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THESIS

IMPROVEMENT OF FROZEN RICE NOODLE BY USING BLENDED FLOUR FROM ANNEALED PRE-GERMINATED BROWN RICE FLOUR

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Frozen rice noodle is normally low stability and poor texture quality after thawing. This research was aimed to modify two pre-germinated brown rice flours, Chai Nat1 and Rice Division 6 (RD6), by annealing process to improve flour quality to make frozen rice noodle to be better freeze-thaw stability. Pre-germinated Chai Nat1 and RD6 paddies at minimum stage (embryo growth length 0.5-1 mm, 60-70% of germination) were done by soaking at 30^{o}C for 12 and 6 hours then incubating at 30^{o}C (85% relative humidity) for 20 and 16 hours, respectively. Both Chai Nat1 pre-germinated brown rice flour at minimum stage (Chai Nat1 PGBR flour_1) and RD6 pre-germinated brown rice flour at minimum stage (RD6 PGBR flour_1) were milled from broken or whole pre-germinated brown rice (PGBR) which were dehusked from pre-germinated paddies at minimum stage. The reducing sugar of both Chai Nat1 PGBR flour_1 (146.50 mg/100g) and RD6 PGBR flour_1 (387.51 mg/100g) were significantly ($p \leq 0.05$) increased when compared to Chai Nat1 white rice flour (85.22 mg/100g) and RD6 white rice flour (344.26 mg/100g) and Chai Nat1 brown rice flour (84.44 mg/100g) and RD6 brown rice flour (341.85 mg/100g). The reducing sugar of Chai Nat1 PGBR flour_1 (146.50 mg/100g) was significantly ($p \leq 0.05$) lower than RD6 PGBR flour_1 (387.51 mg/100g). The syneresis at the fifth freeze-thaw cycles of both multiple steps annealed Chai Nat1 PGBR flour_1 (30.32 %) and RD6 PGBR flour_1 (14.31 %) showed significantly ($p \leq 0.05$) decreased when compared to double and one step annealing processes. The syneresis of multiple steps annealed Chai Nat1 PGBR flour_1 among 1-5 cycles (6.95-30.32 %) were significantly ($p \leq 0.05$) higher than multiple steps annealed RD6 PGBR flour_1 among 1-5 cycles (3.95-14.31 %). Frozen rice noodle making from blended both multiple steps annealed Chai Nat1 and RD6 PGBR flour_1 to Chai Nat1 white rice flour (5:5:90) showed significantly ($p \leq 0.05$) higher tensile strength (33.11 g) after the fifth freeze-thaw cycles when compared to frozen rice noodle making from Chai Nat1 white rice flour (13.59g). Moreover, this frozen rice noodle showed significantly ($p \leq 0.05$) increased firmness (11.45) in sensory evaluation using qualitative data analysis (QDA) when compared to frozen rice noodle making from Chai Nat1 white rice flour (9.65).

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Student's signature Thesis Advisor's signature

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Onamon Chongsrimsirisakhul

March 2012

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LISTS OF ABBREVIATIONS

cP	=	Centipoise
g	=	Gram
PGBR	=	Pre-germinated brown rice
PGBR flour	=	Pre-germinated brown rice flour
PGBR flour_1	=	Pre-germinated brown rice flour at minimum stage (0.5-1.0 mm, 60-70% of pre-germination)
PGBR flour_2	=	Pre-germinated brown rice flour at optimum stage (1.0-2.0 mm, 70-80% of pre-germination)
PGBR flour_3	=	Pre-germinated brown rice flour at maximum stage (2.0-3.0 mm, more than 80% of pre-germination)
RD6	=	Rice Devision6
RVA	=	Rapid Visco Analyzer
TPA	=	Texture Profile Analyzer

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IMPROVEMENT OF FROZEN RICE NOODLE BY USING BLENDED FLOUR FROM ANNEALED PRE-GERMINATED BROWN RICE

INTRODUCTION

In the principle, rice could be modified by pre-germination to improve functionality and bioactive compound (Saman *et al*., 2008). Pre-germination of paddy was approximately between 0.5-3.0 mm of an embryo growth (Dunand, 1993). During pre-germination process, alpha amylase was needed to carry out the starch breakdown and decompose high molecular weight polymer, which lead to the changing of bio-function substances such as gamma-aminobutyric acid (GABA), free amino acid, dietary fiber, inositols, furulic acid, phytic acid, tocotrienols, magnesium, potassium and zinc etc. (Moongngarm and Saetung, 2010). Pre-germination is an inexpensive and effective way to increase nutrition value of rice. However it showed poor functional properties to produce various products due to it showed low pasting temperature and high swelling power when compared to native rice (Panchan and Naivikul, 2009; Musa *et al*., 2011).

Annealing is the hydrothermal treatment that modified starch granules in the presence of excess water for an extended period of time. Annealing performed at temperature between glass transition temperature and gelatinization temperature of starch granules, specifically changes the physicochemical properties of starch by improving crystalline perfection and facilitating interactions among the starch chains (Tester *et al.*, 2000; Hoover and Vasanthan, 1993). The extent of starch chain mobility and the realignment of double helices on annealing might different due to cultivar and the ratio of amylose and amylopectin in starch (Waduge *et al.*, 2006). It was difficult to define what happens to the internal structure of starch granules in response to hydrothermal treatment. The main change in the annealing was reorganization of the granule structures which increased granule stability (Jacobs *et al.*, 1995). Now a day, there are many ways to achieve annealing process for example: one, double and

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multiple steps annealing processes (Nakazawa and Wang, 2003; Shi, 2008). Annealing process could reorganize pre-germinated starch to become more orderly and stable starch molecules structure which could resist heat, shear force and freeze-thaw cycle.

The aim in this study was to find the suitable process to modify pre-germinated starch molecular structure to become stronger molecules structure and blended to white rice flour for making better quality of frozen rice noodle.

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OBJECTIVES

1. To find the condition for pre-germination both Chai Nat1 and RD6 paddies.

2. To find the condition for annealing process both Chai Nat1 and RD6 pre-germinated brown rice flours.

3. To find the ratio for blending both annealed Chai Nat1 PGBR flour and RD6 PGBR flour to Chai Nat1 white rice flour for making frozen rice noodle.

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LITERATURE REVIEW

Rice is a staple food for a large part of the world's especially in Southeast Asia and one of the leading crops in the world which more than 2,000 million people obtain 60-70% of their calories from rice and its by-products (Shih and Daigle, 1997; Zhang *et al.,* 2005).

Rice (*Oryza sativa* L.) is a member of the family Poaceae (formerly Gramineae or grass). Rice grain consists of hull (21%), rice bran (8%), embryo (1%) and endosperm (70%) (Tortayeva, 2009). Rice is harvested as paddy and removed the hull, the outer layer of the rice called brown rice. The brown rice consisted of pericarp, aleurone, grain coat, nucellus and embryo. Removal of pericarp can produce white rice that composed entirely of endosperm, with a starch content of approximately 78% (14% moisture). In addition remove pericarp layer because a decreases in lipid, protein, fiber, ash, reducing sugar and vitamins (Zhou *et al.*, 2002) Protein is the second most abundant constituent of milled rice, and has highest concentration on the surface and decrease toward the center of the kernel. The crude protein and crude fat were found the highest in brown rice followed by milled rice and rough rice. Crude fiber and ash showed the highest value in rough rice than brown rice and white rice. During the milling and polishing process, the majority of the nutrient content contained in white rice is removed and for this reason it is beneficial to consume brown rice instead (Tortayeva, 2009) as seen in Table 1 and 2.

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Table 1 Chemical composition of paddy, brown rice and white rice at 14 percent moisture content.

Nutrients	Paddy	Brown rice	White rice
Protein (N x 6.25%)	5.8-7.7	4.3-18.2	4.5-10.5
Crude fat, %	1.5-2.3	1.6-2.8	0.3-0.5
Crude fiber, %	7.2-10.4	0.6-1.0	0.2-0.5
Crude ash, %	2.9-5.2	1.0-1.5	0.3-0.8
Available Carbohydrates, %	64-73	73-87	77-89
Neutral detergent fiber, %	16.4-19.2	2.9-3.9	0.7-2.3
Free sugars, %	0.5-1.2	0.7-1.3	0.22-0.45
Energy, kj/g	15.8	15.2-16.1	14.6-15.6

Source: Tortayeva (2009)

Table 2 Vitamins and minerals of paddy, brown rice and white rice at 14 percent moisture content.

Vitamins and Minerals	Paddy	Brown rice	White rice
Thiamine (mg/100g)	0.26-0.33	0.29-0.61	0.02-0.11
Riboflavin (mg/100g)	0.06-0.11	0.04-0.14	0.02-0.06
Niacin (mg/100g)	2.90-5.60	3.50-5.30	1.30-2.40
Calcium (mg/100g)	10-80	10-50	10-30
Phosphorus (g/100g)	0.17-0.39	0.17-0.43	0.08-0.15
Iron (mg/100g)	1.40-6.00	0.20-5.20	0.20-2.80
Zinc (mg/100g)	1.70-3.10	0.60-2.80	0.60-2.30

Source: Tortayeva (2009)

In most common types of cereal endosperm starches, the relative weight percentages of amylose and amylopectin range between 72 -82% amylopectin, and 18- 33% amylose. However, some mutant genotypes of maize, barley, and rice contain as much as 70%, amylose whereas other genotypes, called waxy, contain less than 1% (maize, barley, rice, sorghum) (Singh *et al*., 2006).

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Amylose was defined as a linear molecule of (1→4) linked a-D-glucopyranosyl units, but it was today well established that some molecules were slightly branch by (1→6)-α-linkages. Branch linkages were frequently located rather near the reducing terminal end and/or they showed multiple branch side chains. The chain conformation consisted in a left-handed six residues per turn helix. The conformation of free amylose in the native starch granule was not known. It could also be partly involved in double helices with amylopectin short chains in the crystalline regions. Moreover, amylose chains could be involved in amylose–lipid complexes. High-amylose starches were known to contain greater amounts of lipids; the extraction, purification and drying of starch processes might easily induced amylose–lipid complexes upon heating (Buleon *et al*., 1998).

Amylopectin was the highly branch component of starch: it was formed through chains of α-D-glucopyranosyl residues linked together mainly by (1→4) linkages but with 5–6% of (1→6) bonds at the branch points. The basic organization of the chains was described in terms of the A, B and C chains. Thus, the outer chains (A) were glycosidically linked at their potential reducing group through C6 of a glucose residue to an inner chain (B); such chains were in turn defined as chains bearing other chains as branches. The single C chain per molecule likewise carries other chains as branches but contained the sole reducing terminal residue. The ratio of A-chains to B-chains, which was also referred to as the degree of multiple branching, was an important parameter. The general rule was that amylopectin showed rather more A-chains than B-chains, with ratios ranging from 1.0:0 to 1.5:1. Amylopectin showed one of the largest relative molecular weights (10^7–10^9), but mostly in excess of 10^8. Amylopectin was usually assumed to support the framework of the crystalline regions in the starch granule. Branch point did not induced extensive defects in the double helical structure. Only dihedral angles Φ and Ψ of residues adjacent to the (1→6) linkage were modified slightly with regard to their values in the double helix. The conformation of chain segments between branching points was not well known up to now. Amylopectin was usually assumed to support the framework of the crystalline regions in the starch granules. Hence, the short chains with polymerization degrees ranging between 15 and 18 probably showed a double helical conformation. The

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lamellae were believed to represent the crystalline (side-chain clusters) and the amorphous regions (branching regions) of the amylopectin molecule (Buleon *et al*., 1998).

High-performance anion-exchange chromatography with pulsed amperometric detection used to estimate the chain length distributions. Amylopectin was built with three types of chains: short chains, S, consisting of both outer (A) or inner (B) chains with a mean polymerization degree (DP) ranging between 14 and 18, inner (B) long chains, L, of DP 45–55, and a few B-chains of DP above 60. Differences related to the botanical species were mainly concerned with the L: S ratio expressed on a molar basis. This ratio was estimated at 8–10 for normal cereal amylopectin from A-crystalline type (normal genotypes) granules. The chains were fractionated into four different fractions with DP ranging in the intervals 6–12, 13–24, 25–36 and more than 37. Amylopectin with high and low amounts of the DP 6–12 fraction respectively, show A- and B-type X-ray diffractions of starch granules respectively. These S-chains with DP between 6 and 12 determined the starch crystalline allomorph. The DP 6–12 fraction should play an important role in the determination of starch crystallite polymorphs. The localization of the α-(1→6) linkages showed defined only by using a sequential enzymatic method. This analysis showed performed both on native amylopectin together with their corresponding β-limit dextrin, and Naegeli amylodextrins from granular starches. These chains were not linked together randomly but rather were organized in a cluster structure. Branch points were arranged in clusters which were not distributed randomly throughout the macromolecule. Another enzymatic approach to the problem of amylopectin structure was obtained through α-amylolysis (Buleon *et al*., 1998) as shown in Figure1.

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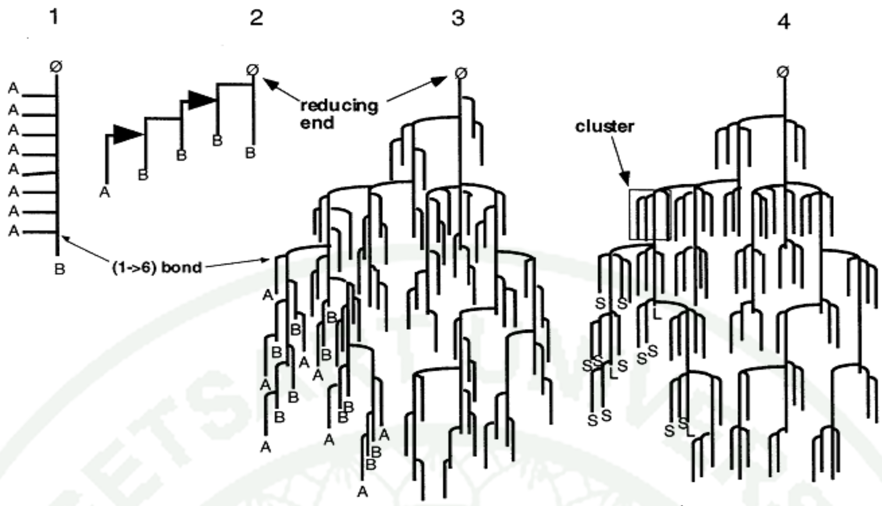

Figure 1 The molecular structure of amylopectin.

Source: Buleon *et al*. (1998)

Starch granule

The shape (round, oval, polyhedral), particle size (2-100 mm) and particle size distribution (unimodal, bimodal, trimodal) of starch granules are typical depend on botanical origin. In rice, several polyhedral small (2-7 μm) granules are produced in one amyloplast. Rice starch have a bimodal distribution comprising large A-type (lenticular, 10-35 μm) and small B-type (spherical, 1-8 μm) granule populations (Stoddard, 1999). Starch granules show “birefringence” when microscopically viewed using polarized light. The refraction of polarized light by the crystalline regions in starch results in a “Maltese cross” characteristic of a radial orientation of the macromolecules. However, the center or hilum of starch granules is less organized than the rest of the granule. Moreover, the peripheral amylopectin helices do not point to a single focus, but to an inner ellipsoid (Waigh *et al*., 1998).

A-chains could be considered as the limiting factor defining crystallite thickness. Assuming classical models for A- and B-crystallites, involvement of S-chains in crystallite thickness leads to a helicoidal length of about 5.7 nm, with 16 glucosyl units, each one giving a repeating distance of 0.35 nm per glucose. The later

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assembly of clusters could explain the formation of crystallites. However, the exact global conformation of amylopectin inside a granule remains unknown (Buleon *et al.*, 1998).

The recent models for A- and B-type structures were based on parallel double-stranded helices, right-handed or left-handed and packed antiparallel or parallel in the unit cell. In the A-structure, these double helices were packed with the space group B2 in a monoclinic unit cell with eight water molecules per unit cell as shown in Figure2. The starch granule organization was very complicated and depends strongly on the botanical origin (Buleon *et al.*, 1998).

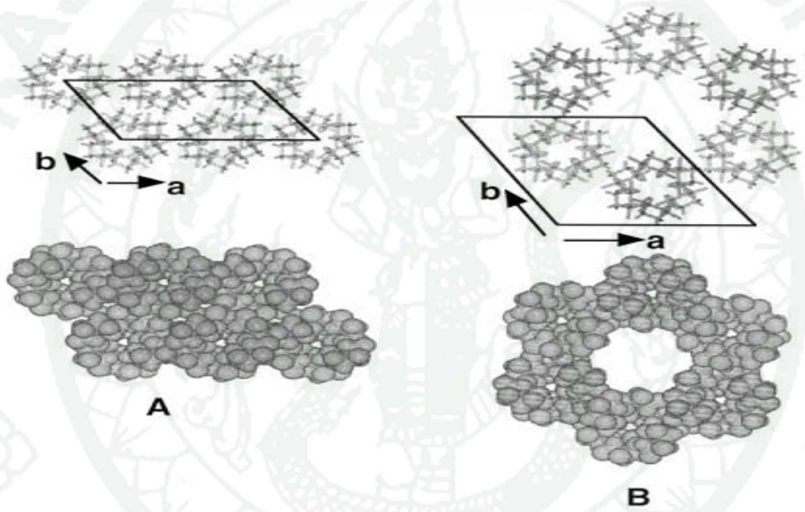

Figure 2 Crystalline packing of double helices in A-type (A) and B-type (B) and projection of the structure onto the (a, b) plane.

Source: Buleon *et al.* (1998)

Native starch granules exhibited two main types of X-ray diffraction diagrams, the A type for cereal starches and the B type for tuber and amylose-rich starches. Another C-type diffraction diagram, which showed been shown to be a mixture of A- and B-type diagrams, was characteristic of most legume starches, and also from cereals grown in specific conditions of temperature and hydration (Buleon *et al.*, 1998).

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Swelling power

Native starch granules are insoluble in cold water but swell in warm water. When starch is heated in excess water, the crystalline structure is disrupted due to the breakage of hydrogen bonds, and water molecules become linked by hydrogen bonding to the exposed hydroxyl group of amylose and amylopectin, the suspension becomes a dispersion of swollen granules and reversible until the heating temperature reach gelatinization temperature the swelling become irreversible. The cooked product is called a starch paste. In general a starch paste can be described as two-phase system composed of a dispersed phase of swollen granules (swelling power) and a continuous phase of leached amylose (solubility) (Singh *et al*., 2000). Swelling power and solubility provide evidence of the magnitude of interaction between starch chains within the amorphous and crystalline regions. The extent of this interaction is thought to be influenced by amylose content, amylose and amylopectin structure, and other factors (Singh *et al*., 2000).

When starch granules are heated in the presence of water, a disorder phase transition occurs. The starch granules can absorb water and irreversible swelling takes place in this process. Amylose and low molecular weight amylopectin leach out from the granules during heating with or without stirring mechanism. In addition, leaching of molecule could occur at temperature below the gelatinization temperature due to its location in amorphous regions and the fact that it is a relatively small, linear molecule that can diffuse out of granules. Starch gelatinization showed change the molecular orderliness in the starch granule such as granular swelling, crystallite melting, loss of birefringence, viscosity development, and solubility property (Eliasson and Gudmundsson, 2006).

Gelatinization

Gelatinization, an energy absorbing process can be examined by differential scanning calorimetry (DSC), which measured both the temperature and the enthalpy of gelatinization. Thermal curves generated by the calorimeter depicting rice starch

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gelatinization are characterized by three temperature; onset (T_o), peak (T_p), and conclusion (T_c) and by the gelatinization enthalpy (ΔH), which is expressed as J/g, which obtained by integrating the area under the curve (Marshall, 1993).

Retrogradation

The retrogradation was a process that occurs when starch molecules re-associate and form an order structure develops viscosity after cooling starch paste (Eliasson and Gudmundsson, 2006). Mechanism of starch retrogradation takes place in two stages. The first and fastest stage in the formation of crystalline regions from regarded amylose. The second stage involves formation of an ordered structure within amylopectin. That results in viscosity increase, gel firming, and textural staling of predominantly starch-containing systems. Amylopectin forms shorter double helices which can be attributed to restrictions by the branching structure of the amylopectin molecules and the chain lengths of the branches. Because the amount of amylopectin in most starches is greater than amylose, most of the crystallites formed during starch retrogradation are related to the association of amylopectin chains. Thus, amylopectin retrogradation proceeds slowly over several weeks of storage and contributes to the long term rheological and structural changes of starch systems (Zhou *et al*., 2002). The retrogradation properties can be measured by DSC and starch gel hardness (Bao and Bergman, 2004).

1. Germination process

White rice or polished rice is manufactured by eliminating the fiber-rich bran layer from paddy, also known as brown rice. Brown rice contains more nutritional components such as dietary fibers, phytic acid, vitamin E and B and GABA than white rice due to the presence of outer bran layer being the main source for the nutritional elements (Musa *et al.*, 2011). Brown rice is produced by removing its external hard husk. Consumption of brown rice became popular in Japan back in the 1970’s because of rice fiber and other nutrients contained in the brown rice such as iron, vitamins and minerals (Patil and Khan, 2011). Although brown rice is more nutritious than white

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rice, its intake is somewhere limited by the chewy texture and reduced digestibility. This problem can be overcome by subjecting brown rice to partial germination, thus producing germinated brown rice (Musa *et al*., 2011). However, the popularity did not last long due to the fact that brown rice showed to be cooked in the pressure cooker and was still hard to chew and less tasty (Patil and Khan, 2011).

The nutrient content of rice can be improved by using the following two techniques. The traditional plant-breeding techniques explore rice germ or cultivars with nutritionally enhanced varieties and breed these with the most commonly grown rice cultivars to enhance the nutrient content of the grain. Recent breakthroughs in scientific technology made it possible to enhance the nutritional value of rice through genetic modification of an existing cultivar (Zhang *et al*. 2005). Second, rice can be modified by germination to improve its functionality. Germination could overcome the problem of brown rice, which can be cooked in an ordinary rice cooker and is soft enough to chew because the outer bran layer becomes soft and more prone to water absorption and has a mellow flavor, soft mouth feel (Musa *et al.,* 2011; Patil and Khan, 2011). Market prices of the germinated brown rice in Japan are at the range of 1,000 Yen (9 US) to 800 Yen (7US) per kg depending upon quality (Patil and Khan, 2011).

The change of staple food from polished rice to germinated brown rice can maintain and promote the healthy life and improve the quality of life (Patil and Khan, 2011). Germinated brown rice or pre-germinated brown rice are considered whole food because only the outermost layer, the hull of the rice kernel is removed which causes least damage to its nutritional value. It is concluded that germination has potential to become innovative rice by preserving all nutrients in the rice grain for human consumption in order to create the highest value from rice (Patil and Khan, 2011).

Germination was developed for marketing in Japan in 1995. Germinated brown rice products were developed first by Domer Co. (Ueda City, Nagano Pref.) and the city government, Mino-cho of Kagawa Pref, was one of the earliest organizations

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engaged in the production of germinated brown rice. It is now produced by several private companies including agricultural cooperatives (Patil and Khan, 2011). Now a day consumption of germinated brown rice is gaining appreciation among health conscious individuals due to its better nutritive, organoleptic value and textural properties than brown rice after cooking easier to chew and tastier (Ito and Ishikawa, 2004).

Starch, non-starch polysaccharides and protein in germinated grains were get partially hydrolyzed to sugar, oligosaccharides and amino acid (Ohtsubo *et al.*, 2005). This phenomenon will create bio-functional substances such as gamma aminobutyric acid (GABA), dietary fiber, inositols, ferulic acid, phytic acid, tocotrienols, magnesium, potassium, zinc and gamma oryzanol (Kayahara and Tukahara, 2000) and improvement palatable texture (Patil and Khan, 2011). GABA has several healthy functions such as neurotransmission, reduced blood pressure, diuretic effects, reducing weight, ameliorating liver, prevent colon cancer , preventing chronic alcohol-related disease, preventing heart disease and preventing Alzheimer's disease (Kayahara and Tsukahara, 2000; Oh *et al.*, 2003; Ito and Ishikawa, 2004; Komatsuzaki *et al.,* 2007).

In plant science, germination means the growth of an embryonic plant contained within a grain, its results in the formation of seeding (Mayer and Mayber, 1982). In food science point of view pre-germinated should have sprout length approximately 0.5-3 mm under suitable environmental conditions (Panchan and Naivikul, 2009; Rattanadee and Naivikul, 2011). Generally, paddy or brown rice can be germinated by anaerobic or aerobic condition.

1.1 Anaerobic condition

Anaerobic condition produced by soaking paddy or brown rice in water at suitable temperature for extent period of times. Many researchers had studied the anaerobic germination process in cereal grain as conclude in Table 3.

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Table 3 Condition for anaerobic rice germination process.

Source	Soaking temperatures (°C)	Soaking times	References
Brown rice	27	5-11 day	Kordan, 1974
Brown rice	30	8, 16 and 24 hours	Watanabe *et al.* (2004)
Brown rice	30	24, 48, 72 and 96 hours	Ohtsubo *et al.* (2005)
Brown rice	25, 35 and 45	12 and 24 hours	Watchraparpaiboon *et al*, (2007)
Brown rice	35	24, 48 and 72 hours	Jiamjariyatam *et al.* (2008)
Brown rice	35	24, 48 and 72 hours	Charoenthaikij *et al.* (2010)
Brown rice	13	28 hours	Sharifi (2010)
Brown rice	30	72 hours	Jannoey *et al.* (2010)

Soaking rice grain is a slow process controlled by the diffusion of water first water penetrated into the ventral side surface and the embryo attachment site of the endosperm, then migrated along to the central line and transverse cracks, and finally diffused to all parts of the endosperm (Bello, 2007).

When grain is soaking in water the moisture content increase rapidly at first but progressively slows down (Saman *et al.*, 2008). Minimum stage of water uptake was mainly absorption of the kernel (Saman *et al.*, 2008; Rattanadee and Naivikul, 2011). Water absorption into the rice kernel can be regarded as a process of diffusion which a proportion of the absorbed water becomes immobilized during water-starch reaction as water diffusion proceeds. The reaction will occur if the activity and affinity of starch with water is high enough. The reduction of water absorption rate at the beginning of soaking process might be due to the siliceous hull in paddy, which does not wet easily and acts as a barrier to water penetration. As air is often trapped between hull and endosperm, this also prevents access of water to the kernel (Bello, 2007).

The maximum stage of soaking process grain was absorbed water slowly and became saturation. During this stage water diffused slowly into an endosperm of the kernel (Bello, 2007; Rattanadee and Naivikul, 2011). The functions of the steep water include initiation of cell elongation, respiration, secretary activity of the embryo

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and activation of enzymes. Many factors influence the water uptake of kernels including size, nitrogenous content, initial moisture content and cultivar of the grain (Saman *et al.*, 2008).

Charoenthaikij *et al.* (2009) studied the chemical and physicochemical properties of brown rice after germination in anaerobic condition at 35°C for 24, 48 and 72 hours. As the soaking time increased germinated brown rice flours had greater reducing sugar and α-amylase activity, while the total starch and viscosity were lower than brown rice.

1.2 Aerobic condition

Aerobic condition was initially produced by soaked grain in water to increase kernel moisture and activate germination then soaken grain was remove from soaking process to germination. Many researchers had studied the aerobic germination process as conclude in Table 4.

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Table 4 Condition for aerobic rice germination process.

Sources	Soaking processes		Germination processes			References
	Temperatures (°C)	Times (hour)	Temperature (°C)	Times (hour)	Relative humidity (%)	
Brown rice	Room temperature	1	30	2-18	-	Akazawa and Fukuchi (1968)
Brown rice	5-45	8-32	10-50	36-144	90	Capanzana and Buckle (1997)
Paddy	30	36	30	4,032	-	Kabeir *et al.* (2004)
Brown rice	35	0.5-5	35	19-23.5	-	Komatsuzaki *et al.* (2007)
Brown rice	25	4-24	30	72	-	Liang *et al.* (2008)
Paddy	Room temperature	60	Room temperature	72	-	Puangwerakul (2008)
Brown rice	Room temperature	24	30	168	-	Saman *et al.* (2008)
Brown rice	30	24	30	72	-	Shu *et al.* (2008)
Brown rice	30	5	30	24	-	Banchuen *et al.* (2009)
Brown rice	30	3	30	8-26		Benjamasuttikul (2009)
Paddy	30	0-36	30	2-36	85	Panchan and Naivikul (2009)
Paddy	20, 25 and 30	24, 48 and 72	20, 25 and 30	24-144	95	Usansa *et al.* (2009)
Brown rice	30	12	30	24-120	-	Veluppillai *et al.* (2009)

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Table 4 (Continued)

Sources	Soaking processes		Germination processes			References
	Temperatures (°C)	Times (hour)	Temperature (°C)	Times (hour)	Relative humidity (%)	
Brown rice	25	16	25	72	65	Mohan *et al.* (2010)
Brown rice	28-30	12	28-30	24	90-95	Moongngarm and Saetung (2010)
Paddy	28-30	48	28-30	48	90-95	Moongngarm and Saetung (2010)
Paddy	Room temperature	24	Room temperature	84	90	Puangwerakul and Klaharn (2010)
Brown rice	Room temperature	24	Room temperature	84	90	Puangwerakul and Klaharn (2010)
Paddy	30	24-168	30	12-24	90-95	Juyjaihoem and Narkrugsa (2011)
Paddy	Room temperature	24, 48 and 72	28-30	24, 48, 72 and 96	90-95	Moongngarm (2011)
Paddy	30	2-24	30	4-28	85	Rattanadee and Naivikul (2011)
Brown rice	30	0.5-12	30	4-28	85	Rattanadee and Naivikul (2011)
Brown rice	30	12	30	24	65	Xu *et al.* (2011)

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1.2.1 Factor affecting aerobic rice germination process

1.2.1.1 Water

The first process which occurs during germination is the uptake water due to the process of imbibition. During imbibition molecules of solvent enter the grain which follows by swelling (Mayer and Maayber, 1982). General, soaking process provides enough water to allow germination (Saman *et al.*, 2008). The moisture content between 30-35% (optimum stage of soaking process) was sufficient for activated enzyme activity and prevent microbial growth (Komatsuzaki *et al.*, 2007; Panchan and Naivikul, 2009; Rattanadee and Naivikul, 2011).

1.2.1.2 Oxygen

Germination is a process related to living cells and requires of energy by these cell. The energy-requirement of living cells is usually sustained by process of oxidation in the absence of oxygen the grain might fermentation (Mayer and Maayber, 1982). Low oxygen tensions have been found to suppress growth in germinated rice seeding (Wongbasg *et al.*, 2009).

1.2.1.3 Temperature

Temperature is known to have an effect on the rate of water uptake. In nature, rice can grows well in a warm and humid environment. Therefore increased temperature enhances many metabolic activities in the rice grain. Different grains have different temperature ranges which they germinate. At very low temperatures and very high temperatures the germination of all grain is delays (Mayer and Maayber, 1982). Temperature stress below 15 °C usually results in reduction of coleoptiles and radical lengths. The decrease in radical length was more pronounced when compared to that in coleoptiles length in the low temperature. The embryonic of grain did not emerge at 40 °C (Mayer and Maayber, 1982). The optimum temperature

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for grain germination was between 25-35 °C (Komatsuzaki *et al.*, 2007; Panchan and Naivikul, 2009; Usansa *et al.,* 2009; Juyjaihoem and Narkrugsa, 2011; Rattanadee and Naivikul, 2011**)**.

The germination temperature was studied by Usansa *et al*. (2009) who produced germination paddy by soaked non-glutinous paddy (Khaw Dok Mali105, Pratum Thani60 and Wild rice) and glutinous paddy (San Pa Tong, RD6 and Khaw Niew Dam) at 20, 25 and 30°C for 24, 48 and 72 hours. The germination at 30°C showed root and shoot length more than germination at 25°C and 20°C, respectively. Because of maximum activities of α-amylase was found at 30°C.Warm temperature causes a faster rate of oxygen depletion. The germinated temperature close to room temperature in Thailand would be useful in terms of saving energy costs in cooling system (Usansa *et al.*, 2009).

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Pre-germination process of paddy can separate into 3 stages by embryo growth lengths, 0.5-1 mm (60-70% of pre-germination, minimum stage), 1-2 mm (71-80% of pre-germination, optimum stage), 2-3 mm (more than 80% of pre-germination, maximum stage) (Panchan and Naivikul, 2009; Rattanadee and Naivikul, 2011) as seen in Table 5.

Table 5 The stage of pre-germination process from paddy.

Stage of pre-germinated paddy	Picture	Definition
Minimum		The germination process is terminated when the embryonic axis is approximately 0.5-1mm (60-70 % of pre-germination).
Optimum		The germination process is terminated when the embryonic axis is approximately 1-2 mm (70-80 % of pre-germination).
Maximum		The germination process is terminated when the embryonic axis is approximately 2-3 mm (more than 80 % of pre-germination).

Source: Panchan and Naivikul (2009); Rattanadee and Naivikul (2011)

1.3 Impact of pre-germination process of rice on chemical properties

1.3.1 α-amylase activity

General activity of α-amylase does not exist in dry rice seed but rapidly increased as the process of germination take place (Charoenthaikij *et al.*, 2009). The increased in α-amylase activity in the grain during germination was due to

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the soaking and germinated condition produced suitable moisture content and develop to activated enzyme activity (Moongngarm, 2011).

The α-amylase activity was increased after germination and were reported in Moongngarm (2011) who germinated paddy rice by soaked RD6 paddy at 30°C for 24 hours then germinated at 30°C (90-95 % relative humidity) for 24 hours, Rattanadee and Naivikul (2011) who pre-germinated paddy by soaked Chai Nat1 paddy at 30°C for 12 hours then pre-germinated at 30°C (85% relative humidity) for 16, 20, 24 hours.

The α-amylase activity continue to increase along with germination temperature as reported from Usana *et al.* (2009) who germinated paddy by soaked non-glutinous paddy (Khao Dawk Mali105, Pratum Thani60 and Wild rice) and glutinous paddy (Sanpatong, RD6 and Khaw Niew Dam) at 20, 25 and 30°C for 24 hours then incubated at 30°C (95% relative humidity) for 1-6 days.

1.3.2 Amylose content

The formation of a helical complex between amylose and iodine results in deep blue color of starch dispersions stained with iodine.This principle was used to quantification of amylose content (Musa *et al.*, 2011). The germination process could reduce the amylose content as a result of hydrolysis enzyme (Xe *et al.*, 2011). The reduction of amylose content after germination was reported from Musa *et al*. (2011) who studied germinated *Indica* brown rice properties, Xe *et al.* (2011) who germinated brown rice by soaked brown rice at room temperature for 12 hours then germinated at 30°C (65% relative humidity) for 24 hours.

1.3.3 Reducing sugar

Before germination process rice showed a small amount of sugars because there were generally converted to starch (Moongngarm and Saetung, 2010). After germination process amylolytic enzyme was activated and hydrolyzed amylose

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and amylopectin to small starch molecule such as reducing sugar (Benjamasuttikul, 2009; Panchan and Naivikul, 2009). When continued germination process the small molecular was decreased due to growing process of rice grain (Saman *et al.*, 2008). Veluppillai *et al*. (2009) reported that the reduced of reducing sugar after prolong germination was due to increase utilization of sugars when compared to rate of production.

The increased in reducing sugar after germination were reported in Benjamasuttikul (2009) who produced pre-germinated brown rice by soaked Khao Dawk Mali105 and RD6 brown rice at 30°C for 3 hours then pre-germinated at 30°C for 8-26 hours, Panchan and Naivikul (2009) who produced pre-germinated Suphan Buri1 paddy by soaked at 30°C for 14 hours then germinated at 30°C (85% relative humidity) for 2-36 hours, Moongngarm and Saetung (2010) who produced germinated RD6 paddy by soaked at room temperature for 48 hours then germinated at 28-30°C (90-95% relative humidity) for 48 hours, Moongngarm (2011) who produced germinated RD6 paddy by soaked at room temperature for 24 hours then germinated at 28-30°C (90-95% relative humidity) for 24 hours, Rattanadee and Naivikul (2011) who produced pre-germinated Chai Nat1 paddy by soaked at 30°C for 12 hours then pre-germinated at 30°C (85% relative humidity) for 16, 20 and 24 hours, Panchan (2011) also reported the increased in reducing sugar after soaked paddy at 30°C for 14 hours then pre-germinated at 30°C (85% relative humidity) for 2-36 hours.

1.4 Impact of pre-germination process on physicochemical properties

1.4.1 Swelling power

The ratio of amylose to amylopectin, molecular weight and distribution, degree of branching, conformation and the length of outer branches amylopectin were factors in swelling power (Whistler and Bemiller, 1999). The germination triggered the production of α-amylase. The α-amylase was able to degrade native starch granules into oligosaccharides and reducing sugar result in higher swelling power (Xe *et al.*, 2011).

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The increased swelling power after germination was reported from Ocheme and Chainma (2008) who produced germinated millet grain by soaked at room temperature for 12 hours then germinated at 32°C for 48 hours. Xe *et al.* (2011) who produced germinated brown rice by soaked at room temperature for 12 hours then germinated at 30°C (65% relative humidity) for 24 hours.

1.4.2 Peak viscosity

Peak viscosity was implied to the intensity of swelling from starch granule after subjected to heat in the excess water (Jane *et al.*, 1999). The peak viscosity was affected by germination because the weakness of intermolecular network by hydrolytic enzyme. When germination the whole starch molecule was hydrolyzed at the α-D (1-4) linkage in random places by α-amylase, convert starch to limit dextrin, oligosaccharide and reducing sugars. These small starches molecular do not contribute to the viscosity after heating and shearing result by low peak viscosity (Xe *et al.*, 2011).

The decreased peak viscosity after germination process were reported from many researchers. Charoenthaikij *et al.* (2009) who produced germinated Khao Dawk Mali105 brown rice and RD6 brown rice by soaked at 35°C for 24 hours, Benjamasuttikul (2009) who produced germinated Khao Dawk Mali105 and RD6 brown rice by soaked at 30°C for 3 hours then pre-germinated for 8-26 hours, Panchan and Naivikul (2009) who produced pre-germinated Suphan Buri1 paddy by soaked at 30°C for 14 hours then germinated at 30°C (85% relative humidity) for 2-36 hours, Mohan *et al.* (2010) who produced germinated *Indica* brown rice and *Japonica* brown rice by soaked at 25°C for 16 hours then germinated at 25°C (65% relative humidity) for 72 hours, Rattanadee and Naivikul (2011) who produced pre-germinated Chai Nat1 paddy by soaked at 30°C for 12 hours then germinated at 30°C (85% relative humidity) for 16, 20 and 24 hours, Panchan (2011) who produced pre-germinated Supaburi paddy, Sinlek paddy and RD6 paddy by soaked at 30°C for 14 hours then pre-germinated at 30°C (85% relative humidity) for 2-36 hours, Xe *et al.* (2011) who produced germinated brown rice (long grain cultivar)

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by soaked at room temperature for 12 hours then germination at 30°C (65% relative humidity) for 24 hours.

1.5 Application of pre-germination cereal grain

In the past people usually consume germinated brown rice in original from that was cooked germinated brown rice (Patil and Khan, 2011). Germinated cereal grains were utilized in products by many researchers as concluded in Table 6.

Table 6 Application for germinated cereal grain.

Products	Formulas	Results	References
Bread	Blended 20% germinated Koshihikari brown rice flour to 80% wheat flour.	Blended bread suppressed staling during storage, increased viscosity of dough and increased water absorption of batter.	Watanabe *et al.* (2004)
Bread	Blended 30% extrude germinated brown rice flour to 70% wheat flour.	Blended bread improved over all sensory properties.	Ohtsubo *et al.* (2005)
Cookie with pineapple filling	Blended 60% germinated Khao Dawk Mali105 brown rice flour to 40% wheat flour.	Blended cookie showed lower brittleness than 100% wheat flour cookie.	Jiamjariyatam *et al.* (2008)
Bread	Blended 30% germinated Khao Dawk Mali105 brown rice flour and 10% germinated RD6 brown rice flour to 60% wheat flour.	Blended bread showed higher retrogradation than 100% wheat flour.	Charoenthaikij *et al.* (2009)
Bread	Blended 30% germinated KDML 105 brown rice flour to 70% wheat flour.	Blended bread showed lower loaf volume and greater hardness than 100% wheat flour.	Charoenthaikij *et al.* (2010)
Bread	Blended 30% gelatinized germinated super hard brown rice flour to 70% wheat flour.	Blended bread became very soft and hardness was markedly retarded.	Nakamura *et al.* (2010)

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Table 6 (Continued)

Products	Formulas	Results	References
Alkaline noodle	Blended 21% gelatinized germinated super hard brown rice flour and 9% gelatinized low amylose white rice flour to70% wheat flour.	Blended noodle showed similar texture properties to durum semolina noodles.	Nakamura *et al.* (2010)
Bread	Blended 35% germinated brown rice flour to 65% wheat flour.	Blended bread showed decreased specific volume, flavor crumb, crumb color, crumb texture, crust color and crust texture when compared to 100% wheat flour.	Veluppillai *et al.* (2009)
Banana cake	100% germinated KDML 105 brown rice flour.	The germinated brown rice flour increased overall acceptation in color, flavor, taste and texture of banana cake.	Chaichaw *et al.* (2011)

2. Annealing process

Annealing is the hydrothermal treatment of starch in the presence of excess water (more than 60% w/w) or intermediate water content (40% w/w) for extend period of time. The annealing process was performed at above the glass transition temperature but below the gelatinization temperature of starch, which a glassy state transforms to a mobile rubbery state (Tester and Debon, 2000). Annealing process specifically changed the physicochemical properties of starch. During annealing, amorphous region absorb water then plasticization provided mobility and allow alignment of starch molecular chains (Shi, 2008) as seen in Figure 3.

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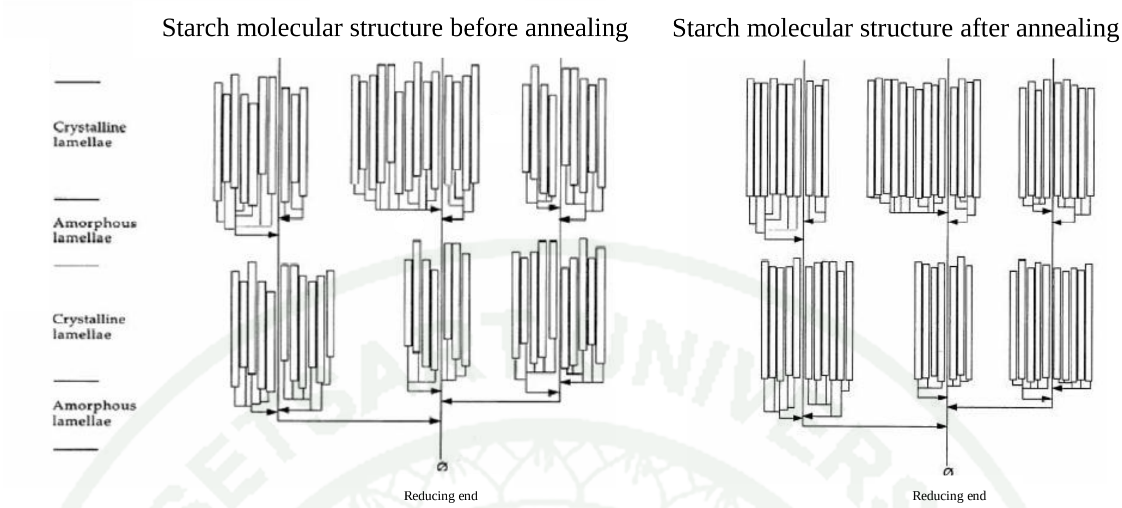

Figure 3 Mechanism and structure of annealed starch.

Source: Tester and Debon (2000)

2.1 Factor affecting annealing process of cereal grain

2.1.1 Temperature

The temperature of annealing process should below gelatinization temperature to prevent the breakdown of crystalline region and above glass transition temperature to induce movement of starch molecule (Tester and Debon, 2000). High annealing temperature showed high impact in starch molecular structure as reported from Dias *et al.* (2010) who annealed rice starch at 45, 50 and 55°C for 24 hours. Siriboon (2007) was also report similar result that after annealed broken rice at 7°C and 30°C below gelatinization temperature for 24 and 72 hours. This was due to amorphous and crystalline regions were more perfect after annealing process, where it was most effective at near gelatinization temperature (Dias *et al.*, 2010).

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2.1.2 Time

Annealing process for a long time showed high impact in starch molecular structure as reported from Intarasiri and Naivikul (2005) who annealed fermented rice flour at 55°C 24 and 48 , Cham and Suwannaporn (2010) who annealed rice starch at 50, 55, 60, 65 and 70°C for 12, 24, 36, 48, 60 and 72 hours. The effect of annealing process could happen until the organized of starch molecular structure reach maximum degree with enough annealing time at specific annealing temperature (Siriboon, 2007).

2.1.3 Moisture content

Siriboon (2007) was reported the effect of annealing process at different moisture content (65% w/w and 75% w/w) in broken rice, the 70% w/w moisture content showed little higher effect in physicochemical properties compared to 65% w/w moisture content. Annealing process in excess water system was appropriate to allow the movement of starch molecule (Tester and Debon, 2000).

Annealing conditions for cereal starch and flour were reported from many researchers as summarized in Table 7.

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Table 7 Condition for annealing process of cereal grains.

Cereal samples	Annealing Step	Annealing Temperatures	Annealing Times	Moisture content	References
Maize starch	Multiple	50, 55, 60, 65 and 70°C	2-72 hours	Water: sample = 1:3	Knutson (1990)
Rice starch	One	Below gelatinization temperature 3-4%	24 hours	Water: sample = 2:1	Jacobs *et al.* (1995)
Maize starch	One	Below gelatinization temperature 10°C	72 hours	More than 96%	Tester *et al*. (2000)
Corn starch	Multiple	40, 45, 50, 55, 60 and 65°C	24 hours for each step	Water: sample = 3:1	Nakazawa and Wang (2003)
Corn starch	Multiple	40, 45 and 50°C	24 hours each step	Water: sample = 3:1	Nakazawa and Wang (2004)
Rice flour	One	55°C	4, 12, 24 and 48 hours	Water: sample = 2:1	Chiewcherngka and Naivikul (2005)
Fermented rice flour	One	55°C	24 and 48 hours	Water: sample = 2:1	Intarasiri and Naivikul (2005)
Barley starch	One	50°C	72 hours	75%	Waduge *et al.* (2006)
Broken rice	One	Below gelatinization temperature 7°C and 30°C	24 and 72 hours	65% and 75%	Siriboon (2007)
Wheat starch	One	Below gelatinization temperature 10°C	72 hours	Water: sample = 3:1	Lan *et al.* (2008)
Rice starch	One	50°C	72 hours	Water: sample = 24:1	Lin *et al.* (2008)
Rice starch	One	77°C	2 hours	Water: sample = 3:1	Shi (2008)

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Table 7 (Continued)

Cereal samples	Annealing Step	Annealing Temperatures	Annealing Times	Moisture content	References
Rice starch	Double	70 and 77°C	16 min for each step	Water: sample = 3:1	Shi (2008)
Rice starch	Multiple	65,70,75 and 80°C	30 min for each step	Water: sample = 3:1	Shi (2008)
Corn starch	One	Below gelatinization temperature 10°C and 15°C	24 hours	70%	Chung *et al.* (2009)
Corn starch	One	30 and 50°C	72 hours	Water: sample = 10:1	Liu *et al.* (2009)
Corn starch	One	50°C	72 hours	Water: sample = 24:1	Lin *et al.* (2009)
Rice starch	One	70°C	12, 24, 36, 48, 60 and 72hours	60 %	Cham and Suwannaporn (2010)
Corn starch	One	50°C	24 hours	Water: sample = 70:30	Chung. *et al.* (2010)
Rice starch	One	45,50 and 55 °C	24 hours	Water: sample = 9:1	Dias *et al.* (2010)
Sorghum starch	One	50°C	24 hours	Water: sample = 4:1	Singh *et al.* (2010)

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One step annealing process was performed at temperature between glass transition and gelatinization for extend period of time in an excess water system (Tester and Debon, 2000). One step annealed rice flour should performed at 55°C for 48 hours (Chiewchergka and Naivikul, 2005; Intarasiri and Naivikul, 2005).

Multiple steps annealing process was performed by annealing at temperature below gelatinization temperature of native starches at a certain prior of time, then re-annealing at temperature below gelatinization temperature of native starch (Shi, 2008). Multiple steps annealed cereal starch should performed between 40-80°C for 0.5-24 hours at each annealing step (Nakazawa and Wang, 2003; Shi, 2008).

2.2 Impact of annealing process of cereal grains.

2.2.1 Chemical property

2.2.1.1 Amylose content

Amylose content was changed after annealing process. The increased apparent amylose content on anneal could be associated with increased number of helical turn and ability to from order helic. Lin *et al.* (2009) were reported that double helix content was increased after annealed acid hydrolyzed corn starch at 50°C for 72 hours. However, the decreased apparent amylose content on anneal could be associated with a decreased the number of helical turns as a result of a change in amylose conformation there by decreasing the color intensity of the amylose-iodine complex (Lan *et al.*, 2008).

2.2.2 Physicochemical properties

2.2.2.1 Swelling power

Swelling power after annealing process was influenced by amylose lipid complex (Hoover and Vansanthan, 1993), intra granular binding forces

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and reinforcement of the granule (Jacobs *et al.*, 1995), amylose content and interaction between starch molecular chains (Tester *et al.*, 2000). The swelling power after annealing was mostly decreased. The reduction in swelling power were reported in annealed barley at 50°C for 72 hours (Waduge *et al.*, 2006), broken rice at 7°C and 30°C below gelatinization temperature for 24, 72 hours (Siriboon, 2007), annealed wheat starch at 10°C below gelatinization temperature for 72 hours (Lan *et al.*, 2008), corn starch annealed at 50°C for 24 hours (Chung *et al*., 2009), rice starch annealed at 45°C, 50°C, 55°C for 24 hours (Dias *et al*., 2010). The reduction was influenced by crystalline perfection, starch molecule interaction, amylose-lipid complex and molecular organization that cause a rigid structure then difficult to swell (Waduge *et al.*, 2006; Dias *et al.*, 2010).

2.2.2.2 Solubility

Solubility after annealing process was influenced by interaction between starch molecular chains (Hoover and Vansanthan, 1993), swelling power (Tester *et al*., 2000), reorientation of molecule (Gomes *et al*., 2005), and amylose lipid complex (Waduge *et al*., 2006). Annealing process generally reduced solubility. The decreased solubility were reported from Intarasiri and Naivikul (2005) who annealed ferment rice flour at 55°C for 24 and 48 hours, Siriboon (2007) who annealed broken rice at 7°C and 30°C below gelatinized temperature for 24 and 72 hours and Dias *et al.* (2010) who annealed rice starch at 45°C, 50°C and 55°C for 24 hours. The strengthening of the bonds between starch molecules and crystalline perfection could prevent solubility (Intarasiri and Naivikul, 2005; Dias *et al*., 2010).

2.2.2.3 Carbohydrate leaching (amylose leaching)

The carbohydrate leaching after annealing process was influenced by degree of swelling power, interaction between starch molecular chains, amylose lipid complex, amylose content and amylopectin structure (Tester *et al.*, 2000; Gomez *et al.*, 2004; Waduge *et al.*, 2006; Siriboon, 2007). The decreased amylose leaching after annealing process was reported from Hoover and Vansanthan

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(1993) who annealed wheat starch at 50°C for 72 hours and Siriboon (2007) who annealed broken rice at 7°C and 30°C below gelatinized temperature for 24 and 72 hours. However, the increased in amylose leaching were reported from Chung *et al*. (2009) who annealed corn starch at 50°C for 24 hours. The reduction in amylose leaching was due to decrease granule swelling and interaction between starch molecule chains (Siriboon, 2007). The increased in amylose leaching after annealing was due to thermal and moisture imparted to loosely packed amylose chains may have increased their mobility thereby facilitating their diffusion out of the granule (Chung *et al*., 2009).

2.2.2.4 Peak viscosity

The peak viscosity was viscosity that developed during heating with water under shear mechanism. The peak viscosity after annealing process was attributed to granule swelling, interaction between leach amylose and friction between swollen granules (Jacobs *et al.*, 1995). Annealing process was reported to decreased peak viscosity in ferment rice flour after annealed at 55°C for 24 and 48 hours (Intarasiri and Naivikul, 2005), broken rice after annealed at 7°C and 30°C below gelatinization temperature for 24 and 72 hours (Siriboon and Tongta, 2007), rice starch after annealed at 70°C for 72 hours (Cham and Suwannaporn, 2010), rice starch after annealed at 45°C, 50°C and 55°C for 24 hours (Dias *et al.*, 2010). However, annealing process was reported to increase peak viscosity in rice starch after annealed at 3-4% below gelatinized temperature for 24 hours (Jacobs *et al*., 1995) and low amylose rice starch after annealed at 55°C for 24 hours (Dias *et al*., 2010).

The peak viscosity decreased after annealing process was due to reduce in swelling power, decreased amylose leaching, increased interaction between starch molecular chains that was difficult for develop viscosity during heating (Intarasiri and Naivikul, 2005; Dias *et al.*, 2010). The peak viscosity increased after annealing process was due to increased granule rigidity then more resistance structure to shear results in a tightly packed array of swollen granules (Jacobs *et al*., 1995).

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2.2.2.5 Syneresis

The annealing process could reduce syneresis after freeze-thaw cycle as reported from Intarasiri and Naivikul (2005) who annealed fermented rice flour at 55°C for 24 and 48 hours. The interaction between starch molecular chains that induced by annealing process could disrupt retrogradation (Intarasiri and Naivikul, 2005).

2.2.3 Physical property

2.2.3.1 Gel hardness

The annealing process showed to increased gel hardness as reported from Hormdok and Noomhorm (2007) who annealed rice starch at 45°C, 50°C and 55°C for 8, 16 and 24 hours, Cham and Suwannaporn (2010) who annealed rice starch at 70°C for 72 hours. The crystalline perfection and rearrangement of starch molecule after annealing process could promote high gel hardness.

2.3 Applications

Annealing process improved thermal, freeze-thaw and gel stability. Therefore annealed sample could be used in frozen rice noodle (Jayakody and Hoover, 2008; Intarasiri, 2007; Cham and Suwannaporn, 2010). The products from annealing process were studied by many researchers as summarized in Table 8.

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Table 8 Rice products from annealing process of rice.

Products	Ratios	Results	References
Dry noodle	Blended 50% annealed rice starch to 50% rice flour	The blended 50% annealed rice starch to 50% rice flour noodle showed higher hardness and tensile strength than rice noodle from 100% rice flour	Hormdok and Noomhorm (2007)
Frozen fermented Kanomjeen	Blended 40% annealed ferment rice flour to 60% ferment rice flour	The frozen fermented Kanomjeen making from blended 40% annealed ferment rice flour to 60% ferment rice flour showed lower cutting force but higher tensile strength than Kanomjeen making from 100% ferment rice flour	Intarasiri (2007)
Fresh noodle	100% annealed rice starch	The texture properties of noodle making from annealed rice starch showed no significantly different with rice noodle making from rice flour.	Cham and Suwannaporn (2010)

However, annealing process showed a little changing on starch molecular structure (Hormdok and Noomhorm, 2007; Cham and Suwannaporn, 2010). The combination of hydrolysis and annealing process could reorganize starch molecular structure better than only annealing process. The short starch molecule could move and realigned starch molecular structure better than long starch molecular (Lin *et al.*, 2009). Pre-germination (enzyme hydrolysis) was a process that hydrolyzed starch molecular structure and might gave high effect to starch molecular structure after annealing process.

3. Rice noodle

Rice noodle is a popular rice product in Thailand. Rice noodle was prepared from milled rice flour, cook to thin rice sheet by steamer and cut to a strip (Phimolsiripol, 2002).

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3.1 Factor affects rice noodle quality

3.1.1 Rice

Rice contain amylose between 27-33% was suitable for noodle products. Because of noodle production required retrogradation process to form noodle sheet. Normally rice noodle prepare by broken white rice that storage more than 6 months due to fresh white rice showed low gel quality (sticky gel) and cannot be used for noodle product (Hormdok and Noomhorm, 2007).

3.1.2 Rice flour

Rice could be milled into rice flour by wet or dry milling processes. Rice flour from different milled method showed different quality and functional property (Suksomboon, 2007).

3.2 Noodle type

3.2.1 Classified by moisture content (Phimolsiripol, 2002)

3.2.1.1 Fresh noodle contained 62-64 % moisture content
3.2.1.2 Semi dry noodle contained 35-37 % moisture content
3.2.1.3 Dry noodle contained 11-13 % moisture content

3.2.2 Classified by size (Phimolsiripol, 2002)

3.2.2.1 Sen Yai was 1.5-2.5 wide
3.2.2.2 Sen Lek was 0.4-.05 wide

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3.3 Rice noodle processing (Phimolsiripol, 2002)

3.3.1 Preparing rice slurry

Concentration of rice slurry showed positive correlation with noodle quality. The rice flour concentration depends on the type of milled rice flour. The concentration of wet milled flour for prepared rice noodle usually between 38-40% (w/w), dry milled flour was prepared with lower flour portion.

3.3.2 Steaming

The rice slurry was cooked with stream. Heat from the stream will make the starch granule changes into a rice sheet gel.

3.3.3 Incubating

The incubation process was used to make the moisture spread across the entire sheet of rice gel. The rice gel sheet will become stronger by retrogradation after subject to cool system.

3.3.4 Cutting

The rice gel sheet after incubation was cut strips to size

The rice noodle industry in Thailand is facing problems with overuse of additives to enhance eating quality. Native rice flour showed poor resistance to shear force and low elastic gel forming ability (Pitiphunpong and Suwannaporn, 2009). Starch was an important role in rice noodle quality and starch functionalities can be improved by replacement with appropriately modified starch (Surojanametakul *et al.*, 2002; Hormdok and Noomhorm, 2007). Annealing process is one of the methods that could produce strong starch molecules structure which can blended to rice flour and improve rice noodle quality (Hormdok and Noomhorm, 2007).

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3.4 Freezing rice noodle

3.4.1 Freezing process

The basic principle of freezing process was reducing the temperature then prevents the microorganism and chemical reaction of food product. In general, freezing process use -18°C or lower to change the state of water in food into ice crystal. The freezing process is dividing into three periods. The first phase is pre-freezing. The temperature of food will decline until the water transition to crystalline ice. The second phase is frozen and the last phase is reduction the temperature to storage, the temperature should not change throughout the system by the heat (Persson and Londahl, 1993).

Frozen food structure was changed by the following procedure. First is nucleation phase second is crystal growth phase. The important factor that affects the size of ice crystal is the rates of heat out of food. If the rate is high the crystal will be small. However if the rates of heating out is low the ice crystal will be large and change food quality (Macdonald and Lanier, 1997).

3.4.2 Freeze-thaw

The starch gel after freeze-thaw was changed to stiff structure and syneresis by retrogradation process. The retrogradation could be reduced by annealing process due to the interaction between starch molecule chains making the structure stable to freezing process (Intarasiri, 2007).

There had been many researchers concern the improvement of frozen rice noodle product quality as summarized in Table 9.

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Table 9 Frozen rice noodle products

Frozen rice noodle	Formula	Results	References
Alkaline rice noodle	Blended 30% pre-gelatinize Chai Nat1flour and 40% heat moisture treatment RD6 flour to 30% Chai Nat1 rice flour.	The texture properties of frozen alkaline rice noodle after freeze-thaw 2 cycles were improve as determined by maximum force, tensile strength and cutting force.	Wadchararat (2006)
Fermented Kanomjeen	Blended 40% annealed ferment rice flour to 60% ferment rice flour	The frozen fermented Kanomjeen making from blended 40% annealed ferment rice flour to 60% ferment rice flour showed lower cutting force but higher tensile strength than Kanomjeen making from 100% ferment rice flour	Intarasiri (2007)

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METERIAL AND METHOD

Materials

1. Rice samples

Thai paddies Chai Nat1, high amylose, was harvested in April 2010 and Rice Division 6 (RD 6), waxy, was harvested in May 2010 were used in this research. Both paddy varieties were obtained from the Bureau of Rice Research and Development, Rice Department, Thailand. The paddy was stored in plastic bags under freezing at -18°C for 3 months before used.

2. Chemical reagents

- Acetic acid
- Ammoniummolybdate
- Anhydrous sodium carbonate
- Anhydrous sodium sulfate
- Copper sulfate
- Disodium arsenate
- Ethyl alcohol 95%
- Iodide
- Potassium iodide
- Potassium meta bisulfide
- Pure potato amylose
- Phenol 5%
- Standard glucose
- Sulfuric acid
- Sodium bicarbonate
- Sodium hydroxide
- Sodium potassium tartrate

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3. Equipments and instruments

- Centrifuge
- Controlled temperature and humidity oven (WTB) (Binder, Germany)
- Dehusker
- Electric streamer
- Freezer
- Hot air oven (Binder, Germany)
- Noodle cutting
- Rapid Visco Analyzer (RVA3D) (Newport Scientific Instruments and Engineer, Austrasia)
- Refrigerator
- Roller mill
- Sieve 100 mesh
- Spectrophotometer (Thermo Genesys 10 series, America)
- Texture Analyzer (TA.XT2) (Stable system, England)
- Tray drier (Reliance Tech-Service. Thailand)
- Viscometer (Brookfield DVII, Brookfield)
- Water bath (UM-WB20L) (UMAC Scientific, Thailand)

Methods

1. Preparation of pre-germinated paddy.

1.1 Determination moisture content of paddy during soaking process.

The Chai Nat1 and RD6 paddies were washed in tap water twice to remove foreign particle and to select young grain from mature grain by soaking in 1.08 specific gravity of sodium chloride solution (Panchan and Naivikul, 2009; Rattanadee and Naivikul, 2011). The clean paddies were steeped at 30°C (Kabeir *et al.*, 2004; Komatsuzaki *et al.*, 2007; Panchan and Naivikul, 2009; Usansa *et al.*, 2009; Juyjaihoem and Narkrugsa, 2011; Rattanadee and Naivikul, 2011) for 2-20 hours

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(Usansa *et al.*, 2009; Puangwerakul and Klaharn, 2010; Moongngarm, 2011; Rattanadee and Naivikul, 2011). Moisture content was determined according to the AACC (2000).

1.2 Determination percent germinated of paddy during pre-germination process

The suitable soaking times of both Chai Nat1 and RD6 paddies were used to select appropriate pre-germination times. The soaked paddies were packed in plastic boxes over wet cheese cloth and cover with a lid then put in a humidity chamber at 30°C (Panchan and Naivikul, 2009; Moongngarm and Saetung, 2010; Juyjaihoem and Narkrugsa, 2011; Rattanadee and Naivikul, 2011) and 85% relative humidity (Panchan and Naivikul, 2009; Rattanadee and Naivikul, 2011) for 4-30 hours (Panchan and Naivikul, 2009; Usansa *et al*., 2009; Moongngarm, 2011; Rattanadee and Naivikul, 2011), until paddies showed embryo length between 0.5-1 mm that was 60-70 % of germination (minimum stage), 1-2 mm that was 70-80 % of germination (optimum stage) and 2-3 mm that was more than 80% of germination (maximum stage), respectively. The three stages of both Chai Nat1 and RD6 pre-germinated paddies were dried at 45°C until moisture content was less than 12% and kept in room temperature for 7 days before dehusking. Three stages of both Chai Nat1 and RD6 pre-germinated paddy were dehusked to be pre-germinated brown rices (PGBR) which were ground and sieved through 100 meshes to be pre-germinated brown rice flours at minimum stage (PGBR flour_1), pre-germinated brown rice flour at optimum stage (PGBR flour_2) and pre-germinated brown rice flour at maximum stage (PGBR flour_3) then stored at 4°C before used (Panchan and Naivikul, 2009; Rattanadee and Naivikul, 2011).

2. Prepare annealed pre-germinated brown rice flour (annealed PGBR flour)

2.1 One step annealing process

Pre-germinated brown rice flour (100 g) was mixed with 300 mL distill water containing 0.5g/100g potassium meta-bisulfite, which acted as antimicrobial

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reagent. The rice solution was incubated at 45, 50, 55°C for 1-6 days in water bath. After annealing process the rice solution were flit through filter paper (whatman No.1) and dried at 40°C until the moisture content were less than 12%, then ground through 100 mesh and stored at 4°C until analyzed (modified from Hormdok and Noomhorm, 2007).

2.2 Double and multiple steps annealing process

Pre-germinated brown rice flour (100 g) was mixed with 300 mL distill water containing 0.5g/100g potassium meta-bisulfite, which acted as antimicrobial reagent. The samples were incubated at 45°C for 1 day and continued to 50°C for 1 day to be double steps annealing process, 45°C for 1 day, 50°C for 1 day and continued to 55°C for 1 day to be multiple steps annealing process. Samples were filt through filter paper (whatman No.1) and dried at 40°C until the moisture content were less than 12%. The samples were ground through 100 mesh and stored at 4°C until analyzed (modified from Nakazawa and Wang, 2003).

3. Chemical properties of rice samples

3.1 Reducing sugar

Rice flour (1.00 g) was placed in a 50 mL centrifuge tube then added 10 mL of 50% ethanol. The sample was centrifuged at 5,000 rpm for 15 min at 25°C. The supernatant was added to an alkaline copper reagent. Sample was heated in boiling water for 15 minutes and immediately cooled in ice bath. Nelson reagent was added and mixed with a vortex mixer. The sample was kept at room temperature for 30 minutes. The sample was then diluted by distilled water before the amount of reducing sugar was measured by a spectrophotometer at 520 nm. The standard used was D-glucose (modified from Chang, 1979; Somogyi, 1951).

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3.2 α-amylase activity

Rice flour was mix with distilled water in the aluminum RVA sample canister. The α-amylase activity of pre-germinated brown rice flour was determined using a Rapid Visco Analyzer following the methods of AACC, 2000. The results were reports as the stirring number (SN).

3.3 Amylose content

Rice flour (0.10 g) was placed in a 100 mL volumetric flask then add alkyl alcohol 95% 1 mL and sodium hydroxide 9 mL shake thoroughly before boiling for 10 min and adjust the volume by distilled water to 100 mL. Pipette 5 mL of adjust solution to another volumetric flask (100 mL) and add 70 mL of distrill water and acitic acid 1 mL with iodine 2 mL adjust the volume by distilled water to 100 mL. The sample was kept at room temperature for 20 minutes then measured amylose content by a spectrophotometer at 620 nm (Juliano, 1985). The standard used was pure corn amylose.

4. Physicochemical properties of rice samples

4.1 Swelling power and solubility

Rice flour (0.5 g) was mixed with 15 mL distilled water in centrifugal tubes. The suspensions were heated at 65, 75, 85, 95°C for 30 min. Then cool at room temperature for 10 min before centrifuged at 2,200 rpm for 20 min. The weight of swollen matter from cooked paste was determined to % swelling power. The supernatants were drain and placed in moisture can and dried at 130°C to constant weight (AACC, 2000). The dried samples were calculated to determine % solubility (modified from Scotch, 1969).

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$$\text{Swelling power (g/g)} = \frac{\text{Weight of swollen matter (g) x 100}}{\text{Weight of sample (g) x (100- Solubility)}}$$

$$\text{Solubility (\%)} = \frac{\text{Weight of soluble matter in supernatant (g) x 100}}{\text{Weight of sample (g)}}$$

4.2 Carbohydrate leaching

The amounts of carbohydrate leached from the granules were measured according to Dubois *et al*. (1956) and Noranizan *et al.* (2010). Rice flour (0.2 g) was suspended in distilled water (6.25 mL) and heat at 95°C. After centrifugation at 3,500 rpm for 10 min, 1 mL of supernatant was pipetted and mixed with 1 mL of 5% phenol solution then followed by rapid added concentrated sulfuric acid 5 mL in screw-capped test glass tube. The samples were allowed to stand in hood for 10 min then shaken and placed at 30°C for 20 min before readings were taken. The absorbance of the yellow-orange color mixture was measured at 490 nm with distilled water as blank. Glucose solution was used as a standard curve.

4.3 Pasting properties

Rice flour was mix with distilled water in the aluminum RVA sample canister. The pasting properties of pre-germinated brown rice flour were determined using a Rapid Visco Analyzer following the methods of AACC (2000).

4.4 Syneresis

Freeze-thaw stability was evaluated by mixed rice flour 25 g with distilled water 280 g and pours into glass beaker, seal with parafilm before heat in boiling bath for 30 min. The slurries were agitated every 10 min and cooled at room temperature for 30 min. Move gel rice into 25 centrifuge tubes (8 mL for each tube) by syringe and stored at -18°C for 23 hours. Then remove centrifuge tube from frozen process and

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thaw at 30°C in water bath for 1 hours, 5 centrifuge tube were subject to centrifuge at 2,800 rpm for 15 min. The rice gel was placed into a filter and allowing the waster to drip out for 2 hours by gravity calculate as % syneresis called freeze-thaw cycle 1. The remained tubes were put back into the freezer and repeat freeze-thaw cycle. Five freeze-thaw cycle was performed for this study (modified from Yuan and Thomson, 1998; Chen *et al.*, 2003; Sodhi and Singh, 2003; Intarasiri, 2007).

$$\% \text{ Syneresis} = \frac{\text{Weight of liquid decanted}}{\text{Weight of the paste before centrifuge}} \times 100$$

5. Physical property of rice samples

5.1 Hardness

The gelatinized rice flour mixture in canister after RVA measurement was transfer to plastic bottom (2.5 x 3.0 cm) and sealed with parafilm then kept overnight at 4°C to allowed formation of solid gel. Gel texture was determined as gel hardness by texture analyzer (modified from Hormdok and Noomhorm, 2007).

6. Frozen rice noodle

6.1 Finding blended rice flour ratio to make rice noodle

PGBR flour and annealed PGBR flour were blended to Chai Nat1 white rice flour and select the appropriate blended ratio by apparent viscosity (modified from Shi and Bemiller, 2002) and peak viscosity (AACC, 2000). The ratio of blended flour was shown in Table 10.

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Table 10 Ratio of blended PGBR flour_1, multiple steps PGBR flour_1 to Chai Nat1 white rice flour.

Chai Nat1 PGBR flour_1 (%)	Multiple steps annealed Chai Nat1 PGBR flour_1 (%)	RD6 PGBR flour_1 (%)	Multiple steps annealed RD6 PGBR flour_1 (%)	Chai Nat1 white rice flour (%)
-	-	-	-	100
5	-	-	-	95
10	-	-	-	90
15	-	-	-	85
20	-	-	-	80
-	-	5	-	95
-	-	10	-	90
-	-	15	-	85
-	-	20	-	80
-	5	-	-	95
-	10	-	-	90
-	15	-	-	85
-	20	-	-	80
-	-	-	5	95
-	-	-	10	90
-	-	-	15	85
-	-	-	20	80
5	-	5	-	90
10	-	10	-	80
15	-	15	-	70
-	5	-	5	90
-	10	-	10	80
-	15	-	15	70

Rice noodle was prepared by mix 30:70 portion of rice flour and water. Flour slurry was stood at room temperature with occasionally stirring at room temperature for 4 hours. The 60 g of rice flour slurry was pour into 18x24 cm stainless steel tray then steamed in an electric streamer for 3.5 min. The gelatinized rice sheet was cool at room temperature for 5 minute at each side and put into the plastic bag

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then kept at 4°C for 6 hours. The retrograded rice sheet was cut to the 0.5 mm wide noodle (Sen Lek). The fresh rice noodle was dried in a hot air oven at 45°C for 4 hours to be dried noodle. Dried rice noodle was soaked in room temperature water for 15 min then boiled for 1.5 min immediately cool in 10°C water for 1 min and drain for 10 min (modified from Israkarn, 2005). The cooked rice noodle was put in plastic boxes and used liquid nitrogen for quick frozen process. Frozen rice noodle was stored at -18°C until analysis.

6.2 Texture analysis

Frozen rice noodle was thawing before analyzed by left at 30°C in incubator for 1 hour and heat in 900 watt microwave for 30 second. The frozen noodle, which was stored at -18°C for 23 hours and thawing, called freeze-thaw cycle 1. This thawed noodle was stored at -18°C for 23 hours and re-thawing called freeze-thaw cycle 2 keep repeat the cycle until reach freeze-thaw cycle 5. The thawed noodle was determined texture profile at freeze-thaw cycle 1, 3 and 5 (modified from Israkarn, 2005).

6.3 Sensory evaluation

Taste panelist was performed a quantitative descriptive analysis (QDA) to evaluate cooked frozen noodles. The attributes evaluated for the cooked frozen noodles were firmness, elasticity and adhesiveness. Perceived intensity was scored on a 15 cm interval scale (modified from Israkarn, 2005; Lorlowhakarn, 2007).

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7. Statistical analysis

7.1 Complete randomize design (CRD) was applied for study rice flour and frozen rice noodle properties. Analysis of variance (ANOVA) was performed to compare treatment means; differences were considered at significant level of 95% ($p \leq 0.05$).

7.2 Randomized complete block design (RCBD) was applied for study the sensory evaluation. Analysis of variance (ANOVA) was performed to compare treatment means; differences were considered at significant level of 95% ($p \leq 0.05$).

8. Experiment place

Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand.

9. Experiment duration

January 2010- February 2012.

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RESULTS AND DISCUSSIONS

1. Pre-germination process of paddy

1.1 Pre-germination condition

1.1.1 Soaking time

The moisture content of both Chai Nat1 and RD6 paddies after soaked in water at 30°C for 2-20 hours were shown in Table 11.

Table 11 Moisture content of both Chai Nat1 and RD6 paddies.

Soaking time (hour)	Moisture content of paddy (% as is basis)[1]	
	Chai Nat 1	RD 6
Before soaked	8.35 ± 0.63iA	8.31± 0.32gA
2	24.97 ± 0.59hB	25.47± 0.53fA
4	26.34 ± 0.18gB	28.34± 0.40eA
6	27.00 ± 0.54fB	32.28± 0.38dA
8	27.66 ± 0.17fB	35.18± 0.26cA
10	28.60 ± 0.40eB	36.19 ± 0.36bA
12	31.05 ± 0.62dB	40.08 ± 0.52aA
14	36.45 ± 1.66cB	40.68 ± 0.28aA
16	39.23 ± 0.18bB	40.98 ± 0.77aA
18	40.28 ± 1.88aB	40.98 ± 0.38aA
20	40.35 ± 0.38aB	41.06 ± 0.18aA

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).

The moisture content of Chai Nat1 and RD6 paddies before soaked was 8.35 % and 8.31 %, respectively. The minimum, optimum and maximum moisture

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content of soaked Chai Nat1 paddy were found at 2-10 hours (24.97-28.60 %), 12 hours (31.05 %) and 14-20 hours (36.45-40.35 %), respectively. The minimum, optimum and maximum moisture content of soaked RD6 paddy were found at 2-4 hours (25.47-28.34 %), 6 hours (32.28 %) and 8-20 hours (35.18-41.06 %), respectively. The moisture contents of RD6 paddy between 2-20 hours (25.47-41.06 %) were significantly ($p \leq 0.05$) higher than Chai Nat1 paddy (24.97-40.35 %).

Rattanadee and Naivikul (2011) were reported that the optimum moisture content of Chai Nat1 paddy was found in 12 hours (30.00%) after soaked at 30°C. The corresponding result was due to the same paddy rice variety also showed a similar structure. The result in soaking behavior of RD6 paddy was reported from Panchan (2011) who soaked RD6 paddy at 30°C the optimum moisture content was found between 4-8 hours and range from 30.83-34.87%. However, Usansa *et al.* (2009) were reported that RD6 paddy after soaked at 30°C the optimum moisture content was found in 48 hours (31.48 %). Eventhough the rice variety was the same but the starch molecular structure could be different by harvest time then the initial moisture content was different followed by the contradict result.

The germination process required water to activate enzyme synthesis as reported from Usansa *et al*. (2009). The moisture content at optimum stage (30-35 %) was suitable to activate enzyme activity as reported from Rattanadee and Naivikul (2011).

RD6 paddy was found to have greater amount of water uptake than Chai Nat1 paddy. Usansa *et al.* (2009) were reported that the moisture content of Pratum Thani60 paddy after soaked at 30°C for 48 hours (28.82 %) showed significantly ($p \leq 0.05$) lower than RD6 paddy (31.48 %). Panchan (2011) was reported that the water absorption of paddy after soaked at 30°C for 36 hours in Suphan Buri1 paddy (33.77 %) was lower than RD6 paddy (38.95 %). The corresponding results were due to the similar amylose range of paddy also showed a similar starch molecular structure. The soaking process of paddy was influence by starch molecular structure.

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Amylopectin structure contained more hydroxyl group and could bind with water higher than amylose structure as reported from Usansa *et al.* (2009).

In summary, the optimum moisture content of soaking process should contain 30-35 % moisture content during 12 and 6 hours in Chai Nat1 and RD6 paddies, respectively. These moisture content was suitable to promote enzyme activity (Rattanadee and Naivikul, 2011).These conditions of both rice varieties were used to further experiments for pre-germination process.

1.1.2 Pre-germination time

Both soaken Chai Nat1 and RD6 paddies that showed 30-35 % moisture content was select to study pre-germination time at 30°C (85% relative humidity) for 4- 30 hours as shown in Table 12.

Table 12 Percent pre-germination of paddy at different embryo growth length.

Time	Chai Nat1			RD6		
(hour)	0.5-1 mm	1-2 mm	2-3 mm	0.5-1 mm	1-2 mm	2-3 mm
4	9.17 ±2.48			14.67 ± 3.08		
6	14.33 ± 2.16			26.50 ± 1.87		
8	29.00 ± 4.20			31.17 ± 2.14		
10	29.17 ± 2.48			47.17 ± 2.42		
12	32.83 ± 1.72			58.33 ± 3.33		
14	43.33 ± 3.27			62.43 ± 3.76		
16	54.83 ± 2.79				64.67 ± 3.98	
18	62.33 ± 2.05				68.33 ± 3.20	
20	64.17 ± 4.58				75.83 ± 3.92	
22		68.42 ± 2.70				78.00 ± 1.10
24		71.17 ± 3.31				86.30 ± 2.09
26			75.50 ± 6.29			89.00 ± 2.83
28			81.17 ± 3.19			92.50 ± 1.87
30			87.17 ± 2.32			93.85± 2.42

[1]Values are means of triplicate measurements ± standard deviation.

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Three stages of subsequent embryo growth length were 0.5-1 mm (60-70 % of germination) called minimum stage, 1-2 mm (70-80 % of germination) call optimum stage, 2-3 mm (more than 80 % of germination) call maximum stage. The pre-germination at minimum, optimum and maximum stages were observed at 18 and 14 hours, 24 and 20 hours, 28 and 24 hours for both Chai Nat1 and RD6 paddies, respectively. The Chai Nat1 paddy showed longer time (18, 24 and 28 hours) to reach three stages of embryo growth length than RD6 paddy (14, 20 and 24 hours).

Rattanadee and Naivikul (2011) were reported that Chai Nat1 paddy after soaked at 30°C for 12 hours then pre-germination at 30°C (85% relative humidity) for 16, 20 and 24 hours to reach three stages of subsequently embryo growth length. Panchan (2011) was reported that the appropriate pre-germinated time of RD6 paddy after soaked at 30°C for 14 hours then pre-germinated at 30°C (85% relative humidity) for 14, 18 and 24 hours to reach three stage of subsequently embryo growth length. Eventhough the pre-germinated condition and paddy verities was the same but the harvest time could make the different starch molecular structure that effect pre-germination times. Activity of enzyme was absent in dry grain but could activated as germination process result in embryo growth as reported from Moongngarm and Saetung (2010).

Okamoto *et al.* (1976) were reported that during the initial stage of rice grain germination, the scutellar epithelium produced multiple form of α-amylase. As germination proceeded enzyme activities continue to increase in the endosperm, while those in the sctellum was not change greatly during germination.

In summary, pre-germinated paddy could separate into three stages by subsequent embryo growth length at 18, 24, 28 hours for Chai Nat1 paddy and 14, 20, 24 hours for RD6 paddy, respectively. The total pre-germination process that showed three subsequently embryo growth length were 30, 36 and 40 hours for Chai Nat1 paddy and 20, 26 and 30 hours for RD6 paddy.

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1.2. Properties of pre-germinated brown rice flour

1.2.1 Chemical properties

1.2.1.1 α-amylase activity

The α-amylase activity before and after pre-germination process were determined by stirring number that was the data from Rapid Visco Analyzer (RVA). The stirring number was indirect variation with α-amylase activity. If the stirring number was high the α-amylase will be low as shown in Table 13.

Table 13 Stirring number (α-amylase activity) of white rice flour, brown rice flour and three stages of PGBR flour.

Rice flour samples[2]	Stirring number (cP)[1]	
	Chai Nat1	RD6
White rice flour	3,189.83 ± 18.21aA	1,180.00 ± 11.39aB
Brown rice flour	3,170.17 ± 15.44aA	1,168.17 ± 14.75aB
PGBR flour _1	1,560.83 ± 19.00bA	902.16 ± 16.00bB
PGBR flour _2	1,338.00 ± 14.84cA	432.83 ± 17.11cB
PGBR flour _3	1,179.17 ± 8.74dA	283.00 ± 10.56dB

[1] Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).
[2] Moisture content of all rice flour were showed in appendix.

All Chai Nat1 rice flour samples (White rice flour, brown rice flour, PGBR flour_1, PGBR flour_2 and PGBR flour_3) showed higher stirring number (3,189.83-1,179.17 cP) and significantly different ($p \leq 0.05$) when compared to RD6 rice flour samples (1,180.00-283.00 cP). The stirring numbers of both (Chai Nat1

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and RD6) white rice flour (3,189.83 cP and 1,180.00 cP) were higher but no significantly different ($p \leq 0.05$) than brown rice flour (3,170.17 cP and 1,168.17 cP).

The α-amylase played major role in germination process and rapidly increased as the process of germination take place. Both PGBR flour (Chai Nat1 and RD6) in all three stages showed lower and significantly different ($p \leq 0.05$) in stirring numbers (1,560.83-1,179.17 cP and 902.16-283.00 cP) than white rice flour (3,189.83 cP and 1,180.00 cP) and brown rice flour (3,170.17 cP and 1,168.17 cP). The amount of stirring number significantly ($p \leq 0.05$) decreased from 1^{st} stage to 3^{st} stage for both Chai Nat1 and RD6, range from 1,560.83-1,179.17 cP for Chai Nat1 and range from 902.16-283.00 cP for RD6..

Rattanadee and Naivikul (2011) were reported that the stirring numbers of pre-germinated Chai Nat1 paddy from three stages (2,583.00-1,417.50 cP) were significantly ($p < 0.05$) lower than brown rice (3,320.00 cP) after soaked at 30°C for 12 hours then pre-germinated at 30°C (85% relative humidity) for 16, 20, 24 hours. The corresponding result was due to the same paddy rice variety also showed a similar structure after pre-germination process.

Moongngarm (2011) was reported that the α-amylase activity of germinated brown rice (21.30 U/g flour) was significantly (p‹0.05) higher than brown rice (1.42 U/g flour) after soaked RD6 paddy at 30°C for 24 hours then germinated at 30°C (90-95 % relative humidity) for 24 hours and using enzyme assay kids to determined α-amylase activity. The corresponding result was due to the same paddy rice variety also showed a similar structure after germination process.

The activity of α-amylase was not exist in dry grain but rapidly increased as the germination. The increased in α-amylase activity during germination was due to the suitable moisture content could develop enzyme activity as reported from Rattanadee and Naivikul (2011).

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In summary, the stirring number after pre-germination process was lower than white rice flour and brown rice flour, translate to high α-amylase activity. The appropriate germination condition could activate α-amylase activity.

1.2.1.2 Amylose content

The amylose content of white rice flour, brown rice flour when compared to PGBR flour was shown in Table 14.

Table 14 Amylose content of white rice flour, brown rice flour and three stages of PGBR flour.

Rice flour sample[2]	Amylose content (%)[1]	
	Chai Nat1	RD6
White rice flour	27.46 ± 0.01aB	5.64 ± 0.13aA
Brown rice flour	27.29 ± 0.06aB	5.59 ± 0.01aA
PGBR flour _1	25.92 ± 0.10bB	5.24 ± 0.05bA
PGBR flour _2	20.90 ± 0.06cB	4.55 ± 0.08cA
PGBR flour _3	15.57 ± 0.04dB	4.02 ± 0.03dA

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different (p≤0.05).
[2] Moisture content of all rice flour samples were showed in appendix.

Both white rice flours (Chai Nat1 and RD6) showed higher but no significantly different (p≤0.05) in amylose content (27.46 and 5.64 %) when compared to brown rice flours (27.29 and 5.59 %). Both PGBR flours_1 (Chai Nat1 and RD6) showed significantly (p≤0.05) lower amylose content (25.92 and 5.24 %) when compared to white rice flours and brown rice flours. The amount of amylose contents were significantly (p≤0.05) decreased from 1st stage to 3st stage for both Chai

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Nat1 and RD6, range from 25.92-15.57 % for Chai Nat1 and range from 5.24-4.02 % for RD6.

Xe *et al.* (2011) were reported that the amylose content of germinated brown rice (121.3 g/kg) was significantly ($p<0.05$) lower than brown rice (173.9 g/kg) after soaked brown rice (long grain cultivar) at room temperature for 12 hours then germinated at 30°C (65% relative humidity) for 24 hours and determined total amylose content on defatted germinated starch. The structure of PGBR flour was not completely hydrolyzed the main structure still be starch then similar result between germinated brown rice starch and PGBR flour could be possible.

Moreover, Xe *et al.* (2011) were reported that starch molecular structures of germinated brown rice were mainly degraded into oligosaccharides by hydrolytic enzymes during germination. The decreased long chain starch molecule could explain low amylose content after pre-germination process.

In summary, pre-germination process produced lower amylose content when compared to white rice flour and brown rice flour. Enzyme activity was increased after pre-germination process at suitable condition and hydrolyzed starch molecular structure then long chain starch molecules such as amylose were decreased.

1.2.1.3 Reducing sugar

The reducing sugar of white rice flour, brown rice flour when compared to PGBR flour in Chai Nat1 and RD6 rice varieties was shown in Table 15.

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Table 15 The reducing sugar of white rice flour, brown rice flour and three stages of PGBR flour.

Rice flour samples[2]	Reducing sugar (mg/100g)[1]	
	Chai Nat1	RD6
White rice flour	85.22 ±5.16dB	344.26 ±20.07dA
Brown rice flour	84.44 ±3.23dB	341.85 ±17.98dA
PGBR flour_1	146.50 ±3.00cB	387.51 ±19.00cA
PGBR flour_2	252.34 ±30.39bB	451.92 ±30.18bA
PGBR flour_3	343.87 ±18.57aB	588.86 ±24.46aA

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).
[2] Moisture content of all rice flour were showed in appendix.

All Chai Nat1 rice flour samples (white rice flour, brown rice flour, PGBR flour_1, PGBR flour_2 and PGBR flour_3) showed lower reducing sugar (85.22-343.87 mg/100g) and significantly different ($p \leq 0.05$) when compared to RD6 rice flour samples (341.85-588.86 mg/100g). The reducing sugars of both (Chai Nat1 and RD6) white rice flours (85.22 and 344.26 mg/100g) were higher but no significantly different ($p \leq 0.05$) than brown rice flours (84.44 and 341.85 mg/100g).

Before germination process rice showed small amount of sugars then after germination process, amylolytic enzyme was activated and hydrolyzed starch to small molecule such as reducing sugar (Moongngarm, 2011). Both PGBR flours_1 (Chai Nat1 and RD6) showed higher and significantly different ($p \leq 0.05$) in reducing sugars (146.50 and 387.51 mg/100g) than white rice flours (85.22 and 344.26 mg/100g) and brown rice flours (84.44 and 341.85 mg/100g). The amount of reducing sugars significantly ($p \leq 0.05$) increased from 1st stage to 3st stage for both Chai Nat1 and RD6, range from 146.50-343.87 mg/100g for Chai Nat1 and range from 387.51-588.86 mg/100g for RD6.

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Rattanadee and Naivikul (2011) were reported that reducing sugar in pre-germinated Chai Nat1 paddy from 3 stages (174.59-329.40 mg/100g db) was higher than brown rice (79.66 mg/100g db) after soaked at 30°C for 12 hours then incubated at 30°C (85% relative humidity) for 16, 20 and 24 hours. The corresponding result of reducing sugar was due to the same paddy rice variety also showed a similar starch molecular structure after pre-germination.

Moongngarm and Saetung (2010) were reported that the germinated RD6 paddy showed higher reducing sugar (0.81 %) than RD6 brown rice (0.19 %) after germinated RD6 paddy by soaked at room temperature for 48 hours then germinated at 28-30°C (90-95% relative humidity) for 48 hours and determined reducing sugar. Moongngarm (2011) was reported that the germinated RD6 paddy showed higher reducing sugar (3.21 %) than RD6 brown rice (0.92 %) after soaked at room temperature for 24 hours then germinated at 28-30°C (90-95% relative humidity) for 24 hours. Panchan (2011) was also reported that the reducing sugar of pre-germinated RD6 paddy from three stages (68.07-80.68 mg/100g db sample) were increased when compared to RD6 brown rice (18.00 mg/100g db sample) after soaked at 30°C for 14 hours then pre-germinated at 30°C (85% relative humidity) for 14, 18, 24 hours. The similar trend of reducing sugar was due to the same paddy rice variety also showed a similar starch molecular structure after germination.

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In summary, pre-germination could produce high value of reducing sugar when compared to white rice flour and brown rice flour. The pre-germination process could activate α-amylase activity then starch molecules were hydrolyzed by α-amylase result of decreased long chain starch molecules (amylose content) and increased small molecular compound such as reducing sugar.

1.2.2 Physicochemical properties

1.2.2.1 Swelling power

The swelling power of white rice flour, brown rice flour and PGBR flour for both Chai Nat1 and RD6 during 65, 75, 85 and 95°C was shown in Table 16.

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Table 16 Swelling powers of white rice flour, brown rice flour and three stages of PGBR flour.

Rice flour samples[2]	Swelling power (%)[1]							
	65°C		75°C		85°C		95°C	
	Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
White rice flour	2.11 ±0.05dB	2.32±0.06dA	3.20±0.08dB	7.24±0.13dA	7.63±0.04dB	9.71±0.22dA	9.36±0.17dB	14.59±0.28dA
Brown rice flour	2.10±0.05dB	2.28±0.07dA	3.15±0.04dB	7.12±0.10dA	7.50±0.11dB	9.57±0.34dA	9.27±0.11dB	14.56±0.17dA
PGBR flour_1	3.34±0.06cB	4.55±0.11cA	4.57±0.08cB	8.32±0.07cA	9.96±0.03cB	10.77±0.07cA	11.50±0.01cB	15.67±0.14cA
PGBR flour_2	4.62±0.09bB	5.75±0.18bA	6.42±0.08bB	10.37±0.11bA	10.11±0.04bB	12.30±0.14bA	12.77±0.15bB	18.83±0.08bA
PGBR flour_3	5.74±0.07aB	6.86±0.12aA	7.23±0.03aB	12.44±0.02aA	10.59±0.02aB	13.66±0.06aA	15.44±0.08aB	20.64±0.04aA

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).

[2] Moisture content of all rice flour were showed in appendix.

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All Chai Nat1 rice flour samples (white rice flour, brown rice flour, PGBR flour_1, PGBR flour_2 and PGBR flour_3) during increased temperature from the range of 65, 75, 85 to 95°C showed lower swelling powers (2.10-5.74 to 9.36-12.44 g/g) and significantly different ($p \leq 0.05$) when compared to RD6 rice flour samples (2.28-6.86 to 14.59-20.64 g/g). Both (Chai Nat1 and RD6) white rice flours during increased temperature from the range of 65, 75, 85 to 95°C showed higher swelling powers (2.11-9.36 and 2.32-14.59 g/g) but no significantly different ($p \leq 0.05$) when compared to brown rice flours (2.10-9.27 and 2.28-14.56 g/g).

The swelling power value of both (Chai Nat1 and RD6) PGBR flour_1 were increased while the temperature was increased from 65, 75, 85 to 95°C of both (Chai Nat1 and RD6) PGBR flours_1 (3.34-11.50 and 4.55-15.67 g/g) were significantly ($p \leq 0.05$) increased when compared to white rice flours (2.11-9.36 g/g and 2.32-14.59 g/g) and brown rice flours (2.10-9.27 and 2.28-14.56 g/g). The value of swelling power was significantly ($p \leq 0.05$) increased while 65, 75, 85 to 95°C from 1st stage to 3st stage for both Chai Nat1 and RD6, range from 3.24-5.74 to 11.50-15.44 g/g for Chai Nat1 and range from 4.55-6.86 to 15.67-20.64 g/g for RD6.

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A similar observation from Xe *et al.* (2011) were reported that the swelling power of germination brown rice starch (16.95 g/g) was significantly ($p<0.05$) increased when compared to brown rice starch (16.24 g/g) after soaked at room temperature for 12 hours then germinated at 30°C (65% relative humidity) for 24 hours. The starch molecular structure of PGBR flour was not completely hydrolyzed by α-amylase then similar result between germinated brown rice starch and PGBR flour could be possible.

In summary, the swelling power was increased after pre-germination process when compared to white rice flour and brown rice flour. Pre-germination process activated α-amylase that hydrolyzed starch molecular structure to small molecule compound likes reducing sugar. The high value of reducing sugar was attributed to high swelling power.

1.2.2.2 Solubility

The solubility of white rice flour, brown rice flour when compared to PGBR flour at 65, 75, 85 and 95°C for both Chai Nat1 and RD6 rice varieties was shown in Table 17.

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Table 17 Solubility of white rice flour, brown rice flour and three stages of PGBR flour.

Rice flour samples[2]	Solubility (%)[1]							
	65°C		75°C		85°C		95°C	
	Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
White rice flour	5.18±0.05dB	8.50±0.06dA	7.31±0.04dB	9.64±0.13dA	8.49±0.04dB	18.57±0.36dA	12.39±0.15dB	31.60±0.32dA
Brown rice flour	5.13±0.05dB	8.25±0.07dA	6.85±0.08dB	9.51±0.11dA	8.15±0.41dB	18.47±0.14dA	11.75±0.11dB	30.49±0.28dA
PGBR flour _1	18.48±0.18cB	20.42±0.11cA	19.62±0.19cB	25.27±0.12cA	21.51±0.22cB	40.76±0.14cA	23.18±0.25cB	57.31±0.28cA
PGBR flour _2	20.19±0.07bB	23.56±0.18bA	20.66±0.28bB	31.27±0.12bA	25.41±0.05bB	61.37±0.24bA	30.38±0.25bB	68.75±0.17bA
PGBR flour _3	25.76±0.18aB	27.31±0.12aA	31.28±0.03aB	43.96±0.15aA	39.26±0.11aB	71.26±0.14aA	41.31±0.28aB	74.12±0.26aA

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).

[2] Moisture content of all rice flour were showed in appendix.

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All Chai Nat1 rice flour samples (white rice flour, brown rice flour, PGBR flour_1, PGBR flour_2 and PGBR flour_3) showed lower solubility during increased temperature from the range of 65, 75, 85 to 95°C (5.18-25.76 to 12.39-41.31 %) and significantly different ($p \leq 0.05$) when compared to RD6 rice flour samples (8.50-27.31 to 31.60-74.12 %). Both (Chai Nat1 and RD6) white rice flours showed higher solubility during increased temperature from the range of 65, 75, 85 to 95°C (5.18-12.39 and 8.50-31.60 %) but no significantly different ($p \leq 0.05$) when compared to brown rice flours (5.13-11.75 and 8.25-30.49 %).

The solubility value of both (Chai Nat1 and RD6) PGBR flour_1 were significantly ($p \leq 0.05$) increased while the temperature was increased from 65, 75, 85 to 95°C (18.48-23.18 and 20.42-57.31 %) when compared to white rice flours (5.18-12.39 and 8.50-31.60 %) and brown rice flours (5.13-11.75 and 8.25-30.49 %). The value of solubility was significantly ($p \leq 0.05$) increased while 65, 75, 85 to 95°C from 1st stage to 3st stage for both Chai Nat1 and RD6, range from 18.48-25.76 to 23.18-41.31 % for Chai Nat1 and range from 20.42-27.31 to 57.31-74.12 % for RD6.

The hydrolysis of starch by germination process may alter the starch molecular structure and produced small starch molecule compound (Moongngarm, 2011; Xe *et al.*, 2011). The sugar and oligosaccharides could be passing through systems that result in high solubility.

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In summary, the solubility was increased after pre-germination process when compared to white rice flour and brown rice flour. Pre-germination process activated α-amylase that hydrolyzed starch molecular structure to reducing sugar and attributed to high solubility because small molecule could easy passing through water systems. Moreover, high swelling power after pre-germinated could also cause high solubility.

1.2.2.3 Carbohydrate leaching

The carbohydrate leaching of white rice flour, brown rice flour when compared to PGBR flour at 65, 75, 85 and 95°C for both Chai Nat1 and RD6 rice varieties was shown in Table 18.

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Table 18 Carbohydrate leaching of white rice flour, brown rice flour and three stages of PGBR flour.

Rice flour samples[2]	Carbohydrate leaching (µg/ml)[1]							
	65°C		75°C		85°C		95°C	
	Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
White rice flour	5.37±0.08dB	8.40±0.24dA	6.47±0.14dB	10.54±0.06dA	11.55±0.18dB	13.58±0.09dA	13.55±0.20dB	14.46±0.06dA
Brown rice flour	4.27±0.09eB	7.50±0.12eA	6.17±0.24dB	9.57±0.28eA	10.55±0.25eB	12.12±0.77eA	12.64±0.33eB	13.14±0.23eA
PGBR flour _1	12.27±0.07cB	20.55±0.26cA	25.38±0.23cB	30.61±0.27cA	41.11±0.27cB	45.79±0.29cA	55.51±0.15cB	68.76±0.28cA
PGBR flour _2	18.37±0.13bB	25.61±0.25bA	33.25±0.14bB	36.44±0.19bA	67.05±0.24bB	78.32±0.15bA	71.24±0.11bB	89.55±0.20bA
PGBR flour _3	27.54±0.29aB	38.77±0.12aA	39.10±0.15aB	49.14±0.27aA	72.77±0.12aB	82.86±0.24aA	80.12±0.21aB	91.93±0.34aA

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).

[2] Moisture content of all rice flour were showed in appendix.

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All Chai Nat1 rice flours samples (white rice flour, brown rice flour, PGBR flour_1, PGBR flour_2 and PGBR flour_3) showed lower carbohydrate leaching during increased temperature from the range of 65, 75, 85 to 95°C (5.37-27.54 to 13.55-80.12 µg/ml) and significantly different ($p \le 0.05$) when compared to RD6 rice flour samples (8.40-38.77 to 14.46-91.93 µg/ml). Both (Chai Nat1 and RD6) white rice flours showed higher carbohydrate leaching during increased temperature from the range of 65, 75, 85 to 95°C (5.37-13.55 and 8.40-14.46 µg/ml) and significantly different ($p \le 0.05$) when compared to brown rice flours (4.27-12.64 and 7.50-13.14 µg/ml).

The carbohydrate leaching of both (Chai Nat1 and RD6) PGBR flours_1 were significantly ($p \le 0.05$) increased while the temperature was increased from 65, 75, 85 to 95°C (12.27-55.51 µg/ml and 20.55-68.76 µg/ml) when compared to white rice flours (5.37-13.55 µg/ml and 8.40-14.46 µg/ml) and brown rice flours (4.27-12.64 µg/ml and 7.50-13.14 µg/ml). The value of carbohydrate leaching was significantly ($p \le 0.05$) increased while 65, 75, 85 to 95°C from 1st stage to 3st stage for both Chai Nat1 and RD6, range from 12.27-27.54 to 55.51-80.12 µg/ml for Chai Nat1 and range from 20.55-38.77 to 68.76-91.93 µg/ml for RD6.

The carbohydrate leaching was increased after pre-germination when compared to white rice flour and brown rice flour. Pre-germination process activated α-amylase that hydrolyzed starch molecular structure to reducing sugar. High value of reducing sugar was respond to high swelling power and solubility that all cause high level of carbohydrate leaching after pre-germination.

1.2.2.4 Peak viscosity

Peak viscosity of white rice flour, brown rice flour when compared to PGBR flour for both Chai Nat1 and RD6 rice varieties was shown in Table 19.

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Table 19 Peak viscosities of white rice flour, brown rice flour and three stages of PGBR flour.

Rice flour samples[2]	Peak viscosity (cP)[1]	
	Chai Nat1	RD6
White rice flour	2,310.00 ± 14.12aB	2,579.00 ± 11.58aA
Brown rice flour	2,307.83 ± 19.10aB	2,536.17 ± 13.34aA
PGBR flour _1	1,643.33 ± 28.31bA	447.50 ± 14.89bB
PGBR flour _2	1,506.67 ± 21.57cA	356.33 ± 4.19cB
PGBR flour _3	1,314.50 ± 22.57dA	239.17 ± 2.33dB

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different (p≤0.05).

[2] Moisture content of all rice flour were showed in appendix.

The peak viscosities of Chai Nat1 white rice flour (2,310.00 cP) and Chai Nat1 brown rice flour (2,307.83 cP) were significantly (p≤0.05) lower than RD6 white rice flour (2,579.00 cP) and RD6 brown rice flour (2,536.17 cP). However, peak viscosities of all three Chai Nat1 PGBR flour stages (1,643.33-1,314.50 cP) were significantly (p≤0.05) higher than all three RD6 PGBR flour stages (447.50-239.17 cP). Both (Chai Nat1 and RD6) white rice flours showed higher peak viscosities (2,310.00 and 2,579.00 cP) but no significantly different (p≤0.05) when compared to brown rice flours (2,307.83 and 2,536.17 cP).

The peak viscosities of both (Chai Nat1 and RD6) PGBR flours_1 (1,643.33 and 447.50 cP) were significantly (p≤0.05) decreased when compared to white rice flours (2,310.00 and 2,579.00cP) and brown rice flours (2,307.83 and 2,536.17cP). The amount of peak viscosities were significantly (p≤0.05) increased from 1st stage to 3st stage for both Chai Nat1 and RD6, range from 1,643.33-1,314.50 cP for Chai Nat1 and range from 447.50-239.17 cP for RD6.

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Rattanadee and Naivikul (2011) were reported that the peak viscosity was decreased after soaked Chai Nat1 paddy at 30°C for 12 hours then incubated at 30°C (85% relative humidity) for 16, 20, 24 hours when compared to brown rice. Xe *et al.* (2011) were reported that peak viscosity of germinated flour (795cP) was lower than brown rice flour (2,275cP) after soaked at room temperature for 12 hours then germinated at 30°C (65% relative humidity) for 24 hours. In addition, peak viscosity of germinated starch (2,726 cP) was lower than brown rice starch (3,239 cP) as reported from Xe *et al.* (2011). The starch molecular structure of PGBR flour was not completely hydrolyzed by α-amylase then similar result between germinated brown rice starch and PGBR flour could be possible.

Moongngarm (2011) was reported that peak viscosity of germinated RD6 (83.91 RVU) was significantly ($p<0.05$) lower than brown rice (89.08 RVU) after soaked at room temperature for 24 hours then germinated at 28-30°C (90-95 % relative humidity) for 24 hours. Panchan (2011) was reported that the peak viscosity of pre-germinated paddy from three stages (1,412.00-1,330.00 cP) were decreased when compared to brown rice (2,017.00 cP) after soaked paddy at 30°C for 14 hours then pre-germinated at 30°C (85% relative humidity) for 14, 20, 24 hours. The similar trend of peak viscosity was due to the same paddy rice variety also showed a similar starch molecular structure after germination.

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In germination, starch molecules were hydrolyzed at the α-D (1-4) linkage in random places by α-amylase, conversion starch to limited dextrin, oligosaccharide and reducing sugars. The small starches molecular do not contribute to the viscosity after heating and shearing and became easy to dissolve resulting in low peak viscosity as reported from Xe *et al.* (2011).

The peak viscosity was decreased after pre-germination when compared to white rice flour and brown rice flour. This was attributed to α-amylase could hydrolyzed starch molecule and produced high reducing sugar value that cause high swelling power, solubility and carbohydrate leaching then peak viscosity was reduced after heat and shear process.

1.2.2.5 Syneresis

Syneresis of white rice flour, brown rice flour when compared to PGBR flour for both Chai Nat1 and RD6 rice varieties was shown in Table 20.

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Table 20 Syneresis of white rice flour, brown rice flour and three stages of PGBR flour.

Rice flour sample[2]	Syneresis (%) [1]									
	Cycle1		Cycle2		Cycle 3		Cycle 4		Cycle 5	
	Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
White rice flour	15.70 ±0.46dB	7.46 ±0.06dA	20.13 ±0.45dB	9.68 ±0.23dA	27.01 ±0.22cB	11.53 ±0.27dA	31.33 ±0.52dB	15.53 ±0.32dA	39.70 ±0.33dB	17.44 ±0.39dA
Brown rice flour	10.72 ±0.33eB	6.26 ±0.68eA	17.28 ±0.39eB	8.58± 0.35eA	26.06 ±0.61cB	10.48 ±0.32eA	30.48 ±0.21eB	12.54 ±0.50eA	38.35 ±0.40eB	16.56 ±0.25eA
PGBR flour _1	19.00 ±0.13cB	10.55 ±0.38cA	25.41 ±0.37cB	19.42 ±0.38cA	37.70 ±0.32bB	22.46 ±0.29cA	40.58 ±0.34cB	25.78 ±0.14cA	52.78 ±0.22cB	35.29 ±0.22cA
PGBR flour _2	20.74 ±0.20bB	12.80 ±0.23bA	30.59 ±0.31bB	21.48 ±0.33bA	42.38 ±0.33aB	25.55 ±0.28bA	45.54 ±0.31bB	29.46 ±0.18bA	57.45 ±0.32bB	40.19 ±0.40bA
PGBR flour _3	22.67 ±0.16aB	13.51 ±0.31aA	32.82 ±0.35aB	25.54 ±0.33aA	42.66 ±0.32aB	28.60 ±0.25aA	47.86 ±0.31aB	32.70 ±0.25aA	62.49 ±0.27aB	52.78 ±0.17aA

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different (*p*≤0.05).

[2] Moisture content of all rice flour samples were showed in appendix.

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All Chai Nat1 rice flours samples (white rice flour, brown rice flour, PGBR flour_1, PGBR flour_2 and PGBR flour_3) showed higher syneresis during increased freeze-thaw cycles range from 1, 2, 3, 4 to 5 cycles (15.70-22.67 to 39.70-62.49 %) and significantly different ($p \leq 0.05$) when compared to RD6 rice flour samples (7.46-13.51 to 17.44-52.78 %). Both (Chai Nat1 and RD6) white rice flours showed higher syneresis during increased freeze-thaw cycles range from 1, 2, 3, 4 to 5 cycles (15.70-39.70 and 7.46-17.44 %) and significantly different ($p \leq 0.05$) when compared to brown rice flours (10.72-38.35 and 6.26-16.56 %).

The syneresis of both (Chai Nat1 and RD6) PGBR flours_1 were significantly ($p \leq 0.05$) increased while the freeze-thaw cycle was increased from 1, 2, 3, 4 to 5 cycles (19.00-52.78 and 10.55-35.29 %) when compared to white rice flours (15.70-39.70 and 7.46-17.44 %) and brown rice flours (10.72-38.35 and 6.26-16.56 %). The value of syneresis was significantly ($p \leq 0.05$) increased while 1, 2, 3, 4 to 5 cycles from 1st stage to 3st stage for both Chai Nat1 and RD6, range from 19.00-22.67 to 52.78-62.49 % for Chai Nat1 and range from 10.55-13.51 to 35.29-52.78 % for RD6.

In agreement with the previous studied from Elmonie *et al.* (2011) who reported that the syneresis of germinated sorghum were increased when compared to sorghum in every freeze-thaw cycles (1-5 cycles) after soaked at 27°C for 3 days. Freeze-thaw stability is the ability of starch paste to maintain its integrity without syneresis when subjected to repeat thermal cycling between ambient and freezing temperature, expressed as the volume of water that separated out. In fact all starches in the frozen state are hardly in process of retrogradation while only during the freezing and thawing process the starch molecular chain may also undergo retrogradation (Chen *et al*., 2003). Elmonie *et al.* (2011) was reported that higher syneresis after germination may be due to short chain starch molecule could move and re-associate easier during freeze-thaw process and provide high among of water leaching.

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In summary, after pre-germination process the syneresis value was increased when compared to white rice flour and brown rice flour. The increased of α-amylase activity after pre-germination process cause high value of reducing sugar that attributed to high swelling power, solubility, carbohydrate leaching and low peak viscosity that correspond to high syneresis after freeze-thaw cycle.

The results showed that after pre-germination the starch molecular structure of both Chai Nat1 and RD6 PGBR flour in all three stages were changed by action of α-amylase results in high reducing sugar and low amylose content. The physicochemical properties were also changed by high swelling power, solubility, carbohydrate leaching and syneresis, while peak viscosity was decreased. All the properties showed that three stages of pre-germination process could affect starch molecule chain lengths. The starch molecular structures in PGBR flour provide various starch molecular chains length in different pre-germination stage. The starch molecular structure could give an advantage to reduce the hardness of food products. However, frozen rice noodle require hardness and freeze-thaw stability properties to make good quality product, these properties were lack in PGBR flour. To improved PGBR flour quality all three PGBR flour stages were select to annealing process to find suitable stage for making frozen rice noodle.

2. One step annealing process

2.1 Chemical property of rice samples

2.1.1 Amylose content

The amylose content of both Chai Nat1 and RD6 PGBR flours after one step annealing treatments at 45°C, 50°C and 55 °C for 1-6 days were shown in Table 21.

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Table 21 Amylose content of PGBR flour from three stages after one step annealed at 45°C, 50°C and 55°C for 1-6 days.

PGBR flours[2]	Annealed time (day)	Amylose content (%)[1] after anneal at 45°C		Amylose content (%) after anneal at 50°C		Amylose content (%) after anneal at 55°C	
		Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
PGBR flour_1	Native	26.28±0.76cA	5.24±0.14bB	26.28±0.76cA	5.24±0.14bB	26.28±0.76cA	5.24±0.14dB
	1	27.05±0.77bcA	5.26±0.20bB	27.36±0.32bA	5.37±0.50bB	28.73±0.61bA	5.49±0.30cdB
	2	27.61±0.40bcA	5.37±0.28bB	27.40±0.18bA	5.59±0.43bB	28.88±0.70bA	5.66±0.59bcB
	3	28.61±0.53abA	5.42±0.50abB	27.59±0.57bA	5.60±0.10abB	29.07±0.93bA	5.99±0.10bB
	4	28.28±0.86aA	5.92±0.10aB	29.03±0.54aA	6.28±0.20aB	30.79±0.43aA	6.42±0.10aB
	5	28.30±0.40aA	5.92±0.10aB	29.23±0.60aA	6.31±0.09aB	30.84±0.50aA	6.50±0.06aB
	6	28.28±0.11aA	5.93±0.15aB	29.22±0.12aA	6.32±0.11aB	30.85±0.47aA	6.51±0.15aB
PGBR flour_2	Native	25.07±0.36cA	5.10±0.07bB	25.07±0.36cA	5.10±0.07cB	25.07±0.36cA	5.10±0.07cB
	1	25.24±0.44bcA	5.22±0.20abB	25.91±0.46bcA	5.24±0.10bcB	26.22±0.80bA	5.50±0.10cbB
	2	25.67±0.80bcA	5.27±0.25abB	25.99±0.70bcA	5.52±0.39bB	26.41±0.80bA	5.52±0.32bB
	3	25.83±0.39bA	5.39±0.10aB	26.02±1.07bA	5.56±0.10bB	26.87±0.83bA	5.59±0.40bB
	4	26.82±0.68aA	5.43±0.20aB	27.87±0.80aA	6.12±0.30abB	28.97±0.82aA	6.31±0.10aB
	5	26.97±0.50aA	5.46±0.08aB	27.99±0.80aA	6.15±0.23aB	28.98±0.60aA	6.38±0.22aB
	6	26.97±0.41aA	5.45±0.11aB	27.98±0.74aA	6.14±0.18aB	28.98±0.58aA	6.37±0.38aB

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Table 21 (Continued)

PGBR flour[2]	Annealed time (day)	Amylose content (%)[1] after anneal at 45°C		Amylose content (%) after anneal at 50°C		Amylose content (%) after anneal at 55°C	
		Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
PGBR flour_3	Native	15.57±0.38cA	4.67±0.26cB	15.57±0.38bA	4.67±0.26cB	15.57±0.38cA	4.67±0.26bB
	1	17.24±0.50bA	4.77±0.32cB	15.70±0.24bA	4.87±0.20bcB	16.52±0.29bA	4.95±0.30bB
	2	17.45±0.20bA	4.95±0.09bcB	15.81±0.30bA	4.95±0.19bcB	16.87±0.70bA	5.03±0.41bB
	3	17.61±0.58bA	5.08±0.30abB	15.99±0.33bA	5.02±0.40bB	16.91±0.19bA	5.11±0.30bB
	4	18.40±0.30aA	5.39±0.20aB	18.51±0.31aA	5.68±0.20aB	19.45±0.47aA	5.99±0.30aB
	5	18.73±0.50aA	5.45±0.10aB	18.52±0.50aA	5.71±0.30aB	19.85±0.80aA	6.01±0.26aB
	6	18.72±0.16aA	5.45±0.11aB	18.51±0.74aA	5.72±0.11aB	19.86±0.44aA	6.03±0.51aB

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).

[2] Moisture content of all rice flour was showed in appendix.

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Amylose content after annealed Chai Nat1 PGBR flour_1 at 45°C, 50°C and 55°C for 4 days (28.28, 29.03 and 30.79 %) were the highest and significantly different ($p \leq 0.05$) when compared to 1 day (27.05, 27.36 and 28.73 %), 2 days (27.61, 27.40 and 28.88 %), 3 days (28.61, 27.59 and 29.07 %) but no significant ($p \leq 0.05$) changed when compared to 5 days (28.30, 29.23 and 30.84 %) and 6 days (28.28, 29.22 and 30.85 %).

In both Chai Nat1 PGBR flour_2 and PGBR flour_3 also observed the highest amylose content after annealing for 4 days at 45°C (26.82 and 18.40 %), 50°C (27.87 and 18.51 %) and 55°C (28.97 and 19.45 %). One step annealing process at 45°C, 50°C and 55°C for 4 days in Chai Nat1 PGBR flours_2 (26.82, 27.87 and 28.97 %) and Chai Nat1 PGBR flours_3 (18.40, 18.51 and 19.45 %) showed lower amylose contents than Chai Nat1 PGBR flours_1 (28.28, 29.03 and 30.79%).

Moreover, the amylose content of annealed Chai Nat1 PGBR flours samples (PGBR flour_1, PGBR flour_2 and PGBR flour_3) for 4 days at 45°C (28.28, 26.82 and 18.40 %), 50°C (29.03, 27.87 and 18.51 %) and 55°C (30.79, 28.97 and 19.45 %) were significantly ($p \leq 0.05$) higher than before annealing process (26.28, 25.07 and 15.57 %).

Amylose content after annealed RD6 PGBR flour_1 samples at 45°C, 50°C and 55°C for 4 days (5.92, 6.28 and 6.42 %) were the highest and significantly different ($p \leq 0.05$) when compared to 1 day (5.26, 5.37 and 5.49 %), 2 days (5.37, 5.59 and 5.66 %), 3 days (5.42, 5.60 and 5.99 %) but no significant ($p \leq 0.05$) changed when compared to 5 days (5.92, 6.31 and 6.50 %) and 6 days (5.93, 6.32 and 6.51 %).

In both RD6 PGBR flour_2 and PGBR flour_3 also observed the highest amylose content after annealing for 4 days at 45°C (5.43 and 5.39 %), 50°C (6.12 and 5.68 %) and 55°C (6.31 and 5.99 %). One step annealing processes at 45°C, 50° and 55°C for 4 days in RD6 PGBR flours_2 (5.43, 6.12 and 6.31 %) and RD6

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PGBR flour_3 (5.39, 5.68 and 5.99%) showed lower amylose content than RD6 PGBR flours_1 (5.92, 6.28 and 6.42%).

Moreover, the amylose content of annealed RD6 PGBR flours samples (PGBR flour_1, PGBR flour_2 and PGBR flour_3) for 4 days at 45°C (5.92, 5.43 and 5.39 %), 50°C (6.28, 6.12 and 5.68%) and 55°C (6.42, 6.31 and 5.99 %) were significantly ($p \leq 0.05$) higher than before annealing process (5.24, 5.10 and 4.67%).

The amylose content of all annealed Chai Nat1 PGBR flours samples (PGBR flour_1, PGBR flour_2 and PGBR flour_3) for 4 days at 45°C (28.28-18.40 %), 50°C (29.03-18.51 %) and 55°C (30.79-19.45 %) were significantly ($p \leq 0.05$) higher than all annealed RD6 PGBR flour samples at 45°C (5.92-5.39%), 50°C (6.28-5.68%) and 55°C (6.42-5.99 %).

Lin *et al.* (2009) were reported that the double helic of annealed acid-methanol corn starch (30-38 %) was increased when compared to acid-methanol corn starch (26-33 %) after acid-methanol hydrolysis at 25°C for 1-30 days then annealing at 50°C for 72 hours by Carbon-13 Cross-Polarization Magic-Angle Spinning Nuclear Manetic (^{13}C CP/MAS NMR).

Although the hydrolysis-annealed sample was not the same but the result could be refer to because annealed hydrolyze samples also contained A-type starch molecular structure. The increased in apparent amylose content on annealing process could be associated with an increase in the number of helical turns and ability to from ordered helix, thereby increasing the color intensity of the amylose-iodine complex (Lin *et al.*, 2009).

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In summary, annealing process could induce the rearrangement of starch molecule and might create a longer starch helic structure that cause high amylose content. Both annealed (Chai Nat1 and RD6) PGBR flour_1 showed higher amylose content when compared to PGBR flour_2 and PGBR flour_3. After annealing at 45°C, 50°C and 55°C for 4 days, both Chai Nat1 and RD6 PGBR flour_1, showed the highest amylose content.

2.2 Physicochemical properties of rice samples

2.2.1 Swelling power

The swelling powers of both Chai Nat1 and RD6 PGBR flour after one step annealing treatments at 45°C, 50°C and 55°C for 1-6 days were shown in Table 22-24.

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Table 22 Swelling powers of PGBR flour from three stages after one step annealing process at 45°C for 1-6 days.

PGBR flour[2]	Anneal time (day)	Swelling power (g/g)[1] 65°C Chai Nat1	65°C RD6	75°C Chai Nat1	75°C RD6	85°C Chai Nat1	85°C RD6	95°C Chai Nat1	95°C RD6
PGBR flour_1	Native	3.34±0.06aB	4.55±0.10aA	4.57±0.08aB	8.23±0.07aA	9.96±0.03aB	10.77±0.07aA	11.50±0.01aB	15.67±0.14aA
	1	1.86±0.08bB	2.34±0.01bA	3.04±0.04bB	6.19±0.07bA	7.86±0.06bB	9.65±0.07bA	10.73±0.15bB	12.69±0.09bA
	2	1.67±0.06cB	2.27±0.07cA	2.57±0.05cB	5.66±0.07cA	7.00±0.12cB	9.09±0.05cA	10.48±0.15cB	12.25±0.06cA
	3	1.56±0.06dB	1.85±0.13dA	2.36±0.14dB	4.53±0.07dA	6.71±0.13dB	8.61±0.10dA	10.05±0.10dB	11.19±0.12dA
	4	1.29±0.17eB	1.43±0.09eA	1.93±0.05eB	3.49±0.16eA	5.57±0.13eB	7.54±0.09eA	8.79±0.07eB	9.68±0.13eA
	5	1.28±0.09eB	1.41±0.12eA	1.90±0.21eB	3.47±0.13eA	5.55±0.08eB	7.52±0.10eA	8.74±0.14eB	9.63±0.11eA
	6	1.28±0.10eB	1.42±0.08eA	1.91±0.15eB	3.46±0.11eA	5.56±0.10eB	7.53±0.05eA	8.73±0.11eB	9.64±0.12eA
PGBR flour_2	Native	4.62±0.09aB	5.75±0.11aA	6.42±0.08aB	10.37±0.11aA	10.11±0.04aB	12.30±0.14aA	12.77±0.15aB	18.83±0.08aA
	1	2.00±0.09bB	2.70±0.05bA	6.30±0.09bB	8.21±0.18bA	9.68±0.14bB	10.21±0.09bA	10.80±0.27bB	14.42±0.28bA
	2	1.82±0.07cB	2.59±0.08cA	5.78±0.19cB	7.85±0.10cA	8.36±0.28cB	9.92±0.09cA	10.59±0.04cB	13.40±0.32cA
	3	1.71±0.09dB	1.75±0.06dA	5.68±0.09dB	7.10±0.13dA	8.00±0.18dB	8.82±0.09dA	10.21±0.06dB	12.74±0.13dA
	4	1.36±0.06eB	1.43±0.18eA	5.04±0.08eB	6.66±0.07eA	7.50±0.06eB	7.77±0.07eA	9.17±0.06eB	11.76±0.13eA
	5	1.30±0.05eB	1.40±0.08eA	4.98±0.09eB	6.64±0.06eA	7.50±0.06eB	7.74±0.08eA	9.15±0.09eB	11.77±0.07eA
	6	1.33±0.10eB	1.41±0.10eA	4.97±0.11eB	6.54±0.12eA	7.51±0.01eB	7.75±0.05eA	9.14±0.11eB	11.77±0.15eA

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Table 22 (Continued)

PGBR flour[2]	Anneal time (day)	Swelling power (g/g)[1]							
		65°C		75°C		85°C		95°C	
		Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
PGBR flour_3	Native	5.74±0.07aB	6.86±0.10aA	7.23±0.03aB	12.44±0.02aA	12.59±0.02aB	13.66±0.06abA	15.44±0.08aB	20.64±0.04aA
	1	2.21±0.12bB	2.80±0.08bA	7.17±0.13bB	9.13±0.25bA	9.84±0.18bB	10.53±0.05bA	11.79±0.17bB	15.52±0.24bA
	2	2.02±0.13cB	2.72±0.12cA	6.99±0.18cB	8.05±1.30cA	8.79±0.14cB	10.52±0.08bA	11.73±0.08cB	15.46±0.13cA
	3	1.81±0.04dB	2.20±0.10dA	6.08±0.05dB	7.43±0.06dA	8.05±0.05dB	10.50±0.21bA	10.89±0.07dB	14.57±0.20dA
	4	1.50±0.12eB	2.11±0.12eA	5.54±0.14eB	6.70±0.11eA	7.69±0.06eB	9.68±0.12cA	9.85±0.09eB	13.55±0.11eA
	5	1.49±0.13eB	2.07±0.15eA	5.53±0.14eB	6.69±0.10eA	7.63±0.06eB	9.62±0.03cA	9.83±0.09eB	13.54±0.16eA
	6	1.48±0.15eB	2.09±0.11eA	5.53±0.12eB	6.65±0.09eA	7.64±0.11eB	9.65±0.04cA	9.84±0.06eB	13.53±0.07eA

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).

[2] Moisture content of all rice flour was showed in appendix.

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Table 23 Swelling powers of PGBR flour from three stages after one step annealing process at 50°C for 1-6 days.

PGBR flour[2]	Anneal time (day)	Swelling power (g/g)[1]							
		65°C		75°C		85°C		95°C	
		Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
PGBR flour_1	Native	3.34±0.06aB	4.55±0.13aA	4.57±0.08aB	8.23±0.07aA	9.96±0.03aB	10.77±0.07aA	11.50±0.01aB	15.67±0.14aA
	1	1.71±0.07bB	1.97±0.05bA	2.93±0.08bB	5.81±0.16bA	7.89±0.08bB	9.67±0.13bA	10.72±0.09bB	12.60±0.13bA
	2	1.37±0.04cB	1.84±0.13cA	2.35±0.08cB	5.57±0.13cA	6.55±0.25cB	8.59±0.27cA	9.90±0.08cB	11.46±0.24cA
	3	1.30±0.06dB	1.75±0.20dA	1.95±0.15dB	3.56±0.14dA	5.45±0.17dB	7.59±0.17dA	9.37±0.17dB	10.57±0.07dA
	4	1.13±0.06eB	1.20±0.21eA	1.66±0.03eB	2.55±0.04eA	4.37±0.26eB	6.12±0.06eA	8.57±0.21eB	9.26±0.05eA
	5	1.11±0.05eB	1.19±0.17eA	1.64±0.07eB	2.51±0.20eA	4.36±0.11eB	6.11±0.07eA	8.55±0.15eB	9.24±0.13eA
	6	1.12±0.07eB	1.18±0.19eA	1.65±0.09eB	2.53±0.17eA	4.35±0.18eB	6.10±0.09eA	8.53±0.18eB	9.25±0.18eA
PGBR flour_2	Native	4.62±0.19aB	5.75±0.11aA	6.42±0.08aB	10.37±0.21aA	10.11±0.04aB	12.30±0.14aA	12.77±0.15aB	18.83±0.08aA
	1	1.74±0.06bB	2.08±0.11bA	6.31±0.24bB	7.73±0.11bA	9.56±0.19bB	9.70±0.05bA	10.57±0.27bB	14.46±0.10bA
	2	1.62±0.13cB	1.85±0.08cA	5.64±0.13cB	6.30±0.07cA	8.73±0.28cB	9.02±0.07cA	10.09±0.22cB	12.99±0.03cA
	3	1.41±0.12dB	1.68±0.04dA	5.52±0.09dB	6.17±0.09dA	7.71±0.19dB	8.66±0.06dA	9.69±0.10dB	12.18±0.10dA
	4	1.34±0.11eB	1.41±0.04eA	4.45±0.10eB	5.98±0.17eA	6.58±0.18eB	7.26±0.08eA	8.98±0.13eB	11.67±0.08eA
	5	1.33±0.09eB	1.40±0.10eA	4.44±0.13eB	5.97±0.14eA	6.56±0.13eB	7.24±0.05eA	8.90±0.12eB	11.63±0.05eA
	6	1.33±0.10eB	1.41±0.18eA	4.43±0.18eB	5.98±0.16eA	6.55±0.15eB	7.25±0.10eA	8.95±0.09eB	11.65±0.06eA

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Table 23 (Continued)

PGBR flour[2]	Anneal time (day)	Swelling power (g/g)[1]							
		65°C		75°C		85°C		95°C	
		Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
PGBR flour_3	Native	5.74±0.07aB	6.86±0.10aA	7.23±0.03aB	12.44±0.02aA	12.59±0.02aB	13.66±0.06aA	15.44±0.08aB	20.64±0.04aA
	1	1.95±0.13bB	2.82±0.14bA	7.07±0.19bB	8.35±0.07bA	9.59±0.20bB	10.57±0.08bA	11.31±0.15bB	14.70±0.12bA
	2	1.73±0.08cB	2.57±0.10cA	6.55±0.07cB	7.68±0.09cA	8.99±0.15cB	10.31±0.09cA	10.60±0.18cB	14.04±0.17cA
	3	1.61±0.18dB	2.01±0.11dA	5.96±0.06dB	6.67±0.17dA	7.93±0.08dB	9.59±0.13dA	9.80±0.24dB	13.30±0.19dA
	4	1.38±0.13eB	1.48±0.18eA	4.63±0.11eB	6.04±0.24eA	7.16±0.17eB	9.07±0.16eA	9.61±0.06eB	12.11±0.12eA
	5	1.35±0.25eB	1.45±0.14eA	4.63±0.09eB	6.03±0.27eA	7.15±0.13eB	9.01±0.15eA	9.58±0.14eB	12.09±0.20eA
	6	1.36±0.26eB	1.46±0.15eA	4.62±0.02eB	6.04±0.28eA	7.15±0.16eB	9.05±0.11eA	9.60±0.13eB	12.10±0.13eA

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).

[2] Moisture content of all rice flour was showed in appendix.

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Table 24 Swelling powers of PGBR flour from three stages after one step annealing process at 55°C for 1-6 days.

PGBR flour[2]	Anneal time (day)	Swelling power (g/g)[1]							
		65°C		75°C		85°C		95°C	
		Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
PGBR flour_1	Native	3.34±0.06aB	4.55±0.13aA	4.57±0.08aB	8.23±0.07aA	9.96±0.03aB	10.77±0.07aA	11.50±0.01aB	15.67±0.14aA
	1	1.58±0.07bB	1.82±0.04bA	2.71±0.07bB	5.49±0.08bA	7.67±0.09bB	9.21±0.05bA	10.29±0.11bB	11.36±0.22bA
	2	1.35±0.04cB	1.72±0.06cA	2.21±0.04cB	4.22±0.09cA	5.19±0.08cB	8.21±0.18cA	8.68±0.30cB	10.75±0.23cA
	3	1.24±0.10dB	1.50±0.11dA	1.84±0.21dB	3.21±0.06dA	4.16±0.07dB	7.28±0.12dA	8.64±0.24cB	10.24±0.10dA
	4	0.88±0.10eB	0.94±0.07eA	1.57±0.22eB	2.16±0.08eA	3.15±0.07eB	5.62±0.11eA	8.31±0.16dB	9.23±0.14eA
	5	0.87±0.13eB	0.92±0.02eA	1.55±0.22eB	2.14±0.07eA	3.12±0.09eB	5.63±0.03eA	8.31±0.21dB	9.20±0.25eA
	6	0.88±0.11eB	0.93±0.05eA	1.55±0.11eB	2.15±0.04eA	3.13±0.05eB	5.64±0.08eA	8.32±0.15dB	9.22±0.14eA
PGBR flour_2	Native	4.62±0.19aB	5.75±0.11aA	6.42±0.08aB	10.37±0.21aA	10.11±0.04aB	12.30±0.14aA	12.77±0.15aB	18.83±0.08aA
	1	1.66±0.07bB	1.93±0.06bA	6.09±0.13bB	7.57±0.27bA	8.05±0.10bB	9.34±0.10bA	10.36±0.18bB	13.62±0.26bA
	2	1.50±0.07cB	1.75±0.05cA	5.32±0.15cB	6.16±0.10cA	7.99±0.15cB	8.72±0.06cA	9.44±0.09cB	12.74±0.24cA
	3	1.39±0.17dB	1.57±0.08dA	5.23±0.10dB	5.89±0.16dA	7.26±0.25dB	7.72±0.06dA	8.79±0.11dB	12.15±0.11dA
	4	0.91±0.08eB	1.09±0.11eA	4.08±0.11eB	4.24±0.15eA	5.10±0.22eB	6.18±0.08eA	8.59±0.15eB	11.44±0.09eA
	5	0.90±0.09eB	1.04±0.09eA	4.08±0.13eB	4.20±0.12eA	5.08±0.08eB	6.16±0.11eA	8.58±0.14eB	11.42±0.05eA
	6	0.91±0.10eB	1.06±0.11eA	4.07±0.12eB	4.22±0.11eA	5.09±0.11eB	6.15±0.18eA	8.57±0.11eB	11.43±0.02eA

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Table 24 (Continued)

PGBR flour[2]	Anneal time (day)	Swelling power (g/g)[1]							
		65°C		75°C		85°C		95°C	
		Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
PGBR flour_3	Native	5.74±0.07aB	6.86±0.10aA	7.23±0.03aB	12.44±0.02aA	12.59±0.02aB	13.66±0.06aA	15.44±0.08aB	20.64±0.04aA
	1	1.86±0.07bB	2.78±0.13bA	6.53±0.14bB	8.15±0.06bA	9.44±0.26bB	10.34±0.09bA	10.75±0.20bB	13.93±0.14bA
	2	1.67±0.21cB	2.55±0.20cA	6.18±0.06cB	6.57±0.17cA	8.33±0.13cB	9.99±0.02cA	10.15±0.07cB	12.96±0.12cA
	3	1.41±0.27dB	2.21±0.07dA	5.66±0.09dB	5.95±0.19dA	7.89±0.09dB	9.15±0.08dA	9.19±0.08dB	12.21±0.13dA
	4	1.09±0.19eB	1.20±0.07eA	4.37±0.11eB	4.87±0.18eA	6.79±0.08eB	8.89±0.10eA	8.70±0.16eB	11.53±0.10eA
	5	1.02±0.07eB	1.18±0.09eA	4.36±0.11eB	4.85±0.10eA	6.77±0.06eB	8.86±0.07eA	8.68±0.17eB	11.52±0.20eA
	6	1.05±0.05eB	1.19±0.10eA	4.35±0.10eB	4.86±0.11eA	6.77±0.05eB	8.88±0.11eA	8.66±0.11eB	11.55±0.11eA

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).

[2] Moisture content of all rice flour was showed in appendix.

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Swelling power over a range of temperature provides information about the relative strength of bonding within the granules. However, when related to noodle quality temperature above 90°C provides a good simulation to predict the quality of noodle (Bhattacharya *et al.*, 1999).

Swelling power (95°C) after annealed (45°C, 50°C and 55°C) Chai Nat1 PGBR flour_1 for 4 days (8.79, 8.57 and 8.31 g/g) were the lowest and significantly different ($p \leq 0.05$) when compared to 1 day (10.73, 10.72 and 10.29 g/g), 2 days (10.48, 9.90 and 8.68 g/g), 3 days (10.05, 9.37 and 8.64 g/g) but no significant (p≤0.05) changed when compared to 5 days (8.74, 8.55 and 8.31 g/g) and 6 days (8.73, 8.33 and 8.32 g/g).

In both Chai Nat1 PGBR flour_2 and PGBR flour_3 also observed the lowest swelling power (95°C) after annealing for 4 days at 45°C (9.17 and 9.85 g/g), 50°C (8.98 and 9.61 g/g) and 55°C (8.59 and 8.70 g/g). The swelling powers (95°C) of all annealed (45°C, 50°C and 55°C for 4 days) Chai Nat1 PGBR flour_2 (9.17, 8.98 and 8.59 g/g) and all annealed Chai Nat1 PGBR flour_3 (9.85, 9.61 and 8.70 g/g) were higher than all annealed Chai Nat1 PGBR flour_1 (8.79, 8.57 and 8.31 g/g).

Moreover, the swelling powers (95°C) of annealed Chai Nat1 PGBR flours sample (PGBR flour_1, PGBR flour_2 and PGBR flour_3) for 4 days at 45°C (8.79, 9.17 and 9.85 g/g), 50°C (8.57, 8.98 and 9.61 g/g) and 55°C (8.31, 8.59 and 8.70 g/g) were significantly ($p \leq 0.05$) lower than before annealing process (11.50, 12.77 and 15.44 g/g).

Swelling powers (95°C) after annealed (45°C, 50°C and 55°C) RD6 PGBR flour_1 for 4 days (9.68, 9.26 and 9.23 g/g) were the highest and significantly different ($p \leq 0.05$) when compared to 1 day (12.69, 12.60 and 11.36 g/g), 2 days (12.25, 11.46 and 10.75 g/g), 3 days (11.19, 10.57 and 10.24 g/g) but no significant (p≤0.05) changed when compared to 5 days (9.63, 9.24 and 9.20 g/g) and 6 days (9.64, 9.25 and 9.22 g/g).

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In both RD6 PGBR flour_2 and PGBR flour_3 also observed the lowest swelling power (95°C) after annealing for 4 days at 45°C (11.76 and 13.55 g/g), 50°C (11.67 and 12.11 g/g) and 55°C (11.44 and 11.53 g/g). The swelling powers (95°C) of all annealed (45°C, 50°C and 55°C for 4 days) RD6 PGBR flour_2 (11.76, 11.67 and 11.44 g/g) and all annealed RD6 PGBR flour_3 (13.55, 12.11 and 11.53 g/g) were higher than all annealed RD6 PGBR flour_1 (9.68, 9.26 and 9.23 g/g).

Moreover, the swelling powers (95°C) of annealed RD6 PGBR flours samples (PGBR flour_1, PGBR flour_2 and PGBR flour_3) for 4 days at 45°C (9.68, 11.76 and 13.55 g/g), 50°C (9.26, 11.67 and 12.11 g/g) and 55°C (9.23, 11.44 and 11.53 g/g) were significantly ($p \leq 0.05$) lower than before annealing process (15.67, 18.83 and 20.64 g/g).

The 1st stage to 3st stage of Chai Nat1 PGBR flours after annealing (45°C, 50°C and 55°C for 4 days) showed significantly ($p \leq 0.05$) lower swelling powers during 65°C (1.29-1.50, 1.13-1.38 and 0.88-1.09 g/g), 75°C (1.93-5.54, 1.66-4.63 and 1.57-4.37 g/g), 85°C (5.57-7.69, 4.37-7.16 and 3.15-6.79 g/g) and 95°C (8.79-9.85, 8.57-9.61 and 8.31-8.70 g/g) than 1st stage to 3st stage of annealed RD6 PGBR flours at 65°C (1.43-2.11, 1.20-1.48 and 0.94-1.20 g/g), 75°C (3.49-6.70, 2.55-6.04 and 2.16-4.87 g/g), 85°C (7.54-9.68, 6.12-9.07 and 5.62-8.89 g/g) and 95°C (9.68-13.55, 9.26-12.11 and 9.23-11.53 g/g).

Intarasiri and Naivikul (2005) were reported that swelling power of annealed ferment rice flour (1.9-17.48) were decreased when compared to ferment rice flour (2.15-23.18) during 65-95°C after annealing at 55°C for 24 and 48 hours. Hormdok and Noomhorm (2007) were reported the decreased swelling power of high amylose rice starch after annealed at 55°C for 24 hours (10.15 g/g) when compared to high amylose rice starch (14.11 g/g). Siriboon (2007) was reported the decreased swelling power after annealed broken rice at 7°C and 30°C below gelatinization temperature for 24 and 72 hours when compared to broken rice. Dias *et al*. (2010) were also reported a similar result on swelling power the annealed rice starches

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showed lower swelling power when compared to rice starches after annealing at 45, 50 and 55°C for 24 hours.

Although, this experiment was not use the same annealing sample and annealed condition but the similar effect of swelling power was observed. The enzyme activity was not completely hydrolyzed PGBR flour so that the starch molecular structure was still remaining. The annealing process modified starch molecular structure that was showed in ferment rice flour, broken rice, rice starch and PGBR flour then the similar effect in swelling power could be observed. Especially in fermented rice flour that has been hydrolyzed by enzyme activity during process and very similar to PGBR flour.

The reduction in swelling power after annealing were attributed to ordering and interaction of molecular chains that made stable and rigid starch molecular structure, which limited starch hydration and thereby decreased swelling power as reported from Siriboon (2007).

In summary, the annealing process could rearrange starch molecular structure to become rigid that increased amylose content and attributed to low swelling power. Both annealed (Chai Nat1 and RD6) PGBR flour_1 showed lower swelling power when compared to PGBR flour_2 and PGBR flour_3. After annealing at 45°C, 50°C and 55°C for 4 days, both Chai Nat1 and RD6 PGBR flour_1, showed the lowest swelling power.

2.2.1 Solubility

The swelling powers of both Chai Nat1 and RD6 PGBR flour after one step annealing treatments at 45°C, 50°C and 55°C for 1-6 days were shown in Table 25-27.

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Table 25 Solubility of PGBR flour from three stages after one step annealing process at 45°C for 1-6 days.

PGBR flour[2]	Annealed time (day)	Solubility (%)[1]							
		65°C		75°C		85°C		95°C	
		Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
PGBR flour_1	Native	18.48±0.18aA	20.42±0.11aA	19.51±0.16aB	25.27±0.12aA	21.51±0.22aB	40.76±0.14aA	23.18±0.25aB	57.31±0.28aA
	1	15.55±0.10bA	19.74±0.21bA	18.57±0.15bB	22.65±0.22bA	19.53±0.15bB	38.65±0.20bA	22.24±0.17bB	51.62±0.26bA
	2	14.68±0.11cA	16.43±0.21cA	17.64±0.08cB	20.58±0.15cA	18.54±0.19cB	35.60±0.12cA	21.93±0.12cB	47.87±0.15cA
	3	14.44±0.07dA	15.47±0.29dA	17.00±0.18dB	19.02±0.14dA	18.06±0.08dB	33.58±0.13dA	20.02±0.17dB	41.94±0.12dA
	4	14.05±0.07eA	14.87±0.12eA	16.59±0.20eB	18.58±0.08eA	17.58±0.09eB	32.21±0.07eA	19.47±0.11eB	40.18±0.10eA
	5	14.02±0.05eA	14.88±0.13eA	16.48±0.18eB	18.53±0.21eA	17.52±0.14eB	32.19±0.12eA	19.43±0.17eB	40.11±0.09eA
	6	14.03±0.18eA	14.86±0.19eA	16.49±0.19eB	18.53±0.17eA	17.55±0.07eB	32.20±0.06eA	19.44±0.19eB	40.12±0.11eA
PGBR flour_2	Native	18.74±0.07aA	20.56±0.18aA	20.66±0.18aB	31.27±0.12aA	22.41±0.05aB	61.37±0.24aA	23.38±0.25aB	68.75±0.17aA
	1	17.54±0.22bA	19.60±0.18bA	20.71±0.19bB	26.65±0.29bA	21.60±0.09bB	57.13±0.13bA	22.63±0.15bB	66.11±0.06bA
	2	17.16±0.22cA	17.46±0.20cA	19.30±0.26cB	25.58±0.19cA	20.50±0.13cB	56.83±0.27cA	21.98±0.14cB	65.25±0.18cA
	3	16.77±0.22dA	16.36±0.24dA	18.61±0.19dB	22.61±0.25dA	19.56±0.18dB	54.64±0.16dA	21.25±0.07dB	63.15±0.05dA
	4	14.32±0.18eB	15.29±0.24eA	17.63±0.28B	20.26±0.16eA	18.55±0.11eB	53.41±0.28eA	20.28±0.11eB	59.72±0.20eA
	5	14.30±0.09eB	15.29±0.13eA	17.57±0.13eB	20.21±0.11eA	18.55±0.18eB	53.43±0.13eA	20.24±0.16eB	59.71±0.20eA
	6	14.31±0.10eB	15.28±0.11eA	17.58±0.11eB	20.25±0.18eA	18.54±0.19eB	53.40±0.19eA	20.25±0.17eB	59.71±0.14eA

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Table 25 (Continued)

PGBR flour[2]	Annealed time (day)	Solubility (%)[1]							
		65°C		75°C		85°C		95°C	
		Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
PGBR flour_3	Native	25.76±0.18aB	27.31±0.12aA	31.28±0.03aB	43.96±0.15aA	39.26±0.11aB	71.26±0.14aA	41.31±0.28aB	74.12±0.26aA
	1	22.16±0.07bB	25.42±0.12bA	24.70±0.18bB	42.46±0.19bA	37.69±0.19bB	68.73±0.13bA	39.62±0.28bB	70.13±0.07bA
	2	20.56±0.24cB	22.22±0.15cA	21.50±0.30cB	40.06±0.13cA	33.56±0.22cB	65.23±0.16cA	37.47±0.18cB	67.58±0.36cA
	3	18.52±0.26dB	20.42±0.24dA	20.57±0.26dB	38.67±0.22dA	31.63±0.20dB	60.57±0.15dA	35.38±0.28dB	66.57±0.22dA
	4	17.57±0.22eB	18.33±0.26eA	19.54±0.17eB	35.52±0.30eA	29.49±0.17eB	58.42±0.24eA	33.05±0.19eB	65.56±0.11eA
	5	17.57±0.29eB	18.26±0.18eA	19.54±0.07eB	35.49±0.41eA	29.46±0.15eB	58.41±0.07eA	32.90±0.23eB	65.56±0.29eA
	6	17.58±0.11eB	18.28±0.21eA	19.54±0.11eB	35.50±0.50eA	29.45±0.19eB	58.40±0.11eA	32.95±0.19eB	65.57±0.32eA

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).

[2]Moisture content of all rice flour were showed in appendix.

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Table 26 Solubility of PGBR flour from three stages after one step annealing process at 50°C for 1-6 days.

PGBR flour[2]	Annealed time (day)	Solubility (%)[1]							
		65°C		75°C		85°C		95°C	
		Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
PGBR	Native	18.48±0.18aB	20.42±0.11aA	19.51±0.16aB	25.27±0.12aA	21.51±0.22aB	40.76±0.14aA	23.18±0.25aB	57.31±0.28aA
flour_1	1	14.51±0.10bB	17.64±0.22bA	18.05±0.24bB	20.39±0.09bA	18.99±0.20bB	29.65±0.17bA	20.52±0.26bB	49.57±0.22bA
	2	13.00±0.04cB	15.67±0.13cA	17.38±0.20cB	19.84±0.14cA	18.06±0.15cB	28.62±0.18cA	19.37±0.20cB	44.25±0.21cA
	3	12.21±0.04dB	14.24±0.17dA	16.21±0.29dB	17.61±0.17dA	17.11±0.26dB	25.44±0.17dA	18.45±0.18dB	43.17±0.13dA
	4	11.13±0.07eB	13.08±0.12eA	15.20±0.12eB	16.57±0.26eA	16.05±0.22eB	24.65±0.19eA	17.20±0.08eB	39.69±0.29eA
	5	11.12±0.09eB	13.02±0.20eA	15.19±0.13eB	16.55±0.08eA	16.03±0.13eB	24.66±0.11eA	17.19±0.14eB	39.65±0.16eA
	6	11.12±0.08eB	13.03±0.19eA	15.18±0.11eB	16.54±0.18eA	16.04±0.13eB	24.61±0.18eA	17.20±0.16eB	39.64±0.17eA
PGBR	Native	18.74±0.07aB	20.56±0.18aA	20.66±0.18aB	31.27±0.12aA	22.41±0.05aB	61.37±0.24aA	23.38±0.25aB	68.75±0.17aA
flour_2	1	16.63±0.19bB	18.67±0.25bA	18.64±0.34bB	25.63±0.28bA	19.28±0.19bB	55.34±0.22bA	20.85±0.18bB	65.78±0.04bA
	2	15.58±0.20cB	17.77±0.11cA	17.67±0.21cB	24.62±0.11cA	18.61±0.23cB	54.77±0.29cA	19.97±0.18cB	64.88±0.14cA
	3	14.21±0.13dB	15.76±0.20dA	16.79±0.13dB	20.30±0.24dA	17.46±0.22dB	53.58±0.19dA	19.02±0.07dB	62.32±0.24dA
	4	13.24±0.16eB	14.71±0.09eA	15.68±0.21eB	19.17±0.12eA	16.63±0.25eB	52.68±0.20eA	18.49±0.08eB	57.30±0.14eA
	5	13.23±0.11eB	14.69±0.17eA	15.67±0.15eB	19.16±0.20eA	16.63±0.23eB	52.66±0.17eA	18.45±0.12eB	57.29±0.20eA
	6	13.24±0.19eB	14.68±0.18eA	15.65±0.18eB	19.16±0.11eA	16.62±0.11eB	52.65±0.11eA	18.46±0.11eB	57.30±0.18eA

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Table 26 (Continued)

PGBR flour[2]	Annealed time (day)	Solubility (%)[1]							
		65°C		75°C		85°C		95°C	
		Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
PGBR	Native	25.76±0.18aB	27.31±0.12aA	31.28±0.03aB	43.96±0.15aA	39.26±0.11aB	71.26±0.14aA	41.31±0.28aB	74.12±0.26aA
flour_3	1	20.69±0.17bB	23.59±0.17bA	22.53±0.19bB	40.08±0.07bA	35.28±0.19bB	58.28±0.37bA	35.47±0.12bB	69.55±0.18bA
	2	19.58±0.06cB	22.48±0.14cA	19.55±0.19cB	37.47±0.13cA	33.66±0.20cB	55.74±0.27cA	32.98±0.12cB	67.77±0.16cA
	3	17.57±0.18dB	20.27±0.22dA	18.47±0.11dB	36.65±0.23dA	30.29±0.16dB	54.17±0.10dA	31.62±0.20dB	65.57±0.24dA
	4	16.27±0.10eB	18.16±0.20eA	17.35±0.22eB	34.50±0.15eA	28.59±0.23eB	53.14±0.08eA	30.95±0.13eB	64.57±0.25eA
	5	16.15±0.12eB	18.14±0.10eA	17.32±0.23eB	34.47±0.30eA	28.65±0.20eB	53.10±0.25eA	30.92±0.25eB	64.39±0.28eA
	6	16.16±0.18eB	18.15±0.11eA	17.33±0.11eB	34.48±0.11eA	28.60±0.11eB	53.11±0.18eA	30.93±0.18eB	64.40±0.18eA

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).

[2] Moisture content of all rice flour were showed in appendix.

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Table 27 Solubility of PGBR flour from three stages after one step annealing process at 55°C for 1-6 days.

PGBR flour[2]	Annealed time (day)	Solubility (%)[1]							
		65°C		75°C		85°C		95°C	
		Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
PGBR flour_1	Native	18.48±0.18aB	20.42±0.11aA	19.51±0.16aB	25.27±0.12aA	21.51±0.22aB	40.76±0.14aA	23.18±0.25aB	57.31±0.28aA
	1	13.48±0.09bB	15.46±0.33bA	17.54±1.60bB	19.63±0.20bA	18.69±0.28bB	28.53±0.25bA	19.43±0.20bB	47.43±0.27bA
	2	12.13±0.06cB	14.18±0.17cA	16.68±0.16cB	17.41±0.21cA	17.74±0.12cB	27.69±0.16cA	18.29±0.22cB	41.29±0.27cA
	3	11.11±0.12dB	13.15±0.07dA	14.99±0.10dB	15.49±0.16dA	16.20±0.09dB	23.65±0.27dA	17.25±0.13dB	37.74±0.18dA
	4	10.12±0.07eB	12.11±0.26eA	13.68±0.25eB	14.42±0.12eA	15.27±0.18eB	22.63±0.20eA	16.09±0.08eB	35.72±0.27eA
	5	10.10±0.11eB	12.10±0.07eA	13.63±0.18eB	14.40±0.19eA	15.23±0.18eB	22.62±0.29eA	16.03±0.06eB	35.69±0.24eA
	6	10.11±0.18eB	12.10±0.09eA	13.65±0.19eB	14.41±0.11eA	15.24±0.19eB	22.62±0.21eA	16.05±0.08eB	35.68±0.11eA
PGBR flour_2	Native	18.74±0.07aB	20.56±0.18aA	20.66±0.18aB	31.27±0.12aA	22.41±0.05aB	61.37±0.24aA	23.38±0.25aB	68.75±0.17aA
	1	15.61±0.24bB	15.49±0.35bA	17.71±0.35bB	23.66±0.25bA	17.60±0.14bB	52.53±0.14bA	19.93±0.24bB	63.83±0.28bA
	2	14.56±0.00cB	14.64±0.22cA	16.88±0.26cB	20.71±0.19cA	17.00±0.17cB	51.51±0.17cA	18.71±0.16cB	60.59±0.17cA
	3	13.86±0.07dB	13.66±0.33dA	15.48±0.26dB	19.52±0.12dA	16.50±0.18dB	50.72±0.32dA	17.85±0.10dB	58.42±0.22dA
	4	12.10±0.06eB	12.45±0.12eA	14.27±0.17eB	18.16±0.17eA	15.43±0.11eB	48.61±0.27eA	17.07±0.08eB	56.41±0.26eA
	5	12.08±0.04eB	12.40±0.38eA	14.26±0.13eB	18.13±0.23eA	15.40±0.19eB	48.59±0.19eA	17.00±0.33eB	56.40±0.23eA
	6	12.09±0.18eB	12.40±0.33eA	14.25±0.18eB	18.14±0.28eA	15.41±0.20eB	48.60±0.18eA	17.01±0.16eB	56.40±0.28eA

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Table 27 (Continued)

PGBR flour[2]	Annealed time (day)	Solubility (%)[1]							
		65°C		75°C		85°C		95°C	
		Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
PGBR flour_3	Native	25.76±0.18aB	27.31±0.12aA	31.28±0.03aB	43.96±0.15aA	39.26±0.11aB	71.26±0.14aA	41.31±0.28aB	74.12±0.26aA
	1	17.24±0.14bB	22.70±0.18bA	20.51±0.34bB	31.67±0.19bA	33.00±0.13bB	55.67±0.29bA	33.63±0.16bB	68.58±0.15bA
	2	16.33±0.24cB	20.57±0.26cA	17.53±0.23cB	30.39±0.21cA	28.52±0.29cB	54.66±0.14cA	31.53±0.10cB	66.52±0.22cA
	3	15.57±0.29dB	18.58±0.18dA	16.45±0.19dB	29.46±0.14dA	26.15±0.08dB	53.62±0.29dA	29.58±0.09dB	64.24±0.16dA
	4	14.56±0.27eB	17.47±0.32eA	15.21±0.10eB	28.40±0.31eA	25.04±0.13eB	52.20±0.13eA	27.47±0.15eB	62.47±0.14eA
	5	14.50±0.22eB	17.44±0.31eA	15.20±0.18eB	28.37±0.22eA	25.00±0.26eB	52.13±0.08eA	27.45±0.14eB	62.44±0.27eA
	6	14.52±0.29eB	17.45±0.19eA	15.20±0.15eB	28.38±0.21eA	25.01±0.18eB	52.12±0.18eA	27.44±0.11eB	62.45±0.20eA

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).

[2] Moisture content of all rice samples were showed in appendix.

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Solubility over a range of temperature provides information about the relative strength of bonding within the granules. However, when related to noodle quality temperature above 90°C provides a good simulation to predict the quality of noodle (Bhattacharya *et al.*, 1999).

Solubilities (95°C) after annealed (45°C, 50°C and 55°C) Chai Nat1 PGBR flour_1 for 4 days (19.47, 17.20 and 16.09 %) were the lowest and significantly different ($p \leq 0.05$) when compared to 1 day (22.24, 20.52 and 19.43 %), 2 days (21.93, 19.37 and 18.29 %), 3 days (20.02, 18.45 and 17.25 %) but no significant ($p \leq 0.05$) changed when compared to 5 days (19.43, 17.19 and 16.03 %) and 6 days (19.44, 17.20 and 16.05 %).

In both Chai Nat1 PGBR flour_2 and PGBR flour_3 also observed the lowest solubility (95°C) after annealing for 4 days at 45°C (20.28 and 33.05 %), 50°C (18.49 and 30.95 %) and 55°C (17.07 and 27.47 %). The solubilities (95°C) of all annealed (45°C, 50°C and 55°C for 4 days) Chai Nat1 PGBR flours_2 (20.28, 18.49 and 17.07%) and all annealed Chai Nat1 PGBR flours_3 (33.05, 30.95 and 27.47%) were higher than all annealed Chai Nat1 PGBR flours_1 samples (19.47, 17.20 and 16.09%).

Moreover, the solubilities of annealed Chai Nat1 PGBR flours samples (PGBR flour_1, PGBR flour_2 and PGBR flour_3) for 4 days at 45°C (19.47, 20.28 and 33.05 %), 50°C (17.20, 18.49 and 30.95 %) and 55°C (16.09, 17.07 and 27.47 %) were higher than before annealing process (23.18, 23.38 and 41.31 %).

Solubilities (95°C) after annealed (45°C, 50°C and 55°C) RD6 PGBR flour_1 for 4 days (40.18, 39.69 and 35.72 %) were the highest and significantly different ($p \leq 0.05$) when compared to 1 day (51.62, 49.57 and 47.43 %), 2 days (47.87, 44.25 and 41.29 %), 3 days (41.94, 43.17 and 37.74 %) but no significant ($p \leq 0.05$) changed when compared to 5 days (40.11, 39.65 and 35.69 %) and 6 days (40.12, 39.64 and 35.68 %).

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In both RD6 PGBR flour_2 and PGBR flour_3 also observed the lowest solubility (95°C) after annealing for 4 days at 45°C (59.72 and 65.56 %), 50°C (57.30 and 64.57 %) and 55°C (56.41 and 62.47 %). The solubilities (95°C) of all annealed (45°C, 50°C and 55°C for 4 days) RD6 PGBR flours_2 (59.72, 57.30 and 56.41 %) and all annealed RD6 PGBR flours_3 (65.56, 64.57 and 62.47%) were higher than all annealed RD6 PGBR flours_1 (40.18, 39.69 and 35.72 %).

Moreover, the solubilities of annealed RD6 PGBR flours samples (PGBR flour_1, PGBR flour_2 and PGBR flour_3) for 4 days at 45°C (40.18, 59.72 and 65.56 %), 50°C (39.69, 57.30 and 64.57 %) and 55°C (35.72, 56.41 and 62.47 %) were higher than before annealing process (57.31, 68.75 and 74.12 %).

The 1st stage to 3st stage of Chai Nat1 PGBR flours after annealing (45°C, 50°C and 55°C for 4 days) showed significantly ($p \leq 0.05$) lower solubilities during 65°C (14.05-17.57, 11.13-16.27 and 10.12-14.56 %), 75°C (16.59-19.54, 15.20-17.35 and 13.68-15.21 %), 85°C (17.58-29.49, 16.05-28.59 and 15.27-25.04 %) and 95°C (19.47-33.05, 17.20-30.95 and 16.09-27.47 %) than 1st stage to 3st stage of annealed RD6 PGBR flours at 65°C (14.87-18.33, 13.08-18.16 and 12.11-17.47 %), 75°C (18.58-35.52, 16.57-34.50 and 14.42-28.40 %), 85°C (32.21-58.42, 24.65-53.14 and22.63-52.20 %) and 95°C (40.18-65.56, 39.69-64.57 and 35.72-62.47 %).

Intarasiri and Naivikul (2005) were reported the decreased solubilities of annealed ferment rice flour (1.79-15.66 %) when compared to ferment rice flour (2.31-21.39 %) during 65-95°C after annealed at 55°C for 24 and 48 hours. Siriboon (2007) was reported the decreased solubility after annealed broken rice at 7°C and 30°C below gelatinization temperature for 24 and 72 hours when compared to broken rice. Dias *et al.* (2010) were also reported the similar result on solubility that the annealed rice starch showed lower solubilities when compared to rice starch after annealing at 45, 50 and 55°C for 24 hours.

Eventhough, this experiment does not use the same annealing sample and annealed condition but the similar effect of solubility was observed. The enzyme

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activity was not completely hydrolyzed PGBR flour so that the starch molecular structure was still remaining. The annealing process modified starch molecular structure that was showed in ferment rice flour, broken rice, rice starch and PGBR flour then the similar effect in solubility could happen. Especially in fermented rice flour that has been hydrolyzed by enzymatic activity during process and very similar to PGBR flour.

The solubility after annealing process was influence by strengthening of the bonds between molecular chains and reduced swelling power impeding starch solubility from the granules as reported from Siriboon (2007).

In summary, the annealing process could induce the movement of starch molecular chain to become rigid and strong cause high amylose content and low swelling power that attributed to low solubility. Both annealed (Chai Nat1 and RD6) PGBR flour_1 showed lower solubility when compared to PGBR flour_2 and PGBR flour_3. After annealing at 45°C, 50°C and 55°C for 4 days, both Chai Nat1 and RD6 PGBR flour_1, showed the lowest solubility.

2.2.3 Carbohydrate leaching

The carbohydrate leaching of both Chai Nat1 and RD6 PGBR flour after one step annealing treatments at 45°C, 50°C and 55°C for 1-6 days was shown in Table 28-30.

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Table 28 Carbohydrate leaching of PGBR flour from three stages after one step annealing process at 45°C for 1-6 days.

PGBR flour[2]	Annealed time (day)	Carbohydrate leaching (µg/ml)[1]							
		65°C		75°C		85°C		95°C	
		Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
PGBR flour_1	Native	12.27±0.07aB	20.55±0.26aA	25.38±0.23aB	30.61±0.27aA	41.11±0.27aB	45.79±0.29aA	55.51±0.15aB	68.76±0.28aA
	1	11.58±0.23bB	18.49±0.20bA	24.79±0.30bB	28.61±0.25bA	39.47±0.26bB	42.67±0.32bA	52.62±0.32bB	66.42±0.38bA
	2	10.91±0.19cB	17.50±0.25cA	23.06±0.27cB	26.52±0.36cA	37.52±0.31cB	40.37±0.32cA	50.59±0.26cB	65.62±0.39cA
	3	9.64±0.26dB	16.95±0.33dA	20.31±0.28dB	25.65±0.14dA	35.42±0.37dB	38.66±0.29dA	48.41±0.36dB	61.67±0.36dA
	4	8.47±0.29eB	15.19±0.28eA	17.25±0.25eB	23.62±0.34eA	32.39±0.36eB	36.56±0.20eA	45.26±0.22eB	58.70±0.38eA
	5	8.45±0.13eB	15.17±0.14eA	17.20±0.27eB	23.51±0.37eA	32.20±0.17eB	36.53±0.31eA	45.22±0.17eB	58.55±0.17eA
	6	8.46±0.18eB	15.16±0.27eA	17.21±0.21eB	23.50±0.18eA	32.20±0.14eB	36.52±0.18eA	45.20±0.19eB	58.52±0.19eA
PGBR flour_2	Native	18.37±0.13aB	25.61±0.25aA	33.25±0.14aB	36.44±0.19aA	67.05±0.24aB	78.32±0.15aA	69.24±0.11aB	89.55±0.20aA
	1	17.55±0.26bB	23.82±0.34bA	32.35±0.26bB	34.50±0.26bA	64.28±0.20bB	76.40±0.25bA	68.43±0.26bB	85.51±0.36bA
	2	16.56±0.29cB	21.45±0.26cA	30.82±0.51cB	32.44±0.32cA	62.10±0.11cB	73.13±1.11cA	65.16±0.10cB	82.49±0.32cA
	3	14.17±0.20dB	20.16±0.23dA	27.58±0.33dB	31.68±0.17dA	57.42±0.33dB	70.48±0.18dA	60.03±0.15dB	78.30±0.24dA
	4	12.43±0.24eB	17.56±0.34eA	25.57±0.32eB	29.47±0.32eA	55.33±0.26eB	67.47±0.28eA	58.55±0.17eB	75.63±0.22eA
	5	12.40±0.24eB	17.49±0.23eA	25.55±0.28eB	29.46±0.31eA	55.29±0.20eB	67.42±0.24eA	58.08±0.27eB	75.55±0.30eA
	6	12.39±0.21eB	17.50±0.22eA	25.54±0.22eB	29.46±0.11eA	55.28±0.14eB	67.45±0.11eA	58.10±0.24eB	75.50±0.18eA

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Table 28 (Continued)

PGBR flour[2]	Annealed time (day)	Carbohydrate leaching (µg/ml)[1]							
		65°C		75°C		85°C		95°C	
		Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
PGBR flour_3	Native	27.54±0.29aB	38.77±0.12aA	39.10±0.15aB	49.14±0.27aA	72.77±0.12aB	82.86±0.24aA	90.12±0.21aB	91.93±0.34aA
	1	26.33±0.34bB	35.51±0.31bA	35.54±0.29bB	45.52±0.25bA	68.47±0.26bB	74.95±0.27bA	85.56±0.30bB	90.51±0.13bA
	2	25.48±0.22cB	32.67±0.60cA	33.39±0.31cB	43.41±0.15cA	64.52±0.16cB	73.93±0.48cA	83.67±0.19cB	87.32±0.31cA
	3	24.04±0.25dB	30.63±0.23dA	29.26±0.18dB	40.00±0.54dA	63.50±0.36dB	71.45±0.35dA	81.16±0.17dB	85.49±0.32dA
	4	23.07±0.25eB	28.66±0.22eA	28.58±0.33eB	37.72±0.26eA	62.59±0.26eB	69.55±0.27eA	80.70±0.29eB	83.64±0.21eA
	5	23.04±0.29eB	28.13±0.19eA	28.59±0.20eB	37.64±0.23eA	62.45±0.24eB	68.62±0.32eA	80.57±0.24eB	83.55±0.39eA
	6	23.05±0.18eB	28.10±0.18eA	28.60±0.18eB	37.65±0.18eA	62.43±0.17eB	68.60±0.17eA	80.58±0.36eB	83.58±0.19eA

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).

[2] Moisture content of all rice flour samples were showed in appendix.

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Table 29 Carbohydrate leaching of PGBR flour from three stages after one step annealing process at 50°C for 1-6 days.

PGBR flour[2]	Annealed time (day)	Carbohydrate leaching (µg/ml)[1]							
		65°C		75°C		85°C		95°C	
		Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
PGBR	Native	12.27±0.07aB	20.55±0.26aA	25.38±0.23aB	30.61±0.27aA	41.11±0.27aB	45.79±0.29aA	55.51±0.15aB	68.76±0.28aA
flour_1	1	10.60±0.28bB	18.18±0.20bA	23.62±0.22bB	26.43±0.31bA	37.37±0.28bB	41.49±0.26bA	48.83±0.73bB	57.62±0.27bA
	2	9.56±0.25cB	16.61±0.27cA	21.63±0.20cB	25.39±0.33cA	35.16±0.09cB	38.95±0.23cA	45.19±0.09cB	53.39±0.34cA
	3	8.40±0.17dB	15.60±0.38dA	19.38±0.23dB	23.37±0.32dA	32.51±0.34dB	36.23±0.20dA	43.33±0.23dB	52.62±0.34dA
	4	7.30±0.12eB	14.46±0.36eA	16.40±0.28eB	22.60±0.29eA	30.74±0.16eB	36.44±0.27eA	40.35±0.34eB	47.37±0.23eA
	5	7.29±0.19eB	14.15±0.20eA	16.39±0.14eB	22.55±0.24eA	30.49±0.24eB	36.56±0.21eA	40.20±0.33eB	47.47±0.30eA
	6	7.28±0.25eB	14.17±0.18eA	16.39±0.17eB	22.56±0.11eA	30.50±0.21eB	36.54±0.18eA	40.22±0.18eB	47.48±0.18eA
PGBR	Native	18.37±0.13aB	25.61±0.25aA	33.25±0.14aB	36.44±0.19aA	67.05±0.24aB	78.32±0.15aA	69.24±0.11aB	89.55±0.20aA
flour_2	1	15.52±0.31bB	20.59±0.55bA	31.61±0.29bB	33.10±0.07bA	55.43±0.16bB	74.87±0.21bA	65.45±0.25bB	84.28±0.28bA
	2	14.60±0.39cB	17.62±0.26cA	28.34±0.27cB	31.56±0.30cA	53.45±0.34cB	70.62±0.21cA	62.16±0.06cB	80.23±0.22cA
	3	13.58±0.27dB	16.43±0.21dA	27.37±1.13dB	29.58±0.26dA	51.71±0.21dB	67.83±0.18dA	58.29±0.26dB	77.56±0.26dA
	4	12.04±0.32eB	15.68±0.20eA	24.58±0.28eB	27.62±0.30eA	50.52±0.39eB	66.40±0.36eA	56.64±0.16eB	74.49±0.30eA
	5	12.01±0.18eB	15.54±0.22eA	24.31±0.23eB	27.55±0.22eA	50.63±0.17eB	66.36±0.27eA	56.53±0.36eB	74.43±0.31eA
	6	12.05±0.17eB	15.53±0.18eA	24.32±0.11eB	27.56±0.19eA	50.64±0.18eB	66.38±0.18eA	56.55±0.17eB	74.45±0.44eA

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Table 29 (Continued)

PGBR flour[2]	Annealed time (day)	Carbohydrate leaching (µg/ml)[1]							
		65°C		75°C		85°C		95°C	
		Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
PGBR flour_3	Native	27.54±0.29aB	38.77±0.12aA	39.10±0.25aB	49.14±0.27aA	72.77±0.12aB	82.86±0.24aA	90.12±0.21aB	91.93±0.34aA
	1	25.19±0.26bB	27.59±0.34bA	33.48±0.26bB	39.68±0.11bA	66.91±0.15bB	75.52±0.27bA	83.41±0.28bB	90.39±0.22bA
	2	24.38±0.27cB	25.43±0.32cA	30.53±0.30cB	37.80±0.15cA	64.35±0.28cB	72.47±0.26cA	81.69±0.19cB	86.44±0.39cA
	3	23.39±0.28dB	23.50±0.20dA	28.51±0.29dB	35.43±0.33dA	62.41±0.30dB	69.43±0.20dA	80.34±0.28dB	84.35±0.30dA
	4	22.54±0.15eB	22.67±0.34eA	26.53±0.30eB	34.82±0.28eA	61.55±0.19eB	66.60±0.27eA	78.56±0.20eB	82.60±0.29eA
	5	22.53±0.21eB	22.63±0.23eA	26.46±0.25eB	34.80±0.24eA	61.23±0.33eB	66.43±0.32eA	78.46±0.13eB	82.54±0.27eA
	6	22.52±0.19eB	22.65±0.18eA	26.45±0.28eB	34.81±0.22eA	61.24±0.28eB	66.45±0.28eA	78.47±0.18eB	82.55±0.29eA

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).

[2] Moisture content of all rice flour samples were showed in appendix.

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Table 30 Carbohydrate leaching of PGBR flour from three stages after one step annealing process at 55°C for 1-6 days.

PGBR flour[2]	Annealed time (day)	Carbohydrate leaching (µg/ml)[1]							
		65°C		75°C		85°C		95°C	
		Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
PGBR flour_1	Native	12.27±0.07aB	20.55±0.26aA	25.38±0.23aB	30.61±0.27aA	41.11±0.27aB	45.79±0.29aA	55.51±0.15aB	68.76±0.28aA
	1	9.91±0.30bB	17.67±0.18bA	20.73±0.13bB	24.55±0.37bA	35.59±0.27bB	40.54±0.31bA	45.62±0.37bB	56.98±0.25bA
	2	9.02±0.32cB	16.83±0.34cA	19.41±0.20cB	23.56±0.10cA	32.36±0.24cB	38.08±0.19cA	43.66±0.32cB	52.50±0.38cA
	3	8.14±0.09dB	15.04±0.27dA	17.14±0.09dB	22.69±0.27dA	30.22±0.15dB	30.46±0.22dA	37.47±0.32dB	49.59±0.32dA
	4	7.20±0.22eB	14.43±0.33eA	15.33±0.12eB	20.40±0.23eA	29.57±0.10eB	30.35±0.23eA	35.28±0.22eB	45.47±0.31eA
	5	7.14±0.20eB	14.37±0.53eA	15.26±0.21eB	20.40±0.31eA	29.43±0.20eB	30.29±0.24eA	35.22±0.25eB	45.42±0.26eA
	6	7.15±0.17eB	14.38±0.19eA	15.28±0.17eB	20.41±0.11eA	29.44±0.18eB	30.28±0.18eA	35.18±0.24eB	45.41±0.11eA
PGBR flour_2	Native	18.37±0.13aB	25.61±0.25aA	33.25±0.14aB	36.44±0.19aA	67.05±0.24aB	78.32±0.15aA	69.24±0.11aB	89.55±0.20aA
	1	14.55±0.31bB	20.48±0.23bA	31.45±0.16bB	30.45±0.35bA	53.33±0.24bB	65.53±0.36bA	63.64±0.24bB	81.44±0.22bA
	2	13.52±0.23cB	17.12±0.21cA	27.13±0.20cB	28.25±0.25cA	49.55±0.24cB	60.78±0.78cA	60.68±0.15cB	79.38±0.29cA
	3	12.35±0.27dB	16.44±0.32dA	26.29±0.39dB	25.36±0.33dA	47.24±0.21dB	59.33±0.29dA	56.53±0.30dB	75.39±0.23dA
	4	10.40±0.22eB	15.60±0.33eA	23.58±0.13eB	23.33±0.22eA	46.47±0.28eB	57.48±0.26eA	54.48±0.19eB	72.72±0.25eA
	5	10.32±0.34eB	15.60±0.24eA	23.25±0.22eB	23.31±0.26eA	46.33±0.33eB	57.54±0.34eA	54.30±0.25eB	72.71±0.24eA
	6	10.33±0.11eB	15.61±0.17eA	23.24±0.18eB	23.30±0.18eA	46.45±0.18eB	57.50±0.11eA	54.31±0.11eB	72.70±0.11eA

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Table 30 (Continued)

PGBR flour[2]	Annealed time (day)	Carbohydrate leaching (µg/ml)[1]							
		65°C		75°C		85°C		95°C	
		Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
PGBR	Native	27.54±0.29aB	38.77±0.12aA	39.10±0.25aB	49.14±0.27aA	72.77±0.12aB	82.86±0.24aA	90.12±0.21aB	91.93±0.34aA
flour_3	1	24.39±0.31bB	27.52±0.26bA	33.52±0.29bB	38.39±0.36bA	64.58±0.32bB	72.61±0.37bA	81.35±0.30bB	85.58±0.23bA
	2	22.60±0.34cB	25.16±0.32cA	28.22±0.27cB	36.50±0.31cA	59.60±0.10cB	71.31±0.26cA	79.55±0.22cB	83.48±0.33cA
	3	20.43±0.12dB	21.52±0.18dA	26.38±0.09dB	34.66±0.22dA	57.49±0.28dB	68.55±0.35dA	75.44±0.28dB	82.53±0.20dA
	4	19.51±0.22eB	20.33±0.30eA	25.33±0.27eB	33.55±0.31eA	55.74±0.34eB	62.62±0.33eA	74.60±0.33eB	78.96±0.30eA
	5	19.26±0.25eB	20.31±0.22eA	25.25±0.31eB	33.59±0.10eA	55.64±0.32eB	62.50±0.33eA	74.49±0.33eB	78.69±0.18eA
	6	19.30±0.18eB	20.32±0.17eA	23.30±0.25eB	33.56±0.17eA	55.65±0.17eB	62.52±0.18eA	74.50±0.17eB	78.70±0.78eA

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).

[2] Moisture content of all rice flour samples were showed in appendix.

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Carbohydrate leaching (95°C) after annealed (45°C, 50°C and 55°C) Chai Nat1 PGBR flours_1 for 4 days (45.26, 40.35 and 35.28 µg/ml) were the lowest and significantly different (p≤0.05) when compared to 1 day (52.62, 48.83 and 45.62 µg/ml), 2 days (50.59, 45.19 and 43.66 µg/ml), 3 days (48.41, 43.33 and 37.47 µg/ml) but no significant (p≤0.05) changed when compared to 5 days (45.22, 40.20 and 35.22 µg/ml) and 6 days (45.20, 40.22 and 35.18 µg/ml).

In both Chai Nat1 PGBR flour_2 and PGBR flour_3 also observed the lowest carbohydrate leaching (95°C) after annealing for 4 days at 45°C (58.55 and 80.70 µg/ml), 50°C (56.64 and 78.56 µg/ml) and 55°C (54.48 and 74.60µg/ml). The carbohydrate leaching (95°C) of all annealed (45°C, 50°C and 55°C for 4 days) Chai Nat1 PGBR flour_2 (58.55, 56.64 and 54.48 µg/ml) and all annealed Chai Nat1 PGBR flour_3 (80.70, 78.56 and 74.60 µg/ml) were higher than all annealed Chai Nat1 PGBR flour_1 (45.26, 40.35 and 35.28 µg/ml).

Moreover, the carbohydrate leaching of annealed Chai Nat1 PGBR flour samples (PGBR flour_1, PGBR flour_2 and PGBR flour_3) for 4 days at 45°C (45.26, 58.55 and 80.70 µg/ml), 50°C (40.35, 56.64 and 78.56 µg/ml) and 55°C (35.28, 54.48 and 74.60 µg/ml) were lower than before annealing process (55.51, 71.24 and 80.12 µg/ml).

Carbohydrate leaching (95°C) after annealed (45°C, 50°C and 55°C) RD6 PGBR flour_1 for 4 days (58.70, 47.37 and 45.47 µg/ml) were the lowest and significantly different (p≤0.05) when compared to 1 day (66.42, 57.62 and 56.98 µg/ml), 2 days (65.62, 53.39 and 52.50 µg/ml), 3 days (61.67, 52.62 and 49.59 µg/ml) but no significant (p≤0.05) changed when compared to 5 days (58.55, 47.47 and 45.42 µg/ml) and 6 days (58.52, 47.48 and 45.41 µg/ml).

In both RD6 PGBR flour_2 and PGBR flour_3 also observed the lowest carbohydrate leaching (95°C) after annealing for 4 days at 45°C (75.63 and 83.64 µg/ml), 50°C (74.49 and 82.60 µg/ml) and 55°C (72.72 and 78.96 µg/ml). The carbohydrate leaching (95°C) of all annealed (45°C, 50°C and 55°C for 4 days) RD6

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PGBR flours_2 (75.63, 74.49 and 72.72 µg/ml) and all annealed RD6 PGBR flours_3 (83.64, 82.60 and 78.96 µg/ml) were higher than all annealed RD6 PGBR flours_1 (58.70, 47.37 and 45.47 µg/ml).

Moreover, the carbohydrate leaching of annealed RD6 PGBR flour samples (PGBR flour_1, PGBR flour_2 and PGBR flour_3) for 4 days at 45°C (58.70, 75.63 and 83.64 µg/ml), 50°C (47.37, 74.49 and 82.60 µg/ml) and 55°C (45.47, 72.72 and 78.96 µg/ml) were lower than before annealing process (68.76, 89.55 and 91.93).

The 1^{st} stage to 3^{st} stage of Chai Nat1 PGBR flours after annealing (45°C, 50°C and 55°C for 4 days) showed significantly ($p \leq 0.05$) lower carbohydrate leaching during 65°C (8.47-23.07, 7.30-22.54 and 7.20-19.51 µg/ml), 75°C (17.25-28.58, 16.40-26.53 and 15.33-25.33 µg/ml), 85°C (32.39-62.59, 30.74-61.55 and 29.57-55.74µg/ml) and 95°C (45.26-80.70, 40.35-78.56 and 35.28-74.60 µg/ml) than 1^{st} stage to 3^{st} stage of annealed RD6 PGBR flours at 65°C (15.19-28.66, 14.46-22.67 and 14.43-20.33 µg/ml), 75°C (23.62-37.72, 22.60-34.82 20.40-33.55and µg/ml), 85°C (36.56-69.55, 36.44-66.60 and 30.35-62.62 µg/ml) and 95°C (58.70-83.64, 47.37-82.60 and 45.47-78.96 µg/ml).

Siriboon (2007) was reported the decreased amylose leaching after annealed broken rice at 7°C and 30°C below gelatinization temperature for 24 and 72 hours when compared to broken waxy rice. Although, this experiment does not use the same annealing sample and annealed condition but the similar effect was observed. The enzyme activity was not completely hydrolyzed PGBR flour so that the starch molecular structure was still remaining. The annealing process modified starch molecular structure that was showed in broken rice and PGBR flour then the similar effect in leaching property could happen.

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The decreased of carbohydrate leaching after annealing process was influence by molecules chain associated, reduction of swelling power and solubility as reported from Siriboon (2007).

In summary, the annealing process could induce the interaction between starch molecular chains then starch molecular structure became strong cause high amylose content, low swelling power and low solubility that prevent carbohydrate leaching. Both annealed (Chai Nat1 and RD6) PGBR flour_1 showed lower carbohydrate leaching when compared to PGBR flour_2 and PGBR flour_3. After annealing at 45°C, 50°C and 55°C for 4 days, both Chai Nat1 and RD6 PGBR flour_1, showed the lowest carbohydrate leaching.

2.2.4 Peak viscosity

The peak viscosity of both Chai Nat1 and RD6 PGBR flour after one step annealing treatments at 45°C, 50°C and 55°C for 1-6 days was shown in Table 31.

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Table 31 Peak viscosity of PGBR flours from three stages after one step annealed at 45°C, 50°C and 55°C for 1-6 days.

PGBR flour[2]	Annealed time (day)	Peak viscosity (cP)[1] after annealed at 45°C		Peak viscosity (cP) after annealed at 50°C		Peak viscosity (cP) after annealed at 55°C	
		Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
PGBR flour_1	Native	1,634.33±28.31aA	447.50±14.89eB	1,634.33±28.31aA	477.50±14.89eB	1,634.33±48.31aA	447.50±14.89eB
	1	1,400.50±10.74bB	2,456.00±29.41aA	1,267.50±24.82bB	2,204.00±26.90aA	1,158.80±28.84bB	2,005.00±16.18aA
	2	1,200.00±20.86cB	1,867.00±26.28bA	1,160.30±19.69cB	1,757.80±10.91bA	1,086.83±13.09cB	1,576.50±10.89bA
	3	1,056.00±13.62dB	1,658.00±12.46cA	1,010.83±13.34dB	1,594.00±14.87cA	869.00±24.45dB	1,382.50±25.17cA
	4	885.83±16.02eB	1,219.00±25.93dA	839.00±10.69eB	1,066.00±24.04dA	657.17±18.97eB	950.80±19.76dA
	5	885.00±16.30eB	1,220.00±17.14dA	835.33±12.81eB	1,072.00±12.94dA	655.50±29.37eB	955.00±11.99dA
	6	880.11±19.19eB	1,215.29±11.16dA	840.39±15.22eB	1,068.33±16.98dA	668.87±15.87eB	953.00±14.36dA
PGBR flour_2	Native	1,506.67±21.57aA	356.33±4.19eB	1,506.67±21.57aA	356.33±4.19eB	1,506.67±21.57aA	356.33±4.19eB
	1	1,134.00±20.41bB	2,012.00±24.11aA	1,197.70±17.39bB	1,908.00±19.89aA	1,090.83±28.54bB	1,874.83±21.25aA
	2	1,005.80±11.00cB	1,862.30±26.42bA	1,080.17±16.97cB	1,665.50±15.21bA	997.33±15.94cB	1,406.00±21.82bA
	3	863.00±18.11dB	1,614.70±28.96cA	694.00±13.08dB	1,505.30±24.86cA	537.50±11.27dB	1,130.30±13.83cA
	4	620.50±16.41eB	1,129.00±15.52dA	533.50±11.39eB	937.80±22.84dA	407.67±19.36eB	801.80±15.12dA
	5	622.00±17.32eB	1,128.00±10.39dA	543.33±27.02eB	923.00±27.63dA	402.83±12.82eB	805.80±19.96dA
	6	620.17±20.58eB	1,130.23±13.11dA	538.37±15.35eB	935.14±14.23dA	410.56±15.35eB	806.48±15.74dA

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Table 31 (Continued)

PGBR flour[2]	Annealed time (day)	Peak viscosity (cP)[1] after annealed at 45°C		Peak viscosity (cP) after annealed at 50°C		Peak viscosity (cP) after annealed at 55°C	
		Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
PGBR flour_3	Native	1,314.50±22.57aA	239.17±2.33eB	1,314.50±22.57aA	239.17±2.33eB	1,314.50±42.57aA	239.17±2.33eB
	1	1,087.17±25.53bB	1,862.30±30.99aA	1,021.00±16.56bB	1,758.20±16.65aA	966.67±23.53bB	1,628.67±12.09aA
	2	894.33±18.98cB	1,720.00±10.53bA	939.33±12.93cB	1,516.00±16.88bA	890.83±18.26cB	1,342.80±10.81bA
	3	753.17±26.67dB	1,361.00±14.04cA	649.67±10.55dB	1,336.30±7.45cA	509.00±9.66dB	1,094.00±12.60cA
	4	546.17±27.16eB	937.30±11.45dA	453.00±8.07eB	807.20±14.48dA	334.83±26.49eB	678.20±5.49dA
	5	550.50±23.01eB	930.30±16.26dA	445.83±18.46eB	805.70±4.59dA	337.83±22.43eB	677.20±8.13dA
	6	549.78±15.11eB	935.11±12.58dA	450.16±16.11eB	810.78±10.55dA	335.15±15.64eB	675.16±4.16dA

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).

[2] Moisture content of all rice flour was showed in appendix.

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Peak viscosity after annealed (45°C, 50°C and 55°C) Chai Nat1 PGBR flours_1 for 4 days (885.83, 839.00 and 657.17 cP) were the lowest and significantly different ($p \leq 0.05$) when compared to 1 day (1,400.50, 1,267.50 and 1,158.80 cP), 2 days (1,200.00, 1,160.30 and 1,086.83 cP), 3 days (1,056.00, 1,010.83 and 869.00 cP) but no significant ($p \leq 0.05$) changed when compared to 5 days (885.00, 835.33 and 655.50 cP) and 6 days (880.11, 840.39 and 668.87 cP).

In both Chai Nat1 PGBR flour_2 and PGBR flour_3 also observed the lowest peak viscosity after annealing for 4 days at 45°C (620.50 and 546.17 cP), 50°C (533.50 and 453.00 cP) and 55°C (407.67 and 334.83 cP). The peak viscosity range of all annealed (45°C, 50°C and 55°C for 4 days) Chai Nat1 PGBR flours_2 (620.50, 533.50 and 407.67 cP) and all annealed Chai Nat1 PGBR flours_3 (546.17, 453.00 and 334.83 cP) were lower than all annealed Chai Nat1 PGBR flours_1 (885.83, 839.00 and 657.17 cP).

Moreover, the peak viscosity of annealed Chai Nat1 PGBR flour samples (PGBR flour_1, PGBR flour_2 and PGBR flour_3) for 4 days at 45°C (885.83, 620.50 and 546.17 cP), 50°C (839.00, 533.50 and 453.00 cP) and 55°C (657.17, 407.67 and 334.83 cP) were higher than before annealing process (1,634.33, 1,506.67 and 1,314.50 cP).

Peak viscosity after annealed (45°C, 50°C and 55°C) RD6 PGBR flours_1 for 4 days (1,219.00, 1,066.00 and 950.80 cP) were the highest and significantly different ($p \leq 0.05$) when compared to 1 day (2,456.00, 2,204.00 and 2,005.00 cP), 2 days (1,867.00, 1,757.80 and 1,576.50 cP), 3 days (1,658.00, 1,594.00 and 1,382.50 cP) but no significant ($p \leq 0.05$) changed when compared to 5 days (1,220.00, 1,072.00 and 955.00 cP) and 6 days (1,215.29, 1,068.33 and 953.00 cP).

In both RD6 PGBR flour_2 and PGBR flour_3 also observed the lowest peak viscosity after annealing for 4 days at 45°C (1,129.00 and 937.30 cP), 50°C (937.80 and 807.20 cP) and 55°C (801.80 and 678.20 cP). The peak viscosity of all annealed (45°C, 50°C and 55°C for 4 days) RD6 PGBR flours_2 (1,129.00, 937.80

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and 901.80 cP) and all annealed RD6 PGBR flours_3 (937.30, 807.20 and 678.20 cP) were lower than all annealed RD6 PGBR flours_1 (1,219.00, 1,066.00 and 950.80 cP).

Moreover, the peak viscosity of annealed RD6 PGBR flour samples (PGBR flour_1, PGBR flour_2 and PGBR flour_3) for 4 days at 45°C (1,219.00, 1,129.00 and 937.30 cP), 50°C (1,066.00, 937.80 and 807.20 cP) and 55°C (950.80, 801.80 and 678.20 cP) were lower than before annealing process (447.50, 356.33 and 239.17 cP).

All annealed Chai Nat1 PGBR flour samples (PGBR flour_1, PGBR flour_2 and PGBR flour_3) at 45°C, 50°C and 55°C for 4 days showed significantly ($p \leq 0.05$) lower peak viscosity (885.83-546.17, 839.00-453.00 and 657.17-334.83 cP) than all annealed RD6 PGBR flour samples (1,219.00-937.30, 1,066.00-807.20, 950.80-678.20 cP).

Intarasiri and Naivikul (2005) were reported that the peak viscosity after annealed fermented Chai Nat1 rice flour at 55°C for 24 and 48 hours (3,427-3,595 cP) were decreased when compared to ferment rice flour (4,034 cP). Hormdok and Noomhorm (2007) were reported the decreased peak viscosity in high amylose rice starch (209.33 RVU) after annealed at 55°C for 24 hours when compared to high amylose rice starch (218.17 RVU). Dias *et al*. (2010) were also reported a similar result on peak viscosity after annealed high amylose rice starch at 55°C for 24 hours the peak viscosity (209.7 RVU) was decreased when compared to rice starch (243.3 RVU).

Although, this experiment does not use the same annealing sample and annealed condition but the similar effect of peak viscosity was observed. The enzyme activity was not completely hydrolyzed PGBR flour so that the starch molecular structure was still remaining. The annealing process modified starch molecular structure that was showed in ferment rice flour, broken rice, rice starch and PGBR flour then the similar effect in peak viscosity could happen. Especially in

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fermented rice flour that has been hydrolyzed by enzymatic activity during process and very similar to PGBR flour.

When annealing process both amorphous and crystalline regions become stronger by the interaction between starch molecular chains then reduction in swelling power and solubility. The reduction phenomena making the granules insufficient to gelatinize result in lower peak viscosity as reported in Intarasiri and Naivikul (2005).

Dias *et al*. (2010) were reported the similar result on peak viscosity, annealed low amylose rice starch showed higher peak viscosity (338.0-420.8 RVU) when compared to low amylose rice starch (332.8 RVU) after annealed at 50°C and 55°C for 24 hours. Eventhough, this experiment does not use the same annealed sample but the similar effect was observed. The enzyme activity was not completely hydrolyzed PGBR flour so that the starch molecular structure was still remaining. The annealing process modified starch molecular structure that was the main structure in rice starch and PGBR flour then the similar effect in peak viscosity could happen. Especially in low amylose rice starch that showed similar amylose content to RD6 rice variety.

Evenif the swelling power is reduced as a result of annealing process a viscosity increase during heating is still possible. The increased viscosity can be described to more resistance of the starch molecular structure to heat and deformation by an enforcement of intra-granular binding forces rather than interaction between leached molecules because the leaching was clearly reduced after annealing process as reported from Jacobs *et al*. (1995).

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In summary, the annealing process provide the movement of starch molecular chains to rearrange and make more order starch molecular structure that cause high amylose, low swelling power, low solubility and low carbohydrate leaching, then the starch molecular structure were insufficient to gelatinize result in low peak viscosity. Both annealed (Chai Nat1 and RD6) PGBR flour_1 showed higher peak viscosity when compared to PGBR flour_2 and PGBR flour_3. After annealing at 45°C, 50°C and 55°C for 4 days, both Chai Nat1 and RD6 PGBR flour_1, showed the lowest peak viscosity.

2.2.5 Syneresis

The syneresis of both annealed Chai Nat1 and RD6 PGBR flour at 45°C, 50°C and 55°C for 1-6 days after freeze-thaw cycle were shown in Table 32-34.

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Table 32 Syneresis of PGBR flour from three stages after one step annealing process at 45°C for 1-6 days.

PGBR flour[2]	Annealed time (day)	Syneresis (%)[1]									
		Cycle 1		Cycle 2		Cycle 3		Cycle 4		Cycle 5	
		Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6
PGBR flour_1	Native	19.00±0.13aA	10.55.±0.38aB	25.41±0.37aA	19.42±0.39aB	37.70±0.31aA	22.46±0.29aB	40.58±0.34aA	25.78±0.77aB	52.78±0.22aA	35.29±0.22aB
	1	17.92±0.51bA	9.89±0.13bB	23.75±0.54bA	17.61±0.63bB	35.33±0.88bA	19.92±0.51bB	39.82±0.30bA	23.04±0.85bB	49.77±0.52bA	34.38±0.36bB
	2	15.22±0.63cA	8.91±0.24cB	21.79±0.82cA	16.72±0.84cB	34.76±0.39cA	18.00±0.58cB	37.32±0.96cA	21.99±1.31cB	47.45±0.26cA	32.76±0.95cB
	3	13.63±0.20dA	7.91±0.51dB	20.61±0.56dA	15.89±0.94dB	32.96±0.37dA	16.61±0.30dB	36.18±0.24dA	20.52±1.31dB	46.44±1.26dA	30.76±0.94dB
	4	12.56±0.38eA	6.72±0.13eB	18.15±0.59A	14.81±0.93eB	30.52±0.41eA	15.91±0.39eB	34.96±0.64eA	18.55±1.18eB	43.87±0.43eA	28.02±1.60eB
	5	12.44±0.24eA	6.67±0.73eB	18.06±0.53eA	14.75±0.21eB	30.48±0.96eA	15.85±0.51eB	34.71±1.10eA	18.37±0.96eB	43.73±0.91eA	28.81±0.78eB
	6	12.45±0.52eA	6.68±0.15eB	18.16±0.21eA	14.68±0.56eB	30.55±1.52eA	15.78±0.24eB	34.52±0.12eA	18.35±1.25eB	43.52±0.44eA	28.54±0.15eB
PGBR flour_2	Native	20.74±0.20aA	12.80±0.25aB	30.59±0.31aA	21.48±0.33aB	42.38±0.33aA	25.55±0.28aB	45.54±0.31aA	29.46±0.18aB	57.45±0.32aA	40.19±0.40aB
	1	19.40±0.58bA	11.06±0.78bB	28.87±0.34bA	19.36±1.48bB	40.07±0.54bA	24.79±0.36bB	43.68±0.89bA	27.42±0.76bB	55.86±1.42bA	38.43±0.39bB
	2	18.90±0.28cA	10.21±1.11cB	27.34±0.84cA	18.19±0.95cB	38.74±0.67cA	23.91±0.75cB	41.77±0.86cA	26.26±0.94cB	53.35±0.66cA	36.95±0.94cB
	3	18.02±0.31dA	9.01±0.62dB	25.93±0.64dA	17.77±0.35dB	36.50±0.50dA	22.78±0.40dB	40.59±0.62dA	24.89±0.95dB	52.00±0.61dA	35.25±0.62dB
	4	17.01±0.43eA	7.87±1.00eB	23.74±0.62eA	16.79±0.26eB	35.73±0.40eA	19.62±0.93eB	37.98±1.05eA	22.28±1.03eB	50.32±0.39eA	32.52±1.06eB
	5	16.99±1.44eA	7.86±0.52eB	23.63±0.82eA	16.67±0.66eB	35.71±0.79eA	19.61±0.66eB	37.87±0.90eA	22.17±0.89eB	50.29±1.03eA	32.30±0.64eB
	6	16.95±0.15eA	7.81±0.16eB	23.54±0.18eA	16.65±0.87eB	35.74±0.11eA	19.58±1.54eB	37.94±0.14eA	22.15±0.14eB	50.24±1.44eA	32.41±0.25eB

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Table 32 (Continued)

PGBR flour[2]	Annealed time (day)	Syneresis (%)[1]									
		Cycle 1		Cycle 2		Cycle 3		Cycle 4		Cycle 5	
		Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6
PGBR	Native	22.67 ±0.16aA	13.51±0.33aB	32.82±0.29aA	25.54±0.33aB	45.66±0.35aA	28.60±0.28aB	47.86±0.32aA	32.70±0.21aB	62.49±0.20aA	52.78±0.17aB
flour_3	1	20.83 ±0.55bA	12.01±0.35bB	30.53±0.80bA	23.28±0.70bB	43.43±0.80bA	27.87±0.44bB	45.63±0.57bA	30.22±0.83bB	58.05±0.28bA	47.98±0.36bB
	2	19.29 ±0.48cA	11.54±0.74cB	28.06±0.42cA	22.78±0.97cB	41.02±0.89cA	26.88±0.12cB	43.92±0.26cA	29.93±0.13cB	57.23±0.16cA	45.49±0.82cB
	3	18.74 ±0.41dA	10.74±0.81dB	26.93±0.23dA	20.99±0.22dB	38.02±3.50dA	25.36±0.84dB	42.35±0.61dA	28.71±0.29dB	56.57±0.33dA	43.39±0.96dB
	4	17.40 ±0.68eA	8.45±0.70eB	25.28±0.78eA	18.03±0.41eB	37.98±0.19eA	23.03±0.47eB	40.06±0.82eA	27.91±0.68eB	54.96±0.81eA	40.05±0.64eB
	5	17.35 ±0.56eA	8.38±0.71eB	25.18±1.12eA	17.94±0.62eB	37.64±0.54eA	22.92±0.58eB	39.99±0.50eA	27.72±0.77eB	54.69±0.78eA	39.92±0.87eB
	6	17.44 ±1.25eA	8.34±0.71eB	25.24±1.47eA	17.84±0.47eB	37.52±0.44eA	22.95±0.78eB	39.87±0.54eA	27.74±0.52eB	54.58±0.57eA	39.84±0.41eB

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).

[2] Moisture content of all rice flour samples were showed in appendix.

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Table 33 Syneresis of PGBR flour from three stages after one step annealing process at 50°C for 1-6 days.

PGBR flour[2]	Annealed time (day)	Syneresis (%)[1]									
		Cycle 1		Cycle 2		Cycle 3		Cycle 4		Cycle 5	
		Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6
PGBR flour_1	Native	19.00±0.13aA	10.55.±0.38aB	25.41±0.37eA	19.42±0.39aB	37.70±0.31aA	22.46±0.29aB	40.58±0.34aA	25.78±0.77aB	52.78±0.22aA	35.29±0.22aB
	1	15.66±0.34bA	8.61±0.27bB	22.55±0.82dA	16.95±0.64bB	35.27±0.66bA	18.28±0.66bB	37.04±0.93bA	22.12±1.07bB	45.66±0.24bA	33.53±0.70bB
	2	12.85±0.49cA	7.75±0.18cB	20.00±2.58cA	15.80±0.44cB	33.47±0.40cA	17.98±1.07cB	35.41±1.47cA	19.42±1.56cB	43.11±0.40cA	30.97±0.91cB
	3	10.94±0.27dA	6.73±0.50dB	18.96±0.62bA	13.59±0.19dB	31.62±0.26dA	15.32±1.05dB	33.79±0.96dA	17.88±1.23dB	40.22±0.85dA	29.02±0.39dB
	4	9.18±0.94eA	5.75±0.29eB	17.06±0.75eA	12.16±0.30eB	29.12±0.40eA	14.83±1.24eB	31.37±0.68eA	15.76±1.09eB	38.82±0.77eA	28.15±0.24eB
	5	9.07±0.41eA	5.72±0.63eB	17.15±0.24eA	12.09±0.41eB	29.05±0.36eA	14.79±1.48eB	31.07±0.81eA	15.59±0.62eB	38.73±0.91eA	28.03±0.37eB
	6	9.05±0.15eA	5.70±0.15eB	17.25±0.58eA	12.10±0.48eB	29.10±0.15eA	14.55±0.58eB	31.02±0.47eA	15.60±0.41eB	38.68±0.47eA	28.10±0.96eB
PGBR flour_2	Native	20.74±0.20aA	12.80±0.25aB	30.59±0.31aA	21.48±0.33aB	42.38±0.33aA	25.55±0.28aB	45.54±0.31aA	29.46±0.18aB	57.45±0.32aA	40.19±0.40aB
	1	15.70±0.97bA	10.73±0.20bB	27.83±0.77bA	18.97±0.91bB	38.99±0.33bA	23.36±0.78bB	41.99±0.91bA	26.87±0.46bB	53.73±1.52bA	35.78±0.72bB
	2	13.96±0.97cA	9.07±0.70cB	25.96±1.00cA	16.78±0.38cB	37.13±0.41cA	20.94±0.99cB	38.86±1.27cA	24.61±0.54cB	50.71±0.81cA	33.15±1.22cB
	3	12.00±0.63dA	8.50±0.60dB	23.60±0.52dA	15.04±0.82dB	35.49±0.82dA	18.47±0.34dB	36.70±1.14dA	23.09±0.55dB	47.57±0.95dA	31.48±1.33dB
	4	10.80±0.52eA	6.26±0.80eB	20.92±0.47eA	13.47±0.70eB	33.24±0.73eA	16.69±0.53eB	34.17±1.44eA	20.39±1.89eB	45.78±1.06eA	29.59±1.48eB
	5	10.75±0.79eA	6.29±0.58eB	20.75±1.26eA	13.49±0.31eB	33.57±0.88eA	16.63±0.92eB	34.10±0.90eA	20.30±1.88eB	45.21±0.48eA	29.35±1.53eB
	6	10.96±0.84eA	6.25±0.74eB	20.85±0.41eA	13.45±0.85eB	33.31±0.18eA	16.65±0.87eB	34.15±0.87eA	20.35±0.24eB	45.31±0.87eA	29.44±0.41eB

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Table 33 (Continued)

PGBR flour[2]	Annealed time (day)	Syneresis (%)[1]									
		Cycle 1		Cycle 2		Cycle 3		Cycle 4		Cycle 5	
		Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6
PGBR flour_3	Native	20.67±0.16aA	13.51±0.33aB	32.82±0.29aA	25.54±0.33aB	45.66±0.35aA	28.60±0.28aB	47.86±0.32aB	32.70±0.21aA	62.49±0.20aA	52.78±0.17aB
	1	18.93±0.70bA	11.19±0.89bB	28.44±1.01bA	23.13±3.21bB	41.84±1.99bA	26.24±0.85bB	43.57±0.45bB	27.29±0.81bA	57.00±0.60bA	45.41±1.10bB
	2	17.14±0.83cA	10.22±1.25cB	26.07±0.69cA	21.06±1.05cB	38.11±0.97cA	24.52±0.78cB	41.41±0.93cB	25.60±1.29cA	55.41±1.14cA	43.72±1.05cB
	3	15.92±1.39dA	9.27±0.56dB	24.55±1.71dA	19.89±0.41dB	36.94±0.39dA	22.77±0.83dB	39.95±0.36dB	24.02±0.80dA	53.77±1.44dA	42.09±0.93dB
	4	14.15±0.67eA	8.16±0.77eB	23.58±0.90eA	17.78±0.81eB	35.73±1.07eA	21.11±0.61eB	38.07±0.38eB	22.21±1.28eA	51.67±1.31eA	38.48±0.91eB
	5	14.07±0.83eA	8.11±1.14eB	23.34±0.48eA	17.64±0.74eB	35.41±1.10eA	21.06±0.85eB	37.95±0.27eB	22.44±1.73eA	51.57±1.33eA	38.39±0.93eB
	6	14.00±0.23eA	8.12±0.23eB	23.31±0.99eA	17.53±0.21eB	35.34±0.12eA	21.00±0.87eB	37.90±0.85eB	22.40±0.25eA	50.54±0.88eA	38.30±0.74eB

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \le 0.05$).

[2] Moisture content of all rice flour samples were showed in appendix.

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Table 34 Syneresis of PGBR flour from three stages after one step annealing process at 55°C for 1-6 days.

PGBR flour[2]	Annealed time (day)	Syneresis (%)[1]									
		Cycle 1		Cycle 2		Cycle 3		Cycle 4		Cycle 5	
		Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6
PGBR flour_1	Native	19.00±0.13aA	10.55.±0.38aB	25.41±0.37aA	19.42±0.39aB	37.70±0.31aA	22.46±0.29aB	40.58±0.34aA	25.78±0.77aB	52.78±0.22aA	35.29±0.22aB
	1	14.55±0.53bA	8.11±0.07bB	20.12±0.61bA	15.41±0.68bB	33.76±0.71bA	17.75±0.51bB	36.27±0.95bA	22.05±0.14bB	43.20±0.66bA	31.31±0.79bB
	2	12.58±0.23cA	6.96±0.30cB	18.00±0.48cA	13.02±0.92cB	30.23±1.39cA	15.61±2.35cB	34.55±0.89c	19.74±0.97cB	41.17±0.57cA	29.01±1.12cB
	3	10.91±0.99dA	5.22±0.50dB	16.80±0.89dA	11.90±0.46dB	28.96±0.37dA	13.67±0.67dB	32.07±1.21dA	17.69±1.29dB	39.92±0.55dA	27.95±1.11dB
	4	8.86±0.45eA	4.77±0.22eB	15.08±0.56eA	9.45±0.77eB	25.98±0.49eA	12.63±0.99eB	28.84±0.80eA	14.64±1.26eB	37.82±0.94eA	25.66±1.06eB
	5	8.56±0.25eA	4.64±0.98eB	15.02±0.66eA	9.29±0.98eB	25.63±0.23eA	12.20±0.45eB	28.80±0.25eA	14.57±0.54eB	37.54±1.84eA	25.55±1.38eB
	6	8.56±0.11eA	4.65±0.87eB	15.05±0.78eA	9.35±0.14eB	25.70±0.11eA	12.35±0.14eB	28.82±0.14eA	14.60±0.58eB	37.05±0.47eA	25.50±0.19eB
PGBR flour_2	Native	20.74±0.20aA	12.80±0.25aB	30.59±0.31aA	21.48±0.33aB	42.38±0.33aA	25.55±0.28aB	45.54±0.31aA	29.46±0.18aB	57.45±0.32aA	40.19±0.40aB
	1	14.60±0.54bA	10.62±0.65bB	26.55±0.35bA	17.00±0.64bB	35.83±1.70bA	20.68±0.99bB	38.77±1.10bA	25.90±1.91bB	50.51±1.52bA	34.75±1.22bB
	2	13.77±0.53cA	8.45±1.16cB	23.29±0.50cA	15.59±1.20cB	31.22±0.86cA	17.48±0.58cB	36.30±0.72cA	23.41±1.13cB	48.53±1.11cA	32.88±0.60cB
	3	11.08±0.13dA	6.62±0.70dB	21.87±0.51dA	14.00±1.50dB	29.95±0.87dA	15.98±0.56dB	34.10±0.14dA	20.43±1.06dB	46.00±0.95dA	30.60±0.95dB
	4	9.69±0.36eA	5.55±0.52eB	19.48±0.20eA	12.45±0.90eB	28.49±0.60eA	13.14±0.62eB	32.28±0.89eA	18.31±1.51eB	43.86±1.28eA	27.12±1.42eB
	5	9.33±0.39eA	5.51±1.55eB	19.39±0.63eA	12.31±1.59eB	28.31±0.37eA	13.02±0.52eB	32.16±0.10eA	18.09±1.67eB	43.66±0.93eA	27.02±1.16eB
	6	9.25±1.52eA	5.50±0.47eB	19.25±0.12eA	12.30±0.18eB	28.41±0.16eA	13.15±0.16eB	32.19±0.58eA	18.21±0.16eB	43.60±0.54eA	27.09±0.18eB

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Table 34 (Continued)

PGBR flour[2]	Annealed time (day)	Syneresis (%)[1]									
		Cycle 1		Cycle 2		Cycle 3		Cycle 4		Cycle 5	
		Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6
PGBR	Native	20.67±0.16aA	13.51±0.33aB	32.82±0.29aA	25.54±0.33aB	45.66±0.35aA	28.60±0.28aB	47.86±0.32aA	32.70±0.21aB	62.49±0.20aA	52.78±0.17aB
flour_3	1	17.87±0.69bA	10.44±0.72bB	27.19±0.39bA	20.34±0.66bB	39.84±2.00bA	25.36±1.11bB	40.54±0.79bA	26.25±0.92bB	55.04±0.67bA	43.34±0.43bB
	2	16.77±1.46cA	8.98±0.66cB	25.85±0.77cA	18.06±1.04cB	37.97±0.59cA	23.02±0.62cB	38.17±2.74cA	24.45±0.95cB	53.07±0.73cA	40.20±1.00cB
	3	14.20±1.14dA	7.82±0.19dB	23.14±0.89dA	16.23±0.38dB	35.11±1.88dA	21.21±0.35dB	35.87±0.60dA	22.53±1.78dB	51.19±0.87dA	37.94±0.34dB
	4	12.14±0.29eA	6.71±0.85eB	21.56±1.54eA	14.34±1.48eB	33.16±0.54eA	18.52±0.56eB	33.29±1.19A	19.71±1.06eB	49.28±2.03eA	35.41±1.08eB
	5	12.06±0.50eA	6.64±0.71eB	21.48±2.16eA	14.06±1.00eB	33.09±1.23eA	18.36±0.85eB	33.11±1.24eA	19.38±1.19eB	49.11±1.88eA	35.38±1.03eB
	6	12.06±0.19eA	6.62±0.47eB	21.39±0.17eA	14.13±0.12eB	33.16±0.13eA	18.40±0.69eB	33.10±0.22eA	19.40±0.69eB	49.10±0.58eA	35.40±0.58eB

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).

[2] Moisture content of all rice flour samples were showed in appendix.

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Syneresis (freeze-thaw 5 cycles) after annealed (45°C, 50°C and 55°C) Chai Nat1 PGBR flour_1 for 4 days (43.87, 38.82 and 37.82 %) were the lowest and significantly different ($p \leq 0.05$) when compared to 1 day (49.77, 45.66 and 43.20 %), 2 days (47.45, 43.11 and 41.17 %), 3 days (46.44, 40.22 and 39.92 %) but no significant ($p \leq 0.05$) changed when compared to 5 days (43.73, 38.73 and 37.54 %) and 6 days (43.52, 38.68 and 37.05 %).

In both Chai Nat1 PGBR flour_2 and PGBR flour_3 also observed the lowest syneresis (freeze-thaw 5 cycles) after annealing for 4 days at 45°C (50.32 and 54.96%), 50°C (45.78 and 51.67 %) and 55°C (43.86 and 49.28 %). The syneresis (freeze-thaw 5 cycles) of all annealed (45°C, 50°C and 55°C for 4 days) Chai Nat1 PGBR flours_2 (50.32, 45.78 and 43.86%) and all annealed Chai Nat1 PGBR flours_3 (54.96, 51.67 and 49.28%) were higher than all annealed Chai Nat1 PGBR flours_1 (43.87, 38.82 and 37.82 %).

Moreover, the syneresis (freeze-thaw 5 cycles) of annealed Chai Nat1 PGBR flour samples (PGBR flour_1, PGBR flour_2 and PGBR flour_3) for 4 days at 45°C (43.87, 50.32 and 54.96 %), 50°C (38.82, 45.78 and 51.67 %) and 55°C (37.82, 43.86 and 49.28 %) were higher than before annealing process (52.78, 57.45 and 62.49 %).

Syneresis (freeze-thaw 5 cycles) after annealed (45°C, 50°C and 55°C) RD6 PGBR flours_1 for 4 days (28.02, 28.15 and 25.66 %) were the lowest and significantly different ($p \leq 0.05$) when compared to 1 day (34.38, 33.53 and 31.31 %), 2 days (32.76, 30.97 and 29.01 %), 3 days (30.76, 29.02 and 27.95 %) but no significant ($p \leq 0.05$) changed when compared to 5 days (28.81, 28.30 and 25.55 %) and 6 days (28.54, 28.10 and 25.50 %).

In both RD6 PGBR flour_2 and PGBR flour_3 also observed the lowest syneresis (freeze-thaw 5 cycles) after annealing for 4 days at 45°C (32.52 and 40.05 %), 50°C (29.59 and 38.48%) and 55°C (27.12 and 35.41%). The syneresis (freeze-thaw 5 cycles) of all annealed (45°C, 50°C and 55°C for 4 days) RD6 PGBR

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flous_2 (32.52, 29.59 and 27.12%) and all annealed RD6 PGBR flours_3 (40.05, 38.48 and 35.41%) were higher than all annealed RD6 PGBR flours_1 (28.02, 28.15 and 25.66 %).

Moreover, the syneresis (freeze-thaw 5 cycles) of annealed RD6 PGBR flour samples (PGBR flour_1, PGBR flour_2 and PGBR flour_3) for 4 days at 45°C (28.02, 35.52 and 40.05%), 50°C (28.15, 29.59 and 38.48%) and 55°C (25.66, 27.12 and 35.41%) were higher than before annealing process (35.29, 40.19 and 52.78%).

Intarasiri and Naivikul (2005) were reported the decreased syneresis of annealed ferment rice flour after 1-5 freeze-thaw cycle (2.75-61.34 %) when compared to ferment rice flour (6.13-67.38 %). The enzyme activity was not completely hydrolyzed PGBR flour so that the starch molecular structure was still remaining. The fermented rice flour was hydrolyzed by enzymatic activity during process and very similar to PGBR flour.

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Freeze-thaw stability is the ability of a starch paste to maintain its integrity without syneresis when subjected to repeat thermal cycling between ambient and freezing temperature, expressed as the volume of water that separated out. The interaction between molecular chain that induced by annealing process made the stable starch molecular structures that disrupt retrogradation Intarasiri and Naivikul (2005).

In summary, annealing process could induce the reordering that cause high amylose while swelling power, solubility, carbohydrate leaching and peak viscosity were low in heating process. All of these properties were attributed to low syneresis after freeze-thaw cycle. Both annealed (Chai Nat1 and RD6) PGBR flour_1 showed lower syneresis when compared to PGBR flour_2 and PGBR flour_3. After annealing at 45°C, 50°C and 55°C for 4 days, both Chai Nat1 and RD6 PGBR flour_1, showed the lowest syneresis.

Annealing process could change the physicochemical properties of PGBR flour in all three stages by provide high stability of starch molecular structure to heating and cooling system.

2.3 Physical property of rice samples

2.3.1 Hardness

Hardness of annealed Chai Nat1 PGBR flour and RD6 PGBR flour at 45°C, 50°C and 55°C for 1-6 days were shown in Table 35.

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Table 35 Hardness of PGBR flour from three stages after one step annealed at 45°C, 50°C and 55°C for 1-6 days.

PGBR flour[2]	Annealed time (day)	Hardness (g)[1] after anneal at 45°C		Hardness (g) after anneal at 50°C		Hardness (g) after anneal at 55°C	
		Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
PGBR flour_1	Native	16.18±0.87eA	10.02±0.63eB	16.18±0.87eA	10.02±0.63eB	16.18±0.87eB	10.02±0.63eA
	1	28.02±0.95dA	12.28±0.41dB	34.39±0.73dA	13.56±0.48dB	39.38±0.95dB	14.12±0.80dA
	2	31.86±0.27cA	13.13±0.71cB	36.55±0.94cA	15.36±0.24cB	41.33±0.21cB	16.06±0.55cA
	3	33.91±0.79bA	15.79±0.60bB	39.73±0.12bA	16.62±0.41bB	43.91±0.79bB	18.32±0.56bA
	4	35.83±0.51aA	18.20±0.74aB	42.26±0.22aA	19.74±0.73aB	44.95±0.92aB	20.63±0.41aA
	5	35.38±0.89aA	18.18±0.49aB	42.76±0.64aA	19.81±0.33aB	45.02±0.49aB	20.70±0.58aA
	6	35.45±0.65aA	18.15±0.50aB	42.78±0.54aA	19.95±0.18aB	45.00±0.47aB	20.75±0.44aA
PGBR flour_2	Native	15.05±0.23eA	7.13±0.56eB	15.05±0.23eA	7.13±0.56eB	15.05±0.23eB	7.13±0.56eA
	1	27.22±0.86dA	9.57±0.48dB	30.91±0.77dA	10.65±0.40dB	37.08±1.15dB	11.47±0.82bA
	2	29.15±0.92cA	10.12±0.36cB	32.42±0.91cA	11.74±0.49cB	39.70±0.49cB	13.95±0.60cA
	3	31.11±0.61bA	13.66±0.65bB	35.52±0.47bA	14.71±0.65bB	42.41±0.96bB	15.72±0.69bA
	4	32.09±0.58aA	15.82±0.52aB	36.13±0.79aA	16.93±0.85aB	44.08±0.13aB	18.86±0.39aA
	5	32.02±0.17aA	15.37±0.73aB	36.66±0.23aA	17.08±0.49aB	44.19±0.18aB	18.97±0.11aA
	6	32.10±0.85aA	15.55±0.57aB	36.78±0.18aA	17.02±0.85aB	44.25±0.32aB	18.95±0.41aA

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Table 35 (Continued)

PGBR flour[2]	Annealed time (day)	Hardness (g)[1] after anneal at 45°C		Hardness (g) after anneal at 50°C		Hardness (g) after anneal at 50°C	
		Chai Nat1	RD6	Chai Nat1	RD6	Chai Nat1	RD6
PGBR flour_3	Native	14.25±0.60eA	3.36±0.46eB	14.25±0.60eA	3.36±0.46eB	14.25±0.60eB	3.36±0.46aA
	1	26.38±0.60dA	5.21±0.60dB	29.13±0.60dA	9.45±0.50dB	32.13±0.09dB	10.98±0.55dA
	2	28.28±0.69cA	7.77±0.50cB	31.56±0.49cA	10.63±0.63cB	34.94±0.42cB	12.20±0.62cA
	3	30.05±0.77bA	8.82±0.40bB	33.91±0.68bA	12.94±0.90bB	36.61±0.52bB	14.16±0.86bA
	4	31.17±0.84aA	9.65±0.68aB	35.29±0.82aA	13.97±0.71aB	38.77±0.55aB	15.98±0.73aA
	5	31.15±0.41aA	9.53±0.83aB	35.30±0.35aA	14.07±0.62aB	39.27±0.68aB	16.72±0.19aA
	6	31.16±0.18aA	9.56±0.48aB	35.28±0.15aA	14.02±0.70aB	39.15±0.15aB	16.85±0.44aA

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).

[2] Moisture content of all rice flour was showed in appendix.

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Hardness after annealed (45°C, 50°C and 55°C) Chai Nat1 PGBR flour_1 for 4 days (35.83, 42.26 and 44.95 g) were the highest and significantly different (*p*≤0.05) when compared to 1 day (28.02, 34.39 and 39.38 g), 2 days (31.86, 36.55 and 41.33 g), 3 days (33.91, 39.73 and 43.91 g) but no significant (p≤0.05) changed when compared to 5 days (35.38, 42.76 and 45.02 g) and 6 days (35.45, 42.78 and 45.00 g).

In both Chai Nat1 PGBR flour_2 and PGBR flour_3 also observed the highest hardness after annealing for 4 days at 45°C (32.09 and 31.17 g), 50°C (36.13 and 35.29 g) and 55°C (44.08 and 38.77 g). The hardnesses of all annealed (45°C, 50°C and 55°C for 4 days) Chai Nat1 PGBR flours_2 (32.09, 36.13 and 44.08 g) and all annealed Chai Nat1 PGBR flours_3 (31.17, 35.29 and 38.77 g) were higher than all annealed Chai Nat1 PGBR flours_1 (35.83, 42.26 and 44.95 g).

Moreover, the hardness of annealed Chai Nat1 PGBR flour samples (PGBR flour_1, PGBR flour_2 and PGBR flour_3) for 4 days at 45°C (35.83, 32.09 and 31.17 g), 50°C (42.26, 36.13 and 35.29 g) and 55°C (44.95, 44.08 and 38.77 g) were higher than before annealing process (16.18, 15.05 and 14.25 g).

Hardness after annealed (45°C, 50°C and 55°C) RD6 PGBR flours_1 for 4 days (18.20, 19.74 and 20.63 g) were the highest and significantly different (*p*≤0.05) when compared to 1 day (12.28, 13.56 and 14.12 g), 2 days (13.13, 15.36 and 16.06 g), 3 days (15.79, 16.62 and 18.32 g) but no significant (p≤0.05) changed when compared to 5 days (18.18, 19.81 and 20.70 g) and 6 days (18.15, 19.95 and 20.75 g).

In both RD6 PGBR flour_2 and PGBR flour_3 also observed the highest hardness after annealing for 4 days at 45°C (32.09 and 31.17 g), 50°C (36.13 and 35.29 g) and 55°C (44.08 and 38.77 g). The hardnesses of all annealed (45°C, 50°C and 55°C for 4 days) RD6 PGBR flours_2 (15.82, 16.93 and 18.86 g) and all annealed RD6 PGBR flours_3 (9.65, 13.97 and 15.98 g) were lower than all annealed RD6 PGBR flours_1 (18.20, 19.74 and 20.63 g)

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Moreover, the hardness of annealed RD6 PGBR flour samples (PGBR flour_1, PGBR flour_2 and PGBR flour_3) for 4 days at 45°C (18.20, 15.82 and 9.65 g), 50°C (19.74, 16.93 and 13.97 g) and 55°C (20.63, 18.86 and 15.98g) were higher than before annealing process (10.02, 7.13 and 3.36g).

The hardness of all annealed Chai Nat1 PGBR flour samples (PGBR flour_1, PGBR flour_2 and PGBR flour_3) for at 45°C (35.53, 32.09 and 31.17 g), 50°C (42.26, 36.13 and 35.29 g) and 55°C (44.95, 44.08 and 38.77 g) were significantly ($p \leq 0.05$) higher hardness than all annealed RD6 PGBR flour samples at 45°C (18.20, 15.82 and 9.65 g), 50°C (19.74, 16.93 and 13.97 g) and 55°C (20.63, 18.86 and 15.98 g).

Hormdok and Noomhorm (2007) were reported that gel hardness of annealed rice starch (60.38 g) was increased when compared to rice starch (49.82 g) after annealing at 55°C for 24 hours. Eventhough, this experiment does not use the same annealing sample but the similar effect of hardness was observed. The enzyme activity was not completely hydrolyzed PGBR flour so that the starch molecular structure was still remaining. The annealing process modified starch molecular structure that was showed in rice flour and PGBR flour then the similar effect in hardness could happen.

The starch gel was formed by the retrograde of leached amylose and rigid swollen granule during cooling. It could state that gel properties are related to the swelling and solubility property. The statement might expected that when solubility decreased the gel would became softer because small among of leached molecule to forms continuous gel matrix. However, the results was opposite to the statement above and could be due to the rigidity of swollen starch granules was the major factor to produces gel hardness as reported from Hormdok and Noomhorm (2007).

In summary, annealing process could change starch molecular structure by rearrangement between starch molecular chains cause high amylose content, while swelling power, solubility, carbohydrate leaching, peak viscosity and

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syneresis were low that showed high starch molecular structure stability to heating and cooling process. The improve starch molecular structure could provide high hardness after annealing process. Both annealed (Chai Nat1 and RD6) PGBR flour_1 showed higher hardness when compared to PGBR flour_2 and PGBR flour_3. After annealing at 45°C, 50°C and 55°C for 4 days, both Chai Nat1 and RD6 PGBR flour_1, showed the highest hardness.

The annealed both Chai Nat1 and RD6 PGBR flour_1 at 45°C, 50°C and 55°C for 4 days were the suitable conditions to made starch molecular structure stable to heat and cooling system.

The high amylose content, low swelling power and low solubility could give the low cooking loss and high tensile strength of rice noodle (Bhattacharya *et al.,* 1999; Fari *et al.,* 2011; Han *et al.,* 2011). The low peak viscosity rice flour could make high hardness rice noodle (Bhattacharya *et al.*, 1999). However, if the peak viscosity was to low the viscosity will not be enough to prepare rice noodle (Gullapanayutt, 2004). The low syneresis could give high stability of frozen rice noodle during freeze-thaw cycle (Intarasiri, 2007).

3. Double and multiple steps annealing process.

The multiple steps annealing process could produce more stable starch molecular structure to heating process more than one step annealing process (Shi, 2008). Both Chai Nat1 and RD6 PGBR flour_1 were subjected to double and multiple steps annealing process to find the suitable annealing process for making frozen rice noodle.

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3.1 Chemical property of rice samples

3.1.1 Amylose content

The amylose content of both Chai Nat1 and RD6 PGBR flour_1 after one step annealing treatments at 45°C, 50°C and 55°C for 4 days, double steps annealing process at 45°C for 1 day follow by 50°C for 1 days, multiple step annealing process at 45°C for 1 day follow by 50°C for 1 days and 55°C for 1 days, white rice flour and brown rice flour was shown in Table 36.

Table 36 Comparison amylose content between three annealing processes to white rice flour, brown rice flour and PGBR flour_1.

Rice flour samples[2]	Amylose content (%)[1]	
	Chai Nat 1	RD6
White rice flour	27.46±0.01eA	5.64±0.13dB
Brown rice flour	27.29±0.06eA	5.59±0.01cB
PGBR flour_1	25.92±0.10eA	5.24± 0.05dB
One step annealed		
45°C	28.28±0.86dA	5.92±0.10cB
50°C	29.03±0.54cA	6.28±0.20bB
55°C	30.79±0.43cA	6.42±0.10bB
Double steps annealed	30.85±0.12bA	6.52±0.41bB
Multiple steps annealed	31.23±0.32aA	6.78±0.32aB

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).

[2] Moisture of all rice flour samples were showed in appendix.

The amylose content of both multiple steps annealed (Chai Nat1 and RD6) PGBR flour_1 (31.23 and 6.78 %) were significantly ($p \leq 0.05$) increased when compared to double steps annealing (30.85 and 6.52 %), one step annealing at 55°C

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(30.79 and 6.42 %), one step annealing at 50°C (29.03 and 6.28 %), one step annealing at 45°C (28.28 and 5.92 %), PGBR flour_1 (25.92 and 5.24 %), white rice flour (27.46 and 5.64 %) and brown rice flour (27.29 and 5.59 %).

All Chai Nat1 flour samples (white rice flour, brown rice flour, one step annealed PGBR flour_1, double steps annealed PGBR flour_1 and multiple steps annealed PGBR flour_1) showed higher amylose content (27.46-31.23 %) and significantly different ($p \leq 0.05$) than RD6 rice flour samples (5.64-6.78 %).

The result was similar to Nakazawa and Wang (2003) were reported that multiple steps annealed acid hydrolyzed corn starch showed higher amylose content (4.7 %) when compared to acid hydrolyzed corn starch (4.4 %). Although the annealed sample was not the same but both acid and enzyme hydrolysis also hydrolyzed A-type starch molecular structure then the similar property after multiple steps annealed could be observed.

Iodine-based determination is based on the ability of the amylose helix to interact with pentaiodide. The increased in apparent amylose content on annealing process could be associated with an increase in the number of helical turns as a result of ability to form more ordered helix, thereby increasing the color intensity of the amylose-iodine complex as reported from Lin *et al.* (2009).

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In summary, multiple steps annealing process used three combination temperatures to improve starch molecular structure. This was advantage factor to induce more movement between starch molecular chains compared to double steps (two annealing temperature) and one step (one annealing temperature) the rearrangement could create longer starch molecular chains result in higher amylose content.

3.2 Physicochemical properties of rice samples

3.2.1 Swelling power

The swelling powers of both Chai Nat1 and RD6 PGBR flour_1 after one step annealing treatments at 45°C, 50°C and 55°C for 4 days, double steps annealing process at 45°C for 1 day follow by 50°C for 1 days, multiple steps annealing process at 45°C for 1 day follow by 50°C for 1 days and 55°C for 1 days, white rice flour and brown rice flour was showed in Table 37.

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Table 37 Comparison swelling power between three annealing processes to white rice flour, brown rice flour and PGBR flour_1.

Rice flour samples[2]	Swelling power (g/g)[1]							
	65°C		75°C		85°C		95°C	
	Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6
White rice flour	2.11±0.05bB	2.32±0.06bA	3.20±0.08bB	7.24±0.13bA	7.63±0.04bB	9.71±0.22bA	9.36±0.17bB	14.59±0.28bA
Brown rice flour	2.10±0.05bB	2.28±0.07bA	3.15±0.04bB	7.12±0.10bA	7.50±0.11bB	9.57±0.34bA	9.27±0.11bB	14.56±0.17bA
PGBR flour_1	3.34±0.06aB	4.55±0.10aA	4.57±0.08aB	8.23±0.07aA	9.96±0.03aB	10.77±0.07aA	11.50±0.01aB	15.67±0.14aA
One step annealed								
45°C	1.29±0.17cB	1.43±0.09cA	1.93±0.05cB	3.49±0.16cA	5.57±0.13cB	7.54±0.09cA	8.79±0.07cB	9.68±0.13cA
50°C	1.13±0.06dB	1.20±0.21dA	1.66±0.03dB	2.55±0.04dA	4.37±0.26dB	6.12±0.06dA	8.57±0.21dB	9.26±0.05dA
55°C	0.88±0.10eB	0.94±0.07eA	1.57±0.22dB	2.16±0.08eA	3.15±0.07eB	5.62±0.11eA	8.31±0.16dB	9.23±0.14dA
Double steps annealed	0.89±0.17eB	0.92±0.11eA	1.42±0.15dB	2.00±0.15eA	3.02±0.22eB	4.95±0.19fA	8.01±0.11eB	8.84±0.19eA
Multiple steps annealed	0.88±0.15eB	0.91±0.23eA	1.38±0.28dB	1.75±0.17eA	2.17±0.17fB	4.02±0.28gA	6.55±0.72fB	8.02±0.25fA

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).

[2] Moisture content of all rice flour samples were showed in appendix.

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The swelling powers (95°C) of both multiple steps annealed (Chai Nat1 and RD6) PGBR flours_1 (7.55 and 8.02 g/g) were significantly ($p \leq 0.05$) decreased when compared to double steps annealing (8.01 and 8.84 g/g), one step annealing at 55°C (8.31 and 9.23 g/g), one step annealing at 50°C (8.57 and 9.26 g/g), one step annealing at 45°C (8.79 and 9.68 g/g), PGBR flour_1 (11.50 and 15.67 g/g), white rice flour (9.36 and 14.59 g/g) and brown rice flour (9.27 and 14.56 g/g).

All Chai Nat1 flour samples (white rice flour, brown rice flour, one step annealed PGBR flour_1, double steps annealed PGBR flour_1 and multiple steps annealed PGBR flour_1) showed lower swelling power and significantly different ($p \leq 0.05$) during 65°C (0.88-2.11 g/g), 75°C (3.20-1.38 g/g), 85°C (7.63-2.17 g/g) and 95°C (9.36-7.55 g/g) than RD6 rice flour samples at 65°C (2.32-0.91 g/g), 75°C (7.24-1.75 g/g), 85°C (9.71-4.52 g/g) and 95°C (14.59-8.02 g/g).

Siriboon (2007) was reported that after annealed broken rice at 7°C below gelatinization temperature the swelling power was lower than annealed broken rice at 30°C below gelatinization temperature. Dias *et al*. (2010) were reported that after annealed rice starch at 55°C for 24 hours the swelling power was lower than annealing at 50°C for 24 hours and annealing 45°C for 24 hours. Hydrothermal treatment, at high temperature could have the energy to reorganize starch molecular structure better than low annealing temperature in one step annealing process. The enzyme activity was not completely hydrolyzed structure of PGBR flour, the starch molecular structure was still remaining. The annealing process modified starch molecular structure that was showed in broken rice, rice starch and PGBR flour then the similar effect in swelling power could happen.

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Double and multiple steps annealing processes were an effective treatment to make crystallites well packed and difficult to destruction by thermal energy or shear force as reported from Shi (2008). The rigid annealed rice starch molecular structures by crystalline perfection were difficult to hydration resulting in lower swelling power. The double and multiple steps annealing processes have combination annealing temperatures, which could have more efficiently to reorganize starch molecule when compared to one temperature.

In summary, multiple steps annealing process used three combination temperatures to improve starch molecular structure. This was advantage factor to induce more movement between starch molecular chains compared to double steps (two annealing temperature) and one step (one annealing temperature) the rearrangement could create rigid starch molecular structure cause high amylose content and result in low swelling power.

3.2.2 Solubility

The solubility of both Chai Nat1 and RD6 PGBR flour_1 after one step annealing treatments at 45°C, 50°C and 55°C for 4 days, double steps annealing process at 45°C for 1 day follow by 50°C for 1 days, multiple steps annealing process at 45°C for 1 day follow by 50°C for 1 days and 55°C for 1 days, white rice flour and brown rice flour was shown in Table 38.

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Table 38 Comparison solubility between three annealing processes to white rice flour, brown rice flour and PGBR flour_1.

Rice flour samples[2]	Solubility (%)[1]							
	65°C		75°C		85°C		95°C	
	Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6
White rice flour	5.18±0.05eB	8.50±0.06eA	7.31±0.04gB	9.64±0.13gA	8.49±0.04gB	18.57±0.36gA	12.39±0.15gB	31.60±0.32g
Brown rice flour	5.13±0.05eB	8.25±0.07eA	6.85±0.08hB	9.51±0.11gA	8.15±0.41gB	18.47±0.14gA	11.75±0.11hB	30.49±0.28h
PGBR flour_1	18.48±0.18aB	20.42±0.11aA	19.51±0.19aB	25.27±0.21aA	21.51±0.22aB	40.27±0.14aA	23.18±0.25aB	57.31±0.28a
One step annealed								
45°C	14.05±0.04bB	14.87±0.12bA	16.59±0.20bB	18.58±0.08bA	17.58±0.09bB	32.21±0.07bA	19.47±0.11bB	40.18±0.10b
50°C	11.13±0.07cB	13.08±0.12cA	15.20±0.12cB	16.57±0.26cA	16.05±0.22cB	24.65±0.19cA	17.20±0.08cB	39.69±0.29c
55°C	10.12±0.07dB	12.11±0.26dA	13.68±0.25dB	14.42±0.12dA	15.27±0.18dB	22.63±0.20dA	16.09±0.08dB	35.72±0.27d
Double steps annealed	10.05±0.67dB	12.07±0.06dA	12.75±0.14eB	13.52±0.04eA	14.77±0.21eB	21.23±0.14eA	15.74±0.18eB	34.79±0.17e
Multiple steps annealed	9.78±0.22dB	12.00±0.31dA	11.82±0.13fB	12.87±0.12fA	13.80±0.16fB	20.78±0.19fA	14.82±0.11fB	33.15±0.24f

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).

[2] Moisture content of all rice flour samples were showed in appendix.

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The solubilities (95°C) of both multiple steps annealed (Chai Nat1 and RD6) PGBR flours_1 (14.82 and 33.15 %) were significantly ($p \leq 0.05$) decreased when compared to double steps annealing (15.74 and 34.79 %), one step annealing at 55°C (16.09 and 35.72 %), one step annealing at 50°C (17.20 and 39.69 %), one step annealing at 45°C (19.47 and 40.18 %), PGBR flour_1 (23.18 and 57.31 %). However, the solubilities (95°C) of both multiple steps annealed (Chai Nat1 and RD6) PGBR flour_1 (14.82 and 33.15 %) was significantly ($p \leq 0.05$) higher than white rice flour (12.39 and 31.60 %) and brown rice flour (11.75 and 30.49 %).

All Chai Nat1 flour samples (white rice flour, brown rice flour, one step annealed PGBR flour_1, double steps annealed PGBR flour_1 and multiple steps annealed PGBR flour_1) showed lower solubility and significantly different ($p \leq 0.05$) during 65°C (5.18-9.78 %), 75°C (7.31-11.82 %), 85°C (8.49-13.80 %) and 95°C (12.39-14.82 %) than RD6 rice flour samples at 65°C (8.50-12.00 %), 75°C (9.64-12.87 %), 85°C (18.57-20.78 %) and 95°C (31.60-33.15%).

Similar finding from Siriboon (2007) was reported that after annealing broken rice at 7°C and 30°C below gelatinization temperature for 24 and 72 hours the solubility of annealed broken rice was decrease when annealing temperature increased from 30°C to 7°C below gelatinization temperature. Dias *et al.* (2010) were reported that annealed rice starch at 55°C showed the lowest solubility when compared to annealing at 45°C and 50°C for 24 hours. The annealing process modified starch molecular structure that was the main structure in broken rice, rice starch and PGBR flour then the similar effect in solubility could happen. Hydrothermal treatment, at high temperature could have the energy to reorganize starch molecular structure more than low annealing temperature as reported from Dias *et al.* (2010). The double and multiple steps annealing have combination annealing temperatures, that could have the efficiently to reorganize starch molecule when compared to one temperature.

In addition, after annealed both Chai Nat1 and RD6 PGBR flour_1 the solubility was higher than those of white rice and brown rice flour. Eventhough the

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swelling power of annealed PGBR flour_1 was lower than white rice flour and brown rice flour, but higher solubility could be possible. The starch molecular chain could interact and reorganize after annealing process. However, small molecular compound such as reducing sugar could be leaching out through the system result in higher solubility when compared to white rice flour and brown rice flour.

In summary, multiple steps annealing process used three combination temperatures to improve starch molecular structure. This was advantage factor to induce more movement between starch molecular chains compared to double steps (two annealing temperature) and one step (one annealing temperature) the rearrangement could create rigid molecular structure cause high amylose content low swelling power result in low solubility.

3.2.3 Carbohydrate leaching

The carbohydrate leaching of both Chai Nat1 and RD6 PGBR flour_1 after one step annealing treatments at 45°C, 50°C and 55°C for 4 days, double steps annealing process at 45°C for 1 day follow by 50°C for 1 days, multiple steps annealing process at 45°C for 1 day follow by 50°C for 1 days and 55°C for 1 days, white rice flour and brown rice flour was shown in Table 39.

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Table 39 Comparison carbohydrate leaching between three annealing processes to white rice flour, brown rice flour and PGBR flour_1.

Rice flour sample[2]	Carbohydrate leaching (µg/ml)[1]							
	65°C		75°C		85°C		95°C	
	Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6
White rice flour	5.37±0.08gB	8.40±0.24fA	6.47±0.14gB	10.54±0.06gA	11.55±0.18gB	13.58±0.09aB	13.55±0.20gB	14.46±0.06gA
Brown rice flour	4.27±0.09hB	7.50±0.12gA	6.17±0.24gB	9.57±0.28hA	10.55±0.25gB	12.12±0.77aB	12.64±0.33gB	13.14±0.23gA
PGBR flour_1	12.27±0.07aB	20.55±0.26aA	25.38±0.23aB	30.61±0.27aA	41.11±0.27aB	45.79±0.29gB	55.51±0.15aB	68.76±0.28aA
One step annealed								
45°C	8.47±0.29bB	15.19±0.28bA	17.25±0.25bB	23.62±0.34bA	32.39±0.36bB	36.56±0.20fB	45.26±0.22bB	58.70±0.38bA
50°C	7.30±0.12cB	14.46±0.36cA	16.40±0.28cB	22.60±0.29cA	30.74±0.16cB	36.44±0.27eB	40.35±0.34cB	47.37±0.23cA
55°C	7.20±0.22dB	14.43±0.33cA	15.33±0.12dB	20.40±0.23dA	29.57±0.10dB	30.35±0.23dB	35.28±0.22dB	45.47±0.31dA
Double steps annealed	6.78±0.16eB	13.18±0.12dA	14.72±0.22eB	18.98±0.27eA	25.36±0.16eB	27.73±0.21cB	32.18±0.11eB	43.34±0.16eA
Multiple steps annealed	5.41±0.14fB	12.42±0.41eA	12.48±0.16fB	15.21±0.13fA	23.41±0.19fB	26.83±0.11bB	30.12±0.18fB	40.87±0.27fA

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).

[2] Moisture content of all rice flour samples were showed in appendix.

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The carbohydrate leaching (95°C) of both multiple steps annealed (Chai Nat1 and RD6) PGBR flours_1 (30.12 and 40.87 µg/ml) were significantly ($p \leq 0.05$) decreased when compared to double steps annealing (32.18 and 43.34 µg/ml), one step annealing at 55°C (35.28 and 45.47 µg/ml), one step annealing at 50°C (40.35 and 47.37 µg/ml), one step annealing at 45°C (45.26 and 58.70 µg/ml), PGBR flour_1 (55.51 and 68.76 µg/ml). However, the carbohydrate leaching (95°C) of both multiple steps annealed (Chai Nat1 and RD6) PGBR flour_1 (30.12 and 40.87 µg/ml) were significantly ($p \leq 0.05$) higher than white rice flour (13.55 and 14.46 µg/ml) and brown rice flour (12.64 and 13.14 µg/ml).

All Chai Nat1 flour samples (white rice flour, brown rice flour, one step annealed PGBR flour_1, double steps annealed PGBR flour_1 and multiple steps annealed PGBR flour_1) showed lower carbohydrate leaching and significantly different ($p \leq 0.05$) during 65°C (5.37-5.41 µg/ml), 75°C (6.47-12.48 µg/ml), 85°C (11.55-23.41 µg/ml) and 95°C (13.55-30.12 µg/ml) than RD6 rice flour samples at 65°C (8.40-12.42 µg/ml), 75°C (10.54-15.21 µg/ml), 85°C (13.58-26.83 µg/ml) and 95°C (14.46-40.87 µg/ml).

Siriboon (2007) was reported that annealing temperature, that close to gelatinization temperature showed lower amylose leaching when compared to annealing temperature that far from gelatinization temperature in annealed broken rice. The enzyme activity was not completely hydrolyzed structure of PGBR flour, the starch molecular structure was still remaining. The annealing process modified starch molecular structure that was showed in broken rice and PGBR flour then the similar effect in leaching property could happen.

The study in carbohydrate leaching could give a clearly understand to the effect of annealing process. The reduction in carbohydrate leaching could assume that the interaction between starch molecular chains really happen in both annealed Chai Nat1 and RD6 PGBR flour_1. The decreased carbohydrate leaching in this research might attribute to interaction between molecular chain and decrease swelling and solubility.

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Double and multiple steps annealing processes were an effective treatment to make crystallites well packed and more re-associate between starch molecular chains as reported from Shi (2008). The different between one, double and multiple steps annealing processes were mainly annealing temperature. Multiple steps annealing process used three combination temperatures and might gave better reorganization.

In summary, multiple steps annealing process used three combination temperatures to improve starch molecular structure. This was advantage factor to induce more movement between starch molecular chains compared to double steps (two annealing temperature) and one step (one annealing temperature) the rearrangement could create rigid molecular structure cause high amylose content low swelling power and low solubility result in low carbohydrate leaching.

3.2.4 Peak viscosity

The peak viscosity of both Chai Nat1 and RD6 PGBR flour_1 after one step annealing treatments at 45°C, 50°C and 55°C for 4 days, double steps annealing process at 45°C for 1 day follow by 50°C for 1 days, multiple steps annealing process at 45°C for 1 day follow by 50°C for 1 days and 55°C for 1 days, white rice flour and brown rice flour was shown in Table 40.

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Table 40 Comparison peak viscosity between three annealing processes to white rice flour, brown rice flour and PGBR flour_1.

Rice flour samples[2]	Peak viscosity[1] (cP)	
	Chai Nat 1	RD6
White rice flour	2,310.00±14.12aB	2,579.00±11.58aA
Brown rice flour	2,307.83±19.10aB	2,536.17±13.34bA
PGBR flour_1	1,643.33±28.31bA	477.50±14.89gB
One step annealed		
45°C	885.83±16.02cA	1,219.00±25.93cB
50°C	839.00±10.69dA	1,066.00±24.04dB
55°C	657.17±18.97eA	950.80±19.76eB
Double steps annealed	604.25±18.94fA	901.74±17.85eB
Multiple steps annealed	511.11±15.11gA	826.63±19.66fB

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).
[2] Moisture content of all rice flour samples were showed in appendix.

The peak viscosity of multiple steps annealed Chai Nat1 PGBR flour_1 (511.11 cP) was significantly ($p \leq 0.05$) decreased when compared to Chai Nat1 PGBR flour_1 (1,643.33 cP), Chai Nat1 white rice flour (2,310.00 cP) and Chai Nat1 brown rice flour (2,307.83 cP). The peak viscosity of multiple steps annealed RD6 PGBR flour_1 (826.63 cP) was significantly ($p \leq 0.05$) decreased when compared to RD6 white rice flour (2,579.00 cP) and RD6 brown rice flour (2,536.17 cP). However, the peak viscosity of multiple steps annealed RD6 PGBR flour (826.63 cP) was significantly ($p \leq 0.05$) higher than RD6 PGBR flour_1 (477.50 cP).

The peak viscosities of both multiple steps annealed (Chai Nat1 and RD6) PGBR flours_1 (511.11 and 826.63 cP) were significantly ($p \leq 0.05$) decreased when compared to double steps annealing (604.25 and 901.74 cP), one step annealing

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at 55°C (657.17 and 950.80 cP), one step annealing at 50°C (839.00 and 1,066.00 cP), one step annealing at 45°C (885.83 and 1,219.00 cP).

Dias *et al.* (2010) were reported that annealing at 55°C caused a reduction in peak viscosity of rice starch than at 50°C and 45°C in. The enzyme activity was not completely hydrolyzed structure of PGBR flour, the starch molecular structure was still remaining. The annealing process modified starch molecular structure that was the main structure in rice starch and PGBR flour then the similar effect in peak viscosity could happen. The decreased peak viscosity on annealing could be attributed to reduction of swelling power, solubility and carbohydrate leaching by strengthening of starch molecular structure then resisted to heat and shear process as reported from Siriboon (2007).

Annealed RD6 PGBR flour_1 showed higher peak viscosity when compared to RD6 PGBR flour_1. The increased viscosity can be a described to more resistance of the starch molecular structure to heat and deformation by an enforcement of intra-granular binding forces rather than interaction between leached molecules because the carbohydrate leaching was clearly reduced after annealing process. The rigidity and resistance starch molecular structure lead to a higher peak viscosity during heating as reported from Jacobs *et al*. (1995). In addition, multiple steps annealed RD6 PGBR flour_1 showed lower peak viscosity cause by low swelling power of multiple steps annealed RD6 PGBR flour_1 could reduce array of swollen granules then leading to less peak viscosity when compared to double and one step annealing processes.

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Double and multiple steps annealing process were an effective treatment to make crystallites well packed and difficult to destruction by thermal energy or shear force as reported from Shi (2008). This explanation might explain the extremely reduction in peak viscosity after multiple steps annealing process on both Chai Nat1 PGBR flour_1 and RD6 PGBR flour_1.

In summary, multiple steps annealing process used three combination temperatures to improve starch molecular structure. This was advantage factor to induce more movement between starch molecular chains compared to double steps (two annealing temperature) and one step (one annealing temperature) the rearrangement could create rigid molecular structure cause high amylose content low swelling power, solubility carbohydrate leaching result in low peak viscosity after apply heating and shearing system.

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3.2.5 Syneresis

The syneresis of both Chai Nat1 and RD6 PGBR flour_1 after one step annealing treatments at 45°C, 50°C and 55°C for 4 days, double steps annealing process at 45°C for 1 day follow by 50°C for 1 days, multiple steps annealing process at 45°C for 1 day follow by 50°C for 1 days and 55°C for 1 days, white rice flour and brown rice flour was present in Table 41.

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Table 41 Comparison syneresis between three annealing processes to white rice flour, brown rice flour and PGBR flour_1.

Rice flour sample[2]	Syneresis (%) [1]									
	Cycle 1		Cycle 2		Cycle 3		Cycle 4		Cycle 5	
	Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6	Chai Nat 1	RD6
White rice flour	15.70±0.46bA	7.46±0.06bB	20.13±0.45bA	9.68±0.23dB	27.01±0.22cA	11.53±0.27eB	31.33±0.52cA	15.53±0.32cB	39.70±0.33cA	17.44±0.39eB
Brown rice flour	10.72±0.33dA	6.26±0.68cB	17.28±0.39dA	8.58±0.35eB	26.06±0.61dA	10.48±0.32fB	30.48±0.21dA	12.54±0.50fA	38.35±0.40dA	16.56±0.25eB
PGBR flour_1	19.00±0.13aA	10.55±0.38aB	25.41±0.37aA	19.42±0.38aB	37.70±0.32aA	22.46±0.29aB	40.58±0.34aA	25.78±0.14aB	52.78±0.22aA	35.29±0.22aB
One step annealing										
45°C	12.56±0.38cA	6.72±0.13cB	18.15±0.59cA	14.81±0.93bB	30.52±0.41bA	15.91±0.39bB	34.96±0.64bA	18.55±1.18bB	43.87±0.43bA	28.02±1.60bB
50°C	9.18±0.94eA	5.75±0.29dB	17.06±0.75dA	12.16±0.30cB	29.12±0.40bA	14.83±1.24cB	31.37±0.68cA	15.76±1.09cB	38.82±0.77dA	28.15±0.24bB
55°C	8.86±0.45fA	4.77±0.22eB	15.08±0.56eA	9.45±0.77dB	25.98±0.49dA	12.63±0.99dB	28.84±0.80eA	14.64±1.26dB	37.82±0.94eA	25.66±1.06cB
Double steps annealing	8.28±0.28fA	4.28±0.13eB	12.11±0.11fA	8.65±0.17eB	24.79±0.24dA	11.15±0.13eB	25.18±0.41fA	13.11±0.10eB	35.41±0.22fA	19.96±0.74dB
Multiple steps annealing	6.95±0.24gA	3.95±0.24fB	9.85±0.27gA	7.18±0.28fB	20.87±0.18eA	9.98±0.07gB	23.99±0.18gA	11.67±0.05gB	30.32±0.04gA	14.31±0.16fB

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).

[2] Moisture content of all rice flour samples were showed in appendix.

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The syneresis (freeze-thaw cycle 5) of both multiple steps annealed (Chai Nat1 and RD6) PGBR flours_1 (30.32 and 14.31 %) were significantly ($p \leq$ 0.05) decreased when compared to double steps annealing (35.41 and 19.96 %), one step annealing at 55°C (37.82 and 25.66 %), one step annealing at 50°C (38.82 and 28.15 %), one step annealing at 45°C (43.87 and 28.02 %), PGBR flour_1 (52.78 and 35.29%), white rice flour (39.70 and 17.44%) and brown rice flour (38.35 and 16.56%).

In freeze-thaw cycle 5, all Chai Nat1 flour samples (white rice flour, brown rice flour, one step annealed PGBR flour_1, double steps annealed PGBR flour_1 and multiple steps annealed PGBR flour_1) showed higher syneresis (39.70-30.32 %) and significantly different ($p \leq 0.05$) than RD6 rice flour samples (17.44-14.31 %).

Intarasiri and Naivikul (2005) were reported that annealed fermented rice flour showed less syneresis (2.75-61.34 g) when compared to fermented rice flour (6.13-67.38 g) after 1-5 freeze-thaw cycles. The enzyme activity was not completely hydrolyzed structure of PGBR flour, the starch molecular structure was still remaining. The annealing process modified starch molecular structure that was showed in ferment rice flour and PGBR flour then the similar effect in syneresis could happen.

The interaction between molecular chain that induced by annealing process made the stable structure that disrupt retrogradation. The double and multiple steps annealing processes have combination annealing temperatures that could have the better efficiently to reorganize starch molecular structure (Shi, 2008).

In summary, multiple steps annealing process used three combination temperatures to improve starch molecular structure. This was advantage factor to induce more movement between starch molecular chains compared to double steps (two annealing temperature) and one step (one annealing temperature) the rearrangement could create rigid molecular structure cause high amylose content low

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swelling power, solubility, carbohydrate leaching, peak viscosity then result of low syneresis after freeze-thaw cycle.

3.3 Physical property of rice samples

3.3.1 Hardness

Hardness of both Chai Nat1 and RD6 PGBR flour_1 after one step annealing treatments at 45°C, 50°C and 55°C for 4 days, double steps annealing process at 45°C for 1 day follow by 50°C for 1 days, multiple steps annealing process at 45°C for 1 day follow by 50°C for 1 days and 55°C for 1 days, white rice flour and brown rice flour was shown in Table 42.

Table 42 Comparison hardness between three annealing processes to white rice flour, brown rice flour and PGBR flour_1.

Rice flour sample[2]	Hardness (g)[1]	
	Chai Nat 1	RD6
White rice flour	35.17±0.56fA	17.84±0.69fB
Brown rice flour	37.88±0.22eA	20.16±0.47cB
PGBR flour_1	16.18±0.87gA	10.02±0.63gB
One step annealed		
45°C	35.83±0.51fA	18.20±0.74eB
50°C	42.26±0.22dA	19.74±0.73dB
55°C	44.95±0.92cA	20.63±0.41cB
Double steps annealed	47.59±0.51bA	22.27±0.25bB
Multiple steps annealed	50.00±0.85aA	24.85±0.74aB

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different and means for each characteristics followed by the different big latter between the column are significantly different ($p \leq 0.05$).

[2] Moisture content of all rice flour samples were showed in appendix.

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The hardness of both multiple steps annealed (Chai Nat1 and RD6) PGBR flour_1 (50.00 and 24.85 g) were significantly ($p \leq 0.05$) increased when compared to double steps annealing (47.59 and 22.27 g), one step annealing at 55°C (44.95 and 20.63 g), one step annealing at 50°C (42.26 and 19.74 g), one step annealing at 45°C (35.83 and 18.20 g), PGBR flour_1 (16.18 and 10.02 g), white rice flour (35.17 and 17.84 g) and brown rice flour (37.88 and 20.16 g).

All Chai Nat1 flour samples (white rice flour, brown rice flour, one step annealed PGBR flour_1, double steps annealed PGBR flour_1 and multiple steps annealed PGBR flour_1) showed higher hardness (35.17-50.00 g) and significantly different ($p \leq 0.05$) than RD6 rice flour samples (17.84-24.85 g).

Cham and Suwannaporn (2010) were reported that the gel hardness of annealed high amylose rice starch was higher when compared high amylose rice starch after annealing at 50 and 55°C for 1-3 days. The enzyme activity was not completely hydrolyzed structure of PGBR flour, the starch molecular structure was still remaining. The annealing process modified starch molecular structure that was showed in ferment rice starch and PGBR flour then the similar effect in hardness could happen.

The starch gel is formed by the leached amylose retrograde during cooling as reported from Cham and Suwannaporn (2010). From the statement might expected that when the solubility decreased the gel would became softer because small among of leached molecule to forms the continuous gel matrix. However, the high hardness in this research might be due to swollen starch granules were very strong by interaction between molecular chains and contributed to hardness. The double and multiple steps annealing processes have combination annealing temperatures, which could have the more efficiently to reorganize starch molecular structure then produced higher gel hardness.

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In summary, multiple steps annealing process used three combination temperatures to improve starch molecular structure. This was advantage factor to induce more movement between starch molecular chains compared to double steps (two annealing temperature) and one step (one annealing temperature) the rearrangement could create rigid molecular structure cause high amylose content low swelling power, solubility, carbohydrate leaching, peak viscosity, syneresis result of high gel hardness.

Annealing process could improve the stability of PGBR flour starch molecular structure by induced the movement and realignment between starch molecular chains. Multiple steps annealing process showed more potential to reorder starch molecular chains than double steps and one step annealing processes as determined by high amylose content and hardness and low swelling power, solubility, carbohydrate leaching, peak viscosity and syneresis after freeze-thaw cycle. The changing properties after applied multiple steps annealing process to PGBR flour_1 could be used for prepared good quality frozen rice noodle.

4. Frozen rice noodle

4.1 Blended rice flour ratio

Viscosity was important factor to noodle quality. However, if the apparent viscosity of rice solution before heating process was too thick or too thin it could not create a thin and firmness gelatinized rice noodle sheet (Lorlowhakarn, 2007). The peak viscosity showed negative correlation to noodle texture and might be a good indicator to predict rice noodle texture (Bhattacharya *et al.*, 1999).

The viscosity of blended PGBR flour_1 to Chai Nat1 white rice flour was shown in Table 43.

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Table 43 Viscosity of blended PGBR flour_1 to Chai Nat1 white rice flour.

Chai Nat1white rice flour (%)	Chai Nat1 PGBR flour_1 (%)	RD6 PGBR flour_1 (%)	Apparent viscosity[1] (cP)	Peak viscosity[1] (cP)
100	-		91.12±2.20a	2,310.00±14.12a
95	5		77.12±0.28b	2,243.48±18.55b
90	10		75.53±0.75c	1,715.41±17.48c
85	15		69.45±0.14d	1,508.52±16.34d
80	20		65.87±0.61e	1,243.98±12.55e
100		-	91.12±2.20a	2,310.00±14.12a
95		5	61.95±0.67b	2,019.01±15.58b
90		10	59.37±0.38c	1,542.58±12.42c
85		15	55.42±1.24d	1,351.08±15.36b
80		20	50.39±0.47e	1,055.11±11.55c
100	-	-	91.12±2.20a	2,310.00±14.12a
90	5	5	65.87±2.55b	2,155.13±17.15b
80	10	10	62.18±0.18c	1,942.33±11.98c
70	15	15	47.95±0.48d	1,248.48±15.44d

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different ($p \leq 0.05$).

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Blended Chai Nat1 PGBR flour_1 to Chai Nat1 white rice flour from 5-20% showed apparent viscosity (77.12-65.87 cP) and peak viscosity (2,243.48-1,243.98 cP), blended RD6 PGBR flour_1 to Chai Nat1 white rice flour from 5-20% showed apparent viscosity (61.95-50.39 cP) and peak viscosity (2,019.07-1,055.11 cP), blended both Chai Nat1 and RD6 PGBR flour_1 from 5-15% to Chai Nat1 white rice flour showed apparent viscosity (65.87-47.95 cP) and peak viscosity (2,155.13-1,248.48 cP) that were all decreased when compared to apparent viscosity (91.12 cP) and peak viscosity (2,310.00 cP) of Chai Nat1 white rice flour. The decreased viscosity hen increased blended ratio was due to the weaker structure of PGBR flour_1 by α-amylase activity could not resisted shear force.

The flour that could make good quality rice noodle should showed appropriate viscosity by not too high or too low that will create very stick and very hard rice noodle, respectively. The 5% blended Chai Nat1 PGBR flour_1 to Chai Nat1 white rice flour, 5% blended RD6 PGBR flour_1 to Chai Nat1 white rice flour and 5% of both Chai Nat1 and RD6 PGBR flour_1 to Chai Nat1 white rice flour showed suitable viscosity that could make thin gelatinized rice sheet noodle.

The viscosity of blended multiple steps PGBR flour_1 to Chai Nat1 white rice flour was show in Table 44.

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Table 44 Viscosity from the blended multiple steps annealed PGBR flour_1 to Chai Nat1 white rice flour.

Chai Nat1 white rice flour (%)	Multiple steps annealed Chai Nat1 PGBR flour_1 (%)	Multiple steps annealed RD6 PGBR flour 1 (%)	Apparent viscosity[1] (cP)	Peak viscosity[1] (cP)
100	-		91.12±2.20a	2,310.00±14.12a
95	5		61.95±0.88b	1,640.30±14.32b
90	10		58.37±0.23c	1,345.17±10.88c
85	15		55.42±0.07d	950.47±18.23d
80	20		50.39±0.98e	902.33±15.81d
100		-	91.12±2.20a	2,310.00±14.12a
95		5	55.48±1.33b	1,947.11±15.68b
90		10	51.22±2.56c	1,547.78±17.22c
85		15	47.12±0.58d	1,174.52±11.69d
80		20	40.90±1.52e	998.72±15.27e
100	-	-	91.12±2.20a	2,310.00±14.12a
90	5	5	58.36±1.83b	1,753.49±15.42b
80	10	10	54.98±1.06c	973.99±18.17c
70	15	15	38.25±1.18d	875.48±13.47d

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different (*p*≤0.05).

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Blended multiple steps annealed Chai Nat1 PGBR flour_1 to Chai Nat1 white rice flour from 5-20% showed apparent viscosity (61.95-50.39 cP) and peak viscosity (1,640.30-902.33 cP), blended multiple steps annealed RD6 PGBR flour_1 to Chai Nat1 white rice flour from 5-20% showed apparent viscosity (55.48-40.90 cP) and peak viscosity (1,947.11-998.72 cP), blended both multiple steps annealed Chai Nat1 and RD6 PGBR flour_1 from 5-15% to Chai Nat1 white rice flour showed apparent viscosity (58.36-38.25 cP) and peak viscosity (1,753.49-875.48 cP) that were all decreased when compared to apparent viscosity (91.12 cP) and peak viscosity (2,310.00 cP) of Chai Nat1 white rice flour. The decreased viscosity when increased blended multiple steps annealed PGBR flour_1 ratio was due to stronger starch molecular structure that resisted to heat and shear force result in lower viscosity.

The flour that could make good quality rice noodle should showed appropriate viscosity by not too high or too low that will create very stick and very hard rice noodle, respectively. The 5% blended multiple steps annealed Chai Nat1 PGBR flour_1 to Chai Nat1 white rice flour, 5% blended multiple steps annealed RD6 PGBR flour_1 to Chai Nat1 white rice flour and 5% blended both multiple steps annealed Chai Nat1 and RD6 PGBR flour_1 to Chai Nat1 white rice flour suitable viscosity that could make thin gelatinized rice sheet noodle.

4.2 Texture properties

Texture properties of frozen rice noodle after freeze-thaw 1, 3 and 5 cycles was shown in Table 45.

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Table 45 Texture properties of frozen rice noodle.

Rice noodles	Cutting force (g)[1]			Tensile strength (g)		
	Cycle 1	Cycle 3	Cycle 5	Cycle 1	Cycle 3	Cycle 5
Chai Nat1 white rice flour	88.42±2.56d	110.35±2.67a	128.11±2.65a	25.58±1.58c	18.11±1.21d	13.59±1.33d
5% Blended Chai Nat1 PGBR flour_1	78.18±4.52f	87.48±4.71c	97.42±4.52d	17.32±2.69d	11.48±2.65f	9.42±1.06e
5% Blended RD6 PGBR flour_1	65.55±3.65g	70.92±1.27d	75.48±3.26e	12.22±1.69e	10.54±1.58f	7.52±1.20f
5% Blended both Chai Nat1and RD6 PGBR flour_1	80.01±3.66e	88.15±2.03c	100.11±1.55d	19.58±1.11d	15.42±1.58e	12.45±1.46d
5% Blended multiple steps annealed Chai Nat1 PGBR flour_1	108.93±1.45b	110.75±2.55a	118.11±1.78b	29.55±1.44b	28.12±1.65b	26.32±1.70b
5% Blended multiple steps annealed RD6 PGBR flour_1	90.18±4.52c	99.89±4.13b	108.52±5.62c	27.98±2.69bc	26.43±1.22c	24.18±1.28c
5% Blended both multiple steps annealed Chai Nat1 and RD6 PGBR flour_1	113.42±1.54a	114.15±1.89a	117.08±2.69b	34.08±2.58a	33.42±1.65a	33.11±1.68a

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different ($p \le 0.05$).

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Texture property (cutting force and tensile strength) of frozen rice noodle was higher after increased freeze-thaw cycle range from 1, 3 to 5 cycles. The cutting force at the fifth freeze-thaw cycle of rice noodle from Chai Nat 1 white rice flour (128.11 g) was the highest and significantly different ($p \leq 0.05$) when compared to rice noodle making from blended multiple step annealed Chai Nat 1 PGBR flour_1 to Chai Nat 1 white rice flour (118.11 g), rice noodle making from blended both multiple step annealed Chai Nat 1 and RD6 PGBR flour_1 to Chai Nat 1 white rice flour (117.08 g), rice noodle making from blended multiple step annealed RD6 PGBR flour_1 to Chai Nat 1 white rice flour (108.52 g), rice noodle making from blended both Chai Nat 1 and RD6 flour_1 to Chai Nat 1 white rice flour (100.11 g), rice noodle making from blended Chai Nat 1 PGBR flour_1 to Chai Nat 1 white rice flour (97.42 g), rice noodle making from blended RD6 PGBR flour_1 to Chai Nat 1 white rice flour (75.48 g).

The tensile strength at the fifth freeze-thaw cycle of rice noodle from blended both multiple steps annealed Chai Nat 1 and RD6 PGBR flour_1 to Chai Nat1 white rice flour (33.11 g), rice noodle making from blended multiple steps annealed Chai Nat 1 PGBR flour_1 to Chai Nat1 white rice flour (26.32 g), rice noodle from blended multiple steps annealed RD6 PGBR flour_1 to Chai Nat1 white rice flour (24.18 g), rice noodle from Chai Nat 1 white rice flour (13.59 g), rice noodle from blended both Chai Nat 1 and RD6 PGBR flour_1 to Chai Nat1 white rice flour (12.45 g), rice noodle from blended Chai Nat 1 PGBR flour to Chai Nat1 white rice flour (9.42 g), rice noodle from blended RD6 PGBR flour_1 to Chai Nat1 white rice flour (7.52 g).

Intarasiri (2007) was reported that of Kanomjeen making from blended annealed fermented rice flour showed a little change in texture properties after freeze-thaw than Kanomjeen making from fermented rice flour.

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In summary, multiple steps annealed Chai Nat1 PGBR flour_1 showed high gel hardness and low swelling power while multiple steps annealed RD6 PGBR flour_1 showed low syneresis. The results could implied that rice noodle structure making from blended both multiple steps annealed Chai Nat1 and RD6 PGBR flour_1 to Chai Nat1 white rice flour was stable and not change by re-associate in a great extent after freeze-thaw cycle when compared to rice noodle making from Chai Nat1 white rice flour.

4.3 Sensory evaluation

The sensory attributed of seven frozen rice noodle after freeze-thaw 5 cycles were established and evaluated by 10 trained panelists. Perceived intensities were scored on a 15 cm interval scale using qualitative data analysis (QDA) was presented in Table 46.

Table 46 Sensory evaluation of frozen rice noodle.

Rice noodles	QDA[1] (15 cm scale)		
	Firmness	Elastic	Adhesiveness
Chai Nat1 white rice flour	9.65±0.85b	8.24±0.85c	5.03±0.92c
5% Blended Chai Nat1 PGBR flour_1	6.52±1.52d	5.98±0.41e	10.74±0.85a
5% Blended RD6 PGBR flour_1	5.55±0.63e	4.21±0.22f	10.85±0.96a
5% Blended both Chai Nat1and RD6 PGBR flour_1	6.87±0.47d	7.02±0.63d	9.41±1.45b
5% Blended multiple steps annealed Chai Nat1 PGBR flour_1	8.77±0.23c	8.16±1.05c	5.21±0.81c
5% Blended multiple steps annealed RD6 PGBR flour_1	9.87±0.87b	8.91±0.87b	4.89±0.45d
5% Blended both multiple steps annealed Chai Nat1 and RD6 PGBR flour_1	11.45±1.26a	9.85±0.23a	4.46±0.23e

[1] Results are expressed as an average of sensory evaluated by 10 trained panelists. Means for each characteristics followed by the different small latter within the same column are significantly different ($p \leq 0.05$).

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The good sensory evaluation of rice noodle should showed high firmness and elasticity while adhesiveness should be low. The result from QDA determination (firmness, elasticity and adhesiveness) of frozen rice noodle making from blended both multiple steps annealed Chai Nat1 and RD6 PGBR flour_1 to Chai Nat1 white rice flour (11.45, 9.85 and 4.46) showed higher sensory evaluation when compared to frozen rice noodle making from blended multiple steps annealed RD6 PGBR flour_1 to Chai Nat1 white rice flour (9.87, 8.91 and 4.89), frozen rice noodle making from Chai Nat1 white rice flour (9.65, 8.24 and 5.03), frozen rice noodle making from blended multiple steps annealed Chai Nat1 PGBR flour_1 to Chai Nat1 white rice flour (8.77, 8.16 and 5.21), frozen rice noodle making from blended both Chai Nat1 and RD6 PGBR flour_1 to Chai Nat1 white rice flour (6.87, 7.02 and 9.41), frozen rice noodle making from blended Chai Nat1 PGBR flour_1 to Chai Nat1 white rice flour (6.52, 5.98 and 10.74) and frozen rice noodle making from blended RD6 PGBR flour_1 to Chai Nat1 white rice flour (5.55, 4.21 and 10.74).

In summary, multiple steps annealed Chai Nat1 PGBR flour_1 showed high gel hardness and low swelling power while multiple steps annealed RD6 PGBR flour_1 showed low syneresis, which improve texture properties and sensory evaluation by high firmness and medium elasticity while adhesiveness was low in frozen rice noodle making from both multiple steps annealed Chai Nat1 and RD6 PGBR flour_1 to Chai Nat1 white rice flour when compared to rice noodle from Chai Nat1 white rice flour.

The overall results could be conclude that the suitable pre-germination condition could change starch molecular structure by α-amylase activity that hydrolyzed starch into a small starch molecule and change physicochemical by increased swelling power, solubility, carbohydrate leaching and syneresis while peak viscosity decreased both Chai Nat1 and RD6 rice varieties. Annealing process could induce the movement between starch molecular changes and create strong starch molecular structure. After subject three stage of PGBR flour into annealing process found that chemical, physicochemical and physical properties were improve by increase amylose content and gel hardness while swelling power, solubility,

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carbohydrate leaching, peak viscosity and syseresis were decreased both Chai Nat1 and RD6 rice varieties. Moreover multiple steps annealing process could rearrange starch molecular changes and produce more stable starch molecular structure to heat and cool process than double and one step annealing processes in both Chai Nat1 and RD6 rice varieties. Especially in multiple steps annealed Chai Nat 1 PGBR flour 1 that showed higher hardness and RD6 multiple steps annealed PGBR flour_1 than white rice flour and brown rice flour, these properties could be used to prepare frozen rice noodle. Frozen noodle quality could be improved by using both pre-germinated and annealing process to modified starch molecular structure. Frozen rice making from blended both Chai Nat 1 and RD6 multiple step annealed PGBR flour_1 to Chai Nat 1 white rice flour showed to decreased changing in texture properties and improve sensory evaluation compared to rice noodle from Chai Nat 1 white rice flour after freeze-thaw cycle.

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CONCLUSIONS AND RECOMMENDATIONS

Conclusions

This research was aimed to improve properties of frozen rice noodle which could be done by making rice noodle from blended both multiple steps annealed Chai Nat1 and RD6 PGBR flour_1 to Chai Nat1 white rice flour (5:5:90).

1. To find the ratio for blended both annealed Chai Nat1 and RD6 PGBR flour_1 to Chai Nat1 white rice flour for making frozen rice noodle.

The frozen rice noodle making from blended multiple steps annealed both Chai Nat1 and RD6 PGBR flour_1 to Chai Nat1 white rice flour could improve texture properties by increased tensile strength after the fifth freeze-thaw cycle (33.11 g) and significantly different ($p \leq 0.05$) when compared to frozen rice noodle making from Chai Nat1 white rice flour (13.59g).

Moreover, frozen rice noodle making from blended both multiple steps annealed Chai Nat1 and RD6 PGBR flour_1 to Chai Nat1 white rice flour was improve sensory evaluation by qualitative data analysis (QDA) which concerned high firmness (11.45), medium elasticity (9.85) and low adhesiveness (4.46) when compared to medium firmness (9.65), medium elasticity (8.24) and medium adhesiveness (5.03) of frozen rice noodle from Chai Nat1 white rice flour.

2. To find the condition for annealing process both Chai Nat1 and RD6 PGBR flour.

The amylose content of both multiple steps annealed (Chai Nat1 and RD6) PGBR flour_1 (31.23 and 6.78 %) were significantly ($p \leq 0.05$) increased when compared to double steps annealing (30.85 and 6.52 %), one step annealing at 55°C (30.79 and 6.42 %), one step annealing at 50°C (29.03 and 6.28 %), one step

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annealing at 45°C (28.28 and 5.92 %), PGBR flour_1 (25.92 and 5.24 %), white rice flour (27.46 and 5.64 %) and brown rice flour (27.29 and 5.59 %).

The swelling powers (95°C) of both multiple steps annealed (Chai Nat1 and RD6) PGBR flours_1 (7.55 and 8.02 g/g) were significantly ($p \leq 0.05$) decreased when compared to double steps annealing (8.01 and 8.84 g/g), one step annealing at 55°C (8.31 and 9.23 g/g), one step annealing at 50°C (8.57 and 9.26 g/g), one step annealing at 45°C (8.79 and 9.68 g/g), PGBR flour_1 (11.50 and 15.67 g/g), white rice flour (9.36 and 14.59 g/g) and brown rice flour (9.27 and 14.56 g/g).

The solubility (95°C) of both multiple steps annealed (Chai Nat1 and RD6) PGBR flours_1 (14.82 and 33.15 %) were significantly ($p \leq 0.05$) decreased when compared to double steps annealing (15.74 and 34.79 %), one step annealing at 55°C (16.09 and 35.72 %), one step annealing at 50°C (17.20 and 39.69 %), one step annealing at 45°C (19.47 and 40.18 %), PGBR flour_1 (23.18 and 57.31 %). However, the solubility (95°C) of both multiple steps annealed (Chai Nat1 and RD6) PGBR flour_1 (14.82 and 33.15 %) was significantly ($p \leq 0.05$) higher than white rice flour (12.39 and 31.60 %) and brown rice flour (11.75 and 30.49 %).

The carbohydrate leaching (95°C) of both multiple steps annealed (Chai Nat1 and RD6) PGBR flours_1 (30.12 and 40.87 µg/ml) were significantly ($p \leq 0.05$) decreased when compared to double steps annealing (32.18 and 43.34 µg/ml), one step annealing at 55°C (35.28 and 45.47 µg/ml), one step annealing at 50°C (40.35 and 47.37 µg/ml), one step annealing at 45°C (45.26 and 58.70 µg/ml), PGBR flour_1 (55.51 and 68.76 µg/ml). However, the carbohydrate leaching (95°C) of both multiple steps annealed (Chai Nat1 and RD6) PGBR flour_1 (30.12 and 40.87 µg/ml) were significantly ($p \leq 0.05$) higher than white rice flour (13.55 and 14.46 µg/ml) and brown rice flour (12.64 and 13.14 µg/ml).

The peak viscosities of both multiple steps annealed (Chai Nat1 and RD6) PGBR flours_1 (511.11 and 826.63 cP) were significantly ($p \leq 0.05$) decreased when compared to double steps annealing (604.25 and 901.74 cP), one step annealing at

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55°C (657.17 and 950.80 cP), one step annealing at 50°C (839.00 and 1,066.00 cP), one step annealing at 45°C (885.83 and 1,219.00 cP).

The syneresis (freeze-thaw cycle 5) of both multiple steps annealed (Chai Nat1 and RD6) PGBR flours_1 (30.32 and 14.31 %) were significantly ($p \leq 0.05$) decreased when compared to double steps annealing (35.41 and 19.96 %), one step annealing at 55°C (37.82 and 25.66 %), one step annealing at 50°C (38.82 and 28.15 %), one step annealing at 45°C (43.87 and 28.02 %), PGBR flour_1 (52.78 and 35.29%), white rice flour (39.70 and 17.44%) and brown rice flour (38.35 and 16.56%).

The hardness of both multiple steps annealed (Chai Nat1 and RD6) PGBR flour_1 (50.00 and 24.85 g) were significantly ($p \leq 0.05$) increased when compared to double steps annealing (47.59 and 22.27 g), one step annealing at 55°C (44.95 and 20.63 g), one step annealing at 50°C (42.26 and 19.74 g), one step annealing at 45°C (35.83 and 18.20 g), PGBR flour_1 (16.18 and 10.02 g), white rice flour (35.17 and 17.84 g) and brown rice flour (37.88 and 20.16 g).

The multiple steps annealing process was the best condition for rearrange starch molecule of PGBR flour_1 when compared to double and one step annealed processes. Both multiple steps annealed Chai Nat1 and RD6 PGBR flour_1 showed higher stability to heating process as determined by swelling power, solubility, carbohydrate leaching and peak viscosity. Besides, both the multiple steps annealed Chai Nat1 and RD6 PGBR flour_1 showed higher freezing stability as determined by syneresis after freeze-thaw.

These properties could be useful for preparing frozen rice noodle. Mixing both multiple steps annealed Chai Nat1 and RD6 PGBR flour_1 to Chai Nat1 white rice flour for making rice noodle could enhance the stability of rice noodle to frozen processing.

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However, the starch chain length was one of the factors that could affect multiple steps annealing process. This process use heating energy and moisture content to induce mobility of rearrange starch chain. If the starch chain length was too long or too short the stability of starch molecular structure will be reduced.

3. To find the condition for pre-germination both Chai Nat1 and RD6 paddies

Pre-germinated Chai Nat1 and RD6 paddies at minimum stage (embryo growth length 0.5-1 mm, 60-70% of germination) were done by soaking at 30^{0}C for 12 and 6 hours then incubating at 30^{0}C (85% relative humidity) for 20 and 16 hours, respectively. Both Chai Nat1 pre-germinated brown rice flour at minimum stage (Chai Nat1 PGBR flour_1) and RD6 pre-germinated brown rice flour at minimum stage (RD6 PGBR flour_1) were milled from broken or whole pre-germinated brown rice (PGBR) which were dehusked from pre-germinated paddies at minimum stage.

Both PGBR flour_1 (Chai Nat1 and RD6) showed lower and significantly different ($p \leq 0.05$) in stirring numbers (1,560.83 and 902.16 cP) than white rice flour (3,189.83 cP and 1,180.00 cP) and brown rice flour (3,170.17 cP and 1,168.17 cP).

Both PGBR flours_1 (Chai Nat1 and RD6) showed significantly ($p \leq 0.05$) lower amylose content (25.92 and 5.24 %) when compared to white rice flours (27.46 and 5.64 %) and brown rice flours (27.29 and 5.59 %).

Both PGBR flours_1 (Chai Nat1 and RD6) showed higher and significantly different ($p \leq 0.05$) reducing sugars (146.50 and 387.51 mg/100g) than white rice flours (85.22 and 344.26 mg/100g) and brown rice flours (84.44 and 341.85 mg/100g).

The swelling powers during increased temperature from the range of 65, 75, 85 to 95°C of both (Chai Nat1 and RD6) PGBR flours_1 (3.34-11.50 and 4.55-15.67 g/g) were significantly ($p \leq 0.05$) increased when compared to white rice flours (2.11-9.36 g/g and 2.32-14.59 g/g) and brown rice flours (2.10-9.27 and 2.28-14.56 g/g).

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The solubility of both (Chai Nat1 and RD6) PGBR flours_1 during increased temperature from the range of 65, 75, 85 to 95°C (18.48-23.18 and 20.42-57.31 %) were significantly ($p \leq 0.05$) increased when compared to white rice flours (5.18-12.39 and 8.50-31.60 %) and brown rice flours (5.13-11.75 and 8.25-30.49 %).

The carbohydrate leaching of both (Chai Nat1 and RD6) PGBR flours_1 during increased temperature from the range of 65, 75, 85 to 95°C (12.27-55.51 µg/ml and 20.55-68.76 µg/ml) were significantly ($p \leq 0.05$) increased when compared to white rice flours (5.37-13.55 µg/ml and 8.40-14.46 µg/ml) and brown rice flours (4.27-12.64 µg/ml and 7.50-13.14 µg/ml).

The peak viscosities of both (Chai Nat1 and RD6) PGBR flours_1 (1,643.33 and 447.50 cP) were significantly ($p \leq 0.05$) decreased when compared to white rice flours (2,310.00 and 2,579.00cP) and brown rice flours (2,307.83 and 2,536.17cP).

The syneresis of both (Chai Nat1 and RD6) PGBR flours_1 during increased freeze-thaw cycles range from 1, 2, 3, 4 to 5 cycles (19.00-52.78 and 10.55-35.29 %) were significantly ($p \leq 0.05$) increased when compared to white rice flours (15.70-39.70 and 7.46-17.44 %)) and brown rice flours (10.72-38.35 and 6.26-16.56 %).

Pre-germination process was one of the methods that hydrolyzed starch chain and could enhance the rearrangement by multiple steps annealing process for making stronger starch molecular structure then improve frozen rice noodle quality.

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Recommendations

1. The mechanism of pre-germination process and annealing process should be studied into the starch molecular structure.

2. The annealed PGBR flour showed yellow color and should be investigated

3. The frozen rice noodle should be checked for sensory evaluation by real person in 9 point hedonic scale.

4. The multiple steps annealed PGBR flour showed low swelling power, solubility and carbohydrate leaching that can be used in food products that involved high temperature such as pasteurized noodle.

5. Frozen rice noodle should be conducted in the form of finished products by adding the meat and vegetable soup.

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APPENDICES

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Appendix A

Chemical analysis

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1. Determination of amylose content (Juliano, 1985)

1.1 Apparatus

- Spectrophotometer
- Boiling water bath
- Hoot

1.2 Reagent

- Ethanol 95%
- Acetic acid 1N
- Sodium hydroxide 1N
- I-KI solution

1.3 Procedure

1.3.1 Weight rice flour 0.1 g into a 20 mL tube

1.3.2 Add ethanol 95% 1 mL and NaOH 9 mL then mixed vigorously on a vortex mixture

1.3.3 Place the tube in the boiling water bath and heat for 10 min

1.3.4 Transfer the solution to the 100 mL volumetric flask and adjust volume to 100 mL with dis trilled water

1.3.5 Pipette 5 mL of solution into another 100 mL volumetric flask

1.3.6 Add CH_3CooH 1 mL and I-KI solution 2 mL

1.3.7 Mixed the solution toughly then adjust to 100 mL with distilled water

1.3.8 After 20 min, read the absorbance at 620 nm against blank (follow all the procedure from 1.3.2-1.3.8)

1.3.9 Amylose content is calculated from amylose standard curve

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1.4 Standard curve

1.4.1 Weigh amylose 0.04 g into a tube then flower the procedure from 1.3.2-1.3.3

1.4.2 Pipette 1, 2, 3, 4, 5 mL of standard glucose solution into 100 mL volumetric flask

1.4.3 Add acetic acid 0.2, 0.4, 0.6, 0.8 and 1.0 mL by order

1.4.4 Add I-KI solution 2 mL then adjust the volume to 100 mL with distilled water, shake thoroughly and stand at room temperature for 20 min before measure absorbance at 620 nm by spectrophotometer

1.4.5 Plot standard curve between standard amylose intensity (X axis) and absorbance (Y axis)

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2. Determined reducing end molecule (Chang, 1979; Somogyi, 1951)

2.1 Apparatus

- Spectrophotometer
- Boiling water bath
- Hoot
- Vortex mixer

2.2 Reagent

2.2.1 Alkaline copper reagent

2.2.1.1 Mixed $Na_2HPO_4.12H_2O$ 71 g with NaK tartrate 40 g and NaOH 4 g into distilled water 700 mL by order and heat in boiling water bath

2.2.1.2 Add $CUSO_4.5H_2O$ 8 g in boil solution keep stirring until all of reagents dissolve

2.2.1.3 Remove from boiling water bath and add Na_2SO_4 180 g stirring thoroughly

2.2.1.4 Adjust volume to 1,000 mL with distilled water keep in brown glass bottom for 24-48 hours at room temperature and filter before used

2.2.2 Nelson reagent

2.2.2.1 Mixed $(NH_4)_6MO_7O_{24}.4H_2O$ 25 g with 450 mL distilled water in 600 mL beaker

2.2.2.2 Add conc. H_2SO_4 21 mL in mixed solution in air flow hoot

2.2.2.3 Add $Na_2HAsO_4.7H_2O$ 6 g in solution and adjust volume with distilled water to 1,000 mL

2.2.2.4 Keep the Nelson reagent solution in brown glass bottom for 24-48 hours at room temperature and filter before used

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2.2.3 Elcohol 50%

2.2.4 D-glucose

2.3 Procedure

2.3.1 Weight rice flour 0.5 g and mixed with ethanol 50% 5 mL into tube and cover with parafilm immediately mixed on a vortex mixer

2.3.2 Centrifuge at 5,000 rpm for 15 min and decant the supernatant carefully

2.3.3 Pipette supernatant 1 mL into glass tube and add alkaline copper reagent 1 mL

2.3.4 Heat solution in boiling water bath for 15 min and immediately cooled down in ice bath

2.3.5 Mix nelson reagent 1 mL with cool solution and mixed on a vortex mixer immediately

2.3.6 Add 10 mL of distilled water after stand the solution at room temperature for 30 min

2.3.7 Read the absorbance at 520 nm after 15 min with distilled water as a blank and calculate to reducing end molecule

2.4. Standard curve

2.4.1 Prepare glucose solution 0.2% then dilute to 0.02% and adjust concentration according to appendix table

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Appendix Table 1 Standard glucose solution 0-200 µg/mL.

Glucose concentration (µg/mL)	Distilled water (mL)	Glucose solution 0.02% (mL)
0	1	0
40	0.8	0.2
80	0.6	0.4
120	0.4	0.6
160	0.2	0.8
200	0	1.0

2.4.2 Follow the procedure from 2.3.3-2.3.7

2.4.3 Plot standard curve between glucose concentration (X axis) and absorbance (Y axis)

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3. Determination of the amylase activity with Rapid Visco analyzer (AACC, 2000)

3.1 Apparatus

Rapid Visco Analyzer (RVA), sample canisters and stirrers

3.2 Instrument preparation

Switch on instrument and computer, and open Rapid Visco analyzer program. Select the test profile for stirring number that consists of 95°C for 3 min

3.3 Determination

3.3.1 Weight 3.00 g rice flour (12% moisture basis) into weighting vessel before transfer into test canister.

3.3.2 Dispense 25.0 mL distilled water (if the moisture content of sample was 12%) into new test canister. Equivalent sample and water mass can be calculated using formula:

$$S = \frac{88 \times 3.0}{100\text{-}M} \quad \text{and} \quad W = 25 + (3.0 - S)$$

Where S= sample weight, W = water weight and M= moisture content of sample

3.3.3 Transfer flour onto water surface in canister. Place paddle into canister and vigorously jog blade through sample up and down 10 times to break flour or meal lumps on water surface.

3.4.4 Place the paddle into canister, and insert paddle and canister assembly firmly into paddle coupling so that paddle is property centered. Initiate measurement cycle by depressing motor tower of instrument.

3.4.5 Record stirring number after 3 min

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4. Determined carbohydrate leaching (Dubois *et al.,* 1956; Noranizan *et al.,* 2010)

4.1 Apparatus

- Spectrophotometer
- Hoot

4.2 Reagent

- Phenol 5%
- Sulfuric acid
- D-glucose.

4.3 Procedure

4.3.1 Weight rice flour 20 mg into centrifuge and add 6.25 mL of distilled water

4.3.2 Given heat treatment at 65, 75, 85 and 95°C for 30 min and immediately cool in ice bath

4.3.3 Pipette 1 mL of supernatant was mix with 5% phenol solution in a screw capped tube and followed by rapid addition of 5 mL concentration sulfuric acid in air flow hoot

4.3.4 The mixture solution was set aside in hoot for 30 min and reading absorbance at 490 nm with distilled water as a blank

4.4 Standard curve

4.4.1 Prepare 0.1% of glucose solution then dilute to 0.01%

4.4.2 Pipette glucose solution in different volume as shown in Appendix table

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Appendix Table 2 Glucose solution 0-100 µg/mL.

Glucose concentration (µg/mL)	Glucose solution 0.01% (mL)	Distilled water (mL)
0	0	1.0
20	0.2	0.8
40	0.4	0.6
60	0.6	0.4
80	0.8	0.2
100	1.0	0

4.4.3 Followed the procedure from 3.3.2-3.3.5

4.4.4 Plot standard curve between glucose concentration (X axis) and absorbance (Y axis)

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Appendix B

Physicochemical analysis

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1. Determination of the pasting properties with Rapid Visco analyzer (AACC, 2000)

1.1 Apparatus

Rapid Visco Analyzer (RVA), sample canisters and stirrers

1.2 Instrument preparation

Switch on instrument and computer, and open Rapid Visco analyzer program.

1.3 Determination

1.3.1 Weight 3.00 g rice flour (12% moisture basis) into weighting vessel before transfer into test canister.

1.3.2 Dispense 25.0 mL distilled water (if the moisture content of sample was 12%) into new test canister. Equivalent sample and water mass can be calculated using formula:

$$S = \frac{88 \times 3.0}{100\text{-}M} \quad \text{and} \quad W = 25 + (3.0 - S)$$

Where S= sample weight, W = water weight and M= moisture content of sample

1.3.3 Transfer flour onto water surface in canister. Place paddle into canister and vigorously jog blade through sample up and down 10 times to break flour or meal lumps on water surface.

1.3.4 Place the paddle into canister, and insert paddle and canister assembly firmly into paddle coupling so that paddle is property centered. Initiate measurement cycle by depressing motor tower of instrument.

1.3.5 Record viscosity, peak viscosity, trough viscosity, breakdown viscosity, final viscosity and setback viscosity.

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Appendix C

Physical analysis

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1. Determination viscosity by Brookfield meter (modified from Lorlowhakarn, 2007)

1.1 Apparatus

Brookfield probe number 21

1.2 Instrument preparation

Switch on instrument, select the test profile at 60 rpm with Brookfield probe number 21

1.3 Apparatus

1.3.1 Rice slurry from ratio in table was incubated at 25°C for 3 hours with stirring every 30 min then measured the viscosity.

1.3.2 Remove rice slurry 8 mL by syringe to Brookfield canister and gripe with the Brookfield meter.

1.3.3 Record the viscosity after turn on the motor probe.

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2. Determination of rice noodle cutting force (modified from AACC, 2000 and Lorlowhakarn, 2007)

2.1 Apparatus

- Texture analyzer
- Knife blade (A/LKB) using 5kg load cell

2.2 Sample preparation

Dried rice noodle 25 g was soaked in 500 mL of distilled water at room temperature for 15 min. The rice noodle was boiling for 1.5 min then removed and rinsed with running water for 2 min. After rinsing the sample was drained in a sieve and left in the sieve for 5 min before textural determination. Cutting force (firmness) of the rice noodle is defined as the maximum force in gram required cutting a rice noodle.

2.3 Determination

The TA-XT2 was set as the condition below

Mode:	measure force in compression
Option:	return to start
Pre test speed:	0.5 mm/s
Test speed:	0.2 mm/s
Post test speed	10.0 mm/s
Stain:	50%
Trigger type:	5g

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3. Determination of rice noodle tensile strength (modified from AACC, 2000 and Lorlowhakarn, 2007)

3.1 Apparatus

- Texture analyzer
- Spaghetti tensile grips (A/SRP) using 5kg load cell

3.2 Sample preparation

Dried rice noodle 25 g was soaked in 500 mL of distilled water at room temperature for 15 min. The rice noodle was boiling for 1.5 min then removed and rinsed with running water for 2 min. After rinsing the sample was drained in a sieve and left in the sieve for 5 min before textural determination. Tensile strength of the rice noodle is defined as the maximum force in gram required breakdown of noodle.

3.3 Determination

The TA-XT2 was set as the condition below

Mode:	measure force in compression
Option:	return to start
Pre test speed:	3.0 mm/s
Test speed:	3.0 mm/s
Post test speed	5.0 mm/s
Distance:	50 mm
Trigger type:	-5g

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Appendix D

Sensory evaluation

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1. Sensory evaluation

Sensory evaluation of three frozen rice noodle after freeze-thaw 5 cycles making from blended PGBR flours to Chai Nat1 white rice flour and three frozen rice noodle after freeze-thaw 5 cycles making from blended annealed PGBR flours to Chai Nat1 white rice flour were determined compared with frozen rice noodle after freeze-thaw 5 cycles making from Chai Nat1 white rice flour.

Ten taste panel was performed a quantitative descriptive analysis (QDA) to evaluate frozen rice noodle after 5 freeze-thaw cycles. The panelists were introduced to the reference rice noodle (Top Brand, purchased from Top supermarket) and asked for characterizing sensory attributes of the product. After that, they were asked to evaluate the reference rice noodle for the intensity of each attribute (firmness, elasticity, and adhesiveness), when the average intensity of each sensory attribute was marked on a 15-cm-interval scale.

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Sensory evaluation from

Product sample: Frozen rice noodle (Sen Lek)

Panelist's Name………………………………………………. Sex……. Date…….

Please describe intensity of sensory characteristics of rice noodle by placing the mark (I) on the scale to locate the intensity of each sensory attribute.

1. Firmness

Fluffy Firm

2. Elasticity

Slightly Elastic

3. Adhesiveness

Non-sticky Sticky

Comments ……………………………………………………………………….

……………………………………………………………………………………...

……………………………………………………………………………………

Thank you

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APPENDIX E

Experimental data

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Appendix Table 3 Moisture content of rice flour.

Rice flour sample	Moisture content[1] (% dry basis)	
	Chai Nat1	RD6
White rice flour	9.15±0.56a	9.61±0.66a
Brown rice flour	9.26±0.22a	9.84±0.31a
PGBR flour_1	10.02±0.14a	9.52±0.51a
PGBR flour_2	9.56±0.84a	9.45±0.22a
PGBR flour_3	9.55±0.94a	9.03±0.47a
Annealed PGBR flour_1 at 45°C for 1 day	9.18±0.24a	9.74±0.61a
Annealed PGBR flour_1 at 45°C for 2 day	9.65±0.36a	9.66±0.94a
Annealed PGBR flour_1 at 45°C for 3 day	9.44±0.57a	9.12±0.62a
Annealed PGBR flour_1 at 45°C for 4 day	9.87±0.13a	9.35±0.31a
Annealed PGBR flour_1 at 45°C for 5 day	9.66±0.87a	9.71±0.55a
Annealed PGBR flour_1 at 45°C for 6 day	10.06±0.45a	9.88±0.47a
Annealed PGBR flour_2 at 45°C for 1 day	9.05±0.66a	9.61±0.61a
Annealed PGBR flour_2 at 45°C for 2 day	9.15±0.51a	9.51±0.31a
Annealed PGBR flour_2 at 45°C for 3 day	9.65±0.87a	9.06±0.84a
Annealed PGBR flour_2 at 45°C for 4 day	9.66±1.06a	10.02±0.66a
Annealed PGBR flour_2 at 45°C for 5 day	9.78±0.69a	9.87±0.41a
Annealed PGBR flour_2 at 45°C for 6 day	9.74±0.33a	9.99±0.77a
Annealed PGBR flour_3 at 45°C for 1 day	9.65±0.46a	9.56±0.68a
Annealed PGBR flour_3 at 45°C for 2 day	9.77±0.51a	9.43±0.64a
Annealed PGBR flour_3 at 45°C for 3 day	9.63±0.97a	9.73±0.52a
Annealed PGBR flour_3 at 45°C for 4 day	9.78±0.21a	9.54±0.31a
Annealed PGBR flour_3 at 45°C for 5 day	9.22±0.64a	9.11±0.68a
Annealed PGBR flour_3 at 45°C for 6 day	9.05±0.52a	9.97±0.66a
Annealed PGBR flour_1 at 50°C for 1 day	9.52±0.97a	9.16±0.44a
Annealed PGBR flour_1 at 50°C for 2 day	10.03±0.66a	9.58±0.81a
Annealed PGBR flour_1 at 50°C for 3 day	9.52±0.31a	9.63±0.13a
Annealed PGBR flour_1 at 50°C for 4 day	9.36±0.52a	9.13±1.06a
Annealed PGBR flour_1 at 50°C for 5 day	9.58±0.91a	9.78±0.69a
Annealed PGBR flour_1 at 50°C for 6 day	9.71±0.64a	9.33±0.87a

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Appendix Table 3 (Continued)

Rice flour sample	Moisture content[1] (% dry basis)	
	Chai Nat1	RD6
Annealed PGBR flour_2 at 50°C for 1 day	9.06±0.66a	9.14±0.99a
Annealed PGBR flour_2 at 50°C for 2 day	9.87±0.57a	9.61±0.15a
Annealed PGBR flour_2 at 50°C for 3 day	9.85±0.61a	9.88±0.61a
Annealed PGBR flour_2 at 50°C for 4 day	9.77±0.33a	9.71±0.58a
Annealed PGBR flour_2 at 50°C for 5 day	9.22±0.87a	9.65±0.33a
Annealed PGBR flour_2 at 50°C for 6 day	9.06±0.64a	9.06±0.87a
Annealed PGBR flour_3 at 50°C for 1 day	9.62±0.33a	9.61±0.56
Annealed PGBR flour_3 at 50°C for 2 day	9.84±0.54a	9.84±0.87
Annealed PGBR flour_3 at 50°C for 3 day	9.22±0.87a	9.62±0.66
Annealed PGBR flour_3 at 50°C for 4 day	9.84±0.26a	9.11±0.21
Annealed PGBR flour_3 at 50°C for 5 day	9.66±0.12a	9.31±0.87
Annealed PGBR flour_3 at 50°C for 6 day	9.85±0.94a	9.42±0.91
Annealed PGBR flour_1 at 55°C for 1 day	9.41±0.51a	9.19±0.95
Annealed PGBR flour_1 at 55°C for 2 day	9.55±0.63a	9.64±0.33
Annealed PGBR flour_1 at 55°C for 3 day	9.06±0.57a	9.26±0.54
Annealed PGBR flour_1 at 55°C for 4 day	9.26±0.61a	9.55±0.16
Annealed PGBR flour_1 at 55°C for 5 day	9.87±0.68a	9.99±0.54
Annealed PGBR flour_1 at 55°C for 6 day	9.63±0.74a	9.71±0.81
Annealed PGBR flour_2 at 55°C for 1 day	9.16±0.63a	10.26±0.96
Annealed PGBR flour_2 at 55°C for 2 day	9.84±0.57a	9.47±0.66
Annealed PGBR flour_2 at 55°C for 3 day	9.06±0.64a	9.33±0.58
Annealed PGBR flour_2 at 55°C for 4 day	10.04±0.55a	8.54±0.61
Annealed PGBR flour_2 at 55°C for 5 day	9.99±0.23a	9.74±0.33
Annealed PGBR flour_2 at 55°C for 6 day	9.74±0.87a	9.66±0.57

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Appendix Table 3 (Continued)

Rice flour sample	Moisture content[1] (% dry basis)	
	Chai Nat1	RD6
Annealed PGBR flour_3 at 55°C for 1 day	8.94±0.66a	9.71±0.88
Annealed PGBR flour_3 at 55°C for 2 day	9.65±0.87a	9.23±0.25
Annealed PGBR flour_3 at 55°C for 3 day	8.77±0.31a	9.06±0.63
Annealed PGBR flour_3 at 55°C for 4 day	9.55±0.28a	9.87±0.74
Annealed PGBR flour_3 at 55°C for 5 day	9.06±0.64a	9.61±0.52
Annealed PGBR flour_3 at 55°C for 6 day	9.78±0.87a	9.35±0.68
Double steps annealed PGBR flour_1	9.66±0.33a	9.33±0.55
Multiple steps PGBR flour_1	9.45±0.47a	9.74±0.67

[1]Values are means of triplicate measurements ± standard deviation. Means for each characteristics followed by the different small latter within the same column are significantly different determined by ANOVA ($p \leq 0.05$).

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