CHAPTER II
LITERATURE REVIEW

2.1 Germinated brown rice

Rice (*Oryza saltiva* L.) is one of the most important crops in term of its contribution of calories to the human diet. It is the main source of food for about two-thirds of the world's population and a staple food in Thailand where it is consumed in all households on a daily basis (29-30). It is generally considered a semiaquatic, annual grass plant, and can grow in a wide range of water-soil regimes. The grain is cultivated by more than 100 countries on every continent (32).

Paddy rice is composed of the hull (16-28% dry mass basis) and the grain (caryopsis) which is enclosed inside the hull. Brown rice is derived from paddy rice by dehulling and is converted to milled rice after removing the bran and embryo (germ) (Figure 2.1) (33).

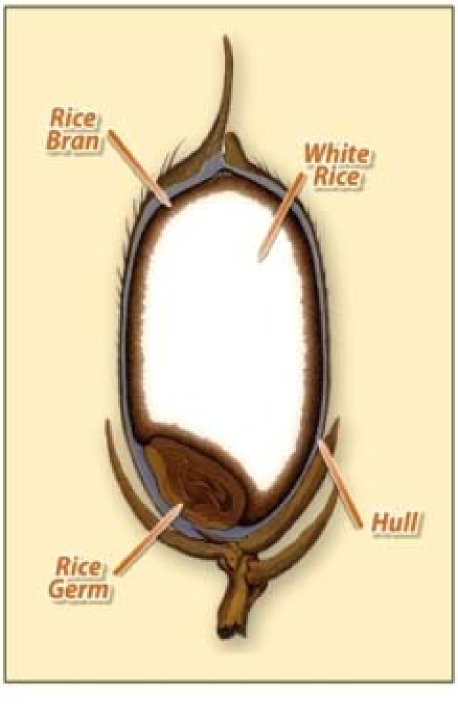

Figure 2.1 Structure of rice grain

Rice can be divided into categories based on their prominent characteristics, i.e. kernel length, shape, variety and amylose content. Based on amylose content, rice is sorted as waxy (1-2%), very low amylose (2-12%), low amylose (12-20%), intermediate amylose (20-25%) and high amylose (25-33%) (34). If amylose contents are high, then cooked rice becomes dry, less tender, and hard upon cooling. In comparison, rice with low amylose contents will cook moist and sticky (35).

Khao Dawk Mali 105 (KDML 105) contains 12-17% amylose content while the amylose content of Chainat 1 is 26-27%. Due to the difference of amylose content, different rices are used in different types of food. 'KDML 105 rice is used directly as consuming or weaning food and Chainat 1 is used for noodle making (36).

From milling, rice can be separated into 2 types, polished rice and brown rice. Rice can be eaten as brown rice or polished rice. Polished rice is polished during milling to remove the brown hull which causes the losing of some of the protein, vitamins, and minerals. Thus, brown rice contains more nutrients than polished rice. It has 4 times more vitamin B1 than polished rice and also higher content of vitamin B2. Iron in brown rice is 2 times higher than in polished rice. Brown rice is also a source of phosphorus and calcium. These elements can prevent beriberi, stomatitis, anemia and are good for the teeth and the bones. Brown rice has more protein, fat, dietary fiber and less carbohydrate than polished rice, so consuming brown rice is good for health, which is compatible with the consumption trend nowadays when consumers pay much more attention to how to get good health (37). Besides, brown rice is directly consumed in food products, for example, noodle, muffin, snack bar and butter cake (37-39).

Although brown rice is really good for health, it is not as popular with consumers as polished rice which has less nutrition. The reason is that it is difficult to cook and hard to chew. Germinated brown rice can solve this problem because after germination, the brown rice has softer texture than non germinated brown rice and cooks more easily (6, 19). Not only the texture, but also the nutrients in germinated brown rice are changed. Total protein, dietary fiber, vitamin (vitamin B, tocotrienols) and mineral (magnesium, potassium, zinc), γ-oryzanol, γ–aminobutyric acid (GABA), inositols, phenolic compound are higher and phytic acid is lower (7, 14-15, 41). Therefore, GBR is considered a functional food (6).

2.1.1 Seed germination

Germination can be defined as the emergence of the radicle through the seed coat or the emergence and development from the seed embryo of the essential structures and can be considered as the resumption of active growth by the embryo resulting in the rupture of the seed coat and the emergency of the young plant. There are two kinds of seed germination, based on the fate of the cotyledons or storage organs (Figure 2.2) (42).

2.1.1.1 Epigeal germination

Epigeal germination is characteristic of bean and pine seeds. During germination, the cotyledons are raised above the ground where they continue to provide nutritive support to the growing points. During root establishment, the hypocotyl begins to elongate and breaks through the soil, pulling the value cotyledons and enclosed plumule through the ground and projecting them into the air. Afterward, the cotyledons open, the plumule grows and the cotyledons wither and fall to the ground (42).

2.1.1.2 Hypogeal germination

Hypogeal germination is characteristic of pea seeds and all grasses such as rice, corn and many other species. During germination, the cotyledons remain beneath the soil while the plumule pushes upward and emerges above the ground. In this germination, the epicotyl is rapidly elongated, and the cotyledons continue to provide nutritive support to the growing points throughout germination (42).

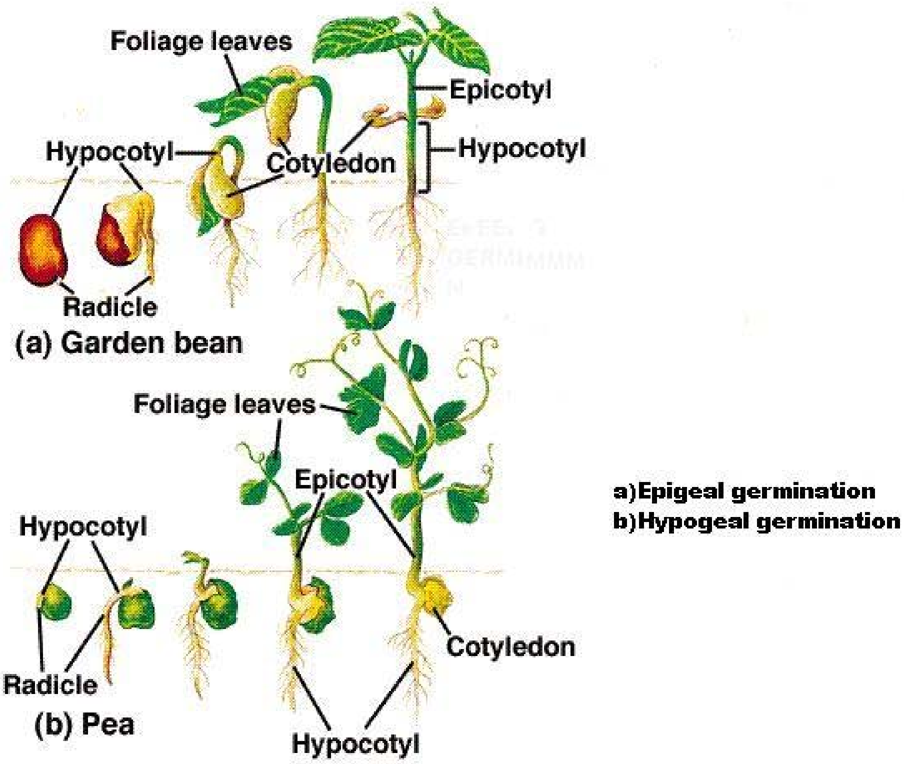

Figure 2.2 Epigeal and hypogeal germination

Germination begins with the uptake of water by the dry seed (imbibition) and is completed when a part of the embryo, usually the radicle, extends to penetrate the structures that surround it. The uptake of water is triphasic (Figure 2.3), with a rapid initial uptake (phase I, i.e. imbibition) followed by a plateau phase (phase II). A further increase in water uptake (phase III) occurs as the embryo axis elongates and breaks through the covering layers to complete germination. The time for all the events to be completed varies from several hours to many weeks, depending on the plant species and the germination conditions (43).

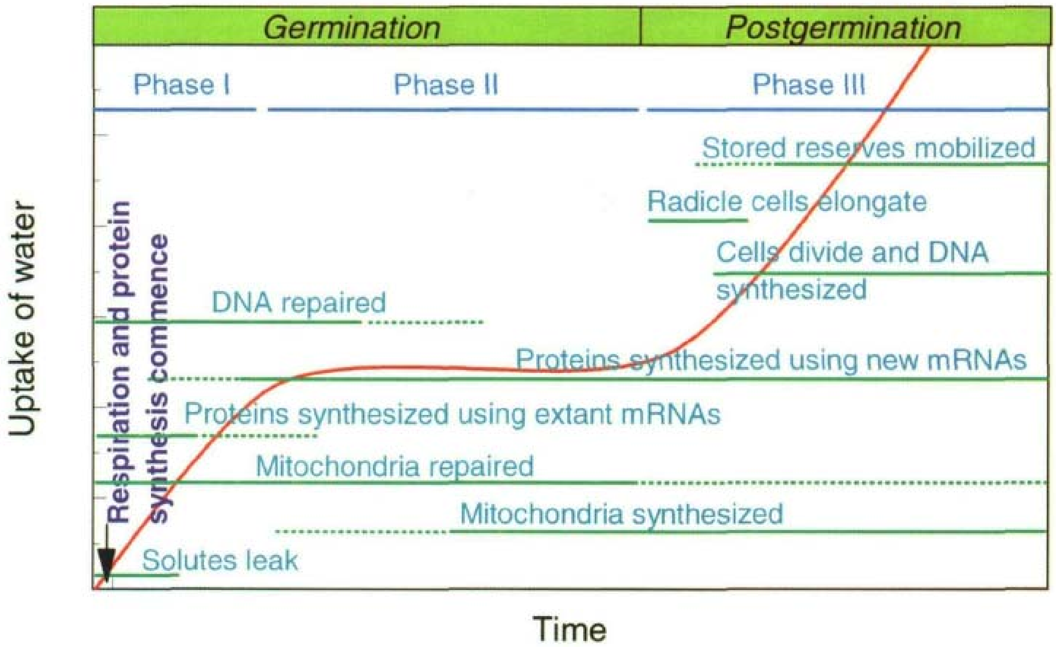

Figure 2.3 Time course of major events associated with germination and post germinative growth

During phase I, the influx of water into the cells of dry seeds results in temporary structural perturbations, particularly to membranes, which lead to a leakage of solutes and low molecular weight metabolites into the surrounding imbibition solution. Within a short time of rehydration, the membranes return to their more stable configuration, at which time solute leakage is diminished (43).

Upon imbibition, the seed rapidly resumes metabolic activity. The structures and enzymes necessary for this occurrence are present within the dry seed, having survived. Reintroduction of water during imbibition is sufficient for metabolic activities to resume, with turnover or replacement of components occurring over several hours as full metabolic status is completed (43).

One of the first changes upon imbibition is the resumption of respiratory activity. After a steep initial increase in oxygen consumption, the rate declines until the radicle penetrates the surrounding structures. At this time, another burst of respiratory activity occurs (44-45). The glycolytic and oxidative pentose phosphate pathways both resume during phase I, and the Kreb's cycle enzymes become activated (46-47). Germinating seeds of many species frequently produce ethanol (48). This is often the result of an internal deficiency in oxygen, caused by restrictions to gaseous diffusion by the structures that surround the seed and by the dense internal structure of most seeds. This oxygen deficiency may result in more pyruvate production than utilization for activities of the Kreb's cycle and electron transport chain (43).

Tissues of the mature dry seed contain mitochondria, and although these organelles are poorly differentiated as a consequence of maturation drying, they contain sufficient Kreb's cycle enzymes and terminal oxidases to provide adequate amounts of ATP to support metabolism for several hours after imbibition (49-50). During germination of embryos, there are two patterns of mitochondrial development. These patterns, which are particularly obvious in cotyledons, depend on the nature of the stored reserves. In starch-storing seeds, repair and activation of preexisting organelles predominate, on the contrary oil-storing seeds typically produce new mitochondria (51-52).

Generally, enzymes that break down carbohydrates, lipids, proteins and phosphorus containing compounds are the first to be activated during phase II of water uptake by seeds. Since the embryonic axis requires energy for growth, storage compounds must be hydrolyzed to soluble forms, translocated from the endosperm to the embryo and transformed to energy molecules that can be immediately utilized by the embryonic axis (42).

Amylopectin and amylose are also hydrolyzed. They are hydrolyzed by α- and β- amylase enzymes. These enzymes split either starch structure yielding the disaccharide maltose, which is split into two monosaccharide glucose units. Some glucose units are converted into the highly mobile disaccharide sucrose for translocation to other sites, after which it is reconverted to glucose or used directly in synthesis (42).

2.1.2 Factors affecting germination process

2.1.2.1 Seed maturity

Seeds of most species are capable of germinating long before physiological maturity. In other cases, maximum seed germination can only be obtained if the seed matures (43).

2.1.2.2 Water

Water is a basic requirement for germination. It is essential for enzyme activation, breakdown, translocation and use of reserve storage material (42).

2.1.2.3 Air

Air is composed of about 20% oxygen, 0.03% carbon dioxide and about 80% nitrogen gas. Oxygen is required for germination of most species. Carbon dioxide concentrations higher than 0.03% retard germination, while nitrogen gas has no influence. During seed germination, respiration increases sharply, thus an adequate supply of oxygen must be available. If the oxygen concentration is reduced below 20% of air, germination of most seed is retarded (47).

There are some plants that can germinate under water where oxygen is present only in limited concentrations i.e. rice, banyard grass and aquatic plants. Rice seeds can germinate even in the complete absence of oxygen. Presumably anaerobic respiration enables these seeds to germinate in the absence of oxygen (43).

2.1.2.4 Light

While moisture, oxygen and favorable temperature are essential for germination of all seeds, certain species also require light. However, some species can not germinate in light condition (43).

2.1.2.5 Osmotic pressure

High osmotic pressures of the germination solution make imbibition more difficult and retard germination. The ability of seeds to germinate under high osmotic pressure differs with variety as well as with species, but all are affected (42).

2.1.2.6 Presoaking

Presoaking seeds in water has been suggested as a means to speed up germination and it has accelerated germination of several grass species. The basis for the acceleration is uncertain. However, it is likely that hydrolytic processes begin during presoaking and the resulting simple sugars that are released can be utilized for synthesis immediately upon germination. It is also possible that membrane repair may occur by enzymes activated during the hydration process (42).

2.1.2.7 Hydrogen-ion concentration (pH)

Germination can proceed over a wide range of pH. The germination of almost all species occurs readily between pH values of 4.0 and 7.6 (45). In 2007, Watchraparpaiboon studied the effect of pH of soaking water in germination process on germination rate of brown rice. The results showed that at pH 6, brown rice had the highest germination rate. The pH does not only affect the germination, but also has influences on nutrients in germinated brown rice. GABA is one of the nutrients that increase during germination. In germination, glutamate decarboxylase enzyme (GAD) is the main enzyme that involved in GABA production. GABA is synthesized from glutamic acid by this enzyme. During germination, GAD becomes activated and glutamic acid is converted to GABA (52).

Previous reports indicated that reduction of pH activates GAD activity and GABA accumulation. Khampang (53) found that at pH 6.5, germinated brown rice contained higher GABA than germinated brown rice soaked in water pH 5.5. Watchraparpaiboon (9) and Khumkah et al. (54) reported that the pH which made germinated brown rice have the highest GABA was pH 6. Horino et al. (55) found that at pH 5.5, the germinated brown rice germ contained the highest amount of GABA which was close to the study of Sunte (3) who reported that germinated brown rice soaked in the water with pH 5 contained the highest GABA. Charoenthaikij et al. (17) showed that lower pH than 5-6 of soaking water can produce high GABA in germinated brown rice, this study showed that at pH 3 germinated brown rice contained the highest GABA.

Other than GABA, pH could affect other nutrients. Khampang (53) showed that germinated brown rice soaked in water at pH 6.5 contained higher protein, vitamin B1, vitamin E, and γ-oryzanol than germinated brown rice soaked in water pH 5.5. Watchraparpaiboon (9) found that at pH 6, germinated brown rice contained the highest protein, fat and lowest carbohydrate. At pH 8, germinated brown rice contained the highest vitamin B1. At pH 4, germinated brown rice contained the lowest phytic acid. Khumkah et al. (54) found that germinated brown rice soaked in water at pH 4 had higher vitamin B1 and carotenoid than germinated brown rice soaked in pH 6. In contrast to other reports, Charoenthaikij et al. (17) found that germinated brown rice soaked in pH 3, 5 and 7 had no difference in crude protein content.

2.1.2.8 Temperature

Many reactions in seed germination are affected by temperature. The optimum temperature for most seeds is between 15 and 30ºC. The maximum temperature for most species is between 30 and 40ºC (16). Taiz and Zeiger (56) found that the appropriate temperature for rice germination was between 30-35 °C and rice can be germinated at 5-10 °C lower and higher than the appropriate temperature.

Many reports confirmed the effect of temperature on seed germination. Sunte (3) showed that soaking 'KDML' 105 brown rice in water at 40 °C gave the highest GABA content of germinated brown rice. Watchraparpaiboon (9) found that at 35 °C, 'KDML' 105 and Chainat 1 germinated brown rice contained the highest GABA, protein, fat and lowest carbohydrate content, at 25 °C, germinated brown rice contained the highest vitamin B1 and at 45 °C, germinated brown rice contained the lowest phytic acid. Khumkah et al. (54) and Khampang (53) reported that soaking 'KDML' 105 brown rice at temperature of 35 °C resulted in a higher protein, GABA, vitamin B1, oryzanol and carotenoid of germinated brown rice.

2.1.2.9 Time

Time is an important factor that also affects germination. Many researchers found that longer germination time resulted in higher GABA content. As well as GABA, protein, fat and vitamins are higher when rice is germinated for a longer time. Jongdee et al. (7) suggested that germinated brown rice from 26 hrs germination time contained higher amount of total amino acids and protein than germinated brown rice from 22 hrs germination time. Wongbasg et al. (18) showed that germinated brown rice soaked for 60 hrs contained the highest protein when compared with germinated brown rice soaked for 12, 24, 36 and 48 hrs. The result of this study was in agreement with that of Khampang (53) who suggested that brown rice germinated for 36, 48 and 72 hrs contained higher protein than brown rice germinated for 24 hrs. Jiamjariyatam (57) found that germinated brown rice soaked for 72 hrs gave the highest protein when compared with germinated brown rice soaked for 24 and 48 hrs. In contrast with other studies, Charoenthaikij et al. (17) found that crude protein was highest when brown rice was soaked for 24 hrs and decreased when used with longer soaking time. Watchraparpaiboon (9) reported that germinated brown rice, soaked for 24 hrs contained higher protein, fat, vitamin B1and lower carbohydrate and phytic acid than germinated brown rice, soaked for 12 hrs. Hirunpong and Tungjaroenchai (41) found that with 24 hrs of germination, germinated brown rice contained higher γ-oryzanol than 6, 12 and 18 hrs germinated brown rice.

However, some studies reported that the nutrients in germinated brown rice did not increase with time. Khampang (53) suggested that higher vitamin B1, vitamin E and γ-oryzanol content was found in 24, 36 and 48 hrs germinated brown rice than in germinated brown rice soaked for 72 hrs germination time. Lerswanichwatana et. al. (58) also reported that vitamin B1 and B2, calcium and iron in germinated brown rice soaked for 48 hrs was higher than in germinated brown rice soaked for 24 hrs but lower than in germinated brown rice soaked for 72 hrs. Kiing et al. (59) found that γ-oryzanol concentrations in germinated brown rice soaked for 16 hrs was higher than in germinated brown rice soaked for 4, 8, 12, 18, 20 and 24 hrs.

Not only do nutrients in germinated brown rice change, but phytochemical such as phenolic compounds in germinated brown rice also change. Phenolic compounds are secondary metabolites synthesized by plants (60). Because of their antioxidant activity (61), intake of phenolic compounds can reduce the risks of chronic diseases such as obesity, heart diseases, diabetes, and cancer (62). Phenolic compounds are located in the lipid–water interface of the membrane because of their lipophilic–hydrophilic structure. This location allows them to scavenge free radicals inside and outside the cell (63). Major amounts of phenolic compounds are presented in the bran portion, whereas the cell walls of endosperm contain very much less (64). The first step that the seed response machinery takes after breaking dormancy is to synthesise phenolic compounds with higher than normal antioxidant activity so as to protect hypocotyl growth against oxidative reactions triggered by environmental factors (65). Cevallos-Casals and Cisneros-Zevallos (65) reported that most of the synthesis of phenolic compounds occurs during imbibition and seed growth. The imbibition stage seems to be an active period of phenolic compounds synthesis for most seeds. After germination, various changes in the phenolic compounds occur which are not only dependent on the type of seeds, but also on the process conditions (65).

Hirunpong and Tungjaroenchai (41) found that germinated brown rice that was soaked in water at 35°C for 24 hrs contained the highest total phenolics which was confirmed by the study of Khumkah et al. (54) who showed that germinated brown rice soaked in pH 6 at 35 °C had high total phenolics content. The results of this study were close to the results of Khampang's study (53) which showed that germinated brown rice soaked in water pH 5.5, 30 ºC for 48 hrs contained highest total phenolic content protein and antioxidant activity. Suwananon and Jiamyangyeun (66) selected a condition for preparing germinated brown rice with high antioxidant activity. The condition was soaking rice in 40° C water for 3 hrs, and further germinating for 18 hrs. In contrast to those three studies, Sawaddiwong et al. (67) suggested that brown rice germinated by soaking in water at 25 °C for 24 hrs had the highest total phenolic content and antioxidant activities when compared to brown rice germinated by soaking in water at the temperatures of 30, 35 and 40 °C.

2.1.3 Physical properties of germinated brown rice

During germination, amylase enzymes are released from the scutellum and become activated. The enzyme causes reduction of amylose content and degradation of starch to shorter chains, yielding dextrin and oligosaccharides, while maltotriose is found to constitute the greatest portion of the oligosaccharides throughout the germination stage (68). Thus a reduction of viscosity occurs as a result of starch saccharification or dextrinification and decreasing of amylose content (69). With all these changes, physical properties of germinated brown rice flour is altered.

Watchraparpaiboon (9) found that gelatinization temperature of germinated brown rice was close to gelatinization temperature of normal brown rice. Germinated brown rice had lower viscosity than normal brown rice, which was in agreement with the study of Jiamjariyatam (16) who found that soaking time had influence on the viscosity of germinated brown rice flour. Longer soaking time caused less peak viscosity, less setback from trough and less final viscosity of the flour. In this study, soaking time did not affect pasting temperature of germinated brown rice flour. Pasting temperature of germinated brown rice flour was not significantly different from pasting temperature of brown rice flour and Charoenthaikij et al. (17) showed that there was no difference in pasting temperatures between germinated brown rice flour and normal brown rice. As the steeping time increased, the values of trough, breakdown, and final viscosity of germinated brown rice flour decreased. The pasting property of germinated brown rice flour that was most affected by germination was the peak viscosity. The peak viscosity of germinated brown rice flour was significantly lower than that of brown rice. Wongbasg et al. (18) compared germinated brown rice soaked in water with germinated brown rice soaked in $CaCl_2$ solution and found that longer soaking time caused less peak viscosity and setback from trough. At the same soaking time, germinated brown rice flour soaked in water had higher viscosity than germinated brown rice flour soaked in $CaCl_2$. Pasting temperature of germinated brown rice flour soaked in both water and $CaCl_2$ solutions was not significantly different from that of brown rice. From the studies above, the pasting temperature of germinated brown rice is not different from that of brown rice. On the contrary, Jiamyangyuen and Ooraikul (19) showed that germinated brown rice had lower pasting temperature, peak viscosity and final viscosity than normal brown rice and when rice was soaked and germinated for longer time, pasting temperature, peak viscosity and final viscosity were decreased.

From the review, pH, temperature and time affect the chemical and physical properties of germinated brown rice. The pH that gives germinated brown rice with high protein, GABA and phenolic compounds is pH 6-7. The temperature suitable for germination is between 30-35 °C, and germination time is between 24 to 72 hrs.

2.1.4 Application of germinated brown rice in food

Other than direct consumption, germinated brown rice can be used in various forms such as flour or ingredient in many food products. Product development is an important role in increasing germinated brown rice utilization (19). There are many papers that study about the application of germinated brown rice in food.

In bakery products, Watanabe et al. (70) reported that bread to which germinated brown rice flour was added had less bread volume and less staling but more viscosity of the dough than bread containing only wheat flour. Jiamjariyatam (16) concluded that the ratio of germinated brown rice flour and wheat flour that gave the best condition for producing cookies was 60:40 but the cookies had less hardness compared to the cookie without germinated brown rice flour. In dairy products, Anawachkul and Jiamyangyuen (71) developed yogurt with enhanced level of GABA, using germinated Munpoo brown rice as an important ingredient. Fifty grams of germinated rice grains were ground in a food blender for 3 minutes. Water (150 ml) was added and the mixture was steamed for 20 minutes. Germinated red rice paste obtained was further used as a yogurt ingredient. The yogurt formula was developed and 30% germinated red rice flour prepared was selected. The enriched yogurt contained 4.09 mg/100 g of GABA. For instant food, Jongdee et al. (7) produced rice porridge from germinated brown rice by steaming the germinated brown rice at 150°C for 30 minutes, drying it in a drum dryer, grinding it into flour and mixing it with seasoning. Sutinium et al. (72) and Lerswanichwatana (58) produced a beverage from germinated brown rice. The process for preparing the flour was similar. Germinated brown rice was heated with water by steaming and drying or using drum dryer and ground into powder. The optimum formulation consisted of 20% and 33.65% of germinated rice powder.

2.2 Flour properties

Flour or starch consists of two glucose polymers: amylose and amylopectin. Both are polymers of α-D-glucose connected by (1→4) linkages. Amylose is a linear polymer of α-1,4 linked glucose units. Amylopectin is a larger highly branched polymer of linear chains of α-1,4 linked glucose units, with α-1,6 branch points every 20 to 25 glucose residues (73). In the starch granule, the amylose and amylopectin are together but when the short linear segments of amylopectin align they become ordered into crystallites. The crystallinity rises from the extensive hydrogen bonding, both intramolecular and to water molecules, of the amylopectin molecules (74).

Granule swelling, gelatinization, pasting and retrogradation are important aspects of flour functionality (75).

2.2.1 Swelling and solubility properties

Flour can not dissolve in water, if it is in the condition where temperature is lower than gelatinization temperature because the flour has hydrogen bonds in between hydroxyl group of each starch granules. When temperature is higher than gelatinization temperature, the hydrogen bonds will break, so water molecule can bond with free hydroxyl group. Starch granule will swell, then flour can be soluble, become clearer and has more viscosity (76).

When flour suspension is heated, starch granules are swollen and some of the starches are dissolved. Swelling of starch granules is also accompanied by leaching of starch molecules from the granules. This material is largely amylose. The molecular weight of the solubilized amylose increases with increasing temperature (75). Swelling power of flour can be expressed in the form of the highest volume or weight of flour, when it swells in water. Solubility properties can be expressed in the form of total weight of soluble solid in water (76).

2.2.2 Gelatinization and retrogradation properties

Gelatinization occurs when starch granules are heated in excess water and progressively gives higher temperatures (75). Gelatinization occurs when flour or starch suspension is heated, resulting in irreversible disruption of molecular order in starch granule (77). First, the granules swell as hydrogen bonds in the amorphous portions are disrupted, then water is absorbed. More hydration and swelling occur in the amorphous regions when the temperature increases, causing the crystallites to disintegrate, and then undergo hydration and melting. Lastly, polymer molecules, particularly amylose, leach out of the granules and viscosity increases (78-81).

Retrogradation is the process in hwhich suspension of flour or starch is cooled below the melting temperature of starch crystallites, amylose and amylopectin reassociate and unite with the swollen starch granules forming a rigid structure, resulting in viscosity increasing, gel firming, and textural staling of predominantly starch-containing systems (78, 82). This is the reason for the increased firmness of cooked food after cooling or storage (78).

Gelatinization and retrogradation properties can be measured using differential scanning calorimetry (DSC). DSC is a widely used tool to investigate thermal properties and phase transition of flour or starch (83). It measures first-order (melting) and second-order (glass transition) transition temperatures and heat flow changing and gives information on order-disorder phenomena of starch granules (84). In the DSC curve of flour or starch at intermediate water levels, three endothermic transitions are usually observed. The first two endotherms correspond to the disorganization of starch crystallites or gelatinization, wherein glass transitions of water-plasticized amorphous portions and then non-equilibrium melting of the microcrystallites of the partially crystalline amylopectin occur (84-85). The third endotherm takes place at higher temperature, and relates to the melting of complexes formed by amylose and lipids (84). Crystallite quality and the overall crystallinity of the starch are measured by the peak temperature (Tp) and the enthalpy of gelatinization (△H), respectively (86). Onset temperature (To) and completion temperature (Tc) determine the boundaries of the different phases in starch (84).

2.2.3 Pasting properties

Continued heating of flour or starch in excess water together with stirring causes the granules to further swell, the amylose to leach more, and the granules to disintegrate more,forming a paste which is a viscous material (81). Pasting occurs after or simultaneously with gelatinization. Pasting properties of starch are important indicators of how the starch will perform during processing and are commonly measured using the Rapid Visco Analyzer (RVA). Figure 2.4 shows a typical RVA pasting curve.

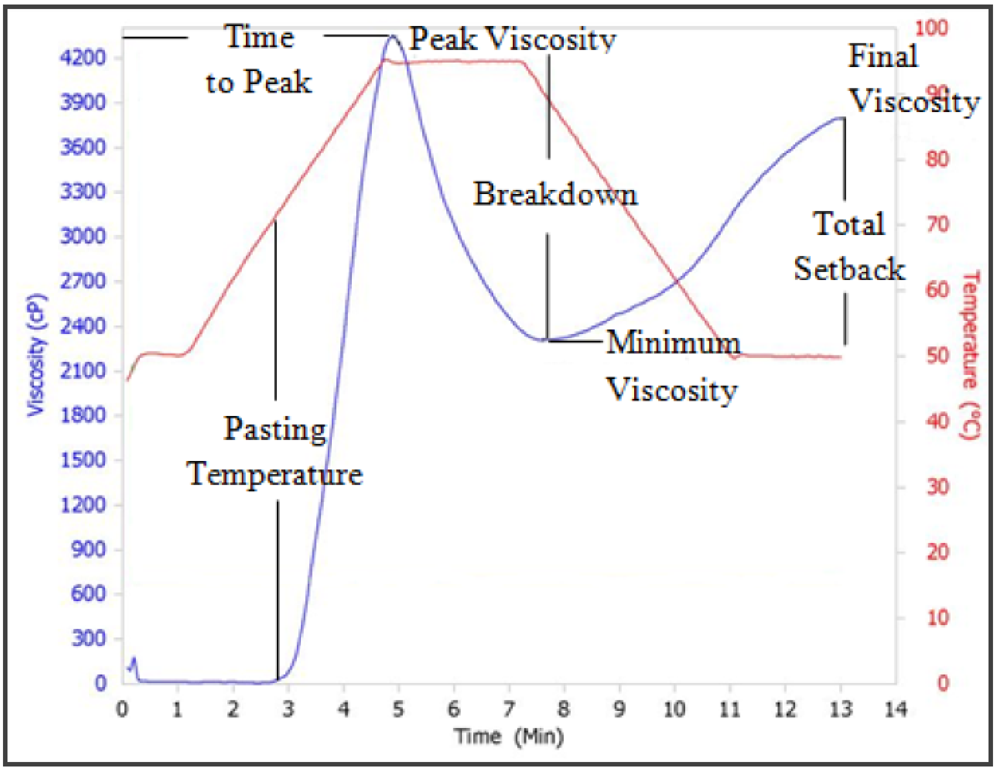

Figure 2.4 Typical Pasting Curve of Starch as Measured by RVA

In the RVA test, flour or starch is mixed with water for hydration and held for a short time above ambient temperature. Heating proceeds resulting in swelling of starch granules. As heating continues, an increase of viscosity can be observed, which indicates the process of pasting. The temperature at the onset of viscosity increase is termed pasting temperature. Viscosity increases with continued heating, until the rate of granule swelling equals the rate of granule collapse, which is referred to as the peak viscosity (PV). PV reflects the swelling extent or water-binding capacity of flour or starch and often correlates with final product quality since the swollen and collapsed granules relate to the texture of the cooked starch. After PV is achieved, a drop in viscosity, or breakdown, is observed as a result of disintegration of granules. Breakdown is a measure of the ease of disrupting swollen starch granules and suggests the degree of stability during cooking (87). Minimum viscosity, also called hot paste viscosity, holding strength, or trough, marks the end of the holding stage at the maximum temperature of the RVA test. Cooling stage begins and viscosity rises again (setback) which is caused by retrogradation of starch. Setback is an indicator of final product texture and is linked to syneresis or weeping during freeze-thaw cycles.

Viscosity normally stabilizes at a final viscosity or cold paste viscosity, which is related to the capacity of starch to form gel after cooking and cooling (88-89).

Table 2.1 Gelatinization and pasting properties of native starches

Property	Rice	Potato	Corn	Wheat	Tapioca	Waxy maize	Sorghum
Gelatinization temperature range (°C)	68-78	58-68	62-72	58-64	59-69	63-72	68-78
Brabender peak viscosity (BU)	500	2900	700	250	1200	1100	700
Swelling power at 95°C	19	1150	24	21	71	64	22
Paste viscosity	Medium-low	Very high	Medium	Medium-low	High	Medium-high	Medium
Paste clarity	Opaque	Translucent	Opaque	Opaque	Translucent	Translucent	Opaque
Retrogradation rate	High	Medium	High	High	Low	Very low	High

2.2.4 Rheological properties

Rheological properties describe the deformation or rupture and flowing of flour under applied stress and temperature. They can be an indication of how the flour is going to behave under various processing conditions. They are of importance in terms of product formulation, quality control, machining properties, scale-up of the process and automation (90-91).

Dynamic rheological properties give information on the flow and elastic properties of materials. In a measurement the sample is put between two round plates or between a cone and a plate. The system is maintained at desired temperature and a sinusoidal deformation at different frequencies is applied. In this process, storage and loss modulus are reported as a function of frequency. Storage modulus (G') represents the energy stored during deformation and relates to the elastic energy of the sample, while loss modulus (G'') represents the energy lost during deformation and relates to the viscose energy. The ratio of loss modulus (G'') and storage modulus (G') is determined as the phase angle or tan δ, which can be an indication of the stiffness and extensibility of flour. A high value of storage modulus and a low value of loss modulus indicate high stiffness. While low value of storage modulus indicates softer and more extensible material (92).

There are many factors that affect the properties of flour. i.e. type of flour, component in flour, modification of flour, size of starch granules and amylose content (30).

Chung and Meullenet (93) determined how protein affected the pasting properties of rice flour and concluded that protein influenced viscosity curve. Peak, hot-paste, and cold-paste viscosities of protease-treated rice flour was significantly decreased compared to the untreated rice flour. Ninchan (36) reported that rice flour had higher pasting temperature, gelatinization temperature and final viscosity than rice starch, due to the role of non-starch components, protein and fat.

Varavinit et. al. (94) investigated the effect of amylose content on gelatinization, retrogradation and pasting properties of flours from different cultivars of Thai rice. Eleven cultivars of Thai rice, consisted of low, medium and high amylose content rice were used. The results indicated that the gelatinization temperature and retrogradation rate increased with increasing amounts of amylose but the viscosity decreased with increasing amounts of amylose. Similar results were presented by Ninchan (36). Singh et. al. (95) reported that higher amylose content caused less swelling and solubility of flour.

2.3 Pregelatinization

Pregelatinization is a process in which suspensions of flour or starch and water were cooked and dried to give products that disperse readily in cold water and form moderately stable suspensions (30). There are many types of equipment used for pregelatinization.

2.3.1 Spray dryer

In spray drying, flour suspension is fed to an atomizer and the droplets are formed. They are mixed with hot gas which evaporates the water from the droplets, leads to formation of particulates of flour. At the same time, hot gas causes gelatinization of starch granules in the flour (96).

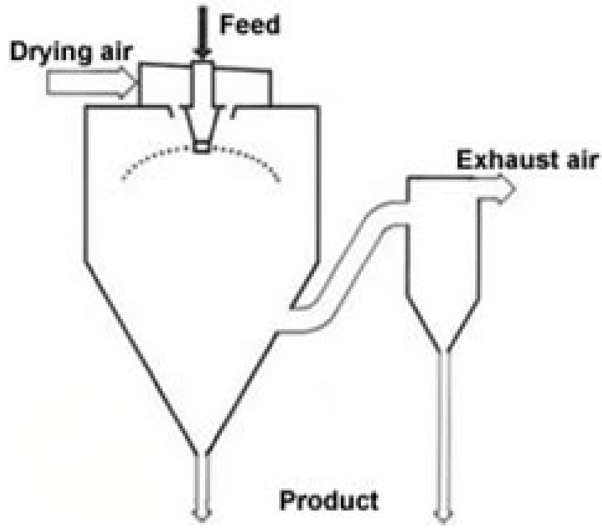

Figure 2.5 Spray dryer scheme

2.3.2 Spouted bed dryer

Spouted bed dryer has been used in many thermal mechanical and chemical processing applications such as drying of varieties of other coarse particles, suspensions, particle coating and granulation, heating, gasification, etc. One major advantage of spouted bed is its obvious low cost due to its mechanical simplicity (96).

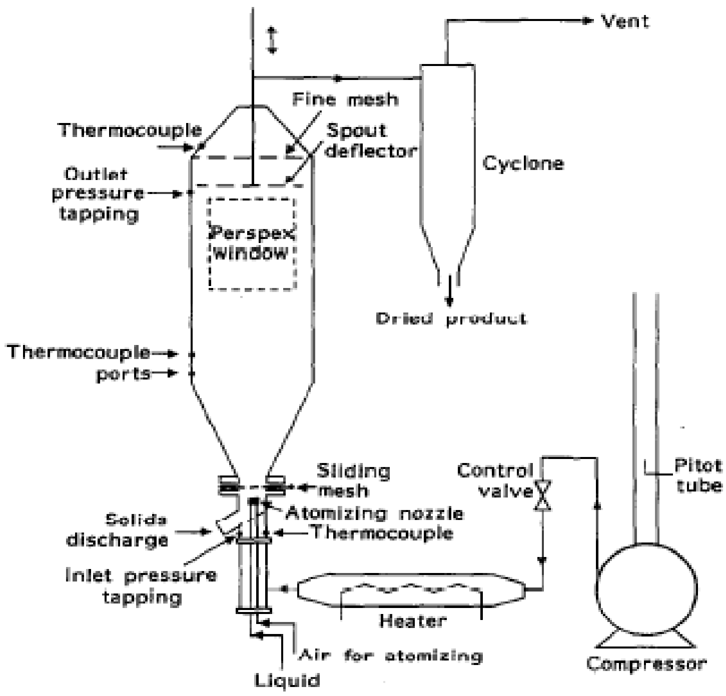

Figure 2.6 Schematic diagram of spouted bed dryer

Basically it comprises a cylindrical vessel with a conical bottom fitted with an inlet nozzle for introduction of the spouting air (drying medium). A draft tube is held in the center to enhance the height for which the granular particles can be spouted. The wet material enters from the side entrance port and undergoes spouting with the assistance of the draft tube. The incoming drying air, introduced through a centrally located opening at the conical base, interacts well with the particles. The particles, upon interaction with the air, rise rapidly through a hollowed central core, namely spout zone, within the vessel. These particles, after being raised a certain height above the bed surface, get separated from the air stream and drop into the annulus region where they move slowly downward due to the angular shape of the bed. As the particles undergo spouting motion, moisture is removed from the particle surface. During the process, flour suspension is gelatinized and dried at the same time (97).

In 2008, Karbassi and Mehdizadeh (98) examined the effects of the fluidized-bed drying, which had similar technique to spray and spouted bed drying, on rice flour. In this study rice flour was pregelatinized at 140 ºC for 2 minutes. Results showed that gelatinization temperature of pregelatinized flour decreased significantly compared to control flour. This is consistent with the report by Timabud et. al. (99) who studied the effect of pregelatinizing rice flour by a fluidized bed dryer. The temperatures used to perform the experiment were 45, 65 and 85 ºC and the drying time was 30 min. The result showed that the increase in temperature decreased viscosity of pregelatinized flour significantly.

2.3.3 Drum dryer

Drum dryer is of two types: single drum dryer and double drum dryer. Heat comes from steam inside the drum and causes gelatinization. When using the drum dryer, adjusting the temperature of the surface of the drum and the speed of the drum must be consistent with the moisture of raw material and ability of gelatinization of the flour (100). For double drum dryer, the gap between the drum must be of equal space all the way of the drum and consistent with the temperature of the drum, speed of the drum and the ability of exchanging heat must be constant (21, 101). The production of pregelatinized flour can be done by preparing the flour solution not over 43% and feeding it to the machine. The heat will cause gelatinization and dry the flour suspension at the same time (100). The production of pregelatinized flour by a drum dryer also can be done by pregelatinizing the flour first, then drying by the drum dryer (102). When the flour dries the blade attached to the drum will scrape the flour sheet, then it will be milled to become pregelatinized flour (96).

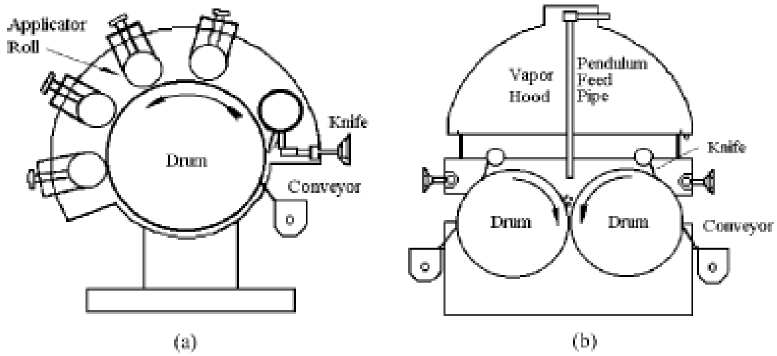

Figure 2.7 Composition of drum dryer (a; single drum dryer b; double drum dryer)

Chainui and Sasakun (103) pregelatinized Cheangphattalung and Lukdangpattani rice flours by a double drum dryer. The effect of the concentration (20, 30, 40 and 50 %) of flour in the suspension and the temperature of the double drum dryer (120, 130 and 140 °C) were investigated. Water Absorption Index (WAI), Water Soluble Index (WSI) and viscosity of pregelatinized flour were analysed. It was found that the solid content in the suspension affected the WAI, WSI and viscosity of pregelatinized flour significantly. They decreased as the solid content increased. The temperature of the drum affected WAI and viscosity significantly. Increasing temperature decreased WAI and viscosity. Similar results were found in the study of Na Nakorn (104) of pregelatinized Glutinous, Jasmine and Chiang rice flour in a double drum dryer at the concentration of 35% at 110, 117 and 123 °C. Viscosity and water absorption index (WAI) were assessed by a Rapid Visco Analyser (RVA). Increasing temperature in pregelatinization decreased viscosity and WAI.

2.3.4 Extruder

Extruder uses single or twin screws to transport, mix, knead, shear, shape, and cook raw materials into food products by forcing the raw materials to mix through shaped dies to produce specific shapes and lengths. Starch will be crushed and deformed due to the shearing inside the moving screw, and heat from friction and cooking causes starch gelatinization. When the flour moves through the dies, pressure and temperature drops, the starch expands and the water evaporates. The pregelatinized flour is dried or semi-dried. Factors affecting the pregelatinized flour are moisture of the flour, temperature and speed of the screw (105).

Extrusion equipment gives many advantages that result in minimizing time, energy, and cost (106). A major difference between extrusion and other food processing method is that gelatinization occurs at lower moisture contents (107).

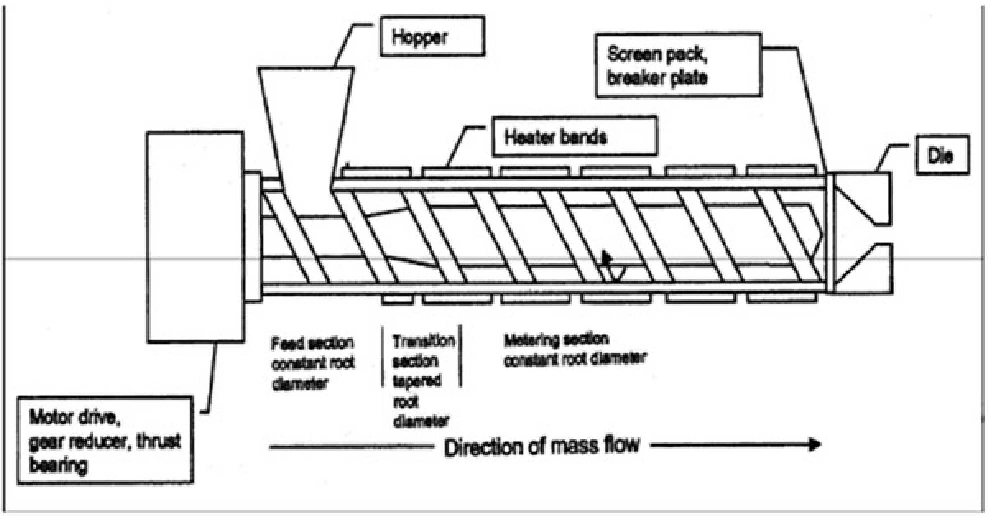

Figure 2.8 Composition of single-screw extruder

Bryant et. al. (108) pregelatinized short grain and long grain rice flours with a twin screw extruder. Three temperatures (100, 120 and 150 °C) and five water feed rates (11, 15, 20, 25, and 30 ml of water/100 g of flour) were studied. Water absorption, water solubility index, fat absorption index, bulk density and viscosity of pregelatinized flour were determined. It was found that extrusion cooking could increase solubility and decrease viscosity. The changes that occurred were related to the cultivar used, moisture content, and temperature conditions of the extruder. Increasing temperature and moisture increased the water absorption, water solubility index, fat absorption index and bulk density but decreased viscosity of flour. Related study was done by Kadan et. al. (109) who studied the functional properties of pregelatinized long-grain and short-grain rice flour by a twin screw extruder at 70 to 120 °C with 22% moisture content. Results showed that water absorption, fat absorption, and water solubility indices increased with an increase in extrusion temperature. The cold-paste viscosities increased, whereas the peak, breakdown, setback, and final viscosities decreased with an increase in extrusion temperature. Chuang and Yeh (110) studied the viscoelastic properties of pregelatinized glutinous rice flour produced by single screw extruder at moisture contents of 45-55% and barrel temperatures of 75-95 °C. It appeared that the moisture content was the main factor influencing the value of G′ and tan δ. Both G′ and G″ decreased due to the disintegration of starch granules with rising temperature and moisture content.

2.3.5 Hot air oven

Hot air oven is one kind of equipment that is most frequently used for food dehydration. It is a method in which heated air is blown over food materials with the aid of fan to remove most of the moisture from the food material. In this process, gelatinization of starch occurs (111).

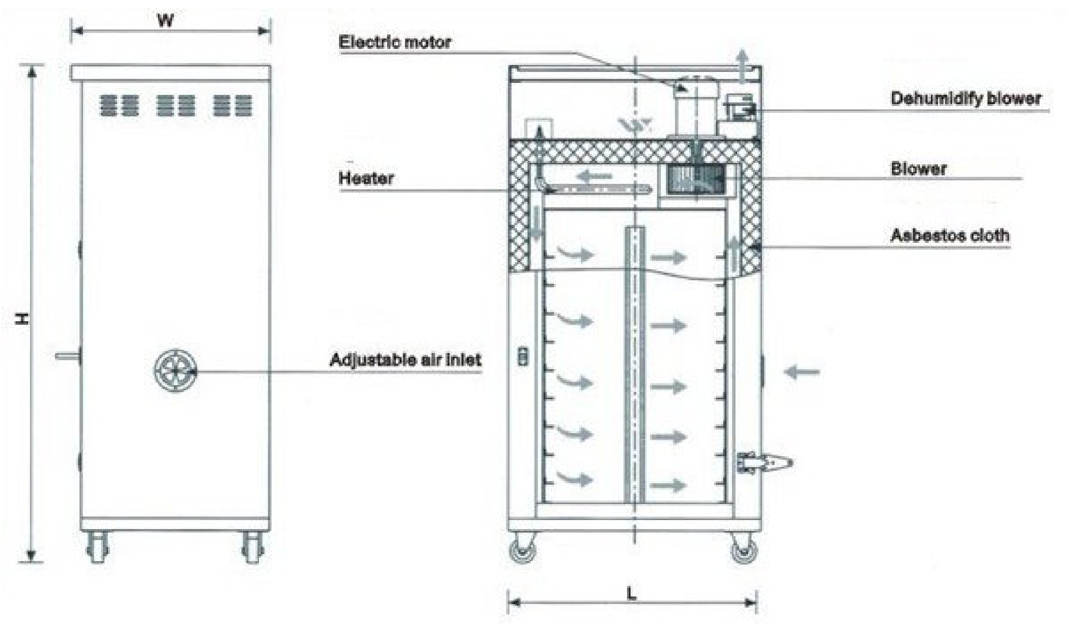

Figure 2.9 Composition of hot air oven

Wadchararat et. al. (24) pregelatinized rice flour of three rice varieties; Chainat 1, Khao Dawk Mali 105 and RD 6 by using hot air oven. The pregelatinized rice flour was prepared by adjusting rice flour initial moisture to 30% and heating at 100 °C for 16 hr. Pregelatinized flour exhibited the increase of the pasting temperature while viscosity decreased. In addition, the swelling power and solubility of pregelatinized flour also decreased. In studies using other cereals i.e. yam, the result showed that viscosity of the pregelatinized yam flour produced from hot air oven at 60 °C decreased significantly compared with control flour. Water absorption capacity, swelling power and solubility index were not significantly different from control flour (111). This was in agreement with Falade and Onyeoziri (112) who demonstrated that pregelatinized yam flour produced by hot air oven at 100 °C for 2 hrs had pasting temperature of pregelatinized flour which was not different from control flour but viscosity was decreased significantly. In sweet potato, Collado and Corke (113) pregelatinized sweet potato flour by adjusting the moisture content of flour to 25%, and placed in a hot air oven for 4, 8 and 16 hrs at 110 °C. Pregelatinized flour had decreased viscosity. As time of pregelatinization increased, viscosity of pregelatinized flour decreased. Lowest viscosity was found in pregelatinized flour with the longest time of pregelatinization.

2.4 Pregelatinized flour properties

Pregelatinized flour has more water binding capacity than normal flour. Because of the deformation of the starch inside, the crystalline of the starch is destroyed (114) but produces no effect on the quantity of amylose and amylopectin content. Thus, pregelatinized flour can dissolve in cold water, and in good absorb water and can give viscosity immediately without heating (115-116). When heating the pregelatinized flour in water, viscosity is less than non-modified flour (96).

One factor that affects the pregelatinized flour properties is milling. When the pregelatinized flour is in the water, it will float on the water better than non-modified flour and will cause lumping easily. If the flour is coarse, it will prevent lumping but the absorb water ability will decrease because the surface area is less, making absorbing water slower (116). If the flour is fine, it will absorb water and give viscosity quickly (117).

In 2004, Lai and Cheng (118) studied the properties of pregelatinized rice flour made by hot air or gun puffing. The results showed that the properties of pregelatinized rice flour are significantly influenced by two major factors, i.e. rice varieties and processing methods. The hot air process does not significantly degrade starch molecules, whereas the gun puffing process causes significant starch degradation. These result in different pasting and hydration properties of pregelatinized rice flour. Pregelatinized rice flour from two methods gave high viscosity when it is added to cold water but the gun puffed rice flour provided a higher initial viscosity than hot air rice flour and the viscosity decreased significantly after cooking.

Rungruangaree (96) produced pregelatinized rice flour by using a double drum dryer and found that the suitable condition for producing pregelatinized rice flour in a drum dryer was flour suspension with 40% concentration, when speed of the drum was 0.25 rpm, temperature of the drums were 130 °C, the space of the drums was 0.01 inch and the pressure was at 20 pound/inch^2. Similar condition was presented by Wadchararat et. al. (24) who prepared pregelatinized flour by the same condition as above except for the temperature which was lower (115 °C). Youngsook (119) prepared pregelatinized flour from glutinous rice. The pregelatinized glutinous rice flour was prepared by the same condition as the condition above. Flour solution containing 35% solid was passed through a drum dryer, the pressure was set at 40 lb/inch^2, and 2.38 rpm of drum speed and 0.04 inch of drum dryer gap were used. From these studies, the results showed that pregelatinized rice flour had higher water soluble index and water absorption index but lower pasting temperature and peak viscosity than normal rice flour.

Hagenimana et. al. (120) prepared pregelatinized flour by a twin screw extruder. Results showed that viscosity values of extruded rice flours were less than that of unprocessed rice flour. Decreasing moisture content of flour and screw speed and increasing barrel temperature resulted in lower viscosity. Water absorption index (WAI) increased with the increase of barrel temperature but low moisture content and high barrel temperature decreased WAI. Higher moisture contents, lower barrel temperatures and screw speeds lead to the lowest values of water soluble index (WSI). Guha et. al. (121) demonstrated that extrudate characteristics were mainly dependent on temperature, whereas the screw speed had less effect. Bulk density decreased with elevation in temperature and screw speed. WAI and WSI increased with the reduction of barrel temperature. Samples extruded at lower screw speeds showed high WAI values than at higher screw speeds. This study conformed to the study of Ding et. al. (122).

Comparison of pregelatinized rice flour produced by different methods was studied. Rungruangaree (96) determined the properties of pregelatinized rice flour by a double drum dryer and a single screw extruder and concluded that pregelatinized rice flour by extrusion process had higher WSI and WAI and lower pasting temperature than pregelatinized rice flour from a drum dryer. Wadchararat et. al. (24) studied the characterization of pregelatinized rice flours and heat moisture treated rice flours. The result showed that pregelatinized rice flour gave a decrease of the pasting temperature, peak viscosity, trough, breakdown, final viscosity and setback. On the other hand, heat moisture treated rice flour exhibited an increase of the pasting temperature and setback with time; while peak viscosity, trough, breakdown and final viscosity decreased, compared to the untreated sample.

2.5 Application of pregelatinized rice flour

Pregelatinized rice flour has been widely used as a bulking agent or thickening agent in baby food, pudding, instant drink, sauce, gravy, soup powder, instant cake mixes due to its easily solubility in cold water property, and is used in meat product as a binder agent to hold water and keep moisture, also to absorb water and absorb air in cake product, and to make the cake moist and have good texture (123).

Youngsook (119) added pregelatinized glutinous rice flour to cookies and cakes. RD 6, RD 10 and Hangyee 71 milled rice were used as materials. In cookies, adding more pregelatinized glutinous rice flour made cookies harder and less brittle. In cakes, adding more pregelatinized glutinous rice flour gave more volume to the cake. Rungruangaree (96) added pregelatinized rice flour and protein isolate to bread made from rice flour. The rice flour in the bread formula was partly replaced with 10%, 15% and 20% of the pregelatinized rice flour and the amount of water was also varied at 90%, 100% and 110% of flour. The most suitable condition for preparing bread was replacing the pregelatinized rice flour at 20% level in the bread formula containing 100% water. The bread had soft crumb, slightly firm texture and showed no crack on crust. However after an overnight storage dried crumb and crust occurred and stale flavor was detected. Wadchararat et. al. (24) used pregelatinized rice flour for freeze noodle making. The noodles contained pregelatinized rice flour, had lower absorption and cooking loss, but higher cooking time. The texture could still be maintained after two freeze-thaw cycles. Quintero-Fuentes et. al. (124) studied the effects of pregelatinized rice flours on the structure and texture of baked corn chips. Results showed that adding 20% of pregelatinized rice flours increased chip strength and thickness of baked chips.

2.6 Effects of heat treatment on nutrients in foods

Heat treatments can cause considerable losses in some essential nutrients, such as protein or water-soluble vitamins and minerals. Degradation depends on specific parameters during food processing e.g. temperature, oxygen, light, moisture, pH and time (31).

Heat treatment generally improves digestibility of proteins due to inactivation of protease inhibitors, which have a harmful effect on the digestion by blocking proteolytic enzymes (125). The biological availability of the amino acids is also affected. Heating in the presence of reducing sugars causes Maillard reaction (126). The Maillard reaction (nonenzymatic browning) is the reaction between reducing sugars and free amino groups in proteins. It leads to a decrease both in protein digestibility and in availability of amino acids involved. Maximum browning occurs at water activities between 0.3 and 0.7 depending on the type of food (127). Thus, dry heat treatment as in roasting or baking is more damaging than autoclaving or pressure cooking where water activities are close to 1 (128). Lysine is the most reactive protein-bound amino acid due to its free ε-amino group. However, arginine, tryptophan, cysteine and histidine may also be affected. Free amino acids are much more sensitive to damage during extrusion cooking than those in proteins (126).

During heat processing the nutritional value of lipids might be affected through different mechanisms such as oxidation, cis-tran isomerization or hydrogenation. A decrease in fat content of processed products has been reported.

Monoglycerides and free fatty acids form complexes with amylose during heating (129), and thus may become more difficult to extract. Other possible explanations of a decrease in fat content are thermal degradation.

Vitamins can be damaged by mechanisms such as heat and oxidation. In addition, conventional boiling in water can lead to considerable loss of water-soluble vitamins, e.g. B-vitamins and ascorbic acid, due to leaching (130).

Thiamin and riboflavin retention during cooking are most studied. During conventional processing, thiamin is most labile to heat while riboflavin is comparatively thermostable. Fadahunsi (131) studied the effect of boiling on the water soluble vitamins in bambara nut flour. It was observed that the thiamin content decreased by 52.4%, riboflavin decreased by 56.2%, folacin decreased by 35.0%, niacin decreased by 70.0% and biotin decreased by 48.3% after boiling for 45 minutes. Related result was shown by Khalil and Mansour (132) who reported that Pyridoxine, pantothenic acid and riboflavin were more stable to heat processing than niacin and thiamine.

During conventional cooking in water, large losses of vitamin C may result from a combination of oxidation and leaching. (133). Boiling for 2 minutes caused 79% destruction of added vitamin C in a maize/soy/groundnut mixture, compared to extrusion cooking which was much less detrimental, only 33% being destroyed (134).

Other than water-soluble vitamin, heating also affected fat-soluble vitamins. Boiling maize/soy/groundnut blend for 2 minutes resulted in greater losses of carotene. (134).

There are many studies that compare the nutrients in food from different heating methods.

Khatoon and Prakash (135) determined the nutrient retention in pressure and microwave-cooked legumes. Both cooking methods resulted in a slight decrease of protein content, iron content, thiamin and ascorbic acid of legumes and no significant difference between the two methods was found. These results were in agreement with the report by Seenaa et. al. (136) who discovered that there was a significant difference between the proximate composition of raw and pressure-cooked legume seeds. In this study, pressure-cooking drained most of the minerals of seeds except for calcium, copper and zinc and when compared with roasting there was no significant difference but pressure-cooked seeds showed better biological indices than roasted seeds. The study also showed that thermal processing decreased total phenolics. Khalil and Mansour (132) supported these results by noticing that protein contents in cooked and pressure-cooked faba bean were not significantly different. Moreover, heat processing increased the contents of leucine, threonine and histidine and there was a slight change in the mineral content by both heating methods with the exception of K and Ca.

In 2010, Kaushik et. al. (137) demonstrated how domestic processing techniques affected the nutritional quality of soybean. The processing consisted of microwave cooking, pressure-cooking and boiling. The results showed that every cooking method greatly reduced starch content and increased crude protein content, which was highest in the case of pressure-cooking. Boiling caused high losses in water soluble vitamin. Microwave cooking resulted in greater retention of minerals and vitamins as compared to pressure cooking and boiling.

It is apparent that heating affects the nutrients. Pregelatinization is a process that involves heating, therefore, nutrients in pregelatinized flour are affected.

Chinnasarn and Manyasi (138) pregelatinized taro flour by boiling at 100 °C for 5 minutes and drying in hot air oven at 65 °C for 7 hours. Results showed that proximate compositions (protein, fat, ash, crude fiber and carbohydrates) of pregelatinized flour were similar to those of native flour. Mbaeyi and Onweluzo (139) also found that pregelatinized sorghum flour produced by steaming at 100 °C had similar proximate compositions when compared to native flour. Jisha et. al. (120) also found that pregelatinized cassava flour by steaming for 30 mins had protein and fat contents similar to native flour (140). These findings were in agreement with a report by Ahmed et. al. (141) who pregeatinized sweet potato flour by heating in hot air oven at 50, 55 and 65 °C. Proximate composition, β-carotene, total phenolic and ascorbic acid of flour were determined. The results showed that proximate composition and total phenolic of pregelatinized flour from every temperature was not different from native flour. β-carotene and ascorbic acid of pregelatinized flour from 65 °C decreased significantly from those of native flour. In addition, Otegbayo et. al. (142) found that pregelatinized rice flour produced by steaming for 15 mins and drying at 45 °C for 8 hrs had drastically lower vitamin B1 content but similar proximate composition compared with native flour. Similar result was found in the report by Karbassi and Mehdizadeh (98) who pregelatinized rice flour using fluidized bed drier at the temperature of 140ºC for 2 mins. Result showed that pregelatinized flour had lower vitamin B1 content compared to native flour. Moreover, Muyonga et. al. (143) produced pregelatinized banana flour by steaming and found that pregelatinized flour had lower content of vitamin C.

From the literature review, pregelatinization affects both chemical and physical properties of flour. Different techniques used in pregelatinization result in pregelatinized flour with different physico-chemical properties. Furthurmore, the investigation of physico-chemical properties of pregelatinized germinated brown rice flour is quite limited. This study thus elucidated the alteration of physico-chemical properties of pregelatinized germinated brown rice flour produced by different techniques: single screw extruder, spray dryer, spouted bed dryer and hot air oven.