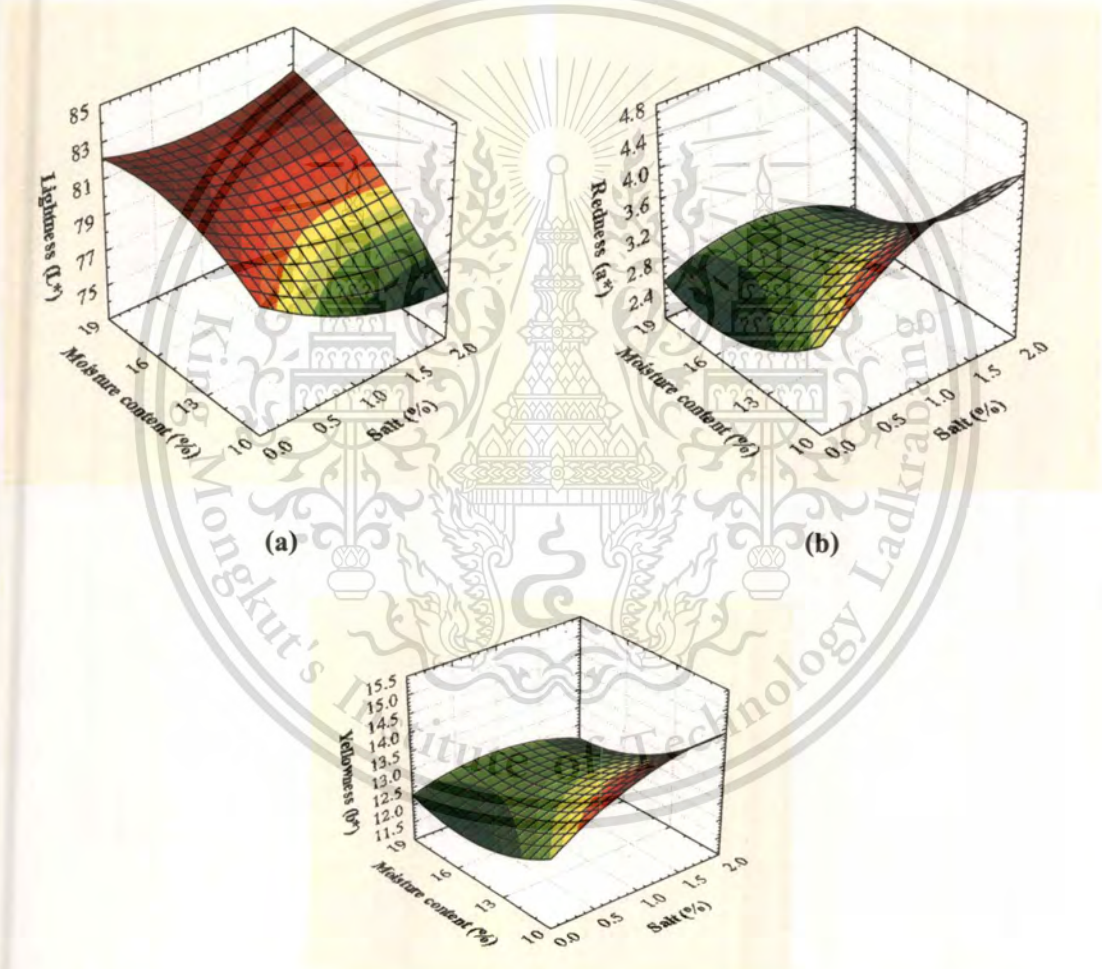


Figure 4.10 Effects of salt solution and moisture content on (a) lightness (b) redness and (c) yellowness of puffed rice puffing at microwave power of 800 watts.

4.2.7 Microstructure of Puffed Rice

The microstructure of puffed rice in Figure 4.11a-d and 4.12e-h, were taken from the paddy rice soaked with water and salt solution at different moisture contents; 10, 13, 16 and 19%, puffed with microwave at 800 watts. The microstructure of the puffed rice from the paddy rice soaked with water had smaller air cells than the paddy rice soaked with salt solution. At 10% moisture content, the microstructure of the two soaking methods was slightly different. However, the size of the air cells in puffed rice kernels seemed to be closely correlated with moisture content. The air cells were smaller in the puffed rice from the lower moisture content rice (Figure 4.11a and 4.12e). The size of the holes on the surface was lager when moisture increased. Besides, the air cell holes were thin. However, at 19 % moisture content, the size of the air cell holes were the largest and more deformed. These results agreed with Jin et al. (1995). They reported that when the extrudate was more expanded, the air cell holes were larger and cell walls were thinner, but when the extrudate was less expanded the air cell were small and the cell wall were thicker. Moraru et al. (2003) proposed that puffing phenomena of the product resulted from the vaporization of superheated water. The simultaneous flash-off of vapor expands the grains, resulting in the porous, sponge like structure within the product. Therefore, moisture content plays an important role on the texture of puffed rice.

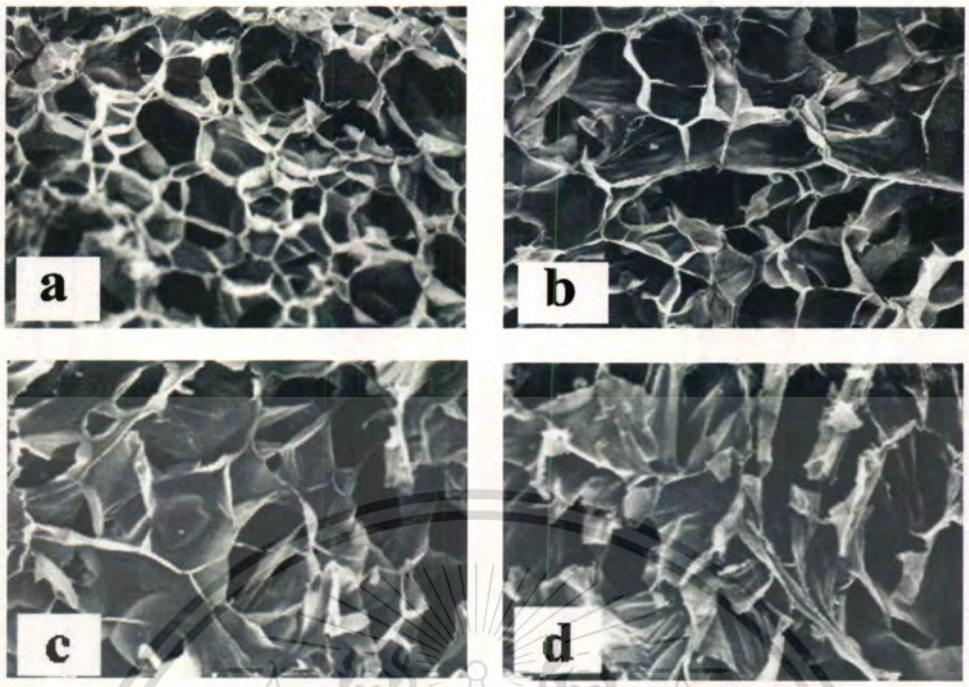


Figure 4.11 Scanning electron micrographs of puffed rice made from at (a) 10, (b) 13, (c) 16 and (d) 19% moisture content of paddy rice soaked with water puffing with microwave power 800 watts.

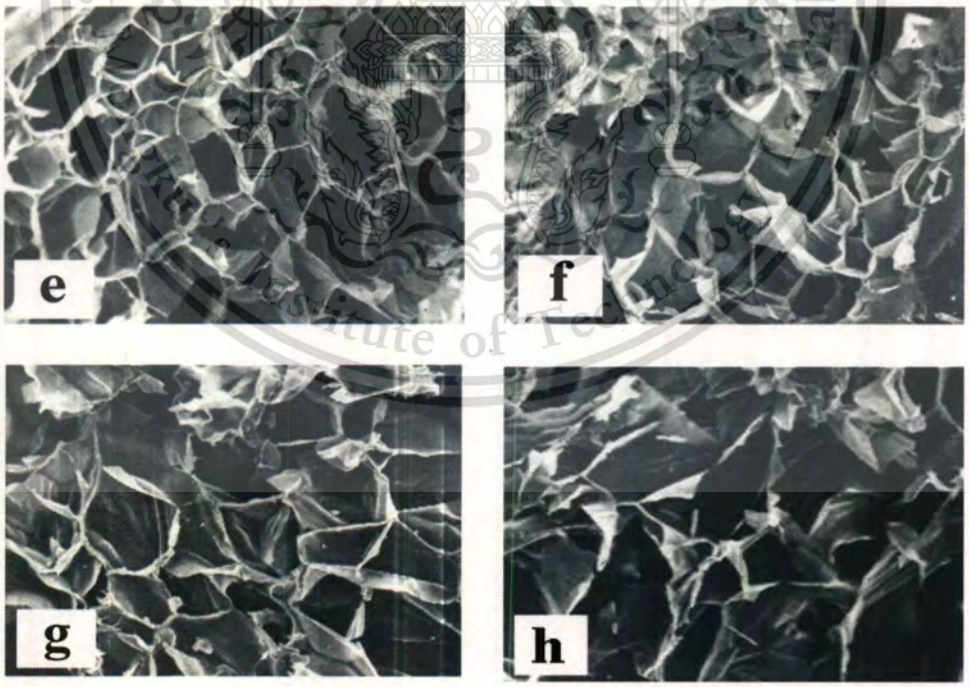


Figure 4.12 Scanning electron micrographs of puff rice made from at (e) 10, (f) 13, (g) 16 and (h) 19% moisture content of paddy rice soaked with salt solution and puffing with microwave power 800 watts.

Table 4.8 Pearson's correlation coefficients (r) between the puffing factors and the qualities of puffed rice.

Puffing factors	Puffing qualities of puffed rice							
	Puffed Yield (%)			Color			Expansion Volume	Hardness
	Total puffed	Fully puffed	Small puffed	L*	a*	b*		
Salt solution	0.487**	0.699**	-0.325**	-0.440**	0.327**	0.289*	0.430**	-0.120
Moisture content	-0.484**	0.460**	-0.701**	0.692**	0.661**	-0.484**	-0.376**	-0.855**
Microwave power	0.208	0.195	-0.047	0.068	-0.174	-0.198	0.278*	-0.194

Note: 1. * means significant at $p<0.05$

2.** means significant at $p<0.01$

Table 4.8 presents the correlations between the puffing factors and the qualities of the puffed rice. Salt solution had high positive relation to the total puffed yield, the fully puffed yield, the expansion volume a* value and b* value while in the small puffed yield and L* value had a negative relation. In contrast, the moisture content trended to have a negative relation to the total puffed yield, small puffed yield, a* value, b* value, expansion volume and hardness of the puffed rice. However, the result illustrated that the fully puffed yield and L* value had positive relation. For microwave power of puffing had only positive relation to expansion volume.

It could be concluded that salt, moisture content and microwave power affected the puffing qualities of the puffed rice. The paddy rice soaked with salt solution gave higher total puffed yield and expansion volume. But, at low moisture (10%) and puffed with high microwave power lower the lightness, while the redness and yellowness value were higher. The moisture content was the most important factor affects the puffing qualities. The suitable moisture content is 13% which produced a puffed rice having the highest puffed yield and the highest expansion volume, with low the hardness. At low moisture content (10%), the small puffed yield was significantly higher than the fully puffed yield. At high moisture content (19%), the puffed yield and the expansion volume were lower, especially with the puffed rice soaked with water. This relation was shown in the microstructure of the puffed rice. Puffed with microwave power at 800

watts, the rice of the highest expansion volume was obtained while puffed at 600 watts gave the rice with the lowest the expansion volume.

4.3 Effect of Germination Process on GABA Content Chemical Compositions, Total Phenolics and Antioxidant Capacity of Waxy Paddy Rice.

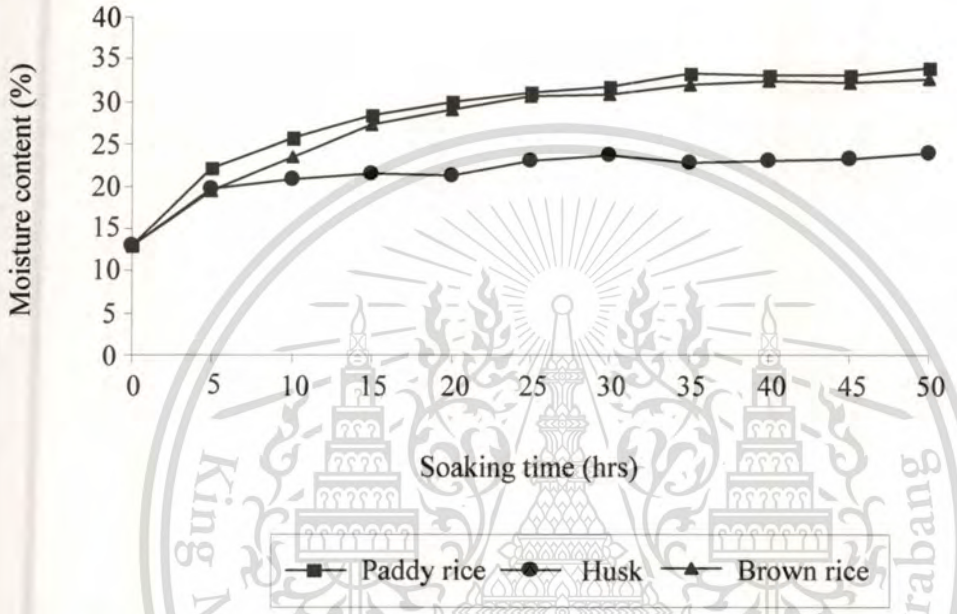


Figure 4.13 Moisture content of paddy rice, husk and brown rice during soaking in water for 0-50 hrs.

4.3.1 Water Hydration of Paddy Rice

Figure 4.13 shows the hydration characteristics of paddy rice, husk and brown rice that were separated from paddy rice. In the early stage of soaking, water absorption rate of the paddy rice rapidly increased until at 15 hrs, the water absorption rate gradually decreased. The initial moisture content of paddy rice is 12.68%. After soaking paddy rice, the moisture content increased to 22.10, 25.78 and 28.31%, when soaked for 5, 10 and 15 hrs respectively. The difference between moisture content of paddy rice and brown rice soaked for the same period of time were 2.67, 2.35 and 1.12% respectively. Soaking more than 20 hrs, the moisture content of the paddy rice and the brown rice were nearly the same. Husk is the barrier for protecting water penetration into paddy grain in the early stage of soaking. After water penetrated through husk of the paddy grain, it was stored spaces between husk and brown rice. Water did not immediately

penetrate into endosperm because of the pericarp and the seed coat (Figure 4.16b and c) (Bello et al., 2004 and Abhay et al., 2006). The first area of which the water penetrate into was the ventral site of embryo and then diffuse through endosperm (Hwang et al., 2009). When soaking time increased, the moisture content of paddy rice, husk and brown rice was up to the equilibrium (>35 hrs): 33.25% for paddy rice, 22.75% for husk and 32.32% for brown rice. The moisture content of the rice grain had a relation with the germination percentage and the germination quality (Puangwerakul, 2007). Lamkin et al. (1983) reported that percentage of germination had a high significant correlation (r=0.916***) with the activity of glutamic acid decarboxylase (GAD) in barley. Paddy rice could germinate at 18-40% moisture content or after soaking for 12-60 hrs. The difference of soaking time and moisture content depend on the paddy rice varieties (Puangwerakul. 2007). besides, if soaking temperature was high, water absorption would be more. However, It depends on the propose of soaking. Moisture content of the paddy rice would cause rice to be anoxia or water stress condition which inducing the accumulation GABA content and changing other constituents.

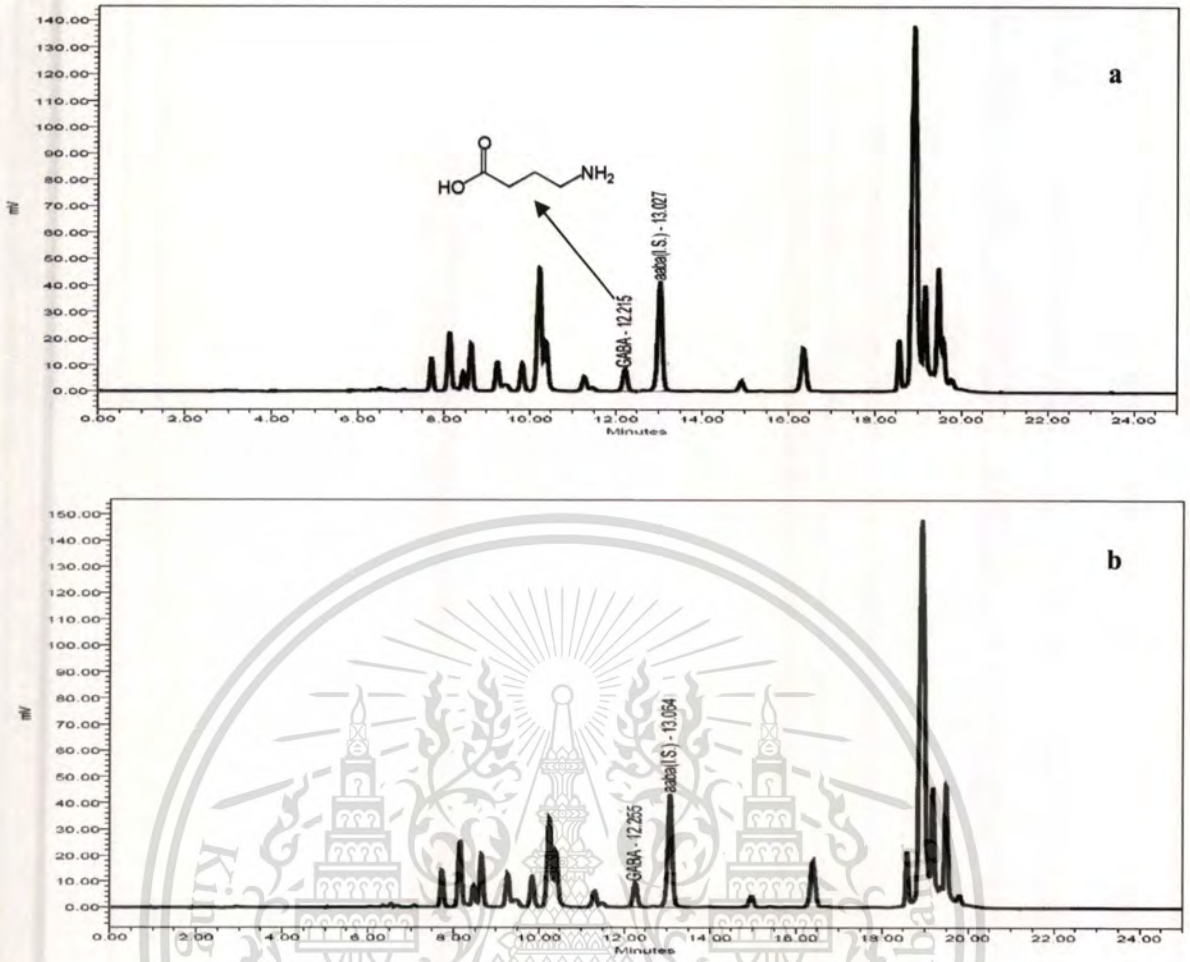


Figure 4.14 Chromatograms of standard GABA 25pmol (a) and paddy rice germ germinated 24 hrs (b).

4.3.2 GABA Content

Embryos of the paddy rice and the germinated paddy rice were determined for the GABA content with HPLC, the chromatogram as shown in Figure 4.14 The GABA content of the embryo of paddy rice could not be detected because of the amount was lower than the minimum value of the standard curve (1-100 pmol). While the paddy rice soaked for 50 hrs, the GABA content increased up to 60 mg/ 100 g(fresh weight) (Figure 4.15). This result indicated that soaking had a contribution in increasing the GABA content. Similar results had been reported for soaked rice germ (Varanyanond et al., 2005; Choi et al., 2006) and brown rice (Saikusa et al., 1994; Ohtsubo et al., 2005; Komatsuzaki et al., 2007). Correspond with Howell et al. (2009) which reported that one hour after rice embryo soaking, the mapping metabolic transcript levels rapidly changes the metabolism, including the increase of hexose phosphates, tricarboxylic acid

cycle (TCA) intermediates, and γ-aminobutyric acid. Later changes in the metabolic, includes those involved in carbohydrate, amino acid and cell wall metabolism. The increase in GABA content during soaking is due to the activation of glutamate decarboxylase (GAD) that catalyzes the decarboxylation of L- glutamic acid to carbon dioxide and GABA cause the glutamic acid to decreased (Oh, 2003; Komatsuzaki et al., 2007). Moreover, soaking could lead to anoxia (Reggiani et al. 1990; Dewar et al., 1997) and suspension cells adapted to water stress (Rhodes et al., 1986). Tissues stress and anoxia, which reduce respiration and the NAD/NADH ratio, can restrict the production of succinate. Such stress may also contribute to the accumulation of GABA by reducing the oxidation of succinic semialdehyde to succinate. Following the removal of the stress, GABA provides an immediate substrate for the Krebs cycle (Wallace et al., 1984).

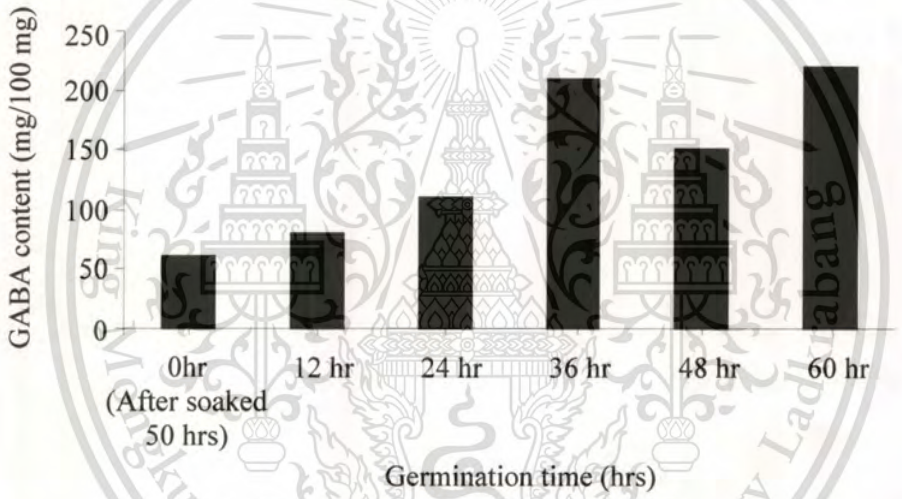


Figure 4.15 GABA content of paddy rice embryos after soaked and various germination time.

In the germination, it was found that the GABA content continually increases with germination time. Germination of paddy rice at 12-60 hrs, GABA content increased from 80 to 220 mg /100 g (fresh weight) (Figure 4.15). The increase of GABA content during germination might be due to the seeding growth and development of a plant (Shelp et al., 1999). Bown et al., (1997) suggested that GABA accumulation and efflux are part of an intercellular signal transduction pathway leading to the regulation of growth and development. GAD is one of the most abundant soluble proteins in plants with a Ca^{+}/Calmodulin-binding domain (Baum et al., 1993; 1996). It was found in all plant tissues, and its level is regulated during development by transcription or posttranscriptional processes (Chen et al., 1994). Baum et al. (1996) reported that transgenic tobacco plants expressing a mutant GAD that lacks the auto-inhibitory Calmodolin

binding domain- binding domain, exhibit higher GABA levels, lower glutamic acid levels, and less stem elongation. While Chung et al. (1992) suggested that the GABA accumulation accompanied by efflux from the cell, might function as an intercellular signaling molecule. (Bown et al., 1997). Aurisano et al. (1995) reported that in seedling rice under the anaerobic condition for 24 hrs induced an accumulation of 3.5 and 6.3 μmolg (fresh weight) in shoot and root, respectively. Thus, the accumulation of GABA occurred in embryo, as a part of GABA content increased when germinating time was which might be from the part shoot and root (Figure 4.15 and 4.16a).

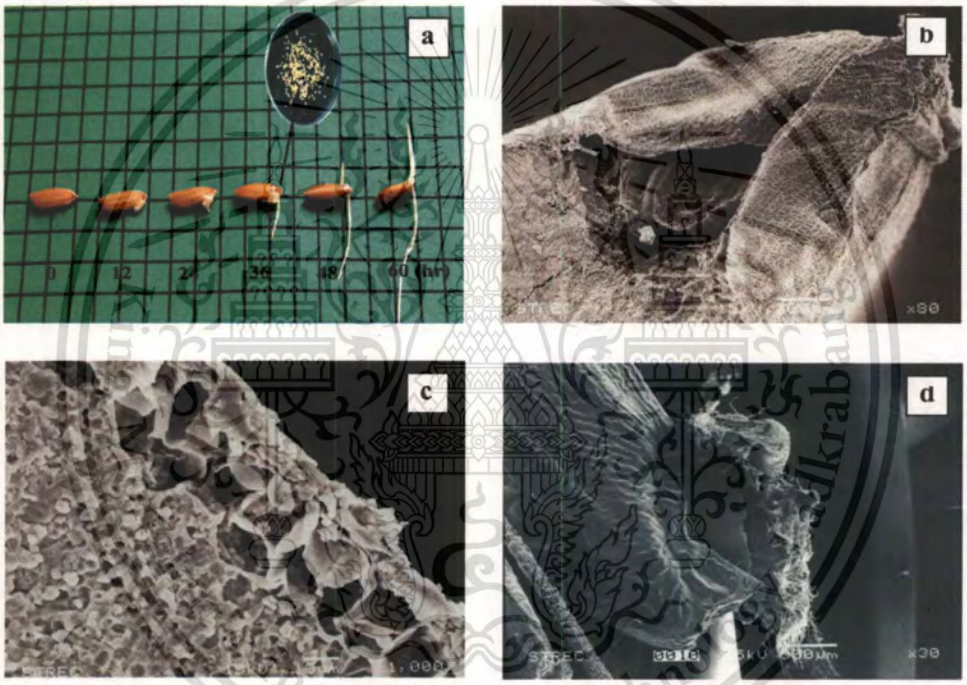


Figure 4.16 The physical characteristic of paddy rice various germination time (a), paddy rice germ (b), aleuron layer at the ventral site(c) and germinated paddy rice germ at 36 hrs (d).

4.3.3 Chemical Compositions

Table 4.9 The chemical compositions of paddy rice at various germination time.

Chemical compositions (%)	Germination time (hrs)					
	0	12	24	36	48	60
Moisture	8.89±0.14^{d}	8.59±0.10^{c}	8.13±0.13^{b}	7.85±0.10^{a}	8.07±0.10^{b}	8.77±0.08^{cd}
Fat	3.08±0.11^{a}	3.06±0.06^{a}	2.97±0.18^{a}	2.92±0.17^{a}	2.86±0.16^{a}	2.81±0.11^{a}
Protein	6.29±0.06^{d}	6.21±0.09^{d}	6.17±0.11^{cd}	6.04±0.11^{bc}	5.96±0.04^{ab}	5.84±0.09^{a}
Ash	1.26±0.08^{a}	1.29±0.03^{a}	1.37±0.06^{ab}	1.46±0.09^{bc}	1.48±0.03^{bc}	1.56±0.06^{c}
Dietary Fiber	2.05±0.01^{a}	2.07±0.06^{a}	2.51±0.01^{b}	2.69±0.00^{c}	2.83±0.04^{d}	2.92±0.06^{d}
Amylose	6.34±0.06^{c}	6.28±0.23^{c}	6.08±0.25^{c}	5.68±0.15^{b}	5.18±0.06^{a}	5.11±0.20^{a}
Starch	75.72±0.77^{b}	73.34±0.49^{ab}	73.97±0.22^{ab}	72.94±1.04^{ab}	72.39±2.19^{ab}	72.05±1.99^{a}

Note: means in row followed by different superscript are significantly different ($p<0.05$).

The chemical compositions of the samples were presented in Table 4.9, germination process affected the changing of the chemical compositions. The protein, fat, amylose and starch content continually decreased when increasing germination time, especially a significant difference of germination time at 48 hrs ($p<0.05$) was found. While dietary fiber content significantly increased ($p<0.05$) when geminating at 36 hrs. Similar results had been reported for germinated paddy rice (Evelyn et al., 1972; Ayernor et al., 2007) and germinated brown rice (Choi et al., 2006; Watchraparpaiboon et al., 2007). During germination metabolism in the grains rapidly increased due to the action of enzymes leading to the utilization of protein fat and carbohydrate for energy and growth (Evelyn et al., 1972). Ayernor et al. (2007) reported that the decrease in the starch content of the grains during germination was also due to the action of hydrolytic enzymes such as α and β-amylases, which hydrolyze starch into low molecular weight carbohydrates such as maltose, glucose and dextrin.

4.3.4 Total Phenolics and Antioxidant Capacity

Table 4.10 Total phenolics and antioxidant capacity during germination.

Germination time (hr)	Total Phenolics (mg Gallic acid/g db.)	Antioxidant capacity	
		DPPH (mg Trolox/g dry db.)	FRAP (mg Trolox/g dry db.)
After soaking	0.4785 ± 0.03^{a}	0.2712 ± 0.01^{a}	0.6888 ± 0.00^{a}
12	0.4848 ± 0.01^{ab}	0.2714 ± 0.00^{a}	0.6949 ± 0.01^{a}
24	0.5572 ± 0.01^{c}	0.2868 ± 0.01^{ab}	0.7461 ± 0.00^{c}
36	0.6245 ± 0.02^{d}	0.2977 ± 0.01^{b}	0.7592 ± 0.00^{d}
48	0.5307 ± 0.00^{bc}	0.2823 ± 0.0^{ab}	0.7234 ± 0.00^{b}
60	0.6432 ± 0.03^{d}	0.3035 ± 0.01^{b}	0.8570 ± 0.00^{e}

Note: ^{abc} means in column followed by different superscript are significantly different ($p<0.05$).

The total phenolics and antioxidant capacity of germinated paddy rice were presented in Table 4.9. After soaking, the total phenolics content in the germinated paddy rice was 0.4785 mg gallic acid/g which was significantly different ($p<0.05$) from the germination time at 24, 36, 48 and 60 hrs, which similar to the report of Tain et al. (2004). When germination time increased, total phenolics increased as well. The highest increased of total phenolics was 0.6432 mg gallic acid/g found at germination time of 60 hrs. Cereal grains contained with phenolics acids and glycosides, existed in soluble and insoluble form, and bound with polysaccharides at the cell wall. Most of phenolic compounds were insoluble form (Miller et al., 2000). However, in brown rice, its soluble phenolics contain free phenolic acids and hydroxycinnamate sucrose ester consisting of feruloylsurose and sinapolysucrose. But insoluble phenolics contain mostly ferulic acid and ρ-coumaric acid (Sosulski et al., 1982; Adom et al., 2002).

During germination the brown rice with about 70% of feruloylsurose and the sinapolysucrose were decreased whereas the sinapinic acid increased nearly 10 times, and insoluble phenolics, the ferulic acid and ρ-coumaric acid increased at about 1-2 times. Tain et al. (2004) explained that the increase of the free forms phenolics in the brown rice is due to the decomposition of the cell wall during germination. In the case of increasing the insoluble form, it might be intended to increase the availability of hydrolyze insoluble phenolic during the germination (Tain et al., 2004; Adom et al., 2002).

Antioxidant capacity, measured by DPPH radical scavenging activity and ferric reducing ability power (FRAP), estimate an antioxidant capacity based on the reaction of the reagent with

electron donating or hydrogen radical producing antioxidant compounds. Germination time modified the antioxidant capacity as shown in Table 4.9. Both of the methods change the trend during germination as for the antioxidant capacity continually increased with germination time. The antioxidant capacity of germinated paddy rice was directly related to the total phenolics. This result was similar to Velioglu et al. (1998). The capacity of DPPH radical scavenging of the germinated paddy rice range from 0.2712 to 0.3035 mg Trolox/g. The ferric reducing ability power or FRAP of the germinated paddy rice reach the values from 0.6888 to 0.8570 mg Trolox/g. Increasing germination time, scavenging free radical activity of DPPH slightly increased while ferric reducing ability power or FRAP tend to significantly increased ($p<0.05$) during the germination at 24 to 60 hrs. However the antioxidant capacity depended on the chemical structure of substrates that reacted with reagent. Adom and Lui (2002) reported that insoluble phenolics were the major contributor to the antioxidant capacity about 71% in rice.

Rice bran is a rich source of vitamin E, the major components are α-tocopherol, γ-tocopherol, α-tocotrienol, γ-tocotrienol and γ- oryzanol, all components exhibited significant antioxidant capacity in the inhibition of cholesterol oxidation (Xu et al., 2001). Kayahara and Tukahara (2000) reported that during the germination of brown rice, vitamin E (tocopherol, and tocotrienol) increase nearly 4 times. Moreover, Butsat and Siriamornpun (2009) reported that the rice husk showed a greater phenolics concentration than rice barn, brown rice and milled rice. The phenolics might diffuse into the rice grain during soaking, which was a part of increasing antioxidant capacity of germinated paddy rice.

The germination process of waxy paddy rice significantly ($p<0.05$) affected on GABA content, chemical compositions, total phenolics and antioxidant capacity. The GABA content, dietary fiber, total phenolics and their antioxidant capacity dramatically increased during the germination. While fat, protein, amylose and starch contents slightly decreased. The results suggested that the germinated paddy rice could be considered as a source of GABA and dietary fiber with a high antioxidant capacity.

4.4 Effect of Germination Process on Puffing Qualities and GABA Retention of Germinated Puffed Rice by Microwave Heating.

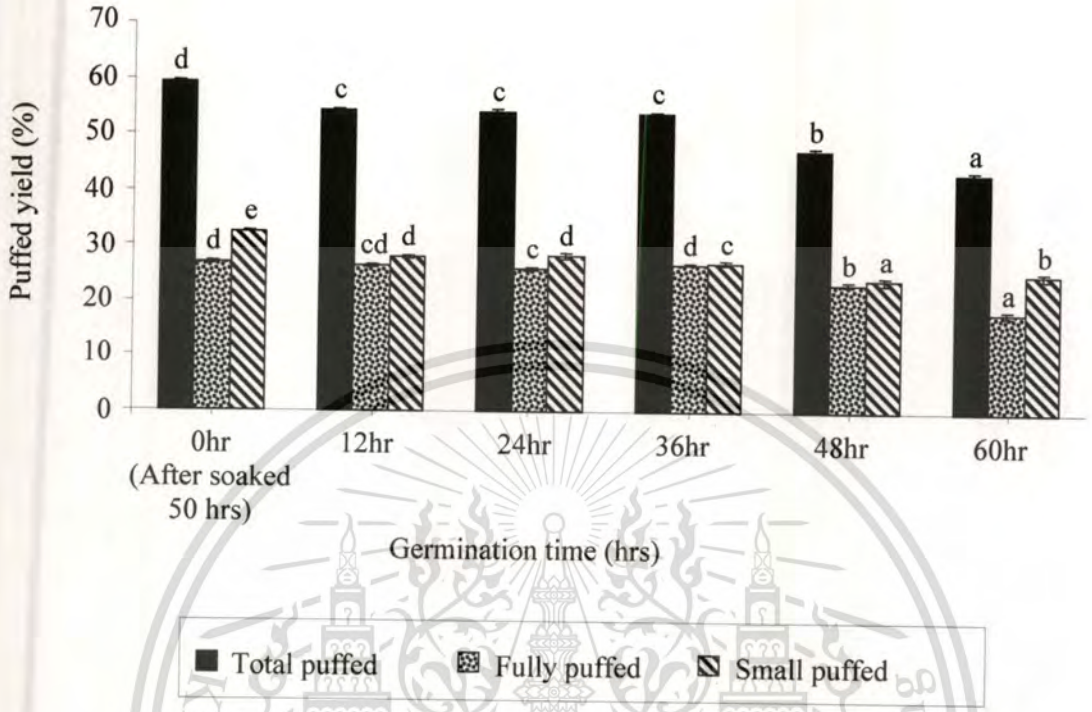


Figure 4.17 Total puffed yield, fully puffed yield and small puffed yield of germinated paddy rice various germination time.

4.4.1 Puffed Yield

It was found that germination time affected the total puffed yield, fully puffed yield and small puffed yield of the germinated puffed rice as shown in Figure 4.17. Total puffed yield trended to decrease with increasing germination time. Total puffed yield of germinated puffed rice produced from germinated paddy rice at 12, 24 and 36 hrs was significantly different ($p<0.05$) and different from the germinated puffed rice produced from the germinated paddy rice at 48 and 60 hrs. During soaking and germination of paddy rice, water started penetrating into the rice kernel, the enzymes (amylase, protease, phytase, and β-glucanase) were produced or activated, which might degrade components such as starch and protein. This made the physical structure change during the increasing germination stage (Figure 4.18a-l) and markedly changed at 48 and 60 hrs as shown in Figure 4.18 e, f, k and l. The action of enzymes directly affected the physical structure and chemical of rice such as the husk (lemma-palea) inter locking (Figure 4.18a-l) and starch. These reasons might be the important factors of decreasing the total puffed

yield, corresponding with Mariotti et al. (2006) mentioned that the effect of puffing was strongly influenced by the morphology and the composition of kernel.

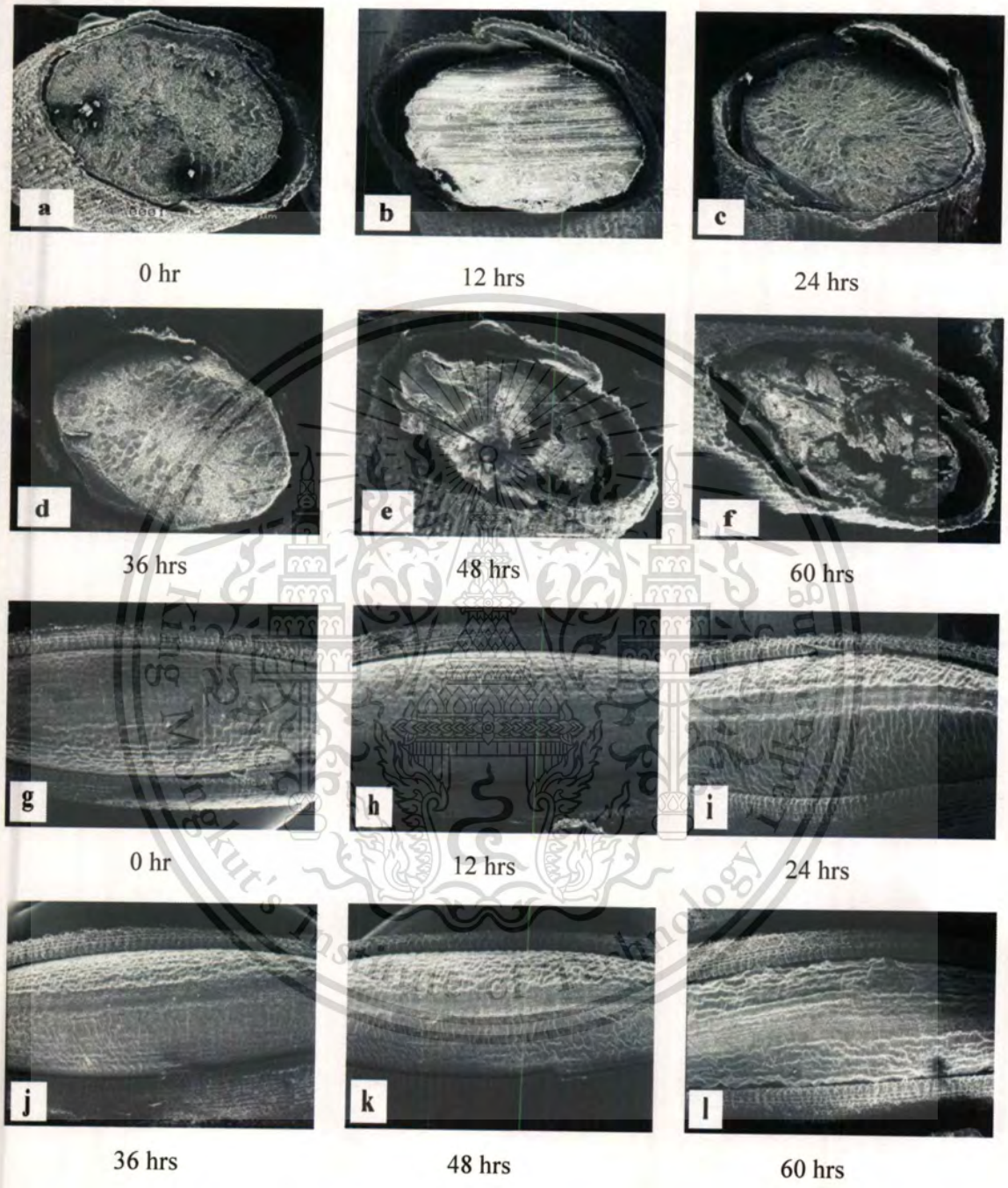


Figure 4.18 The SEM image of cross section 500 μm x 40 (a-f) and longitude section 500 μm x 30 (g-l) of germinated paddy rice at 0-60 hrs.

Our previous work supported the result of husk (lemma-palea) inter locking, because it hold an internal steam pressure until the threshold level required to expand the grain. So, the loose husk directly affected the capacity to maintain vapor pressure before the kernel exploded. For the ratio of fully puffed yield and small puffed yield were different when the germination time increased. All of the germinated puffed rice had small puffed yield than fully puffed yield, and trended to increase with increasing germination time. The above reasons dealt with the ratio of fully puffed yield and small puffed yield. Besides, the shape of germinated puffed rice was observed and compared in our previous work, the shape of fully puffed was smaller than the puffed rice with adjusted moisture content by spraying method. Small puffed yield, its shape was not cylinder and the surface was not smooth and crack. On the other hand Maisont and Narkrugsa (2009b) reported that the ratio of fully puffed yield and small puffed of puffed rice depended on the moisture content in rice kernel before puffing, high moisture content produced a lot of fully puffed yield, because moisture directly converted to steam and pressure vapor for driving force inside kernel explodes. In the case of the moisture content of the germinated paddy rice adjusted at the same level (13% mc.) might be caused from the changes of physical structure and chemical components.

4.4.2 Expansion Volume

The expansion volume of germinated puffed rice was the indicator in showing the effect of germination of germinated puffed rice. The expansion volume decreased when increasing the germination time, especially significantly decreased ($p<0.05$) at 36 hrs. Hence, the changes of physical structure of paddy rice affected expansion volume. Comparing the physical structure of germinated paddy rice at 0, 48 and 60 hrs, as showed in the Figure 4.18a-l. Loose of husk inter-locking and the damaged aleuron layer, they led the kernel not able to keep enough vapor to produce high pressure for exploding the rice kernel. It was known that during germination, the starch was degraded by the action of enzymes. Figure 4.18e and f shows the characteristic of starch granules of germinated paddy rice at 48 and 60 hrs, respectively. At 48 and 60 hrs germinated paddy rice, the partial of starch granules were damaged the loose compact and became holes, which it might the cause to change the starch in the kernel. Schwartberg et al. (1995) mentioned that starch as the major polymer responsible for whole cereal kernel expansion. Assuming that melted starch acts as pseudoplastic power-law fluid and used a mathematical model to describe the bubble expansion in moltent starch or the porous size of the puffed

structure. Degradation of starch granules were responsible to reduce starch swelling (Ali and Bhattacharya, 1980).

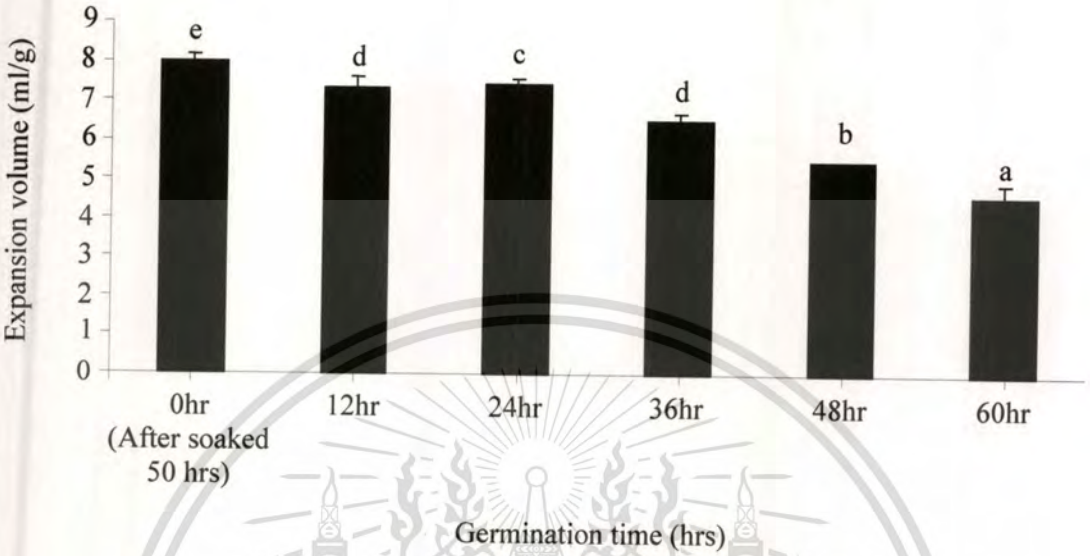


Figure 4.19 The expansion volume of germinated puffed rice various germination time.

4.4.3 Hardness

The hardness of germinated puffed rice was shown in Figure 4.20. The hardness of the non-germinated puffed rice was significantly different from the germinated puffed rice ($p<0.05$). The hardness of the germinated puffed rice significantly decreased. The porous, number, size and thickness of the structure affected on puffing of the whole cereal kernel (Schwartzber et al., 1995). Thus, the characteristic of germinated puffed rice; such as shape, size, the ratio of fully puffed and small puffed; contributed to the hardness too. Due to unfirmness of both the starch granules and the husk interlocking during germination, especially at 48 and 60 hrs, water vapor inside the kernel could easily vent out. It could be said that the texture of puffed rice produced from short germination time was harder than long germination time.

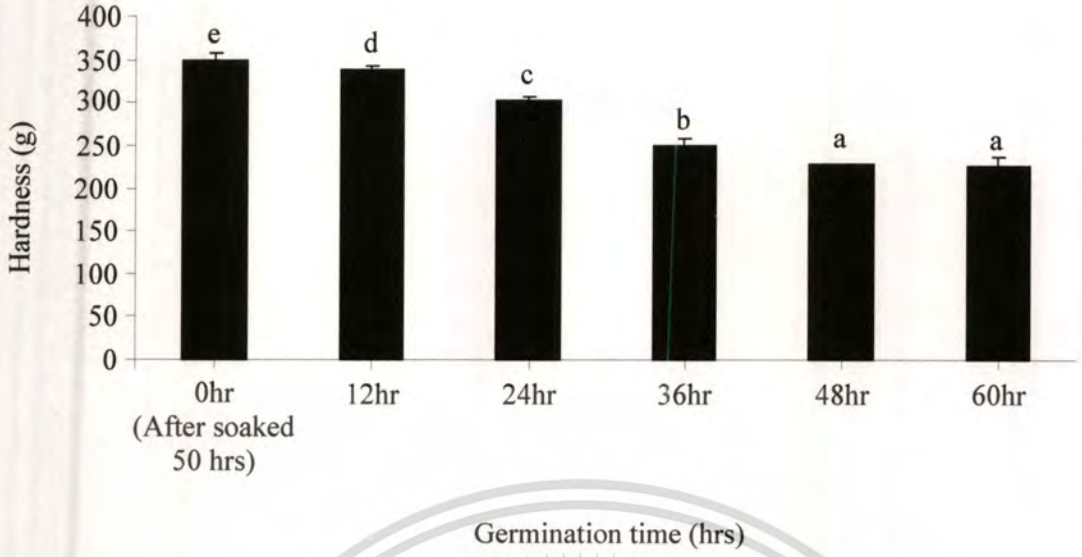


Figure 4.20 The hardness of germinated puffed rice at various germination time.

4.4.4 Color

It was found that germination times had significantly affected color of germinated puffed rice including the L*, a* and b* values as shown in Figure 4.21. The L* values or brightness of puffed rice was decreased during germination time. On the other hand a* value and b* values increased. During germination time the components of rice grain changed, especially increasing of reducing sugar (Ohtsudo et al., 2005; Ayernor and Ocloo, 2007). Germinated brown rice at 24 hrs, fructose and reducing sugar increased 3.4 and 2.75 times, respectively (Choi et al., 2006) and germinated brown rice (KDML105) at 96 hrs, reducing sugar increased 5 times (Trakulpiboonchai, 2006). During microwave puffing, the germinated paddy rice was highly heated (173-197°C), there was a chance to combine other components such as protein and reducing sugar leading occur browning reaction, this browning effect contributed to decrease in brightness while increasing in the redness and the yellowness of germinated puffed rice.

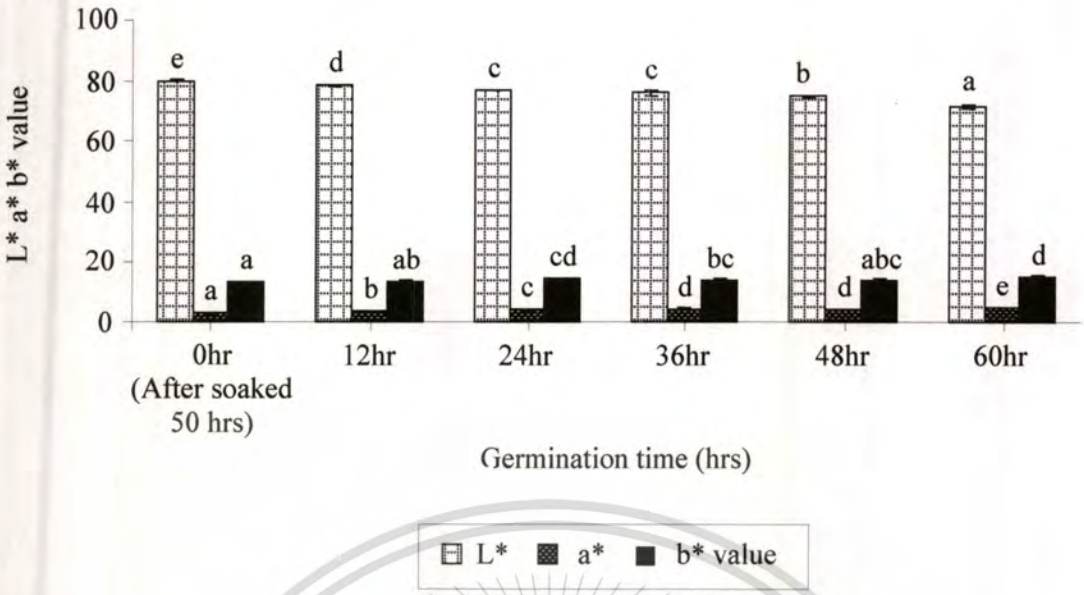


Figure 4.21 The L* a* and b* values of germinated puffed rice from various germination time.

4.4.5 Water Absorption (WAI) and Water Solubility (WSI) Index

The WAI and WSI characteristics of germinated puffed rice were showed in Figure 4.22. WAI of germinated puffed rice was less than the WAI of non-germinated significantly different ($p<0.05$). Increasing germination time WAI of germinated puffed rice trended to decrease respectively. While WSI of germinated puffed rice at 12 and 24 hrs slightly decreased comparing to non-germinated puffed rice. WSI trended to significantly increase ($p<0.05$) at 36, 48 and 60 hrs. Anderson et al. (1969) mentioned that WAI, measured the amount of water absorbed by starch. The germinated puffed at 36, 48 and 60 hrs was measured WAI which has low value, this means that the starch was low as well. On the other hand, Kirby et al. (1988) indicated that WSI, measured the amount of soluble components released from starch, and often used an indicator of degradation of molecular components. The germinated puffed rice at 36, 48 and 60 hrs was measured WSI which has high value. This indicated that it has more soluble components during germination such as reducing sugars and free sugars.

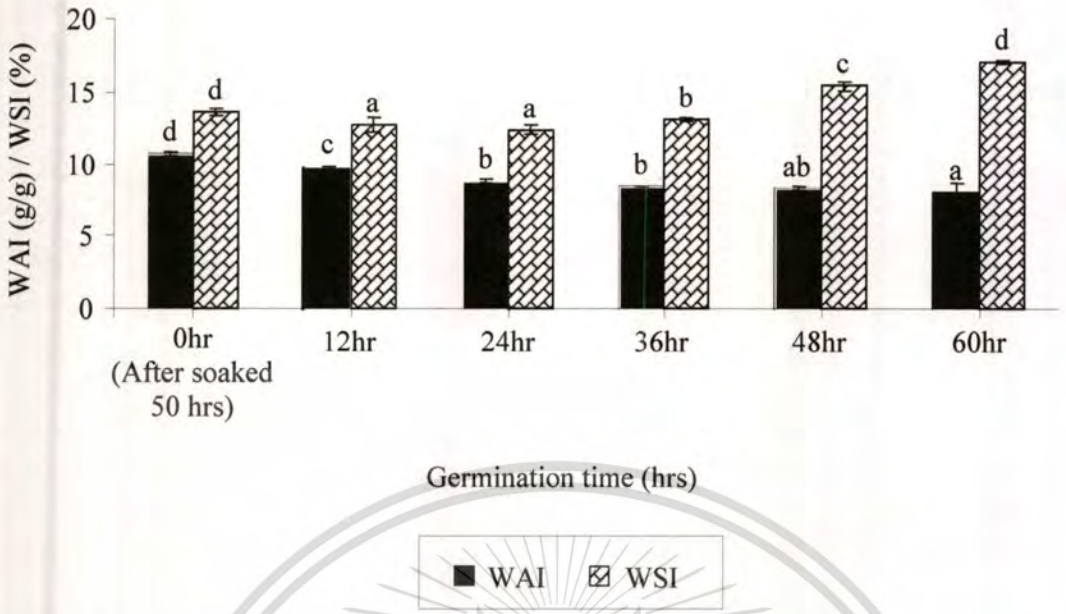


Figure 4.22 The water absorption (WAI) and water solubility (WSI) index of germinated puffed rice various germination times.

4.4.6 The Effects of Drying and Puffing on GABA Retention

Soaking and germination paddy rice induced GABA accumulation, GABA increased with germination time. Paddy rice germinated at 36 hrs containing GABA content 276.31 mg/100 germ (db.) was the condition which could increase higher GABA and had a good puffing qualities (puffed yield and expansion volume) when compared to germinated paddy rice at 48 and 60 hrs. Therefore germinated paddy rice at 36 hrs was selected to study the effect of drying and puffing process. It was found that drying germinated paddy rice with tray-dryer at 50 °C for 4.5-5 hrs and puffing with microwave 700 watts for 110 sec. influenced the decreased GABA content. GABA content in germinated paddy rice germ decreased from 276.31 mg/100 germ (db.) to 186.07 mg/100 germ (db.) about 32.66%. Puffing dried germinated paddy rice GABA content decreased from 276.31 mg/100 germ (db.) to 146.75 mg/100 germ (db.) about 46.89 %. The result was similar to Sootthiboon (2006) who mentioned that GABA was sensitive to heat treatment (cooking and drying process) especially drying at low temperature for a long time(40 ^{o}C 15 hrs). In the baking process, Watanabe et al. (2004) reported that GABA decomposed about 98% in pre-germinated brown rice 30% substituting bread, moreover he suggested that the oryzanol was more stable in pre-germinated brown rice than brown rice, in the part of ferulic acid was quite stable both in 30% pre-germinated brown and 30% brown rice breads. From this results Germination

process affected the qualities of paddy rice such as puffed yield, expansion volume decreased, especially at 48 and 60 hrs, but the water solubility index (WSI) was higher. So its the germination time at suitable range at 12–36 hrs. Drying and puffing process affected GABA decreased, but it still retained rather high content when compare to brown rice.

CHAPTER 5

CONCLUSIONS

5.1 Conclusions

The objective of the study was to investigate the effects of physicochemical properties of paddy rice varieties on puffing qualities by microwave heating at frequency 2450 MHz. The changes of chemical compositions, GABA content, antioxidant activity and antioxidant capacity of waxy paddy rice during germination were determined.

5.1.1 The physicochemical properties of ten paddy rice varieties were determined the degree of husk interlocking, the shell ability, the husk content, the length and width, the ventral region thickness white belly, morphology of starch granule as well as the protein and the amylose content. The amylose content (range 5.58-21.24%) was strongly negatively correlated with all qualities of puffed rice namely puffed yield ($r=-0.95^{**}$), expansion volume($r=-0.82^{**}$), expansion ratio($r=-0.79^{**}$) and bulk density($r=-0.78^{**}$). The degree of husk interlocking or tightness of husk (lemma-palea) interlocking had a relation to the expansion volume. While the thickness of ventral region layer, husk content, width and length of brown rice was unrelated to puffing qualities.

5.1.2 The simple linear regression showed that amylose content could explain 91% of the puffed yield, 67% of the expansion volume, 62% of the expansion ratio and 61% of the bulk density. The highest puffed yield was 57.38%. The suitable paddy rice varieties for puffing were RD6, RD10 and Niaw-San-Pah-Thwng. Thus, waxy paddy rice RD 6 was selected for further study.

5.1.3 The waxy paddy rice RD6 was selected to study the effects of salt (0 and 2%), moisture content of paddy rice before puffing (10,13,16 and 19%) and microwave power (600, 700 and 800 watts) on puffing qualities of puffed rice. The results showed that all the main factors and their interaction significantly ($p<0.05$) affected the puffing qualities namely total puffed yield, fully puffed yield, small puffed yield, expansion volume, texture, color and microstructure.

5.1.4 Paddy rice soaked with 2% salt solution showed higher puffed yield than paddy rice soaked with water. The higher moisture content and microwave power produced puffed rice with

higher puffed yield and expansion volume but lower hardness. Puffing paddy rice which low moisture content (10%) and at low microwave power (600 watts), the texture of puffed rice was the hardest. While puffing it with low moisture content and at high watt power, the lightness of puffed rice was decreased. Puffing paddy rice at 19% moisture content with 600-700 watts showed the highest fully puffed yield but the lowest in the total puffed yield. The results suggested that puffing conditions could produce high puffed yield and expansion volume but moderate hardness were produced from soaked paddy rice at 2% salt solution 13% moisture content and puffed with 700 or 800 watts microwave power.

5.1.5 The waxy paddy rice (RD6) was used to study the effect of germination at 0, 12, 24, 36, 48 and 60 hrs on the changes of GABA content (gamma aminobutyric acid), chemical compositions, total phenolics and antioxidant capacity. It was found that germination time significantly increased GABA, dietary fiber, total phenolics and antioxidant capacity at $p<0.05$. While fat, protein, starch and amylose contents trended to decrease slightly. It has a little changed in the early stage of germination and it markedly increased after 36 hrs. Exception to GABA content, after soaking it increased gradually from 80 to 220 mg/100 g (fresh weight) from 12 to 60 hrs. From the results, it could be suggested that germinated paddy rice can be an alternative source of GABA and dietary fiber content with high total phenolics and high antioxidant capacity.

5.1.6 The effect of germination on puffing qualities and the GABA content retention after drying and puffing was studied. After soaking the waxy paddy rice and germinated in the cabinet incubator (28-30°C) at 0, 12, 24, 36, 48 and 60 hrs, dried at 50±2°C in the tray-dryer 4.5-5 hrs until moisture content decreased to 8-10 % (wb.) and rehydrated with 2% salted solution up to 13% moisture content. Puffing with microwave at frequency of 2450 MHz at 700 watts 110 sec. It was found that germination significantly affected all puffing qualities ($p<0.05$) at 36, 48 and 60 hrs but it had a little different at 12 and 24 hrs. The total puffed yield, fully puffed yield, small puffed yield, expansion volume, hardness, L* value and WSI decreased while WAI, a* and b* value increased. Besides, after drying and puffing significantly decreased the GABA content about 32.66 and 46.89%, respectively. Germinated paddy rice at 36 hrs contained GABA 276.31 mg/100g germ decreased to186.07 and 146.75 mg/ 100g (db.). From these results, germinated paddy rice could be puffed by microwave heating and the germination time need to be to more than 36 hrs. The qualities of puffed rice, the puffed yield and expansion volume trended to

decrease. Drying and puffing decreased the GABA content. However, germinated puffed rice product could still retain the GABA content of more than 50%.

5.2 Recommendations

The following recommendations are made for further study:

5.2.1. The suitable conditions in puffing of brown rice and parboiled rice with microwave heating should be study.

5.2.2. The suitable conditions of each rice variety, the variety investigated as waxy paddy rice and low amylose paddy rice with microwave heating in the industrial level needed to developed.

5.2.3. The germination condition for further study should include more soaking, germination time and temperatures in order to increase the GABA content.

5.2.4. Effect of keeping conditions for germinated paddy rice before puffed should be investigated.

5.2.5. The apparatus for screening and classifying of puffed rice and germinated should be invented

5.2.6. Puffed rice and germinated puffed rice application to the industrial use such as infant food, bakery and beverage should be investigated.

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APPENDIX A

Methods for Determination Paddy Rice and Germinated Paddy Rice Properties

1. Amylose Content Determination (Juliano, 1971)

1.1 Apparatus

- Spectrophotometer
- Hot plate
- 100 ml volumetric flasks
- Pipettes
- Cylinders, etc.

1.2 Chemicals and Reagents

- Amylose, from potato
- Ethanol, 95%
- Sodium hydroxide, 1.0 N
- Sodium hydroxide, 0.09 N
- Acetic acid, 1.0 N
- Iodine solution, 0.2% I2 and 2% KI in distilled water
- Hydrochloric acid

1.3 Procedure

1.3.1 Weigh 100 mg starch sample and transfer to 100 ml volumetric flask.

1.3.2 Add 1 ml of 95% ethanol, carefully washing down any sample adhering to side of the flask.

1.3.3 Add of 9 ml 1 N sodium hydroxide to each sample and heat in a boiling water for 10 minutes and cool to room temperature.

1.3.4 Make the solutions to 100 ml volume with distilled water and votex vigorously and obtain 1 mg/ml solution. Let it stand for at least 2 hours before continuing next steps.

1.3.5 Pipette 5 ml of the sample solutions into 100 ml into a volumetric flask, containing about 50 ml of distilled water.

1.3.6 Add 1.0 ml N acetic acid and mix together.

1.3.7 Add 2 ml Iodine solution.

1.3.8 Make volume up to 100 ml with distilled water, mix and let stand for 20 minutes.

1.3.9 Measure the absorbance at 620 nm.

1.4 Procedure for standard curve preparation

1.4.1 Weigh 40 mg of amylose transfer to 100 ml volumetric flask and the next step as the same procedure of 1.2.1 to 1.2.4 for the sample preparation above.

1.4.2 Prepare working solutions of mixed amylose for standard curve

1.4.3 Pipette 1,2,3,4 and 5 ml aliquots in 100 ml volumetric flasks, each containing about 50 ml of distilled water.

1.4.4 Repeat the steps of 1.2.6 to 1.2.9.

1.4.5 The amylose content expressed as the percentage of dry matter calculating by using the linear equation from standard amylose. (Figure A1)

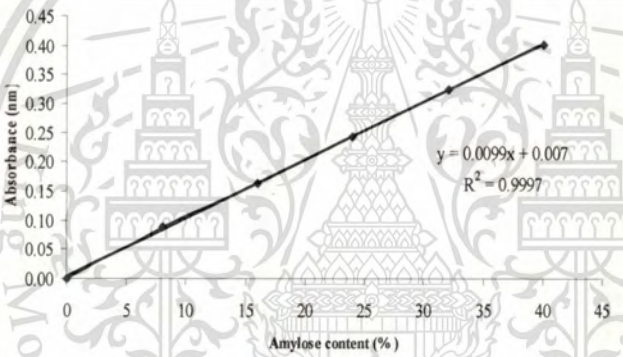

Figure A1 Standard curve for amylose content determination.

2. Protein Content Determination (AOAC, 1990)

2.1 Apparatus

- Digestion block tubes
- Distillation unit
- Distillation titration flask
- Burette
- Cylinders, etc.

2.2 Chemicals and Reagents

- Sulfuric acid 95-98 %

- Catalyst consisting of, copper sulfate ($CuSO_4$) anhydrous and potassium sulfate (K_2SO_4) anhydrous

- Sodium hydroxide

- Methyl red / bromocresol green indicator solution: dissolve 0.2 g methyl red and dilute to 100 ml in 95% ethanol. Dissolve 1.0 g bromocresol green and dilute to 500 ml in 95% ethanol. Mix 1 part of methyl red solution with 5 parts of bromocresol green solution (combine all of both solution)

- Boric acid solution, 4%

- Hydrocholoric acid standard solution 0.1 M

2.3 Procedure

2.2.1. Weigh sample 1 g put into a digestion tube.

2.2.2 Add K_2SO_4 Anhydrous 4.5 g and $CuSO_4 \cdot 5H_2O$ 0.5 g as for a catalyst and add Glass beads about 2–3 pieces.

2.2.3 Add sulfuric acid 20 mL.

2.2.4 Put a digestion tube on the digestion block for digesting until become clear liquid and leave for cooling.

2.2.5 Add distilled water 250 mL, boric acid (4%) 50 mL, sulfuric acid 25 mL and add 4 drops of Methylene Blue for an indicator.

2.2.6. Bring the solution to distill.

2.2.7. Distill solution titrate with hydrochloric acid 0.01 M, until the solution changes from green into soft pink, record the volume of used hydrochloric acid in order to determine nitrogen.

2.2.8. Repeat procedure the steps of 2.2.1 to 2.2.9 for making a blank, calculated results as follows:

$$\text{Nitrogen, (\%)} = \frac{1.47007 \times (Vs - Vb) \times M}{W}$$

Where V_s and V_b = volume of HCL titrant used for sample and blank (mL), respectively.

M = molarity of HCL solution,

W = weight of sample (g).

Protein (%) = % nitrogen x F

Where: F = factor for converting nitrogen to protein in flour 5.95.

3. GABA Content Determination (Liu et al., 1995)

3.1 Apparatus

- HPLC system with heater, a AccQ-Tag Column (3.9 I.D. x 150 mm, particle size 4 μm), a multi λ fluorescence detector (EX: 250 nm, EM: 395 nm).
- 0.22 μm nylon filter
- 50 μl syringe
- 10 mL syringe
- 16 × 150 mm test tube
- LC vial, etc.

3.2 Chemicals and Reagents

- Standard GABA
- Mobile phase solution (AccQ-Tag Eluent A, acetronitrile and deionized water)
- Acetonitrile
- Methanol, etc.

3.3 Sample Preparation

3.3.1. Weigh 100 mg sample put into a test tube and add hydrochloric acid 6 N 5 mL, heat with heating block at 110 C for 22 hrs.

3.3.2. Cooling and add standard amino acid such as γ-amino butyric acid (GABA) which is Internal standard , dry with nitrogen at 50 C, dilute the sample digesting with deionized water and filter.

3.3.3. Pipette the sample 10μl mixed with AccQ fluor derivatization buffer 70 μl and add AccQ fluor reagent 20 μl, heat the sample at 55 C with heating block.

3.4 Procedure

3.4.1. Inject 5 μl digested and extracted sample, use Acetronitile ,Deionized water and AccQ Tag Elient A is mobile phase.

3.4.2 The peak of GABA extracted compare to the retention time of the sample standard and determine the GABA. (Figure A2).

Individual Sample Report

Reported by User: System

Project Name: CIF010_2552

SAMPLE INFORMATION

Sample Name:	H-STD+GABA 5 pmol	Acquired By:	System
Sample Type:	Standard	Date Acquired:	20/5/2552 21:58:24
Vial:	7	Acq. Method Set:	GABA_fluro_25
Injection #:	1	Date Processed:	21/5/2552 11:45:46
Injection Volume:	5.00 ul	Processing Method:	GABA 25 min
Run Time:	25.0 Minutes	Channel Name:	SATIN
Sample Set Name:	gaba 25 min	Proc. Chnl. Descr.:	

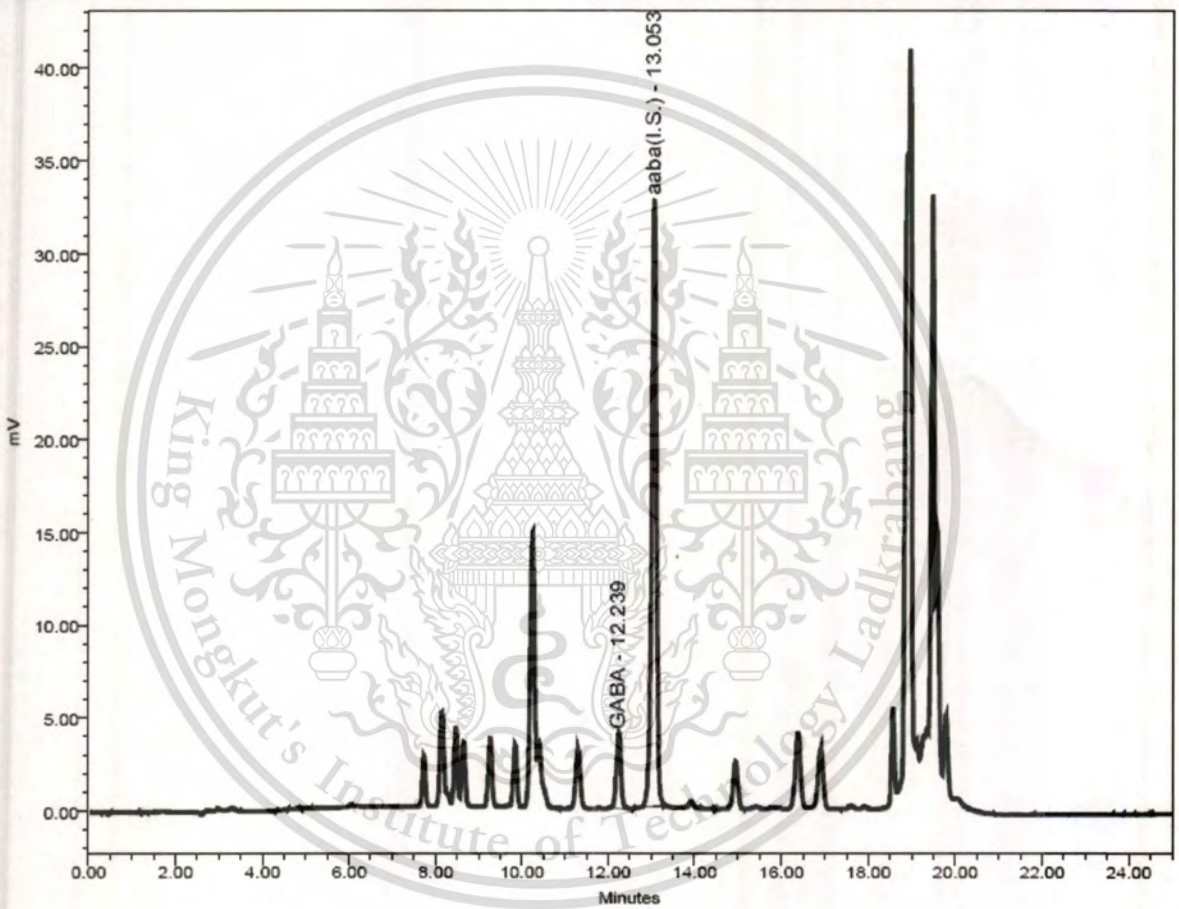

Figure A2. Chromatogram of standard GABA.

4. **Dietary Fiber Determination** (AOAC, 2000)

4.1 Apparatus

- Fritted crucible
- Vacuum pump
- Hot air oven
- Muffle furnace
- Shaking water bath
- pH meter
- Analytical balance, etc.

4.2 Chemicals and Reagents

- Phosphate buffer pH 6.0, 0.08 M: dissolve 1.4 g sodium phosphate dibasic, anhydrous (Na_2HPO_4). Dilute to 1 L with distilled water check pH with pH meter.
- Protease, keep refrigerated
- Amyloglucosidase, keep refrigerated
- 0.275 M Sodium hydroxide solution,
- 0.325 M Hydrochloric acid solution,
- 95% Ethanol
- Celite, acid washed
- Acetone, etc.

4.3 Procedure

4.3.1. Weigh 2 g sample in duplicate test portions (difference in weight shold not >20mg) in the flask.

4.3.2 Add phosphate buffer (0.08 M, pH 6) 50 mL, and add α-amylase 0.1 mL and close the flask with aluminium foil, bring it to heat in shaking water bath the temperature is about 95-100 °C for 30 minutes.

4.3.3 The solution cools down until room temperature and adjust the pH of solution is 7.5±0.2 with Sodium Hydroxide 0.275 M which is about 10 mL.

4.3.4 For remove protein, add protease 0.1 mL and close the flask with aluminium foil, and heat up the temperature is at about 45-55 °C , stir with magnetic for 30 minutes and cooling.

4.3.5 Adjust pH of the solution up to 4.5±0.2 with hydrochloric 0.325 M by using dropper about 30 drops.

4.3.6 For remove starch, add amyloglucosidase 0.3 mL and close the flask with aluminium foil, heat up to 60-65 $^{\circ}$C, stir all the time with magnetic for 30 min, and cooling. (final solution is about 70 mL)

4.3.7 Add 95% ethyl alcohol 280 mL, let precipitate form at room temperature for 60 min.

4.3.8 Collect the residues (soluble fiber+insoluble fiber) in pre-weight crucibles.

4.3.9 Filtration, one test portion for protein, using N x 6.25 as conversion factor.

4.3.10 Incinerate second test portion at 525°C for 5 hours.

4.3.11 Calculate the dietary fiber content as follows:

$$TDF(\%) = \frac{(R - P - A - \text{blank}) \times 100}{W}$$

where:

R = average weight residue of duplicate test portion determinations(g),

P = weight of protein from test portion residue (g),

A = weight of ash from test portion residue (g),

W = average weight of two test portions (g).

Determination of blank:

$$\text{Blank, g} = R_B - P_B - A_B$$

where:

R_B = average weight residue of duplicate blank determinations (g),

P_B = weight of protein from blank residue (g),

A_B = weight of ash from blank residue (g).

5. Starch Content Determination (AACC, 1990)

5.1 Apparatus

- Spectrophotometer
- Autoclave
- Water bath with shaker for glucoamylase reaction
- Erlenmeyer flask, 100 mL
- Pipettes 1-5 mL
- Test tubes, 18 x 150 mm

5.2 Chemicals and Reagents

- Glucoamylase free of transglucosidase activity under assay conditions, dissolved in distilled water (10 mg/mL).
- Acetate buffer 4 M, pH 4.8 (120 mL) (glacial acetic acid and 164 g anhydrous sodium acetate per liter).
- Standard D- glucose solution, 400 mg pure anhydrous D-glucose/L. Allow 4 hours for complete mutarotation before use.
- Tris-phosphate buffer. Dissolve 36.3 g tris (trihydroxy methylaminomethane) and 50 g $NaH_2PO_4H_2O$ in or 45.5 g anhydrous $NaH_2PO_4H_2O$ in 500 mL water. Adjust pH to 7 with phosphoric acid at 37 ^{o}C before bringing to 1 Lit with distilled water.
- Enzyme- buffer-chromogen mixture, (dissolve in 100 mL tris-phoaphate buffer: 30 mg glucose oxidase (Type II); 3 mg peroxidase (Type I); 10 mg o-dianisidine dihydrochloride. Disperse o-dianisidine with rest of enzyme-buffer mixture. This mixture can be stored at 4^{o}C for 10 days
- H_2SO_4 18 N

5.3 Procedure

5.3.1 Grind the sample to particle size smaller than 0.5 mm or 40 mesh.

5.3.2 Determine moisture content in ground grain to correct starch data to dry weight basis.

5.3.3 Weigh sample 0.5 g in Erlenmeyer flask.

5.3.4 Add 25 mL water with strring to disperse starch and adjust pH between 5 and 7. Suspension is then boiled with gentle stirring for 3 minutes and pressure heated at 135 ^{o}C (autoclave) for 1 hr.

5.3.5 Remove from autoclave, maintain temperature near 55°C, and 2.5 mL acetate buffer and sufficient water to adjust tatol weight of solution to 45°C 1 g.

5.3.6 Immerse Erlenmeyer flask in water bath with shaker at optimal temperature of glucoamylase used and add 5 mL glucoamylase solution.

5.3.7 Hydrolyze 2 hrs with continuous shaking, filter through folded filter paper into 250 mL volumetric flask, wash quantitatively, and dilute to volume.

5.3.8 Transfer 1 mL aliquots containing 20-60 μg D-glucose to test tubes. To obtain this rang of glucose concentrations, it may be necessary to dilute hydrolysate of step 5.3.7.

5.3.9 Add 2 mL enzyme-buffer-chromogen mixture, shake tubes, and place in dark at 37±1°C exactly 30 min to develop color.

5.3.9 Stop reaction with 2 mL H_2SO_4 18 N and measure absorbance at 540 nm.

5.3.10 Prepare standard D-glucose curve from 0 to 60 μg mL and blank for each series of analyses.

5.3.11 A control sample of starch of know purity and from a similar source should be included in analysis.

5. 3.12 Starch content calculate as follows:

$$\% \text{ Starch} = 0.9 \times \frac{M}{106} \times \frac{V_1}{1} \times \frac{250}{V_0} \times \frac{100}{E} \times \frac{100}{MS} = \frac{2.25 \times M \times V_1}{V_0 \times E \times MS}$$

Where:

E = weight in grams of sample,

M = weight in μg of D-glucose obtained from standard curve,

V_o = volume in mL of aliquot 250 mL flask,

MS = percentage dry weight of sample

V_1 = volume in mL if extra dilution is done in step 5.3.8 Value of V_1 is 1.0 when no extra dilution is done.

6. Total Phenolics Content Determination (Singleton et al., 1999)

6.1 Apparatus

- Spectrophotometer
- Test tubes
- Vertex mixer

6.2 Chemicals and Reagents

- Gallic acid
- Folin-Ciocalteu
- Sodium carbonate 10%
- Distilled water, etc

6.3. Standard Curve of Gallic Acid

6.3.1 Prepare standard Gallic acid solution 400 µg/ mL

6.3.2 Pipette the standard Gallic acid solution and put into a test tube 0, 0.05, 0.20, 0.30 and 0.35 mL, respectively , adjust with distilled water up to 10 mL in each test tube.

6.3.3 Add Folin-Ciocalteu solution 0.5 mL and mix together with vortex mixer, let stand for 5 minutes at room temperature.

6.3.4 Add sodium carbonate solution 10 % 2 mL mix together with vortex mixer, let stand for 10 minutes at room temperature and measured at 730 nm.

6.4 Procedure

6.4.1 Pipette the samples extracted 0.5 mL in test tube, adjust the volume with distilled water up to 10 mL.

6.4.2 Add Folin-Ciocalteu solution 0.5 mL and mix together with vortex mixer, let stand for 5 min. at room temperature.

6.4.3 Add sodium carbonate solution 10 % 2 mL , mix together with vortex mixer, let stand for 1 hour at room temperature and measure at 730 nm.

The concentration of total phenolics content expressed as mg of Gallic acid equivalents/g dry matter, using the linear equation of standard curve of Gallic acid (Figure A3)

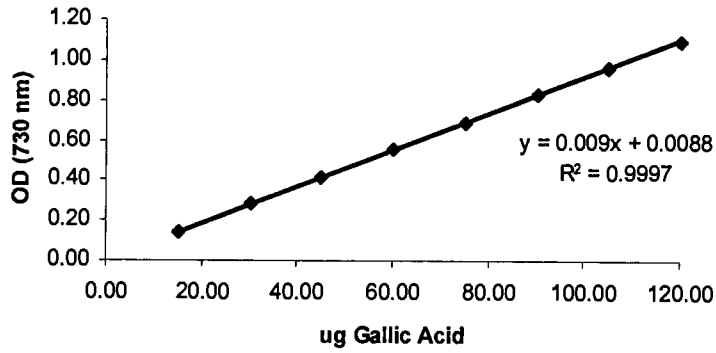

Figure A3 Standard curve of Gallic acid for total phenolics acid calculation.

7. DPPH Radical Scavenging Activity (DPPH-RSA) (Ragaee et al., 2006 and Choi et al., 2006)

7.1 Apparatus

- Spectrophotometer
- Test tubes
- Vertex mixer

7.2 Chemicals and Reagents

- 2,2-Diphenyl-1-picryl-hydrazyl (DPPH)
- 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox)
- Methanol 40%
- Distilled water

7.3 Procedure

7.3.1 Pipette the samples extracted (0.4 mL) into the test tube, 5 ml of 40% methanol and 0.6 mL of 0.8 $mmolL^{-1}$ of DPPH solution.

7.3.2 The solution mixes together with vortex mixer, let it stand for 30 min under subdued light.

7.3.3 The absorbance of each sample extract containing DPPH (A_1), measured at 517 nm using a spectrophotometer.

7.3.4 The absorbance of each sample extract without DPPH (A_s), and only DPPH solution without extract sample (A_0, called control). The percentage of DPPH-RSA of each sample extract was calculated using the equation.

$$\text{DPPH} - \text{RSA}\ (\%) = \left[\frac{A_0 - (A_1 - A_s)}{A_0}\right] \times 100$$

where:

A_0 = The absorbance of the control solution (containing only DPPH),

A_1 = The absorbance in the presence of the samples extracted in DPPH solution and

A_s, = The absorbance of each sample extracted without DPPH used for error correction arising from unequal color of the sample solutions, is the absorbance of the samples extracted solution without DPPH.

The DPPH-radical scavenging activity expressed as 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) equivalents in mg/g dry matter was determined using the linear equation from standard curve of trolox standard (Figure A4).

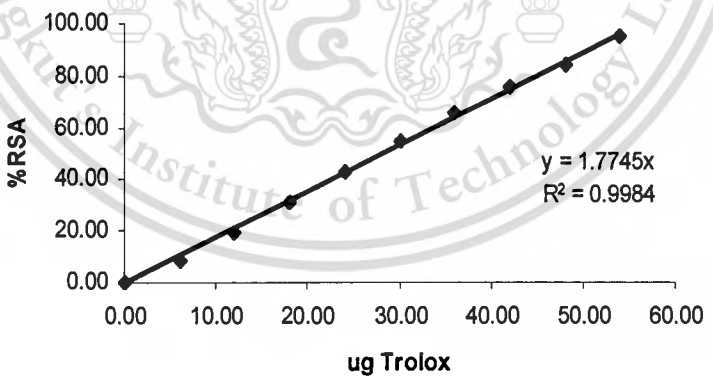

Figure A4 Standard curve of Trolox for DPPH calculation.

8. Ferric Reducing Ability Power (FRAP) (Wong et al., 2006)

8.1 Apparatus

- Spectrophotometer
- Test tubes
- Vertex mixer

8.2 Chemicals and Reagents

- Acetrate buffer, pH 3.6, 0.1 M (weigh sodium acetrate trihydrate 3.1 g. mixed with glacial acetic acid 16 mL adjusting with distilled water up to 1 L)
- TPTZ (2,4,6-Tris (2-pyridyl)-s- triazine) 10 $mmolL^{-1}$ in HCL 40 $mmolL^{-1}$ (weigh 0.156 g dissolve in HCl 40 $mmolL^{-1}$, adjust volume up to 50 mL).
- $FeCl_3$. $6H_2O$ 20 $mmolL^{-1}$ (weigh $FeCl_3$. $6H_2O$ 0.27 g dissolve in distilled water and adjust volume up to 50 mL).
- Hydrochloric acid
- Trolox
- Glacial acetic acid
- Sodium acetrate trihydrate
- Distilled water
- FRAB reagent: (mix the solutions by the ratio of Acetrate buffer: TPTZ : $FeCl_3$. $6H_2O$ is equal to 10:1:1 by volume, respectively).

8.3 Procedure

8.3.1 Pipette the extracted samples 0.1 mL.

8.3.2 Add FRAP reagent 3 mL.

8.3.3 Mix together with vortex mixer, and let to stand for 8 min. at room temperature.

8.3.4 Measure with spectrophotometer at absorbance 593 nm.

8.4 Standard Curve Trolox

8.4.1 Pipette standard trolox into a test tube which each test tube has 6,12,18,24,30, and 36 µg/mL, and adjust the volume by distilled water for total volume in each test tube as 0.1 mL.

8.4.2 Add FRAP reagent 3 mL.

8.4.3 Mix together with vortex mixer, and let to stand for 8 minutes. at room temperature, measure with spectrophotometer at absorbance 593 nm.

The FRAP expressed as mg 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) equivalents/g dry matter was calculated using the linear equation from standard curve of trolox standard (Figure A5).

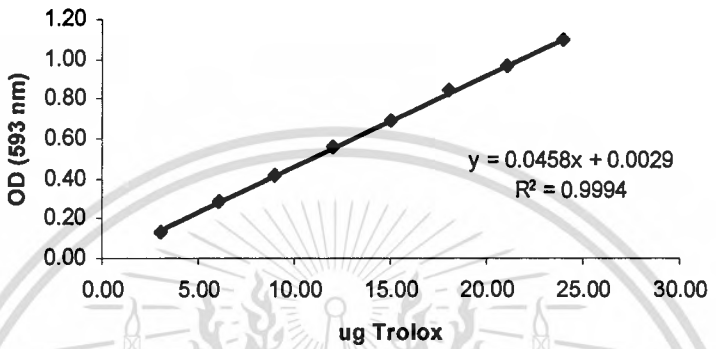

Figure A 5 Standard curve trolox for FRAP calculation.

APPENDIX B

1. Condition for Measuring the Texture Analyzer (Stable Micro System, 1995)

The texture analyzer was calibrated at the beginning of each testing session using 5 kg calibration weight. Six samples of puffed rice were measured in triplicate for characteristics of hardness (g force). The condition for measured as follows:

Conditions of TA–XTi Plus with Ottawa Cell with Wire Plate probe

Objection	:	Comparison of hardness of puffed rice cereal by bulk compression
Mode	:	Measure Force in Compression
Option	:	Return to Start
Pre-Test-Speed	:	N/A
Test-Speed	:	5.0 mm/s
Post-Speed	:	10.0 mm/s
Distance	:	70 mm
Trigger Type	:	Button
Break Mode	:	off
Stop Plot At	:	Start Position
Tare Mode	:	off
Advanced Options	:	on
Data Acquisition Rate	:	200 pps

Figure B1 Measurement of textural characteristics of puffed rice and germinated puffed rice.

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