THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Doctor of Philosophy (Agro-Industrial Product Development)

DEGREE

Agro-Industrial Product Development | Product Development

FIELD | **DEPARTMENT**

TITLE: Development of Bread from Composite Wheat-Germinated Brown Rice Flour

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THESIS

DEVELOPMENT OF BREAD FROM COMPOSITE WHEAT-GERMINATED BROWN RICE FLOUR

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A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Doctor of Philosophy (Agro-Industrial Product Development)
Graduate School, Kasetsart University
2010

Phantipha Charoenthaikij 2010: Development of Bread from Composite Wheat-Germinated Brown Rice Flour. Doctor of Philosophy (Agro-Industrial Product Development), Major Field: Agro-Industrial Product Development, Department of Product Development. Thesis Advisor: Associate Professor Kamolwan Jangchud, Ph.D. 175 pages.

The objectives of this study were to determine the effect of germination conditions on physicochemical properties of germinated brown rice flour and incorporate those flours into bread formulations that were acceptable to consumers. A 4x3 factorial arrangement in a completely randomized design (CRD) with four pH levels of steeping water (3, 5, 7 and as-is: DI, distilled water) and three levels of steeping time (24, 48, and 72 h for brown rice, GBRF; 24, 36 and 48 h for glutinous brown rice, GNBRF) was investigated. Pasting profiles, α-amylase activity, and free GABA were identified as variables that discriminated among germinated brown rice. GBRFs from germination conditions (pH 3 or 6.8; 24 or 48 h) contained a higher GABA concentration (14.8–67.0 mg/100g flour), and exhibited lower peak-viscosity and set-back than the control. Bread could be formulated with composite flour containing 40% germinated flour obtained from steeping at pH 3 for 24 h [GBRF(30):GGNBRF(10)] without negatively affecting sensory acceptability. Combination of microcrystalline cellulose [Fibrotech^{TM} at 0, 0.5, 1.0%, w/w] and diacetyl tartaric acid ester of monoglyceride [DATEM at 0, 1.0, 2.0%, w/w] were used to improve the quality of composite-flour bread following a 3 x 3 factorial in the CRD. The bread formulation with 0.5% Fibrotech^{TM} and 1.0% DATEM yielded breads with softer texture than the one without additives. The developed bread contained free GABA content of 1.91 mg/serving (2 pieces of bread, 54g). Regarding consumer acceptability (*n* = 114 and 116 for Thai and the U.S. consumers, respectively), mean overall liking score of the fresh bread containing germinated flour was slightly lower than the wheat bread (6.7 vs. 6.3 and 7.1 vs. 7.6 for Thai and US. consumers, respectively). At least 75% of both Thai and US. consumers would purchase the fresh formulated bread if commercially available. Bread was stored for 0, 3, and 5 days and evaluated for physicochemical properties and consumer acceptability (US. consumers) compared to the control (0-day stored wheat bread). During storage, moisture content drastically decreased with increasing crumb hardness (from 4.16 N to 10.37 N) of composite-flour breads. Consumer liking of all sensory attributes significantly decreased as storage time increased. In conclusion, this study demonstrated the feasibility of incorporating of germinated flour in bread formulation up to 40% that was acceptable to both Thai and US consumers. However, the quality of developed bread, particularly texture-related attributes, was less desirable with increasing storage time.

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ACKNOWLEDGMENTS

I would like to express my sincerest gratitude to Assoc. Prof. Kamolwan Jangchud, Assoc. Prof. Anuvat Jangchud, Dr. Kuakoon Piyachomkwan, and Prof. Witoon Prinyawiwatkul for providing their time and their guidance throughout the study and in the preparation of this thesis. I would sincerely like to thank Assoc. Prof. Penkwan Chompreeda for serving as the chairman of my defense and Arjan Patcharee Tungtrakul for her kind support.

I would also extent my acknowledgement to: the Royal Golden Jubilee Ph.D. program, Fulbright-TRF, and Graduate School, Kasetsart University for providing financial support throughout the study and the Dept. of Product Development, Kasetsart University, Bangkok, Dept. of Food Science, Louisiana State University (LSU), Agricultural Center, LA, U.S.A. for providing research facilities and collaboration; Prof. John Finley, Prof. Joan King, Prof. Marlene Jane, Dr. Mark LeBlanc, Bonnie Mann, and Terri Gilmer for the much appreciated support throughout my research conducted at LSU;

Pamarin Waimaleongora-Ek, Wannita Jirangrat, Karen Gacia, Damir Torrico, Wisdom Wardy, Reshani Senevirathne, Ravirajsinh Jadeja, Yenjit Rareung and all friends who have not been mentioned for their assistance and friendship throughout my research conducted in Dr.Witoon's lab, LSU. I also wish to thank Chanjira Phimpharian, Ratchanee Charoen, Bisri Soison, Sriwiang Thipkanon, Rongrat Rattanathamawat, Phodchanee Pongpa-ngan, Saowapark Wattanapahu, Juraiphon Keawsritong, Surasidh Boonchunone, officers, and friends who have not been mentioned for their support and friendship throughout my research conducted in Dept. of Product Development.

My appreciation goes to my parents and my brother and sisters for their encouragement, constant support, and unconditional love.

Phantipha Charoenthaikij
April 2010

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DEVELOPMENT OF BREAD FROM COMPOSITE WHEAT-GERMINATED BROWN RICE FLOUR

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food of more than half of the world's population, including Thailand. It is associated with almost every aspect of Thai way of life. Consumer preferences are recently shifting towards healthier products. Thus, the awareness of cereal intake was increased and recommended world-wide (Wang *et al*., 2002) according to its nutritional quality. The consumption of whole grain including brown rice was reported to have many health benefits related to reduction of blood pressure, coronary heart disease, colon cancer, and diabetes (Slavin, 2004; Bakke and Vickers, 2007).

In germinated grains which have been studied for over a decade, hydrolytic enzymes are activated to hydrolyze the high molecular weight polymers, resulting in the generation of bio-functional components in barley (Agu and Palmer, 1997), finger millet (Nirmala *et al.*, 2000), lentil (Frias *et al.*, 1997; Kuo *et al.*, 2004), and brown rice (Saikusa *et al.*, 1994; Watanabe *et al.*, 2004; Ohtsubo *et al.*, 2005). Germination improves the nutrients in the grains and makes them easier to be digested and absorbed (Tian *et al.*, 2004; Komatsuzaki *et al.*, 2007).

Gamma-aminobutyric acid (GABA), an important non-protein amino acid, is also found in germinated brown rice (Ohtsubo *et al.*, 2005). It functions as the predominant inhibitory neurotransmitter in the central nervous system (Kinnersley and Turano, 2000). It also has been proven to be effective in decreasing blood pressure of animals and humans (Zhang *et al.*, 2006), and has been used for the treatment of epilepsy (Marrosu *et al.*, 2003). GABA accumulation in germinated brown rice was found to increase according to various conditions such as germination time (Ohtsubo *et al.*, 2005) and soaking and gaseous treatment (Komatsuzaki *et al.*, 2007). Many researchers have previously reported that GABA synthesis in response to H^+ is a pH-regulating mechanism (Bown and Shelp, 1997; Kinnersley and Turano, 2000). In addition, during germination of rice, various factors such as variety, germination time, pH, and other substances in the steeping water are involved. However, studies on the relationships between biochemical and physicochemical changes in germinated brown rice flour (GBRF) and those factors are limited.

Bread is a staple food in many parts of the world and varies widely in its formulation. Flour is the most important and basic ingredient in bread making. To improve the nutritional profile of wheat bread, substitution of wheat flour with other flours, such as barley (Skrbic *et al.*, 2009), chickpea (Utrilla-Coello *et al.*, 2007), oat (Flander *et al.*, 2007), and rice flour (Watanabe *et al.*, 2004; Nakamura *et al.*, 2009; Renzetti and Arendt, 2009), has been of much interested (Skrbic *et al.*, 2009). However, substituted products have not been much attractive to consumers because of less desirable quality, particularly sensory properties, of the finished products (Hung *et al*., 2007).

The manufacture of rice bread without gluten is still limited, because gluten is an important structure-forming protein and responsible for elastic characteristics of dough, and contributes to the appearance and crumb structure of the product (Gallagher *et al*., 2004). The composite wheat-rice flour blends have been used for bread making. Additionally, flours from germinated cereals were reported to retard staling of bakery products including bread (Watanabe *et al.*, 2004). Accordingly, developing bread containing GBRF with more desirable nutritional quality and sensory acceptability is worth investigation.

Moreover, the quality of bread was undergone changes during storage time, particularly due to the staling process. The addition of various enzymes and additives (Gujral and Rosell, 2004; Renzetti and Arendt, 2009) was used to improve the quality of bread. However, the inferior quality of developed breads is still a significant issue and needs to be further investigated.

The alternative flour such as waxy flour was investigated by many researchers to improve the quality of wheat bread. Waxy flour such as waxy wheat flour (Morita *et al*., 2002; Hayakawa *et al*., 2004; Hung *et al*., 2007; Peng *et al*., 2009), waxy barley flour (Gill *et al*., 2002), or waxy corn starch (Hibi, 2001) was applied in bread making with the objective of bread staling retardation and shelf life extension by reducing the firming of bread crumb. The addition of waxy wheat flour alone in bread making was reported to yield a soft and glutinous bread crumb (Hung *et al*., 2007) which is an inferior quality of bread. Thus, the incorporation of waxy and regular flours was desirable to use to improve the texture and functionality of bread.

Sensory perceptions are what consumers associate with food quality and they have great influence in determining consumer acceptance and purchase intent for food products (Herrera-Corredor *et al*.,2010). When purchasing bread, consumers may have certain quality criteria including appearance, color, freshness, flavor, texture, and overall and consistent quality. Loss of freshness during storage of bakery products has a negative impact on overall product quality and consumer acceptance (Curic *et al.*, 2008). In order to satisfy consumer demands for bread products, it requires not only successful product development but also understanding of factors underlying consumer perception and acceptance. There is very limited information in the literature on the effects of composite germinated brown rice flours prepared from brown rice and glutinous brown rice on bread quality during storage.

The major aims of this study were to increase the utilization of rice by provide information of appropriate germination conditions to produce the germinated brown rice flour as well as offer an alternative nutritious choice of bread with acceptable levels of germinated brown rice flour to consumers. Therefore, this thesis was undertaken to examine and reveal information of the germination conditions affecting selected physicochemical properties of flour prepared from two varieties of brown rice including normal and glutinous brown rice, development of bread from composite wheat-germinated brown rice flour, and the consumer acceptability of fresh and stored composite bread.

OBJECTIVES

1. To investigate the effects of germination conditions on selected physicochemical properties of flour prepared from two varieties of brown rice.

2. To develop the formulation and the process of bread from the composite wheat-germinated brown rice flours.

3. To evaluate the quality of the bread from composite wheat-germinated brown rice flours and study the consumer acceptance of the developed product.

4. To determine the effect of storage time on the selected physicochemical properties and consumer acceptability of bread from composite wheat-germinated brown rice flours.

LITERATUR REVIEW

1. Rice

Rice (*Oryza sativa* L.) plays a fundamental role as the staple food of more than half of the world's population and of the entire Kingdom of Thailand. As the importance of rice-based food system increases, the United Nations General Assembly named 2004 the International Year of Rice with the theme "Rice is Life" (Fresco, 2005). In addition, more than 90% of the world's rice is produced in Asia and 2.5 billion people are dependent on it (Zemin, 2003). Thus, it is not surprising that there is growing concern about rice production being able to deal with an increasing world population.

Rice which belongs to the *Porceae Gramineae* or grass family has been consumed by humans for at least 5000 years (Bao and Bergman, 2004). It is a semi-aquatic, annual grass which can be grown under a broad range of climatic conditions. Thailand has long been known as the "Rice Bowl of Asia", not just because of its importance in the country's trade, but also because of the importance of rice in every aspect of Thai life. Thailand is one of the world's biggest rice producers with paddy output of 27 million tons in 2003. Thai rice exports made up about 27% of the world market (Cheaupun *et al*., 2004). Thailand's success in the international rice trade is due to its high quality which has characteristics described as follows.

1.1 Rice classifications

Generally, rice is classified into many categories based on their quality, for example milling quality, cooking and processing quality, nutritive quality, and purity (Webb, 1991). However, rice can be used in many different ways, so that the characteristics desired vary considerably, being ultimately related to final consumer acceptance of each rice or rice-containing products. There are many characteristics influencing the quality of rice related to genetic and non genetic control (Luh, 2001).

Rice can be mainly divided into two sub-species, indica and japonica; the former category consists of cultivar grown on approximately 80% of all rice acreage (Bao and Bergman, 2004). Both sub-species contain genotypes that vary greatly in terms of starch properties. Specifically, rice that is long grain, thin and cooks firm and fluffy is marketed with the name indica-rice grown in the tropical area of the Asian countries such as Thailand, India, and the Philippines. This rice has unique aroma which is similar to roasted nuts (Luh, 2001) and a compound present in pandanus leaves (Dendy, 2001). A major constituent responsible for this unique characteristic of this type of rice is 2-acetyl-1-pyrroline (Luh, 2001). Additionally, that which is short, wide, and has a soft grain and sticky texture is called japonica-rice (Bao and Bergman, 2004) which is commonly grown in semi-warm area such as Japan, Korea, and North of China. Waxy rice, whose endosperm opaque and contains little to no amylose exists in both sub-species.

Rice is also primarily classified according to its grain shape. However, within grain shape categories there are differences in cooking qualities that are determined by the chemical compositions of the grain and affect cooked grain texture (Bergman *et al.*, 2000). The quality of rice is mainly defined as long, slender and translucent grain which is preferable quality for consumption. Moreover, different varieties produce a different quality of cooked rice, for example waxy (glutinous) rice is very sticky while non-glutinous rice is slightly soft or hard in its texture when cooked. The factor responsible for this property is amylose content.

Depends on the amylose content, rice can be classified into 3 groups. The low-amylose type (less than 20% amylose) always has a soft texture and sticky when cooked. The intermediate-amylose type (21–25%) produces slightly soft cooked rice, whereas the high-amylose type (> 25 %) has a hard texture (Cheaupun *et al.*, 2004). On the basis of gelatinization temperature, rice varieties can be classified into three groups: low, intermediate, and high gelatinization temperature. Thai rice has a great variation in cooked-rice as shown in Table 1.

Table 1 Characteristics of some Thai rice varieties

Varieties	Grain length (mm)	Amylose (%)	Gelatinization temperature
Soft –sticky cooked rice			
KDML105 [a]	7.4	12-18	Low
RD15	7.5	14-18	Low
RD21	7.3	17-19	Low
Khao Jow Hawm	7.8	18-19	Low
Pathum Thani [a]	7.6	14-19	Low
Slightly soft cooked rice			
Khao Pahk Maw	7.7	24-26	Intermediate
RD23	7.3	23-27	Intermediate
Suphanburi 60	7.5	19-26	Low
Hard cooked rice			
Leuang Pratew 123	7.4	28-32	Low-intermediate
Suphanburi 1	7.3	29	Intermediate
Suphanburi 90	7.4	27-30	Intermediate
Chai Nat 1	7.4	27-30	Intermediate
Pathum Thani 60 [a]	7.5	27-30	Low

[a] Aromatic rice

Source: Cheaupun *et al*. (2004)

In addition, many of the uses for rice flour and starch exist because rice is low sodium, low fat (compared to other cereals), hypo-allergenic, gluten-free, colorless, and bland in flavor (Deis, 1997; Gujral and Rosell, 2004). In its native form, it exists with many different functional characteristics. Since milled rice consists of about 90% starch, the structure of this fraction and its physicochemical properties are the primary characteristics used to select rice cultivars and rice starch for specific industrial application (Bao and Bergman, 2004) which will be described further.

1.2 Structure of rice grain

Freshly harvested rice is called paddy grain or rough rice. The white starch grain used for cooking is the centre of the rice seed and is protected by the hull. Inside the hull, the white grain is covered by a layer called bran. The embryo is contained within the bran layer and together, the grain, embryo, and bran are called brown rice. It consists of the outer layer of pericarp, testa, germ and the endosperm. The endosperm consists of the aleurone layer and the endosperm proper, consisting of the subaleurone layer and the starchy endosperm (Juliano, 1993). The aleurone layer encloses the embryo are rich in protein, lipid, vitamin, and phytic acid (Dendy, 2001). The rice grain morphology is shown as Figure 1.

The starch granules are polyhedral and mainly 3 to 9 µm in size, with unimodal distribution. Protein occurs mainly in the form of spherical protein bodies 0.5 to 4 µm in size throughout the endosperm (Figure 2), but crystalline protein bodies and small spherical protein bodies are localized in the subaleurone layer (Juliano, 1993).

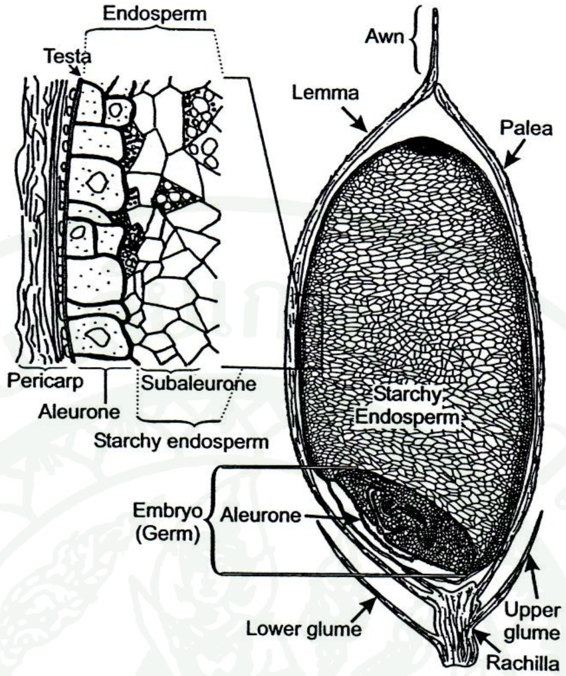


Figure 1 Illustration of a longitudinal section of a rice spikelet.

Source: Champagne *et al*. (2004)

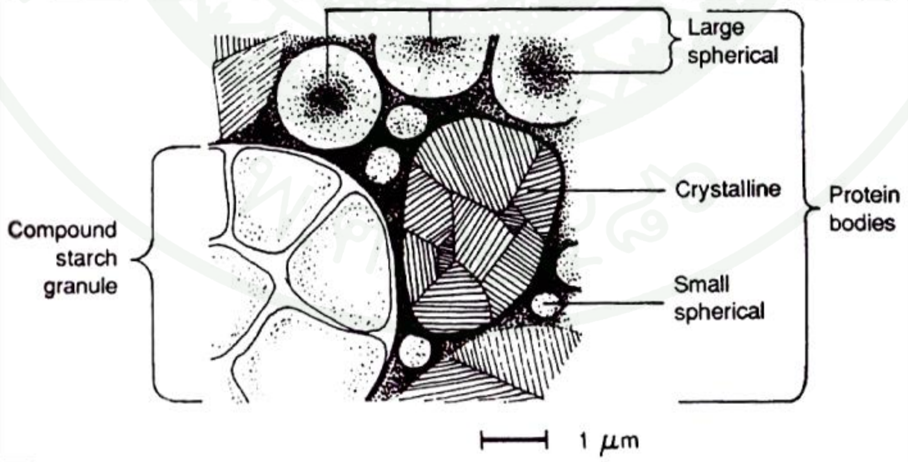


Figure 2 Schematic diagram of various protein bodies and compound starch granule in the endosperm subaleurone layer.

Source: Juliano (1993)

Available carbohydrates, mainly starch, are about 90% of the dry matter in milled rice which is higher than in brown rice. The gradients for various substances are illustrated in Figure 3. Dietary fiber is highest in the brand layer. Various bio-functional substances such as thiamine, riboflavin, niacin, *alpha* -tocopherol, calcium, phosphorus, phytin p, iron and zinc are also concentrated in the outer layer of brown rice. Friction milling to remove the pericarp, seed-coat, testa, aleurone layer and embryo to yield milled rice results in loss of those high nutritional compositions (Juliano, 1993). However, lipid content of rice is mainly in the bran fraction (20%, dry basis), since then brown rice is susceptible to lipid oxidation led to presenting of off-odors, off-flavors and shortened shelf life depending on their extent of surface damage, moisture content, and temperature of storage (Champagne *et al*., 1992).

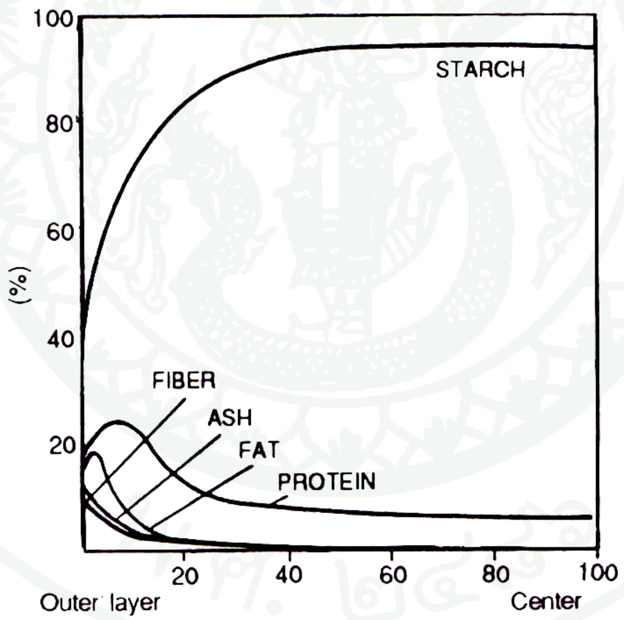


Figure 3 Distribution pattern of major constituents of brown rice determined using a tangential abrasive mill.

Source: Juliano (1993)

1.3 Rice flour

Broken rice, a by-product of rice milling, from high to low amylose-types and waxy-types is to produce industrial flours of different granulations and functionality (Bao and Bergman, 2004). There are, however, differences in protein, lipid, starch contents, and the amylose and amylopectin ratios in the starch. Composition differences contribute to the diversity of chemical and physical properties of various rice flours, such as viscosity, starch gelatinization temperatures, and water absorption (Luh and Liu, 1991). The amylograph of different rice varieties are presented in Figure 4.

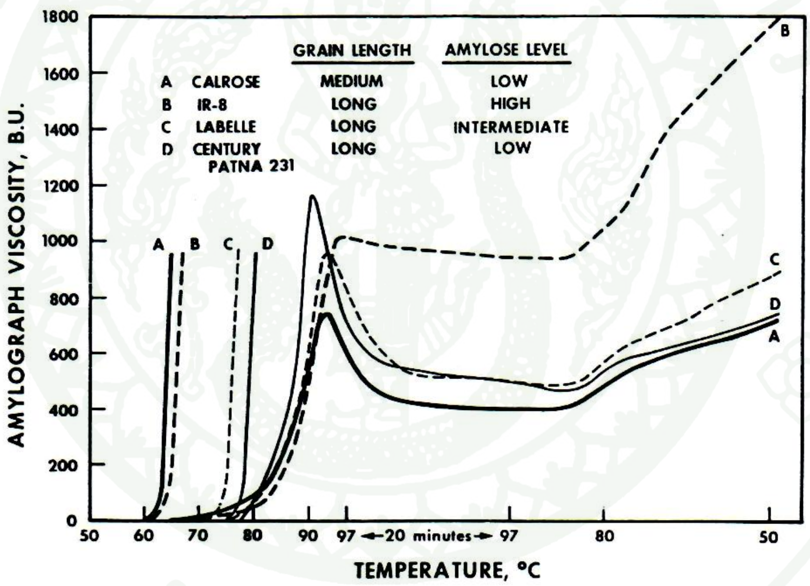


Figure 4 Amylograph curves of roller-milled flour from four rice varieties. Short curves, 20% slurries; full curve, 10% slurries.

Source: Luh and Liu (1991)

In general, there are three methods used to prepare rice flour such as wet, semidry, and dry milling (Yeh, 2004), as illustrated in Figure 5. Soaking, milling, drying, and regrinding are involved in wet milling. The key purpose of wet milling is to prepare flour with minimal damage starch and finer particle size than dry milling process (Juliano, 1985; Solanki *et al.*, 2005). Flour prepared from wet milling is usually used for cake, rice cracker, and rice noodle. Regarding semidry milling, rice is soaked, drained, and ground. The application of semidry milled flour is similar to those for wet-milled flour. Although, it can produce high quality of flour but it also produces high amount of waste water as well. On the other hand, rice can be directly ground in a process designated as dry milling including rolling, pounding, shock, stone, and lateral steel. The disadvantage of this process is more damage starch than another process. Dry-milled rice flour are used for baked product, baby food, extrusion-cooked product and high-protein rice flour (Yeh, 2004).

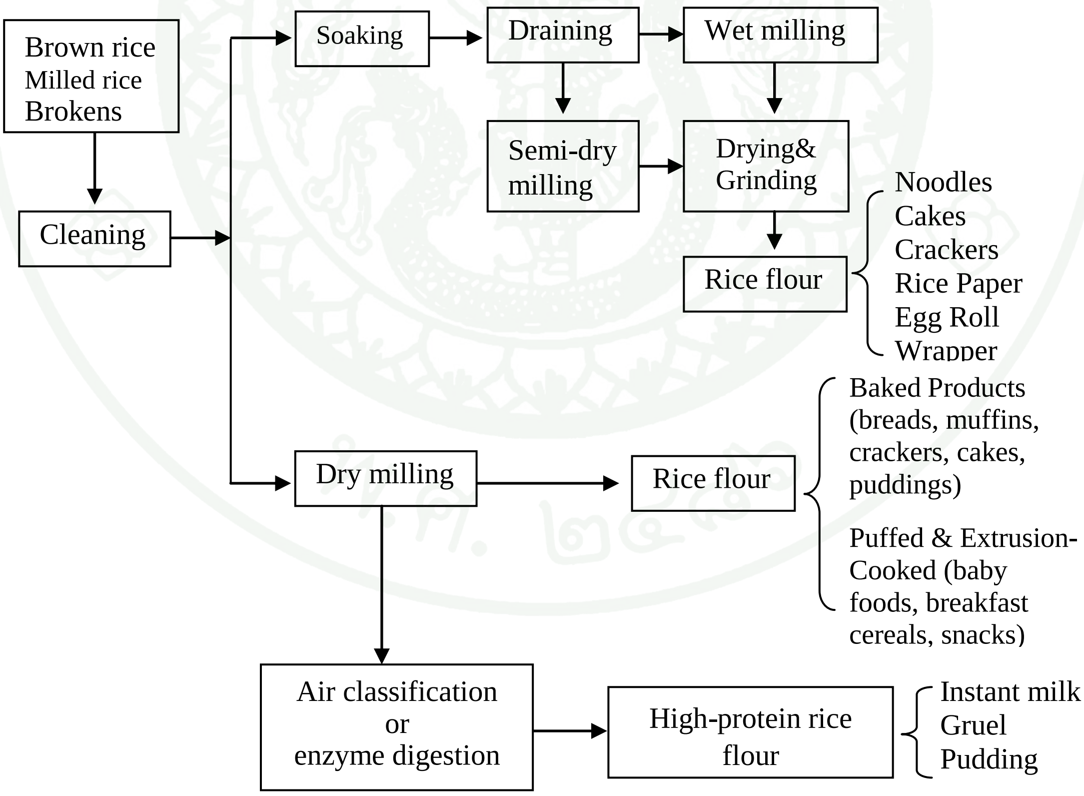


Figure 5 Manufacture of rice flours and their applications.

Source: Yeh (2004)

Rice is not only consumed as cooked rice but it can also be consumed in other forms. Rice varieties with certain amylose-amylopectin ratios are used for various rice flour based products. For example, across the world a demand for rice flour for use in baby food, breakfast cereal, crackers, candy, noodle, bread, and snack food exist (Bao and Bergman, 2004). Rice flour can be used as dusting powder and breading mixes (Ito and Ishikawa, 2004). It is also used as a separating powder for refrigerate-preformed-unbaked biscuit and pizza, as an extender in powdered cheeses and sugar, and as coating for French fries and in mixes for pancakes and waffle. Rice flour in deep fat frying batters added crispness to the coating, reduced oil-pick-up (Shih and Daigle, 1999; Bao and Bergman, 2004), retarded moisture transmission, and extended holding time (Bao and Bergman, 2004).

Recently, waxy-rice flour is also available commercially. It has the common viscosity characteristics of waxy-type flours from corn or sorghum. It contains less than 2% amylose in the starch and an appreciable amount of *alpha* –amylase. This flour has a lower peak viscosity than some of the short-grain rice, probably because of its amylolytic activity and it has practically no setback viscosity (Luh and Liu, 1991). Waxy-rice flours and starches also have substantial freeze-thaw stability than any other flours or starches. This behavior may be attributable to the virtual lack of amylose starch which caused the formation of fewer inter-molecular associations (Luh and Liu, 1991; Bao and Bergman, 2004). Waxy rice flour is frequently used as a thickening agent for white sauces, gravies, and puddings (Bao and Bergman, 2004).

2. Nutritional changes of brown rice during germination

Brown rice is simply white rice that has not had the bran covering the rice grains removed. Since brown rice still has the bran intact, it is a much better source of fiber and contains more nutrients than does milled or white rice (Kulp and Ponte, 2000; Watanabe *et al.*, 2004; Ohtsubo *et al.*, 2005). These components exist mainly in the germ and bran layers which are removed during polishing or milling. However, its dark appearance and hard texture, brown rice is not considered suitable for table rice (Varanyanond *et al*., 2005).

Malting is the germination of cereal grain in moist air under controlled conditions, the primary objective being to promote the development of hydrolytic enzymes which are not present in the nongerminated grain (Dewar *et al*., 1997). The malting process can be divided into three physically different operations, for example steeping, germinating, and drying or kilning step. Steeping, the soaking of grain in water is widely recognized as the most critical stage of the malting process. This is a consequence of the importance of initiating germination such that modification of the endosperm structure will progress at a rate producing malt of the desired quality (Dewar *et al*., 1997).

In most cases, there must be sufficient oxygen to allow aerobic respiration, suitable temperature to permit various metabolic processes to continue at an adequate rate, and enough moisture for growth and development (Capanzana and Buckle, 1997; Dewar *et al*., 1997). Germination induces biochemical changes which produce an increase in free limiting amino acid and changes in the functionality and sensory characteristics (Fernandez and Berry, 1989; Frias *et al*., 1997) of seed components. Therefore, the nutrition of germinated grains has been studied up to today for over decades ago. In germinated cereal grains, hydrolytic enzymes are activated during germination. The breakdown of the high molecular weight polymers leads to generation of bio-functional in barley (Bhatty, 1996), finger millet (Nirmala *et al*., 2000), lentil (Frias *et al*., 1997; Kuo *et al*., 2004) and rice (Saikusa *et al*., 1994; Watanabe *et al*., 2004). Amylases also play a significant role in seed germination and maturation are instrumental in starch digestion in animals resulting in the formation of sugars, which are subsequently used for various metabolic activities (Muralikrishna and Nirmala, 2005).

Accordingly, germinated flour from cereals has been reported to have better nutritional properties than flours from nongerminated cereals (Capanzana and Buckle, 1997). Generally, supplementary foods made from germinated flours have low viscosity and high nutrient density and have properties acceptable to weaning infants in developing countries. Germinated flour can also be used in bakery products including bread (Pyler and Thomas, 2000; Watanabe *et al*., 2004). Recently, germinated brown rice (GBR) is very much of interest to public attention, including in Thailand. This product is simply brown rice steeped in excess water and required germination period until it begins to bud (Watanabe *et al*., 2004; Tian *et al*., 2004). This process makes the internal minerals change, and the brown rice becomes more nutritious, and tastier (Ito and Ishikawa, 2004). Currently, GBR has been studied using japonica rice such as *Koshihikari* (Watanabe *et al*., 2004) and *Nipponbare* (Komatsuzaki *et al*., 2007). However, indica rice, the most important variety of Thai rice, could also be suitable for a production of GBR (Ito and Ishikawa, 2004).

Some nutritional components such as γ-aminobutyric acid (GABA), are produced in pre-germinated brown rice (PGBR) with small bud length of 0.5-1.0 mm (Saikusa *et al*., 1994). GABA has several physiological functions such as predominant inhibitory neurotransmission (Kinnersley and Turano, 2000) and induction of hypotensive effects, diuretic effects, and tranquilizes effects (Jakobs *et al*., 1993). It also has been proven to be effective in decreasing blood pressure of animals and humans (Zhang *et al.*, 2006), treatment of epilepsy (Marrosu *et al.*, 2003), and sleeplessness improvement (Ito and Ishikawa, 2004). Toxic levels of GABA have not been reported. However, very high intake of GABA may cause anxiety, tingling of extremities, shortness of breath as well as a numb feeling around the mouth (Anonymous, 2010).

GABA, an important non-protein amino acid, is produced primarily by the decarboxylation of L-glutamic acid, catalyzed by the enzyme, glutamate decarboxylase (GAD) [EC4.1.1.15] (Komatsuzaki *et al*., 2007). Subsequently, GABA is metabolized via GABA transaminase (GABA-T) and succinic semialdehyde dehydrogenase (SSADH) to succinate. The succinate produced is utilized by the Krebs cycle. This pathway is referred to as the GABA shunt (Figure 6).

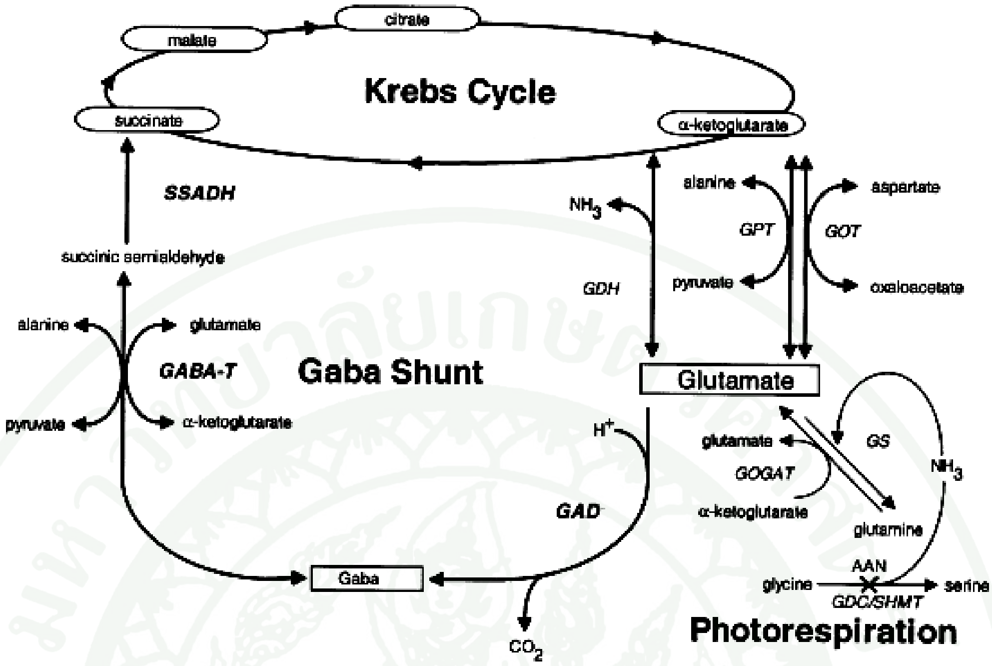


Figure 6 Pathways of glutamate (Glu) metabolism, showing the relationships between the Gaba shunt, the Krebs cycle, GS:GOGAT and photorespiration. In the Krebs cycle, metabolites under consideration here, and the direction of overall carbon flux are shown. *Abbreviations* – AAN, aminoacetonitrile; Gaba, g-aminobutyric acid; Gaba-T, Gaba-transaminase; GAD, Glu decarboxylase; GDC, Gly decarboxylase; GDH, Glu dehydrogenase; GS, Gln synthetase; GOGAT, Gln:a-ketoglutarate aminotranferase (ferredoxin-dependent; NADPH-dependent; NADH-dependent); GOT, Glu:oxaloacetate transaminase; GPT, Glu:pyruvate transaminase; a-KGDH, a-ketoglutarate dehydrogenase; MSO, methionine sulfoximine; SHMT, Ser hydroxymethyltransferase; SSADH, succinic semialdehyde dehydrogenase. GS: Gln synthetase, Glu:oxaloacetate transaminase (GOT) and Glu:pyruvate transaminase (GPT).

Source: Scott-Taggart *et al.* (1999)

GABA synthesis also increases rapidly in response to a variety of environmental signals, including hypoxia, acidosis, mechanical stress, or cold stress, as a result of a stimulation of GAD activity by Ca^{2+}/calmodulin or a reduction in cytosolic pH (Scott-Taggart *et al*., 1999).

GABA accumulation in germinated brown rice was found to increase according to various conditions such as germination time (Ohtsubo *et al.*, 2005), soaking and gaseous treatment (Komatsuzaki *et al.*, 2007). Many researchers have previously reported that GABA synthesis in response to H^{+} is a pH-regulating mechanism (Bown and Shelp, 1997; Kinnersley and Turano, 2000).

Those changes in textural properties and functional quality influenced on rheological properties and other physical and sensory characteristics are very important in determining the acceptability of the products, especially for bread industry.

3. Bread and its qualities

Bread is a classic handicraft which has a long tradition (Stampfli and Nersten, 1995). Around the world, bread is the principal food, provides more nutrients than any other single food source and varies widely formulations.

3.1 Wheat bread

Wheat is grown mostly in the northern hemisphere in Europe and North America but large quantities are also grown in China, Australia, Northern India, Russia, and South America. Approximately 70% of world wheat production is used as food, mainly as bread and other baked products such as cakes and cookies (Dobraszczyk, 2001).

In bread making, each ingredient acts differently and has its own specific functions affecting bread quality. According to their functions, they could be classified into various groups, for example structure creation, hydration, leavening, flavor enhancement, energy source of yeast, lubrication, nutrition and crust color enhancement, and enhancement of flour strength which were demonstrated in Table 2.

Table 2 Functions of some ingredients used in bread

Ingredient	**Function**
	Structure
Flour	1. Protein (gliadin and glutenin) and water form viscoelastic material, called gluten. Gluten retains gas formed by sugar fermentation and contributes to structure of dough and bread.
	2. Starch + water + heat forms a viscous paste that sets to a gel after baking. During bread storage the starch crystallizes (retrogrades and contributes to firming major part of staling) of bread.
	Hydration
Water	1. Combines (hydrates) protein to form gluten.
	2. Hydrates flour gums (pentosans) and mill-damaged starch granules.
	3. Solvent, dispersing agent, and medium for chemical and biochemical reactions.
	4. Aids dough mobility.
	Leavening
Yeast	1. Produces carbon dioxide, ethanol by fermentation of fermentable sugars.
	2. Conditions dough biochemically.
	3. Forms flavor precursors (by-products of alcoholic fermentation).
	4. Rate of fermentation is controlled by temperature, nutrient supply, water level, pH, sugar concentration, salt, and level and type of yeast.

Table 2 (Continued)

Ingredient	**Function**
	Flavor Enhancer
Salt	1. Helps control fermentation.
	2. Toughens dough by interaction with gluten.
	3. Extends required dough development (delayed addition in dough mixing decreases mixing time by (10- 20%).
	Energy source of yeast
Sugar	1. Fermentable carbohydrates (fermentation).
	2. Flavor – residual sugar (sweeteners), fermentation by-products, Maillard-type compounds during baking.
	3. Crust color – results of caramelization (sugars and heat) and nonenzymatic browning (reducing sugar plus amino group of proteins, amino acid, etc.).
	4. Extends shelf life by increasing hygroscopicity due to presence of residual sugars and tenderizing the crumb.
	Lubrication
Shortening	1. Ease of gas cell expansion in doughs.
	2. Lubricates slicing blades during bread slicing.
	3. Extends shelf life.
	4. Tenderizes crust.
	Nutrition and Crust Color Enhancement
Dairy products	1. Protein (high in lysine) and calcium.
	2. Flavor enhancement.
	3. Crust color (browning reaction and caramelization).
	4. Buffering effect in doughs and liquid ferments.

Table 2 (Continued)

Ingredient	Function
	Enhancement of Flour Strength
Wheat gluten	1. Increases dough strength (1% gluten increases protein content by 0.6%). 2. Increases water absorption [1% added gluten (flour basis) enhances absorption by 1.5% (flour basis)]. 3. Improves dough mixing of fermentation tolerance. 4. Increases bread loaf volume. 5. Especially used in formulation of specialty breads.
Diastatic malt	1. Contributes fermentable sugar maltose. 2. Contains amylase, which converts starches to sugars. 3. Enhance flavor. 4. Improves crust color. 5. Improves dough handling. 6. Extends shelf life.
Nondiastatic malt	1. Contributes sugar (maltose). 2. Enhances flavor. 3. Improves crust color.
Fungal enzymes	A. Amylase: 1. Convert starches to sugars. 2. Aid crust color. 3. Improve dough handling. 4. Extend shelf life. 5. Less heat stable than cereal amylases (they are inactivated before starch gelatinizes in the oven). B. Protease: 1. Weaken doughs due to cleavage of peptide bonds in wheat proteins. 2. Reduce dough-mixing time. 3. Increase pan flow.

Source: Modified from Kulp (1991)

In addition, bakers have different requirements from the miller, particularly consistent quality flour to produce bread without problems in the bakery production (Dobraszczyk, 2001). The quality of flour used as ingredients for bread making was determined by a variety of characteristics which divided into chemical, enzymatic, and physical properties (Rasper, 1991). Moisture content, protein (11-13% protein for bread flour, 14% moisture basis), mineral, fiber, and starch content were parameters usually used to ensure the chemical quality of flours. The activity of amylolytic activity, starch-hydrolyzing enzymes, is considered of primary importance of flour. Some level of this enzyme activity is desired in wheat flour for a sufficient development of gas during fermentation and early stages of baking of yeasted dough (Rasper, 1991). Insufficient or excessively high level of this enzyme resulted in a reduction in quality of both dough and final baked product (Rasper, 1991; Hallen *et al*., 2004) which will be described further.

Dough rheology is complex and developed by high-speed mixing to give a viscoelastic network that is sufficiently elastic to retain gas during rising and viscous to flow rather than rupture. Thus, rheological measurements on dough being mixed are indicative of proving and baking performance. The origin of dough rheology is the wheat gluten proteins which can retain gas for much longer and up to higher temperature (72 ^{o}C) than the other cereals (Dobraszczyk, 2001). Gluten protein with in wheat is a mixture of glutenins and gliadins, which make different contributions to the viscoelasticity of gluten. Dough made from glutenins alone exhibit substantial strength and elasticity while dough made from gliadins alone behave like a highly viscous liquid but have little resistance to deformation (elasticity). Thus the combination of these two proteins imparts the unique viscoelastic properties responsible for gas retention in wheat flour dough (Dobraszczyk, 2001). Collectively, the gluten matrix is a major determinant of the important rheological characteristics of dough including elasticity, extensibility, resistance to stretch, mixing tolerance, and gas holding ability (Lazaridou *et al*., 2007).

It is a reason that physical properties of dough have a pronounced effect on the quality of the finished product. The Barbender-farinograph is one of the most widely used investigating the rheological properties of dough. A farinogram was recorded which obtained the commonly measured indices as shown in Figure 7. Water absorption of flour is a property defined as the amount of water required for dough to reach a definite consistency (500 BU) at the point of optimum development. Flour with higher protein and greater gluten quality are characterized by higher absorption (Rasper, 1991).

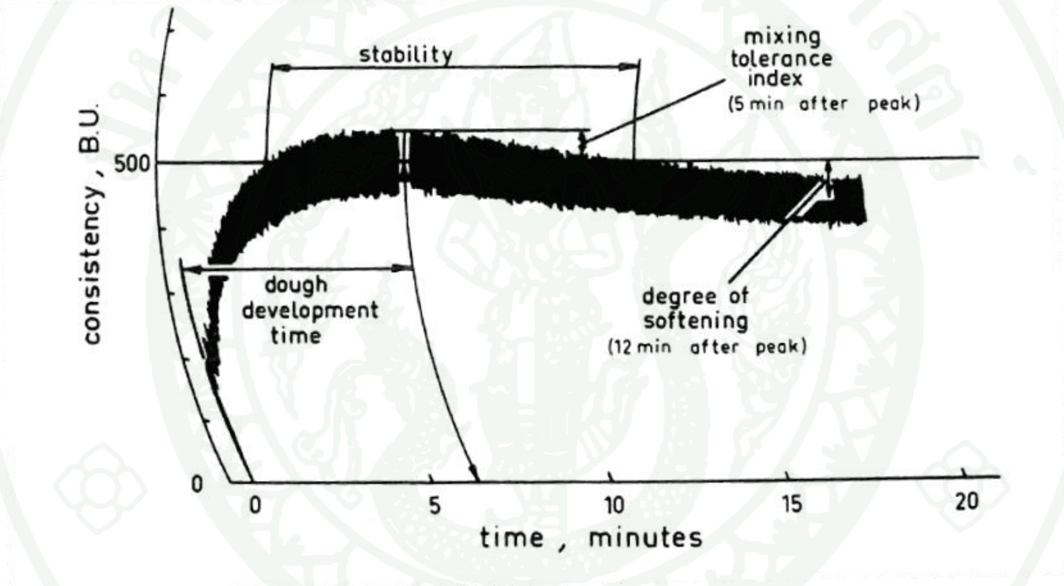


Figure 7 Representative farinogram showing some commonly measured indices.

Source: Rasper (1991)

Values other than absorption are frequently derived from farinograph curves. Some parameters have been proposed as the following (AACC, 2000, Rasper, 1991);

(a) Dough development time (DDT) is interval. It is the required time for dough development to reach 500 BU of dough consistency

(b) Mixing tolerance index (MTI) is the difference in BU from top of curve at peak to top of curve measured at 5 min after peak is reached.

(c) Stability is defined as time difference between point where top of curve first intersects 500-BU line (arrival time) and point where top of curve leaves 500-BU line (departure time).

Dough development time (DDT) and stability increase with increasing strength of flour, while mixing tolerance index and degree of softening conversely related to increasing strength (Rasper, 1991).

3.2 Composite flours for bread making

Consumers may have certain quality criteria for purchasing bread, including appearance, freshness, taste, flavour, and overall and a consistent quality. It is a great challenge for the baking industry to meet these criteria, for several reasons. The first reason is the main ingredient which is flour, the single most important and basic ingredient in bread making varies due to variety, growing season, and milling technology. Another reason is the difference in bread preferences. Consumer preferences are shifting towards healthier products. It is sometimes possible to make new bread varieties by simply adjusting the formulation or baking procedure (Si and Drost-Lustenberger, 2002).

Composite flours may be considered as the first option to modify the quality of wheat bread, particularly in countries where other flour sources are commonly grown. It has been utilized in bread making to reduce the proportion of wheat flour being used if economic conditions required the reduction of wheat imports (Dendy, 2001). In addition, those flours which contribute to enhance the nutritional quality of white wheat bread have been of much interested (Dendy, 2001; Cauvin, 2007). In some cases it is possible to make a product which has some of the attributes of wheat breads with mixtures of some of cereals by utilizing the gelatinization properties of their starches to form a bread-like, aerated structure. In these situations, the lack of gas-holding capabilities of any proteins present in the flour must be compensated for by adding other stabilizing materials (Cauvain, 2007) which will be described further.

Rice flour has unique nutritional and hypoallergenic properties. It is naturally gluten-free, low level of sodium, and high amounts of easily digested carbohydrate with colorless and bland taste (Deis 1997; Gujral and Rosell, 2004; Renzetti and Arendt, 2009). Therefore, its use in many different food products, especially in developing of food for gluten intolerant consumers (Sae-Ew *et al.*, 2007) has been increasing.

Rice flours can be used in baking application in many different forms. They can be either white rice flour or whole-grain brown rice flour. In addition, rice bread became possible to make by the creation of fine rice flour (Ito and Ishikawa, 2004). However, the use of rice flour in bread making is still limited because rice proteins are unable to retain the gas produced during the fermentation process (Gujral and Rosell, 2004). The manufacture of rice bread without gluten presents considerable technological difficulties, because gluten is the important structure-forming protein.

According to those qualities, gluten-free rice bread using only rice flour mainly also has established and studied by many researchers (Lazaridou *et al*., 2007). However, the inferior quality of rice bread compared with wheat bread was still a problem. Therefore, a number of researches on the improving quality of gluten-free bread were focused. The high quality gluten-free bread can be achieved from a blend of brown rice flour and other starches such as corn and potato starch incorporated with the suitable hydrocolloid (Moore *et al*., 2006). Nevertheless, the presence of rice flour in baking application has impact on the texture of a product. Optimal rice bread formulations have been developed using carboxymethyl cellulose (CMC) and hydroxypropylmethyl cellulose (HPMC) which has been improved bread quality in terms of specific volume, crust and crumb color, firmness, and moisture content (Collar *et al*., 1999; Gallagher *et al*., 2004).

The composite wheat-rice flour blends have been used for bread making. Some researchers indicated that substitution of wheat flour with rice flour up to 30% (Watanabe *et al.*, 2004; Nakamura *et al.*, 2009) in bread is acceptable without compromising the sensory quality. Newly developed rice flours was studied by Nakamura *et al.* (2009) that bread containing 40% of rice flour of total flour without any additives were maintained well specific volume and qualities. Additionally, flours from germinated cereals were reported to retard staling of bakery products, especially bread (Watanabe *et al*., 2004).

Besides rice flour, new efforts have been undertaken to replace wheat flour by other starch sources. Various kind of flours such as maize, barley, oats, and sorghum (Flander *et al*., 2007; Utrilla-Coello *et al*., 2007; Nakamura *et al*., 2009; Renzetti and Arendt, 2009; Skrbic *et al*., 2009) have also found to apply into bread products. Modified starches have been investigated and developed for more than a century and have various applications in food. It could be used to substitute up to 20% for wheat flour without diminishing bread quality (Miyazaki *et al*., 2006).

Normally, dough containing substituted flour revealed less stability than dough with wheat flour. Nevertheless, modified starch played the different roles in functionality and quality of bread. For example, heat-moisture treated maize starch can apply in bread because it possessed a high emulsifying ability (Miyazaki and Morita, 2005). However, the specific volume of bread decreased by the addition of this flour. The firmness of bread substituted with hydroxypropylated starch was lower than bread substituted with other modified starch or wheat flour alone. The cross-linking waxy starches retarded the firmness of bread crumb (Miyazaki *et al*., 2006).

3.3 Bread staling and improvers

Bread is subjected to changes since after baking and storing. It causes the significant financial losses to both consumers and producers. Bread staling is a general term that describes the time-dependent loss in quality (Gray and BeMiller 2003; Gimenez *et al.*, 2007). This process involves three factors impacted the freshness of products which are firming of the crumb, loss of flavour, and loss of crispness in the crust (Stampfli and Nersten, 1995; Azizi and Rao, 2005; Orthoefer, 2008) resulting in the discard of baked products.

Crumb softness and crumb elasticity are important characteristics for the description of crumb freshness perceived by consumers. The two texture characteristics may not necessarily correspond to each other. Bread staling is also responsible for a reduction of crumb elasticity and an increase of crumb firmness. Moreover, staling of bread is an important factor to evaluate the quality of bread and the retrogradation of starch is believed to be responsible for this phenomenon (Hung *et al.*, 2007). The crystallization of amylopectin and changes in water mobility due to re-association of polymers had been related to increase in bread staling during prolonged storage (Curic *et al.*, 2008; Lodi and Vodovotz, 2008; Goesaert *et al.*, 2009a). If the branching chain becomes short, the possibility of crystallization will be reduced.

Therefore, bread staling is a very complex process that cannot be explained by a single effect, amylopectin retrogradation, reorganization of polymers within the amorphous region, loss of moisture content, distribution of water content between the amorphous and crystalline zone, and the crumb macroscopic structure participated in this process (Guarda *et al*., 2004). The schematic representation of this mechanism proposed by Schoch and French was shown in Figure 8.

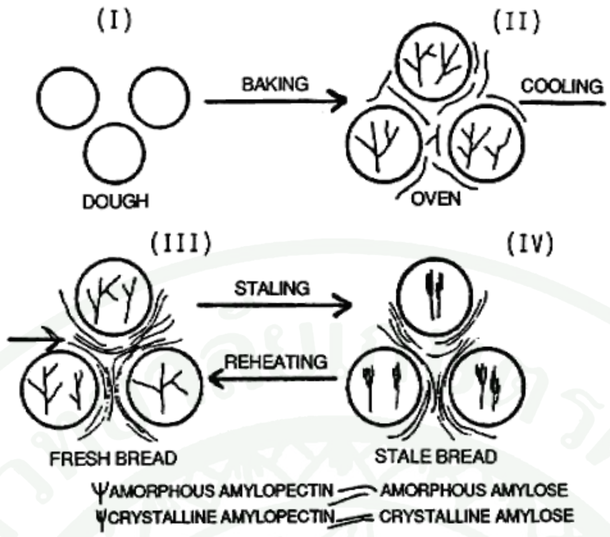


Figure 8 Schoch’s mechanism of bread staling. The intact starch granule (I) swell and gelatinize during baking, which transforms the starch polymers (amylose and amylopectin) into an amorphous state (II). During cooling, the amylose crystallizes (retrogrades) and contributes to the initial crumb firmness of bakery foods (III). During storage, amylopectin retrogrades (IV), which causes firming of breads.

Source: Modified from Kulp (1991)

For the baking industry, the optimization of dough properties and the quality improvement of the finished product are first of all of the interest while the sensorial appeal is the most important for consumer. Consumers demand products of better quality and longer shelf life therefore the search for solutions to meet those requirement has been parallel to the development of different additives and technological aids that modify dough rheology and improve bread quality. Therefore, there are a lot of bread improvers available in the market such as emulsifiers, hydrocolloids, or enzymes. Moreover, the replacement of wheat flour presents a major technological challenge because gluten which is an essential structure-building protein contributes to the appearance and crumb structure of baked products.

3.3.1 Emulsifiers

Emulsifiers are in the general class of compounds called surface active agent or surfactants. It is fatty substance possessing both lipophilic and hydrophilic moieties (Stampfli and Nersten, 1995; Gomez *et al*., 2004). This specific chemical structure enables emulsifier to concentrate at the oil in water interphase promoted the stability of a thermodynamically unstable system and possibility of forming complexes with starch and protein (Forssell, *et al*., 1998). However, emulsification is often of secondary importance. Starch complexing, protein strengthening, and aeration may be the primary function as well as fat sparing effects are also of importance (Orthoefer, 2008).

Emulsifiers are multifunctional ingredients and have been widely used to improve the quality of baked products. Food emulsifiers can be classified on the basis of several characteristics (Kamel and Ponte, 1993) such as origin: either synthetic or natural, solubility properties, the presence of functional groups, hydrophilipophilic balance (HLB), potential for ionization (nonionic versus ionic). In practice, the most widely used methods are the HLB index and the potential for ionization. The HLB index is based on the relative percentage of hydrophilic to lipophilic groups within the emulsifier molecule (Stampfli and Nersten, 1995).

The major functions of emulsifier in baked products are as follows (Orthoefer, 2008).

(a) To assist in blending and emulsification of ingredients
(b) To enhance the properties of shortening
(c) To beneficially interact with the components of the flour and other ingredients in the mix

Emulsifiers can improve both of dough quality and finished product by function as dough conditioners, resulting in improving of dough machining and handling properties (Stampfli and Nersten, 1995; Gomez *et al*., 2004;

Sawa *et al*., 2009) such as improved tolerance to resting time, shock and fermentation, greater dough strength, improved rated of hydration and water absorption, improved gas retention resulting in lower yeast requirements, better ovenspring, faster rate of proofing, and improved symmetry. Moreover, starch-complexig surfactants retard retrogradation of starch resulting in the prevention of side-by-side stacking of starch helices. Thus, nucleation sites for retrogradation or recrystallization are reduced (Hasenhuettl, 2008). Consequently, the finished products obtained superior quality such as greater loaf volume, brighter crumb, more uniformity in cell size, and longer shelf life (Stampfli and Nersten, 1995).

According to the required properties in bread making, the emulsifiers are normally divided into dough strengtheners and crumb softeners (Stampfli and Nersten, 1995; Gomez *et al*., 2004) as shown in Table 3.

Table 3 Classification of some emulsifiers based on their properties

Classification	Emulsifier	Abbreviation	EEC No	Softening	Strengthening
Amphoteric					
	Lecitihin	None	E322	Good	None
Ionic					
- cationic	Not used in foods				
- anionic	Diacetyl tartaric acid esters of monoglycerides	DATEM	E472e	Fair	Excellent
	Sodium stearoyl-2-lactylate	SSL	E481	Very good	Excellent
	Calciumstearoyl-2-lactylate	CSL	E482	Good	Excellent

Table 3 (Continued)

Classification	Emulsifier	Abbreviation	EEC No	Softening	Strengthening
Nonionic					
	Monodiglycerides	MDG	E471		
	Distilled monoglycerides	DMG		Excellent	None
	Ethoxylated monoglycerides	EMG		Poor	Very good
	Sucrose esters of fatty acids	SE	E473	Good	Excellent
	Polysorbate-60	Poly-60	E435	Fair	Very good

Source: Stampfli and Nersten (1995)

Emulsifiers include compounds with a completely different chemical structure and diverse mechanisms of action performing different effects in dough and bread. The action of the emulsifier begins with dough preparation and ends with oven baking and storage (Figure 9). Additionally, the emulsifiers increase dough stability as concentration-dependent (Gomez *et al*., 2004). Thus, several studies have been done to optimize bread quality with different emulsifiers.

Dough conditioning refers to the development of less tacky, more extensible doughs which help dough was processed through machinery without tearing or sticking.

3.3.1.1 Dough strengthener

In the production of yeast-raised products, the mixing of the dough results in gluten-gluten bonding through disulfide linkages. Development of the linkage is often incomplete resulting in weak dough structure. The gas produced by the yeast escapes through the weak portion of the gluten films. Gas cells having weak gluten cell walls have a tendency to collapse. The rheological properties of the dough are very important characteristics in bread production (Stampfli and Nersten, 1995; Bollain and Collar, 2004). Therefore, emulsifiers are used as dough conditioners to obtain a good machine tolerance.

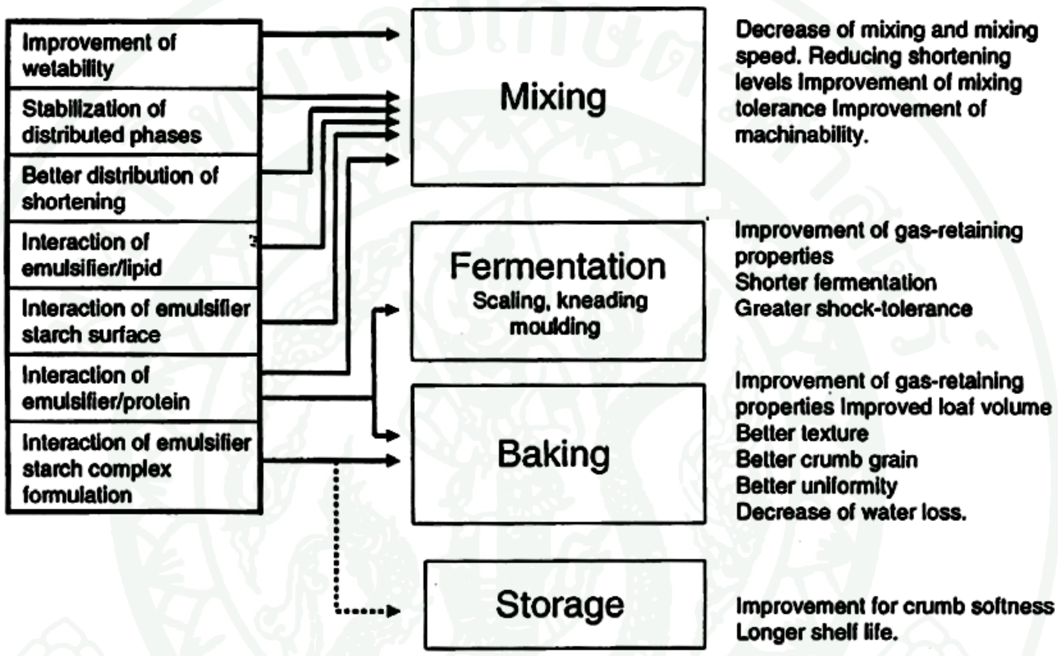


Figure 9 Influence of emulsifiers on production and quality of baked products.

Source: Orthoefer (2008)

Succinylated monoglyceride (SMG), diacetyl tartaric acid esters of monoglyceride (DATEM) and other lactic acid derivatives, sodium stearoyl lactylate (SSL) and the calcium form are the most commonly used as dough strengtheners (Orthoefer, 2008). Their effects were performed during fermentation, mechanical handling, shaping and transport, and proofing resulting in higher volume and better crumb structure of finished product. The structure of some dough strengtheners are shown as follows (Figure 10).

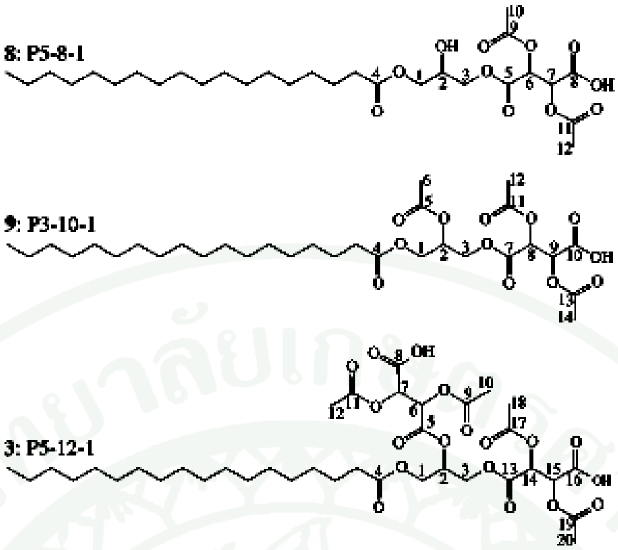


Figure 10 Structures of major DATEM components.

Source: Stampfli and Nersten (1995)

The good dough strengtheners should be able to form liquid films of lamellar structure in the interphase between gluten strands and starch. This interaction reduced electrostatic repulsion and/or increased hydrophilic/hydrophobic interactions (Stampfli and Nersten, 1995; Sawa et al., 2009) allowed gluten to form film which retains the gas produced by yeast (Stampfli and Nersten, 1995). DATEM showed a positive influence on the fermentation and strong CO_2 retention coefficient. It was effective with regard to forming amylose lipid complexes. However, some emulsifiers had no improving effects at low levels such as sucrose fatty acid, ester, DATEM, and SSL (Stampfli and Nersten, 1995).

3.3.1.2 Crumb softener

Emulsifiers that complex with starch are referred to as "crumb softeners". During bread preparation and baking, amylose polymers associate upon cooling forming a strong gel after 10-12 h. After baking, amylopectin crystallizes more slowly resulting in firming of the bread in 3-6 days (Orthoefer, 2008). Emulsifiers do not soften the bread but they inhibit the firming of the bread crumb, associated with staling by retarding the rate of starch crystallization, particularly interaction with the linear amylose fraction, or slow the rate of bread firming by forming the complex with the amylopectin fraction within the starch granule (Kamel and Ponte, 1993).

The use level of crumb softeners is varied widely. The most commonly use crumb softeners are the water emulsions, hydrates, or monoglycerides (Orthoefer, 2008). Mono-glycerides are widely used as anti-staling agents and account for approximately one third of the emulsifiers used in the baking industry (Stauffer, 2000). The anti-staling properties of monoglycerides are attributed to their ability to complex with amylose. This complex is insoluble in water; therefore some part of the amylose does not participate in the gel formation during baking process (Knightly, 1988; Stampfli and Nersten, 1995). Most studies have shown that monoglycerides reduce the rate of staling rather than influence the initial crumb firmness and vary according to their ability to form inclusion complexes with amylose (Knightly, 1988). The monoglycerides containing longer chain saturated fatty acid are reported to have greater anti-staling properties than those containing unsaturated fatty acids. In addition, some long chain saturated monoglycerides (C16:0, C18:0, and C22:0) had less impact on mixing properties (Sawa *et al*., 2009).

3.3.2 Hydrocolloids

Hydrocolloids is a term referred to a range of polysaccharides and protein that are widely used in a variety of industrial sectors to perform several functions such as thickening and gelling aqueous solutions, stabilizing foams, emulsions and dispersions, inhibiting ice and sugar crystal formation and the controlled release of flavours (Williams and Phillips, 2000). In bread making, it was used to induce structural changes in main components of wheat flour systems along bread making step were shown to affect pasting properties, dough rheological behavior, and bread staling (Bollain and Collar, 2004). Hydrocolloids strengthen dough trough different mechanism. Thus, in bread industries hydrocolloids are of increasing importance as bread making improvers.

They are widely used in the bakery industry to modified texture and appearance properties to cereal-based foods (Gallagher *et al*., 2004). Hydrocolloids affect bread making performance and keepability of stored bread. Their effects on the functional performance of dough and subsequent bread quality depend on their nature, origin, and particle size. The presence of hydrocolloids influenced melting, gelatinization, and retrogradation characteristic of starch which impacted pasting properties, dough rheological behavior and bread staling (Collar *et al*., 1999). Thus, a number of studies have used a range of starches or hydrocolloids for making gluten-free bakery products. Hydrocolloids when used only in small amounts are expected to increase loaf volume and water retention because of their strong water binding abilities (Gallagher *et al*., 2004) but decrease firmness and starch retrogradation (Collar *et al*., 1999). In this section, some hydrocolloids were described subsequently for their properties including xanthan gum, hydroxy methyl cellulose, and microcrystalline cellulose.

Xanthan gum, at low concentrations provides storage stability and water binding capacity. Its rheological property is important in bakery products during dough preparation such as prevents lump formation during kneading process and improves dough homogeneity during moulding (Collar *et al*., 1999). It was reported that xanthan induced desirable dough strengthening as the resistance to extension of dough. Collar *et al*. (1999) reported the incorporation of xanthan with pectin, *alpha*-amylase, and sucrose in to dough formula improved the quality of dough by the reduction of the induced softening effect. The effects of xanthan on dough rheology and quality of gluten-free formulations were evaluated by Lazaridou *et al*. (2007). They reported that xanthan had pronounced effect on viscoelastic properties yielding strengthened doughs that resistance to deformation than other hydrocolloids such as carboxymethylcellulose, pectin, agarose and β-glucan and can improved the whiteness of bread crumb. However, some study found a decrease in loaf volume of gluten free bread with increasing xanthan gum level (Schober *et al*., 2005; Lazaridou *et al*., 2007).

Cellulose derivatives, mainly hydroxypropylmethyl cellulose (HPMC) had proved to increase water absorption and to give softer doughs and breads with improved sensory characteristics and longer shelf-life. Synergistic effects with carboxymethylcellulose such as decreased cohesiveness and increased dough hardness had been described (Collar *et al*., 1999). The results from Barcenas and Rosell *et al*. (2001) confirmed the ability of the HPMC for improving fresh bread quality and for delaying staling. The presence of HPMC decreased the hardening rate of the bread crumb and also retarded the amylopectin retrogradation. Furthermore, HPMC showed the best results to improved rice flour dough (Kadan *et al*., 2001; Gujral *et al*., 2003). The dough retained the carbon dioxide produced during leavening and to form a bread crumb-like structure compared to others different hydrocolloid such as locust bean gum, guar gum, carrageenan, and xanthan gum. Sivaramakrishnan *et al*. (2004) also stated that the rheological measurements from the oscillation tests and creep tests showed that the rice dough with 1.5% and 3.0% HPMC had similar rheological properties to that of wheat flour dough and was suitable for making rice bread.

Microcrystalline cellulose (MCC) is a unique ingredient used mainly in the pharmaceutical and food industries to solve products or processing problems (Imeson and Humphreys, 1997; Iijima and Takeo, 2000). It is a hydrophilic, water-insoluble, linear, and high molecular weight polymer consisting of crystalline areas. According to its characteristic, MCC can be called a multifunctional ingredient because it can work as a viscosity controller, gelling agent, texture modifier, suspension stabiliser, fat mimetic, ice crystal suppressant, water absorber, non-adhesive binder, emulsifier, etc. (Iijima and Takeo, 2000).

MCC is a purified, partially depolymerised fraction of α-cellulose. It cosists of anhydroglucose units linked together through a β-(1-4) glycosidic bond (Figure 11) (Imeson and Humphreys, 1997). It prepared from naturally occurring cellulose that has been purified. The hydrolysis under controlled conditions brings out the stable cellulose microcrystals, which are composed of tight bundles of cellulose chains in a rigid linear arrangement. MCC has been used widely as an additive for pharmaceuticals, foods, cosmetics and general industrial use. In addition, its application for dietary fibre has been drawing much attention in the food industry field (Iijima and Takeo, 2000).

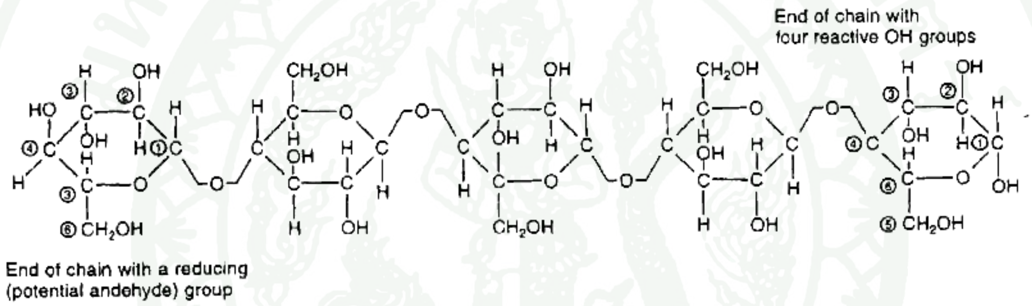
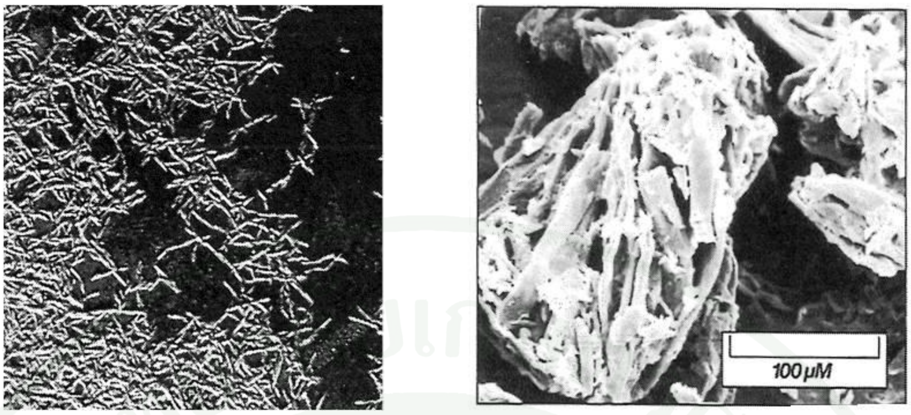


Figure 11 The molecular structure of microcrystalline cellulose.

Source: Imeson and Humphreys (1997)

There are three types of commercialised MCC used in food such as (a) powder type MCC (b) colloidal type MCC and (c) cream paste type MCC (Imeson and Humphreys, 1997). The scanning electron micrograph of MCC was shown in Figure 12. The process for manufacturing these three types of MCC comprises three stages shown in Figure 13 (Iijima and Takeo, 2000).



(a) (b)

Figure 12 Scanning electon micrograph of a 3-D network of colloidal micro-crystalline (a) and particle of powdered microcrystalline cellulose (b).

Source: Imeson and Humphreys (1997)

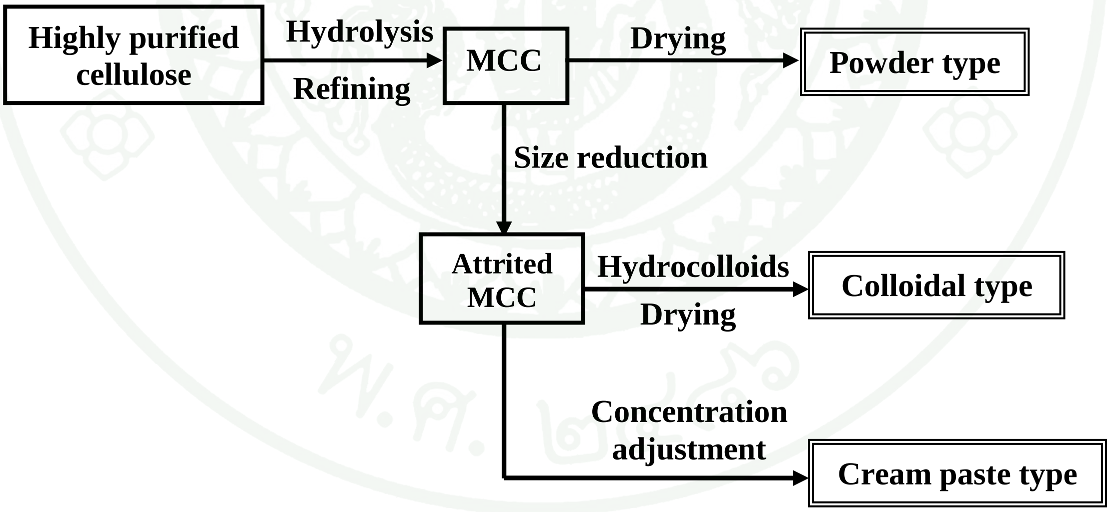


Figure 13 Process for manufacturing three types of microcrystalline cellulose.

Source: Iijima and Takeo (2000)

3.3.3 Enzymes

For decades enzymes such as malt and microbial *alpha*-amylases have been used for bread making. Due to the changes in the baking industry and the demand for more variety and natural products, enzymes have gained more and more importance in bread formulations. The use of enzymes instead of chemical oxidants is a very interesting option to improve bread making performance of dough because they are not only perceived as natural products but also as non-toxic food components which could be a clean label declaration (Rosell *et al*., 2001).

Through new and rapid developments in biotechnology, a number of enzymes have recently been made available to the baking industry. For example, xylanase used as dough-conditioning enzyme can improve the dough handling with single activity instead of traditional hemicellulase preparation. A lipase has a gluten strengthening effect that results in more stable dough and improved crumb structure similar to DATEM or SSL and a maltogenic *alpha*-amylase in particular from *Bacillus stearothermophilus* that has a unique and very effective anti-staling effect (Si and Drost-Lustenberger, 2002; Goesaert *et al.*, 2009b). The reaction of enzyme on bread dough improved their viscoelastic and structural properties, therefore, better performance for bread making. Enzymes are specific biological catalysts able to react under mild conditions of temperature and pH, contributing to the formation of covalent bonds between polypeptide chains. Many enzymes have been studied and applied in bread product such as *alpha*-amylase, glucose-oxidase as well as transglutaminase.

(a) *Alpha* amylase can be extracted from animal, plant and microbial kingdoms. They have particular importance in the brewing, distilling and baking product (Muralikrishna and Nirmala, 2005). Several *alpha*-amylases are reported to reduce distinctly the rate of hardening of bread under appropriate conditions. It hydrolysed amylose and amylopectin to produce the particular length of dextrins, especially soluble intermediate-size dextrins of DP2 – DP12 (Si and Drost-Lustenberger, 2002). Its functionality affects dough properties such as gassing power and consistency. The addition of *alpha*-amylase which has not only a substantial anti-staling effect but also improves the elasticity of bread crumb is quite popular and necessary on bread making. Kim *et al*. (2006) reported that bread contained *alpha*-amylase have been reported in improvement of gas cell distribution and softness of breadcrumbs without lowering the loaf volume. The amylase functionality may also be related to the reduction of dough viscosity during starch gelatinization, thus prolonging oven rise and resulting in an increased loaf volume. The addition of amylases increase the level of fermentable and reducing sugars in flour and dough, promoting yeast fermentation and the formation of Maillard reaction products (Goesaert *et al.*, 2006) resulted in better crust color and improved flavor of bread (Si and Drost-Lustenberger, 2002). In addition, Watanabe *et al*. (2004) have indicated that the bread containing pre-germinated brown rice can suppressed staling during storage time.

However, if bread contained an overdose of enzyme, it will commonly lead to excessive liquefaction and dextrinization, yielding bread with a wet sticky crumb (Si and Drost-Lustenberger, 2002; Gujral *et al*., 2003; Hallen *et al*., 2004) and too little causes dry, crumbly and high loaf density (Hallen *et al*., 2004). In addition, *alpha* – amylase in the form of malt flour increase the flour capacity to hydrolyze the starch. The activity of *alpha* –amylase as well as the levels of maltose and glucose increased during germination. Hence, the addition of flour from malted grains also enhances the growth yeast in dough. Therefore, it seems important to study the potential application of malt as a source of *alpha* – amylase to enhance viscosity and dough properties. However, limited studies have reported on the effect of malt on dough rheology.

Maltogenic *alpha*-amylase, a thermostable enzyme, is able to hydrolyse the glycosidic linkages of the gelatinized starch during the baking process. It acted as an exo-acting amylase with more pronounced endo-action at higher temperatures. This enzyme caused extensive degradation of the crystallizable amylopectin side chains and thus limited amylopectin recrystallization and network formation and water immobilization during storage (Si and Drost-Lustenberger, 2002). The major advantage of this enzyme is its tolerance to overdosing during bread making. Fungal *alpha*-amylase can cause the sticky dough or over browning of the crust when using in excess amount but this enzyme has low activity at a temperature under 35 ^{o}C. It is highly active only at a temperature during starch gelatinization while it does not excessively degrade the starch by produces small soluble dextrins. The overdosing risk is, therefore, lower than another type of amylase (Si and Drost-Lustenberger, 2002). The functionality of maltogenic alpha-amylase was proposed in Figure 14.

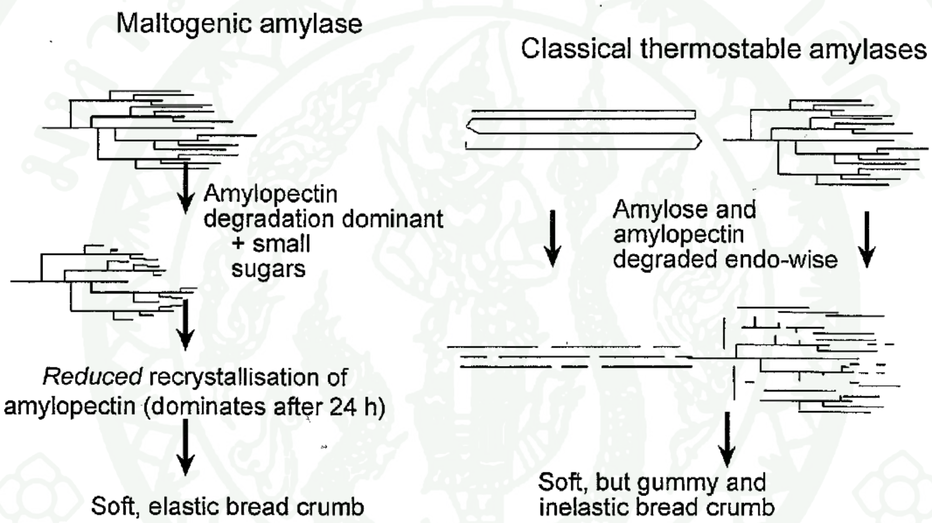


Figure 14 A model suggestion of modified amylopectin by maltogenic and thermostable bacteria *alpha*-amylase.

Source: Si and Drost-Lustenberger (2002)

(b) Glucose Oxidase (GO) (EC 1.1.3.4) catalyses the oxidation of β-D-glucose to gluconic acid and hydrogen peroxide. Many researches indicate that hydrogen peroxide produced during GO reaction causes the oxidation of the free sulfhydryl units from gluten protein giving disulfide linkages and the gelation of water soluble pentosans, changing rheological properties of wheat flour dough (Gujral and Rosell, 2004; Bonet *et al*., 2006).

According to its good oxidising effects, loaves of bread will increase in volume and had better appearance. GO caused the crosslinking of water soluble protein (albumin and globulin) fraction in wheat flour, involving in both disulfide and non-disulfide linkage (Rasiah *et al*., 2005). Normally, rice has very little prolamin (2.5-3.5%), as a result a dough is not formed when rice flour is kneaded with water. Therefore, the gases produced during proofing and baking cannot be retained and resulting product has a low specific volume. GO can modify the vicoelastic properties of rice flour promoted lowering the thiol and amino group concentration resulting in less stiff dough with better specific volume and texture bread (Gujral and Rosell, 2004). GO is the currently preferred enzyme alternative to chemical oxidising agents for bread improvement. The use of GO in combination with other enzymes and surfactants for the production of wheat bread has been reported. However, the excessive addition of GO produced an excessive crosslinking in the gluten network resulting in the negative effect on the bread making properties (Bonet *et al*., 2006).

(c) Transglutaminase (protein-glutamine γ-glutamyltransferase, EC 2.3.2.13) (TG) has been reported extensively for the ability to crosslink different food proteins. TG is able to improve the functionality of flour proteins through the formation of large insoluble polymers (Caballero *et al*., 2007). It catalyzes acyl-transfer reactions, introducing covalent crosslinks in proteins. Crosslinks form between lysine residues and glutamine residues to form an ϵ-(γ-Glu)-Lys bond without decomposing the nutritional quality of the lysine residue. Since it is a natural enzyme that is destroyed during cooking, microbial transglutaminase has been approved as a processing aid and unnecessary to be declared on ingredients labels (Gerrard *et al*., 1998).

Nevertheless**,** combining enzymes to maximise shelf life is feasible to use with the aims of improving the bread quality parameters such as volume, dough stability, and crumb structure. The synergistic effects of combination enzymes such as *alpha*-amylases, xylanase and protease significantly affected to viscoelastic properties of dough and exhibited a significantly anti-staling effect (Caballero *et al*., 2007) as the polysaccharide and gluten degrading enzymes.

4. Multivariate approaches

The quality of food products is determined by their certain characteristics for example, sensory attributes, chemical compositions, and physical properties. Among those factors the sensory attributes have the crucial influence on the market success of the product (Probola and Zander, 2007). Evaluation of production variability requires a large number of various data. Therefore, multivariate statistical methods are employed to reduce variables and give presentation of the results in a clear graphical way (Næs *et al*., 1996).

There are many techniques used to solve that problem such as principal component analysis (PCA), cluster analysis (CA), etc. Generally, there are often significant correlations between variables. The presence of such covariation means that the significant information can be described by means of a smaller number of variables than those actually measured.

4.1 Principal component analysis (PCA)

PCA is a frequently applied method for multivariate overview analysis of sensory data. The main purpose is to interpret any latent factors spanned by many characteristics such as flavor, odor, appearance and texture as well as to find products that are similar or different, and what discriminate those profiles (Westad *et al*., 2003).

The data is arranged into a matrix X with one column for each variable and one row for each object. This matrix is then decomposed such that

$$X = TP' + E$$

Where T and P are score and loading matrixes, respectively, with one column for each principal component (PC) included. The residual matrix, E, includes the variance not explained by any PC, and is usually considered as noise. The PCs are orthogonal and describe independent variation structures in the data. The first PC always explains the greatest part, and the following PCs successively explain smaller parts of the original variance. Graphical overviews of the samples and variables are obtained by score- and loading-plots, respectively, ideally showing a large part of the variance in two dimensions.

The score plots shows how the samples relate to each other. The samples with similar values of many characteristics will appear close to each other. The pattern of variation is visualized by loading plots. Variables which appear close together in loading plot are positively correlated, whereas those explained by different PCs vary independently. Both sample and loading plots can be interpreted together for better explanation.

The PCA method can be considered as a special tool that data are integrally used without any loss of information. Several researches had been determined using PCA technique to classify or differentiate products based on their characteristics. The research related to bread had also been conducted using PCA to identify a consumer segment (Heenan *et al*., 2008; Kihlberg and Risvik, 2007).

Moreover, the popularity of preference mapping techniques has increased in recent years. The key aspect of this method is the ability to relate sensory profiles and acceptability data explaining intrinsic characteristics of the product that drive consumer preference. Therefore, a considerable number of preference mapping applications are reported in the literatures of many kind of food products, for example

kiwifruit (Jaeger *et al*., 2003), cracker (Martinez *et al*., 2002), mayonnaise (Santa Cruz *et al*., 2002), and some starchy food (Monteleone *et al.*, 1998).
This methodology can be used to address a wide range of problems. It is, therefore, a useful technique in new product development.

4.2 Descriptive Discriminant Analysis (DDA)

Discrimination was defined as the using of information in a learning set of labeled observations to construct a classifier that will separate the predefined classes as much as possible (Izenman, 2008).

Descriptive discriminant analysis (DDA) was then a multivariate technique used to identify explanatory variables that caused significant differences among samples (Huberty, 1994). When numerous variables are available for analysis, stepwise discriminant analysis can be used to identify the variables that have significant discriminating power for classification purposes, and to build a discriminant model based on these variables (Worth and Cronin, 2003).

In consumer research, when all attributes were compared, DDA can be used to classify significant attributes that account for differences among the products in terms of consumers' perceptions. It was also applied for the product classification based on sensory evaluations in quality control assessment (Resurrecion, 1998; Granitto *et al*., 2008).

Several studies have used this method for identifying the discriminant parameters for product differentiation. For example, Scriven and Seaman (1990) used this method to investigate consumer perception of bananas treated with ethylene or allowed to ripen naturally and indicated that the decreasing liking of skin color and softness were essentially the only attributes consumers could use to distinguish between bananas. Martin *et al*. (2001) reported that discriminant analysis and PCA allowed discrimination between green and roasted *arabica* and *robusta* coffees using fatty acid profile as discriminant parameters.

4.3 Logistic Regression Analysis (LRA)

Logistic regression is a predictive analysis which based on the assumption that a logistic relationship (i.e. a sigmoidal dependency) (Figure 15) exists between the probability of group membership [E(Y)] and one or more predictor variables (X) (Worth and Cronin, 2003). If there are two groups, binary logistic regression (BLR) is used.

This analysis involves the prediction of the likelihood of the outcome, a dichotomous dependent variable (yes/no), based on the predictor variables which are quantitative or categorical. It is not limited to a single predictor, making it suitable for use in consumer study, in which the acceptability and purchase intent (dependent variables) are predicted by the sensory attributes.

This methodology determines the probability of success (event) over the probability of failure (non event) called 'odds ratio' with the results in the form of likelihood. It use as a descriptive statistic and plays an important role in logistic regression. The odds ratio can be any nonnegative number with the value that is greater than 1.0 when a success is more likely to occur than the failure. For example, when odds ratio is 2.0, it means that an event is two times as likely as non event (Agresti, 1996).

LRA is applied in consumer test to predict both product acceptability and purchase intent based on the odds ratio point estimate of consumer perception. Ares *et al*. (2008) studied the shelf life estimation of minimally processed lettuce and reported that results from LRA could suggest consumers' consideration and decision making whether to purchase or to consume the lettuce during the different storage conditions.

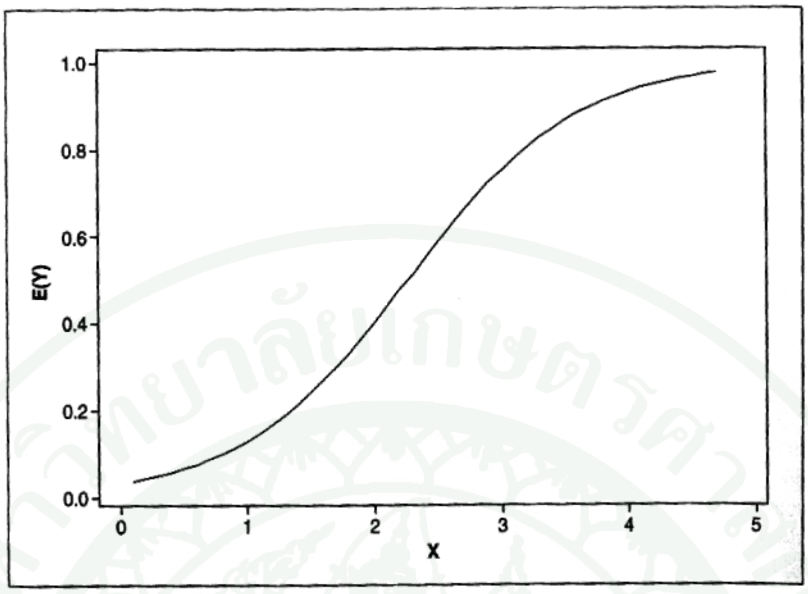


Figure 15 Typical function graph for logistic regression.

Source: Ryan (2009)

5. Consumer research

In the process of new product development which can originate from new technology or new market opportunities, consumers are the ultimate judge for a new product success. Consumers also play an important role as the most appropriate tool for determining food product quality and shelf life (Gimenez *et al*., 2007). Therefore, a deep understanding of 'the voice of the consumer' (van Kleef *et al*., 2005) is the most important key of the success. Accordingly, consumer research required systematic controls and the use of standard practices to access the accurate personal responses (Resurrecion, 1998).

The perceive freshness of foods is a complex process that involves interactions of sensory sensations attributed to appearance, odour, taste, flavour, and oral texture of products (van Kleef *et al*., 2005). Those characteristics and perceived freshness affect consumers' purchase of bread (Ginon *et al*., 2009). In addition, consumer perceptions of bread may be influenced by social, demographic, and product experiences which depend on different sensory characteristics to form a judgement of freshness. For this purpose, multivariate techniques have been commonly applied to investigate complex consumer perceptions and the sensory drivers. In addition, several studies have used a multivariate approach combined with sensory descriptive analysis to help interpret consumer perceptions of products (Heenan *et al*., 2008).

Just-about-right (JAR) rating scale has been included in the questionnaire in sensory consumer test and marketing research (Gacula *et al*., 2007). These scales, categorical variables, are an approach to the measurement of the perceived attribute intensities that assess whether there is too little, too much, or a JAR level of a particular attribute (Lawless and Heymann, 1999; Gacula *et al.*, 2007). It is usually expressed as the percentage of respondents who consider the product according to those scales. The JAR method can also be used to quantify on a non-linear scale which used for quantitative and qualitative analysis of the typicality concept (Cadot *et al.*, 2010). However, Popper *et al*. (2004) reported that the presence of JAR questions in the questionnaire affects overall liking.

The analysis based on signal-to-noise ratio (SNR) provides robustness of the product evaluated for quality control with the goals of maximize the product effect and minimize the random variability or noise effect (Gacula *et al.*, 2007). The SNR statistic can be applied to JAR rating scale where the target value is the middle category of the scale. Gacula *et al*. (2007) stated that this analytic method provide a new way to look at the JAR data, especially with respect to hedonics and product improvement.

For a data set with *n* panelists (i = 1, 2, . . , n), the SNR for a particular treatment or product is computed by the following formula,

$$SNR = \Sigma\{-10 \times \log[(Yi - 3)^2 + k]\}/n$$

Where;

1. The constant, value “3” in this formula, is the JAR scale category value.
2. The constant k value can range from 0.1 to 1.0, and is arbitrary, but should be fixed for any set of comparisons.

A graphical plot of intensity versus SNR forms an inverted letter V. It indicate that a desirable SNR should be large, suggesting a robust product or production process (Figure 16). Therefore, the condition that generates the highest possible value of SNR is close to the target value of 3 on the 5-point scale or JAR (Gacula *et al*., 2007).

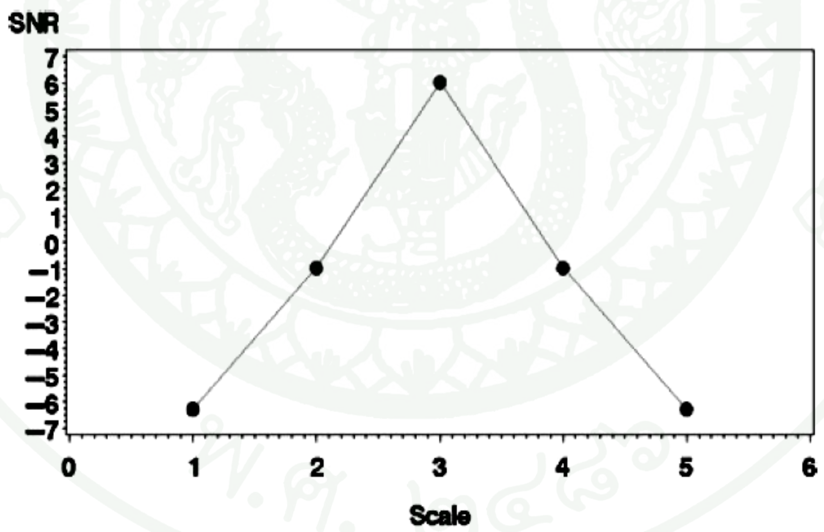


Figure 16 The expected relation between signal-to-noise ratio (SNR) and 5-point just- about-right scale.

Source: Gacula *et al*. (2007)

Penalty analysis or mean drop analysis is a method for determining it the rating of JAR of respondents for a specific attribute are associated with a drop in choice measure, most commonly overall liking. The penalties are calculated by subtracting the mean hedonic score for the JAR group from either of the means of the too weak (not enough) or the too strong (too much) groups (Cavitt *et al.*, 2005). Penalty analysis is an effective tool for linking attribute performance to overall liking (Schraidt, 2009).

McNemar's test, a chi-square statistic for dichotomous variable (two classes), is most often applied to categorical variables independent observations (Agresti, 1996; Lawless and Heymann, 1999). It is a simple way to test marginal homogeneity for matched binary responses in a two-by-two matrix. The test may have a one-tailed or two-tailed alternate hypothesis. This is a test originally designed for before and after experiments such as the effect of information on attitude change. It can be used in the situation that consumers examine products and their responses are two classes. For example, it could be applied to observe if consumers' liking score of the product change after providing consumers with some information about a product such as nutritional quality (Lawless and Heymann, 1999). The subsequent table (Table 4) showed the test classified responses in a two-by-two matrix, with the same responses categories as rows and columns. The two cells with the same values of row and column variable are not included in the calculation because the test was designed to examine differences (eq. 1).

Moreover, this method allows the researcher to determine whether there is a significant difference in JAR scores distributions between two products when JAR scale values have been combined in two categories and the same assessors evaluated both products (Rothman and Parker, 2009).

Table 4 McNemar's test

After information presented	Before information presented	
	Number of consumer liking the product	Number of consumer disliking or neutral
Number of consumer liking the product	a	b
Number of consumer disliking or neutral	c	d

Source: Lawless and Heymann (1999)

$$\text{Chi-square} = \frac{(|b-c|-1)^2}{b+c} \qquad \text{eq. 1}$$

MATERIALS AND METHODS

Materials

1. Raw materials

1.1 Brown rice

Two cultivars of Thai rice: Khao Dawk Mali 105 (*Oryza sativa* L. cv. KDML 105) and RD6 (*Oryza sativa* L. cv. RD6, glutinous brown rice) harvested in 2008 and 2009 were purchased from Department of Agriculture, Thailand. These two cultivars were selected because they are commercially available and accumulate a high level of GABA during germination (Varanyanond *et al*., 2005). They were packed in plastic bags made of linear low density polyethylene (LLDPE), placed in containers and stored at 8 ^{o}C before the experiment was carried out. For the quality evaluation of the finished product and consumer acceptance test, rice cultivars harvested in 2009 were used in the study.

1.2 Ingredients for bread making

The ingredients used in the study were food-grade obtained from local market and their suppliers are listed as follows.

1.2.1 Ingredients for bread making in Thailand

(a) Wheat flour (White swan®, United Flour Mill Public Co., Ltd., Samut Prakarn, Thailand)

(b) Instant dried yeast (Fermipan® Brown, GB Ingredients Ltd., Suffolk, UK)

(c) Salt (Prung Thip®, Pure Salt Industry Co., Ltd., Nakhonratsima, Thailand)

(d) Sugar (Lin® sugar, Baanrai Sugar Industry Co., Ltd., Uthai Thani, Thailand)

(e) Fresh CP® eggs, Size No. 2, Bangkok Agro-Industrial Products Plc., Co., Ltd. (Nakornluang Farm), Chonburi, Thailand)

(f) Unsalted butter (Orchid®, Thai Milk Industry Co., Ltd., Ayutthaya, Thailand)

(g) Fibrotec™ HV591 (Microcrystalline cellulose colloid grade with carboxymethyl cellulose, provided by Nova Nutraceutical Technology, LLC. Portland, Oreg., U.S.A.)

(h) Diacetyl tartaric acid ester of monoglycerides (DATEM) (provided by Kerry Bio-Science, Zwijndrecht, the Netherlands)

1.2.2 Ingredients for bread making in the U.S.

(a) Wheat flour (16.9% protein and 11.4% moisture content, Conagra Food Ingredients, Omaha, Ne., U.S.A.)

(b) Instant dried yeast (Fleischmann's®, Ach Food Companies, Inc., Memphis, Tn. U.S.A.)

(c) Iodized salt (Morton International Inc., Chicago, Ill., U.S.A.)

(d) Powdered sugar (Domino FoodsInc., Yonkers, N.Y., U.S.A.)

(e) Butter (Great value™, Wal-mart Stores, Inc., Bentonville, Ar., U.S.A.)

(f) Fresh grade A large eggs (Cal-Maine Foods Inc., Pine Grove, La., U.S.A.)

(g) Fibrotec™ HV591 (Microcrystalline cellulose colloid grade with carboxymethyl cellulose, provided by Nova Nutraceutical Technology, LLC. Portland, Oreg., U.S.A.)

(h) Diacetyl tartaric acid ester of monoglycerides (DATEM) (provided by Kerry Bio-Science, Zwijndrecht, the Netherlands)

2. Reagents

2.1 Buffer

2.1.1 Citrate buffer
2.1.2 Phosphate buffer

2.2 Reagent for chemical analysis

2.2.1 Sodium hydroxide
2.2.2 Dabsyl-Cl acetonitrile solution
2.2.3 Alkaline copper reagent
2.2.4 Nelson reagent
2.2.5 Ethyl alcohol
2.2.6 D-Glucose

3. Test kit

3.1 3M® test kit for aerobic count plate and yeast & mold (3M Petrifilm™ , 3M Company, St. Paul, MN, USA)

3.2 Total starch assay test kit (Megazyme Intl. Ireland Ltd.,Co Wicklow, Ireland)

3.3 Amylose/amylopectin assay test kit (Megazyme Intl. Ireland Ltd.,Co Wicklow, Ireland)

4. Equipments

4.1 Equipments for preparing germinated brown rice flour

4.1.1 Commercial incubator (Siam Incubators System Co., Ltd, Bangkok, Thailand)

4.1.2 Commercial sieve shaker (Kluay Nam Thai Co., Ltd., Bangkok, Thailand)

4.1.3 Commercial tray drying oven (Model HA 200, K.S.L. Engineering Co., Ltd., Bangkok, Thailand)

4.1.4 Pin mill (Alpine Mill, Augsburg, Germany)

4.1.5 Plastic box (18x28x10 cm^3)

4.2 Equipments for bread preparation

4.2.1 Equipments for bread making in Thailand

(a) Automatic home bakery machine (SEV-3983, Severin Elektrogeräte GmbH, Sundern, Germany)

(b) Aluminum pan (19.0 x 10.0 x 7.4 cm^3)

(c) Commercial incubator with humidifier (Siam Incubators System Co., Ltd, Bangkok, Thailand)

(d) Electric oven (Klauy Num Tai Oven, Bangkok, Thailand)

4.2.2 Equipments for bread making in the US.

(a) Aluminum pan (19.0 x 10.0 x 7.4 cm^3)

(b) Electric oven (Turbofan32, Moffat Ltd., Christchurch, New Zealand)

(c) KitchenAid mixer (KitchenAid, Professional 6, St. Loseph, Mich., U.S.A)

(d) Proofing machine (Deluxe equipment Co., Inc., Bradenton, Fl, U.S.A.)

4.3 Analytical equipments

4.3.1 Benchtop Centrifuge (Model 2-16, Sigma, Germany)

4.3.2 Drying oven (Model FD115, WTB BINDER, Germany)

4.3.3 Differential scanning calorimeter (DSC 822^{e} module, Mettler-Toledo, GmbH, Switzerland)

4.3.4 Farinograph (Brabender, Duisburg, Germany)

4.3.5 HPLC (Agilent 1100 Series, Agilent Technologies, Calif., U.S.A) equipped with a column (Supelcosil-LC-DABS 4.6 mm i.d. x 150 mm)

4.3.6 Incubator (Yamato® IC 1800, Yamato scientific Co.,Ltd. Japan)

4.3.7 Novasina (Aw sprint, Model TH-500, Switzerland)

4.3.8 Novasina (Aw TH 200, Novasina, Pfaffikon, Switzerland)

4.3.9 Protein analyzer (Leco model FP528, St Joseph, Mich., USA)

4.3.10 Rapid Visco Analyzer (RVA) (4D, Perten Instruments Group, Narrabeen, Australia)

4.3.11 Spectrophotometer (CM-3500d, Minolta Co., Ltd., Osaka, Japan)

4.3.12 Spectrophotometer (CM-508d, Minolta Co., Ltd., Osaka, Japan)

4.3.13 Spectrophotometer (UV-160A, Shimadzu Co., Japan)

4.3.14 Texture Analyzer (Lloyd TA-500, Lloyd instruments Ltd., UK)

4.3.15 Texture Analyzer (TA XT plus, Stable Micro system, Texture Technologies Crop, NY., U.S.A.)

4.3.16 Ultra centrifugal mill (Retsch model ZM100, Haan, Germany) with sieve shaker

4.3.17 Water Bath (WB22, Memmert, Germany)

5. Packaging

5.1 Linear low density polyethylene (LLDPE) plastic bag

5.2 Polyethylene (PE) bag

5.3 Ziploc® double zipper Fresh Shield^{TM} Freezer bags (Ziploc®16 , S.C. Johnson & Son, Inc., Racine, Wi, U.S.A)

Methods

1. The study of germination conditions affecting physicochemical properties of germinated brown rice flour

1.1 Preparation of brown rice and germination procedure

Brown rice from two cultivars of Thai rice: Khao Dawk Mali 105 (*Oryza sativa* L. cv. KDML 105), designated as BR, and RD6 (*Oryza sativa* L. cv. RD6, glutinous brown rice), designated as GNBR, were purchased from Department of Agriculture, Thailand. They were packed in plastic bags made of linear low density polyethylene (LLDPE), placed in containers and stored at 8 ^{o}C before the experiment was carried out.

The experiment was carried out by steeping 200 g of brown rice grains in 800 ml of 80 ppm of peroxyacetic acid (Tsunami®100, Ecolab Inc., St Paul, Minn., USA) for 15 min, washing with tap water, and rinsing with distilled water to neutral pH. Subsequently, grains were soaked in steeping water with a grain-to-water ratio of 1:5 w/v at 35 ^{o}C. The steeping water was 50 mM citrate buffer (pH3 and pH5), 50 mM phosphate buffer (pH7) or distilled water (DI, pH 6.8). The non-germinated brown rice flour of each rice variety served as the control. The grains after reaching the required germination period were dried at 55 ^{o}C using a tray dryer (Model HA 200, 15 K.S.L. Engineering Co., Ltd., Bangkok, Thailand) until the flour had reached the final moisture content of 10 $\pm$ 2% and finely ground using an ultra centrifugal mill (Retsch model ZM100, Haan, Germany) with a 0.25 mm screen to produce uniform-size flour. All flour samples were packed in plastic bags made of LLDPE and stored at 8 ^{o}C until further analyses.

1.2 To study the effects of germination conditions on the qualities of germinated brown rice flour

A 4x3 full factorial arrangement in a completely randomized design (CRD) with four pH levels of steeping water (3, 5, 7 and as-is: DI, distilled water) and three levels of steeping time (24, 48 and 72 h for BR; 24, 36 and 48 h for GNBR) were investigated. Two separate batches of GBRFs for both rice varieties were prepared and measured the physicochemical properties as follows.

1.2.1 Chemical analyses

1.2.1.1 Moisture content was determined in duplicate according to the AOAC method 945.14 (AOAC, 1990).

1.2.1.2 Crude protein was determined in duplicate by a combustion method using a protein analyzer (Leco model FP528, St Joseph, Mich., U.S.A.). Percentage of protein was calculated by multiplying %N with a factor of 5.95 (Capanzana and Buckle, 1997).

1.2.1.3 Free GABA content was determined in triplicate using HPLC according to the method of Cohen and Michaud (1993). Two hundred to five hundred milligrams of GBRF were weighed into a plastic tube and 2 mL of deionized water was added. The mixture was centrifuged at 2264 *x g* for 10 min. One mL of supernatant was pipetted and mixed with 200 µL of 0.4 M $NaHCO_3$ and 400 µL of 6 mM Dabsyl-Cl acetonitrile solution. The reaction was performed at 70^{o}C for 20 min. After derivatization, the sample was filtered into a vial and injected into an HPLC (Agilent 1100 Series, Agilent Technologies, Calif., U.S.A.) equipped with column (Supelcosil-LC-DABS 4.6 mm I.D. x 150 mm). Acetonitrile was used as the mobile phase with a flow rate of 1.0 mL/min and injection volume of 10.0 µL. The column temperature was 40 ^{o}C and the ultraviolet detector was set at 315 nm.

1.2.1.4 *Alpha*-amylase activity was measured in duplicate using a Rapid Visco Analyzer (RVA) (4D, Perten Instruments Group, Narrabeen, Australia), following the AACC method 22-08 (AACC, 2000) as shown in the Appendix C. The results were reported as the stirring number (SN).

1.2.1.5 Reducing sugar from GBRF was extracted by using aqueous ethanol (80% v/v). Reducing sugar content was then determined in duplicate according to the Nelson-Somogyi's method (Somogyi, 1951) as shown in the Appendix C.

1.2.1.6 Total starch content was determined in duplicate using a total starch assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) with a pre-treatment before analysis as described below. One hundred milligram of GBRF was put into a centrifuge tube, followed by adding 5.0 mL of aqueous ethanol (80% v/v), and incubated at 80–85°C for 5 min. After incubation, suspensions were mixed using a vortex stirrer, another 5 mL of 80% aqueous ethanol was added, and the mixture was centrifuged at 1000 *x g* for 10 min. Supernatant was carefully poured off, and 1 mL of 1 M NaOH was added to the tube. Tube contents were boiled for 5 min and neutralized with 1 mL of 1 M HCl. Thermostable *alpha*-amylase and amyloglucosidase hydrolysis and glucose measurement using glucose oxidase-peroxidase-4-aminoantipyrine (GOPOD) reagent were conducted according to the standard assay procedure of Magazyme assay kit (Megazyme International Ireland Ltd.).

1.2.2 Physical measurements

Pasting profiles of GBRF passing through a 80 mesh sieve was analyzed in triplicate using the RVA (4D, Perten Instruments Group) following the AACC method 61-20 (AACC, 2000).

1.2.3 Statistical analyses

All physicochemical data of GBRF obtained from each rice variety were analyzed by analysis of variance (ANOVA). The Duncan's Multiple Range Test (DMRT) was performed for post-hoc multiple comparison. Statistically significant difference was established at $P<0.05$. Principal component analysis (PCA) was carried out to examine the visualization of the differences among GBRFs based on the physicochemical properties by using XLstat2007 software (Addinsoft, Paris, France).

2. The study of germination conditions affecting selected quality of composite wheat-germinated brown rice flour and bread formulations

The GBRFs with a high free GABA concentration and desirable pasting properties were selected for bread making and evaluated the quality as the following procedures.

2.1 To study the effects of germination conditions on *alpha*-amylase activity and pasting properties of composite flour

Composite flours were prepared by blending wheat flour with BRF or GBRFs at a 70:30 ratio followed a completely randomized design. These composite flours were subjected to further analyses and compared to wheat flour (Control). All composite flours were determined for *alpha*-amylase activity and pasting characteristics in triplicate using the RVA (4D, Perten Instruments Group) as the same procedure described in the method sections 1.2.1.6 and 1.2.2, respectively.

4.3 To study the effects of germination conditions on the quality of bread containing composite flours

4.3.2 Bread making procedure

Bread were prepared either with 450 g flour of 100% wheat flour (control), wheat:BRF or wheat:GBRFs at 70:30 ratios and mixed with others ingredients (Table 5). Bread doughs were mixed in duplicate using an automatic home bakery machine (SEV-3983, Severin Elektrogeräte GmbH, Sundern, Germany). All ingredients were mixed for 30 min and the dough was removed and divided into 300 g portion. Each portion was then manually rounded and rested for 10 min. Dough was punched, rolled, put into a well greased aluminum pan (19 x 10 x 7.4 cm^3), and proofed at 35 ^oC and 75% RH for 75 min using a commercial incubator with humidifier. The baking process was carried out at 180^oC for 30 min using an electric oven. After the bread had been cooled, it was placed in a polyethylene bag and stored at room temperature (28 ^oC) for 24 h before subjected to further analyses. It should be noted that the bread formulation used in this study was a very rich (high sugar and high fat with egg product) formulation, not a standard research bread formulation.

Table 5 Bread formulations (g) made with wheat flour or composite wheat-germinated brown rice flour prepared from various germination conditions

Flour,	Formulation No.					
pH/Steeping time (h)[A]	1	2	3	4	5	6
Wheat flour	100	70	70	70	70	70
BRF	-	30	-	-	-	-
GBRF, 3/24	-	-	30	-	-	-
GBRF, 3/48	-	-	-	30	-	-
GBRF, 6.8/24	-	-	-	-	30	-
GBRF, 6.8/48	-	-	-	-	-	30
Others[B]	120.25	120.25	120.25	120.25	120.25	120.25

[A] BRF = nongerminated brown rice flour; GBRF = germinated brown rice flour
[B] Others (%, w/w) were instant dried yeast, 1.25 %; salt, 1%; sugar, 34 %; fresh whole egg, 10 %; butter, 20 %; water, 54 %.

4.3.3 Physical measurements

4.3.3.1 The surface of bread crumb was photocopied to compare the gas cell distribution.

4.3.3.2 Water activity (a_w) of bread crumb was evaluated in duplicate using a_w meter (Aw TH 200, Novasina, Pfaffikon, Switzerland).

4.3.3.3 Loaf volume was measured in duplicate using the rapeseed displacement method, and specific volume was subsequently calculated as loaf volume/weight (mL/g). Expansion ratio (%) was calculated from the mean value of specific volume of each sample/mean specific volume of the control x 100.

4.3.3.4 Crumb color was measured in duplicated using a spectrophotomer (CM-3500d, Minolta Co., Ltd, Osaka, Japan), and reported as L*, a*, b*, C* (Chroma) and h* (Hue angle).

4.3.3.5 Crumb texture was determined in triplicate by texture profile analysis (TPA) method using a texture analyser (TA-500, Lloyd instruments Ltd., UK). The bread slice of 7x 8 x 2.5 cm^3 and 7 x11 x 2.5 cm^3 for bread containing composite wheat-GBRF and the control, respectively was compressed to 40% deformation at a cross-head speed of 2 mm/s with a trigger force of 20 g to compress the middle of the bread (Moore *et al*., 2006) using a compression probe (diameter of 50 mm). Results [hardness (N), springiness (mm) and cohesiveness] were the mean of six measurements from two different sets of breads.

4.3.4 Sensory evaluation

Consumer acceptance test was preliminarily conducted in this study. Consumers (N = 60; 70% female and 30% male; 18-45 years of age) were recruited from Kasetsart University, Bangkok, Thailand. Criteria for recruitment were that they were regular bread consumers and not allergic to wheat flour and other ingredients used in bread making. The test was conducted in a sensory laboratory with a partitioned booth for an individual consumer at controlled temperature of 25 ^{o}C. Consumers were provided with water to rinse their mouth before testing and between samples. Each consumer was presented with 6 coded pieces of bread (each slice was 5 x 5 x 1.25 cm^3 without the crust).

The serving order was counter balanced, followed the randomized block design. The wheat bread served as the control. Prior to the consumer test, consumers were briefed about the questionnaire and instructed to visually evaluate product acceptability for overall appearance. Each sample was, then, sniffed prior to evaluating aroma acceptability. Afterwards, they were instructed to bite a half piece of bread and masticate the product before providing an acceptability rating for softness, overall flavor, and overall liking. The consumers evaluated the products using the 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely) (Peryam and Pilgrim, 1957), and the paper ballot was used.

4.3.5 Statistical analyses

All data were subjected to analysis of variance (ANOVA). The Duncan's Multiple Range test (DMRT) was performed for post-hoc multiple comparisons. Statistically significant difference was established at $P<0.05$. Principal component analysis (PCA) was carried out to demonstrate visualization of all bread formulations as affected by germination conditions based on the physicochemical properties and sensory acceptability using XLstat2007 software (Addinsoft, Paris, France).

3. The study of incorporation of germinated glutinous brown rice flour affecting the selected properties of composite wheat-germinated brown rice flour and its application in bread

As the utilization of germinated glutinous brown rice flour (GGNBRF) in bread has not been reported at the present time. In addition, waxy flour has been used to improve the quality of wheat bread with the objective of bread staling retardation and shelf life extension by reducing the firming of bread crumb (Hung *et al*., 2007). Therefore, this study was conducted to incorporate the GGNBRF in composite flour wheat-germinated brown rice flour.

3.1 To study the effects of incorporation of GGNBRF in composite wheat-GBRF on *alpha*-amylase activity and pasting profiles of composite flours

GGNBRF obtained from condition leading to high GABA content was used in this experiment. Composite flours were prepared by blending wheat flour with GBRF and GGNBRF at different ratios as follows: Wheat:GBRF:GGNBRF at 70:30:0, 60:30:10, 60:40:0 and 50:40:10 ratios using a completely randomized design. These four composite flours were subjected to *alpha*-amylase activity and pasting characteristics measurement analysed in duplicate and triplicate, respectively using the RVA (4D, Perten Instruments Group) as the same procedure as in the items 1.2.1.6 and 1.2.2, respectively.

3.2 To study the feasibility of using composite flours in bread formulations

Bread formulations were prepared with wheat flour (control) or composite flours as the same ratio as in the item 3.1. Ingredients and procedures for bread making used in this study were the same as of the method section 2.2.1.

To determine the feasibility of using those composite flours in bread making, the consumer acceptance test was preliminary conducted in this study. Consumers (N = 30) were recruited from Kasetsart University, Bangkok, Thailand.

Each consumer was presented with 5 coded pieces of bread (each slice was 5 x 5 x 1.25 cm^3 without the crust). Criteria for recruitment, instructions, serving orders and sample evaluations were conducted following the same procedure as the method section 2.2.2.

3.3 Statistical analyses

All data were subjected to analysis of variance (ANOVA). The Duncan's Multiple Range test (DMRT) was performed for post-hoc multiple comparisons. Statistically significant difference was established at P<0.05.

4. The study of functionality of selected bread improvers on the quality of dough and wheat-germinated brown rice bread

Bread improvers have been reported to help alleviate the quality of bread, especially in terms of textural quality. Fibrotec™ and DATEM were selected improvers according to their water holding capacity and anti-staling effect, repectively. Thus, in this study the functionality of selected improvers would be studied in bread containing composite wheat-germinated brown rice flour obtained from the study 3.

4.1 To study the effects of selected improvers on the quality of bread dough

Dough was prepared in duplicate with the amount of water giving a consistency of 500 Brabender Units, BU. Combinations of Fibrotec™ (0.0, 0.5, and 1.0%, w/w) and DATEM (0.0, 1.0, and 2.0%, w/w) were used following a 3 x 3 factorial arrangement in CRD. Doughs were mixed in a Brabender farinograph mixer (300 g flour capacity) (Brabender, Duisburg, Germany) to dough development.

The rheological profiles of dough formulated with bread improvers were performed in duplicate using a Brabender farinograph (Brabender, Duisburg, Germany) according to AACC method 54-21 (AACC, 2000) as shown in the

Appendix C. The results were reported as dough water absorption (WA, %), dough development time (min), mixing tolerance index (MTI, BU), and stability (min).

4.2 To study the effect of selected improvers on the quality of bread

Breads were prepared from wheat flour (control) or composite flour (wheat:GBRF:GGNBRF) at optimum ratios selected from the previous study (study 3). The ingredients and processes to produce bread were the same as in the previous section (method section 2.2). The quality of bread was evaluated as the subsequence measurements.

4.2.1 Physical measurements

4.2.1.1 The surface of bread crumb was photocopied to compare the gas cell distribution.

4.2.1.2 Loaf volume was measured in duplicate using the rapeseed displacement method (Curic *et al.*, 2008). Specific volume was subsequently calculated as loaf volume/weight (mL/g). Expansion ratio (%) was calculated from the mean value of specific volume of each sample/mean specific volume of the control x 100.

4.2.1.3 Crumb color was measured in duplicated using a spectro-photomer (CM-3500d, Minolta Co., Ltd, Osaka, Japan).

4.2.1.4 Crumb texture was determined in triplicate by texture profile analysis (TPA) method using a texture analyser (TA-500, Lloyd instruments Ltd., UK). The bread slice of 7x 8 x 2.5 cm^3 and 7 x11 x 2.5 cm^3 for bread containing composite wheat-GBRF and the control, respectively was compressed to 40% deformation at a cross-head speed of 2 mm/s with a trigger force of 20 g to compress the middle of the bread (Moore *et al.*, 2006) using a compression probe (diameter of

50 mm). Results [hardness (N), springiness (mm) and cohesiveness] were the mean of six measurements from two different sets of breads.

4.2.2 Sensory evaluation

Consumer acceptance test was conducted in this study. Consumers (*n* = 30) were recruited from Kasetsart University, Bangkok, Thailand. Criteria for recruitment, instructions, sampling orders, and evaluations were the same procedures as mentioned in the previous method section 2.2.2.

4.2.3 Statistical analyses

All data were subjected to analysis of variance (ANOVA). The Duncan's Multiple Range test (DMRT) was performed for post-hoc multiple comparisons. Statistically significant difference was established at $P<0.05$.

5. Determination of the developed bread qualities from composite wheat-germinated brown rice flour

After obtaining the final formulation of bread based on the results of all studies (Item no. 2 – 4), main ingredients including wheat flour and germinated flour and the developed bread were subjected to subsequence analysis.

5.1 Proximate and mineral composition of developed bread were analysed in duplicate as follows. Crude protein was determined by Kjeldahl method according to the AOAC method 976.06 (AOAC, 2000). Crude fat was determined by the ether-extraction method using soxtec system HT (Soxtec System HT6, Tecator, Sweden) according to the AOAC method 920.39 (AOAC, 2000). Crude fiber was measured using fiber analyzer (ANKOM 2000, ANKOM Technology, NY, U.S.A.) according to the AOAC method 962.09 (AOAC, 2000). Ash was determined by the muffle furnace method according to the AOAC method 930.22 (AOAC, 2000). Mineral content was measured by inductively coupled plasma-optical emission spectrometer (Optima 4300DV, Perkin Elmer, Massachusetts, U.S.A.) according to AOAC method 985.01 (AOAC, 2000).

5.2 GABA content of the finished product was determined using HPLC according to the method of Cohen and Michaud (1993) as mentioned in the method section 1.2.1.3.

5.3 Microbiological quality measurements

To ensure the quality of bread, the microbiological quality including aerobic plate count and yeast and mold using 3M ® test kit (3M Petrifilm™, 3M Company, St. Paul, MN, USA) were determined.

5.4 The cost estimation of bread from composite wheat-germinated brown rice flour

The cost of bread containing composite wheat-germinated flour was estimated including the cost of raw materials and another variable cost such as a man power and an electricity expense (excluded a packaging cost).

6. The study of the consumer acceptance and purchase intent to the developed bread from composite wheat-germinated brown rice flour

In order to satisfy consumer demands for bread products, understanding factors underlying consumer perception and acceptance are very important. Therefore, in this study, the consumer acceptance of developed bread was conducted both in Thailand and in the US. The developed bread was prepared and evaluated with the control. However, the formulation and ingredients used in bread making were different according to the availability in each market.

6.1 Bread formulation and bread making procedure

6.1.1 Bread making procedure in Thailand

Bread formulation was the final formulation obtained from the study 4.

6.1.2 Bread making procedure in the US.

Ingredients and the bread making machine were changed according to the availability in the US. market. However, the amount of each ingredient, except water (49% and 51.8 % for the control and bread made from composite flour) remained the same as the bread formulated in Thailand.

Bread doughs were prepared in duplicate using KitchenAid mixer (KitchenAid, Professional 6, St. Loseph, Mich., U.S.A). All ingredients were mixed using a KitchenAid mixer (KitchenAid, Professional 6, St. Loseph, Mich., U.S.A) for 18 min and the dough was divided into 300 g portions. Each 300-g portion was manually rounded and rested for 10 min. Dough was then punched, rolled, put into a well greased aluminum pan (19 x 10 x 7.4 cm^3), and proofed at 35 $\pm$ 2 ^{o}C and 85% RH (Deluxe equipment Co., Inc., Bradenton, Fl, U.S.A.) for 85 min. The baking process was carried out at 180 ^{o}C for 25 min using an electric oven (Turbofan32, Moffat Ltd., Christchurch, New Zealand). After the bread had been cooled on the rack for 1 h, it was packed in a gallon-size Ziploc® double zipper Fresh Shield™ Freezer bags (Ziploc®, S.C. Johnson & Son, Inc., Racine, Wi, U.S.A) before further analyses.

6.2 Consumer acceptance and purchase intent

In Thailand, consumers (*n* = 114) were recruited from Kasetsart University. Criteria for recruitment, instructions, sampling orders, and evaluations were the same procedures as mentioned in method section 2.2.2., The central location test (CLT) was conducted in a laboratory room with control temperature (25 ^{o}C) and fluorescent light. Questions (in Thai) in the ballot were the same questions as of questionnaire used for the US. consumers which were described subsequently.

While conducting consumer acceptance test in the US., the experimental consumer test protocol was approved by the Louisiana State University (LSU) Agricultural Center Institutional Review Board. Consumers (n = 116) were recruited from Baton Rouge, La., USA. They were prescreened for potential food allergies to wheat flour, rice flour, and all other ingredients used in bread formulations. The CLT was performed in a large conference type room illuminated with cool, natural, fluorescent lights.

Prior to the taste test, all consumers thoroughly read and signed a research consent form, and were briefed on the questionnaire, particularly the sensory attributes and their meanings. Each consumer was presented with 2 coded pieces of bread (7 x 8-11 x 1.25 cm^3). The wheat bread served as the control. They were instructed to (1) visually evaluate acceptability for overall appearance, (2) sniff to evaluate aroma acceptability, and (3) bite a half piece of bread and masticate the product before scoring acceptability for moistness, smoothness, softness, overall flavor, and overall liking, all on a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely) (Peryam and Pilgrim, 1957). The Just-About-Right (JAR) scale (1 = not enough, 2 = just about right, JAR, and 3 = too much) was also used to evaluate moistness, smoothness and softness. Purchase intent before and after acquiring health benefits of GBRF and GGBRF was evaluated using a binomial (yes/no) scale. Consumers were provided with room temperature distilled water to rinse their mouth before testing and between samples. Additional samples were given upon request. To avoid biases, consumers did not receive any monetary incentive for participation.

6.3 Statistical analyses

Data were subjected to analysis of variance (ANOVA) and the Tukey's studentized range test performed for post-hoc multiple comparisons. The McNemar's test was applied to determine if significant difference existed between consumers' purchase intent before vs. after having gained information about health benefits of the GBRF and GGBRF in the products. Statistically significant difference was established at $P < 0.05$.

7. Determination of the stability of bread from wheat-germinated brown rice flour during storage times

Bread was subjected to changes during storage. To reveal the stability, the effect of storage time on the quality of developed bread was determined. This section was conducted in the US.

Breads as a whole loaf were kept in Ziploc® double zipper Fresh Shield™ Freezer bags stored at room temperature (25 ^{o}C) with 70 $\pm$ 5% RH for 0, 3, and 5 days at and stored were evaluated for the selected physicochemical properties compared to the wheat bread (control).

7.1 Physicochemical measurements

7.1.1 Water activity (a_w) of crumb was evaluated in duplicate using a_w meter (Aw sprint, TH-500, Novasina, Pfaffikon, Switzerland).

7.1.2 Moisture content of bread crumb was evaluated in duplicate following the AACC method 44-15 (AACC, 2000).

7.1.3 Crumb color was measured in triplicate using a spectro-photometer (CM-508d, Minolta Co., Ltd., Osaka, Japan) and reported as L*, a*, b*, C* (Chroma) and h* (Hue angle).

7.1.4 Crumb texture was determined by texture profile analysis (TPA) method using a texture analyser (TA.XT. plus Texture Analyser Stable Micro Systems, Texture Technologies Crop., Scarsdale, NY, U.S.A). The bread slice of 7x 8 x 2.5 cm^3 and 7 x11 x 2.5 cm^3 for bread containing composite wheat-GBRF and the control, respectively was compressed to 40% deformation at a cross-head speed of 2 mm/s to compress the middle of the bread (Moore *et al.*, 2006) using a cylindrical probe with a diameter of 37.5 mm. Results [hardness (N), springiness (mm) and cohesiveness] were the mean of six measurements from two different sets of breads.

7.1.5 The thermal properties of freeze-dried bread crumb grounded with a grinder (Cyclone sample mill, UDY corporation, Fort Collins, Co, U.S.A) was performed in duplicate for different batches of bread using a differential scanning calorimeter (DSC) (DSC 822^e module, Mettler-Toledo GmbH, Switzerland). About 10 mg of the freeze-dried powdered samples were weighted directly into DSC stainless steel pans and distilled water was added to obtain a water:bread of 2:1 using micropipette. After sealing and standing for 1 h, the pan was gradually scanned from 25 ^oC to 140 ^oC at a constant rate of 5 ^oC/min. An empty pan was used as a reference. The parameters measured were the onset temperature (T_o), the peak temperature (T_p) and the conclusion temperature (T_c). The enthalpy associated with starch retrogradation (ΔH) was calculated as the area enclosed by the straight line between T_o and T_c and the endotherm curve expressed in J/g of dry sample.

7.2 Microbiological quality measurements

To ensure the quality of bread before conducting the consumer test, bread was determined for the microbiological quality including aerobic plate count and yeast and mold using 3M ® test kit (3M Petrifilm™ , 3M Company, St. Paul, MN, USA) with the incubator (Yamato® IC 1800, Yamato scientific Co.,Ltd. Japan).

7.3 Consumer acceptance test

Each consumer (US. consumer) was presented with 4 coded pieces of bread (slices of 7x 8 x 2.5 cm^3 and 7 x11 x 2.5 cm^3 for bread containing composite wheat-GBRF and the control, respectively) following the randomized complete block design with a counter balance serving order. The procedure of consumer acceptance test was described as in the study 6.

7.4 Statistical analyses

Data were subjected to analysis of variance (ANOVA) and the Tukey's studentized range test performed for post-hoc multiple comparisons. Multivariate statistical analyses were applied on all physicochemical properties and consumer data. Multivariate analysis of variance (MANOVA), principal component analysis (PCA) and descriptive discriminant analysis (DDA; Prinyawiwatkul and Chompreeda, 2007) were applied to evaluate factors that could mathematically distinguish all bread samples. The PCA biplot was carried out based on physicochemical properties and sensory acceptability using XLstat2007 software (Addinsoft, Paris, France). To identify potential directions for product improvement, the JAR data was analyzed using penalty analysis and the signal-to-noise ratio (SNR) analysis (Gacula *et al.*, 2007). Logistic regression was performed to identify sensory attributes influencing consumer purchase intent, and the McNemar's test was applied to determine if significant difference existed between consumers' purchase intent before vs. after having gained information about health benefits of the GBRF and GGBRF in the products. Statistically significant difference was established at $P < 0.05$.

RESULTS AND DISCUSSION

1. Effects of germination conditions on physicochemical properties of germinated brown rice flour (GBRF)

1.1 Effects of germination conditions on the qualities of germinated brown rice flour

1.1.1 Chemical properties

1.1.1.1 Moisture and protein content

Table 6 shows the changes in physicochemical properties of GBRF compared to those of the control (nongerminated brown rice flour). Steeping brown rice grains under controlled conditions led to changes in selected physicochemical properties of GBRF. In this study, different steeping time intervals of both brown rice varieties were investigated because the rate of germination of glutinous brown rice (GNBR) was faster than that of brown rice (BR). The 72 h steeping time of GNBR was not carried out in this study due to the occurrence of fermented odour. The moisture content of all flour samples was in the range of 7.74% to 9.29 %.

At pH 5 and 7, increasing the steeping time from 24 to 72 h caused a slight change in protein content in GBRF prepared from BR. GBRF from BR (KDML105) obtained under pH3 during 24, 48 and 72 h of steeping time contained 7.07, 5.92 and 5.37% protein (dry weight basis: db), respectively, compared to that of the control (6.74% protein, db) and those with DI (6.75-6.94%, db) (Table 6). The protein content of GBRF from GNBR ranged from 6.97 to 7.97%, db. Traoré *et al.* (2004) found that malting slightly increased protein content in red sorghum, millet and maize. However, this was not observed for BR and GNBR at pH 3.0, where the protein content tended to be decreased with the steeping time.

Table 6 Crude protein, reducing sugar, free GABA and stirring number (SN) of germinated brown rice flour (GBRF) as affected by various steeping conditions

Rice variety and Treatment^{A}	Steeping conditions		Crude Protein (%dry basis)	Reducing sugar (mg/100g flour, dry basis)	Free GABA (mg/100g flour, dry basis)	SN^{B}
	Steeping time (h)	pH of steeping water				
BR^{C}						
(1)	Control		6.74 ± 0.09^{bc}	14.98 ± 1.22^{g}	2.11 ± 0.13^{k}	2,890^{a}
(2)	24	3	7.07 ± 0.04^{a}	560.05 ± 9.46^{c}	32.70 ± 0.16^{d}	1,004^{c}
(3)	48	3	5.92 ± 0.07^{e}	1,217.89 ± 47.32^{b}	67.00 ± 2.01^{a}	62^{d}
(4)	72	3	5.37 ± 0.08^{f}	3,130.63 ± 72.90^{a}	63.24 ± 0.19^{b}	50^{d}
(5)	24	5	7.04 ± 0.08^{a}	31.38 ± 2.19^{g}	16.17 ± 0.16^{g}	2,883^{a}
(6)	48	5	6.98 ± 0.13^{ab}	60.35 ± 2.18^{g}	21.14 ± 0.70^{f}	2,055^{b}
(7)	72	5	7.06 ± 0.14^{a}	238.99 ± 7.18^{f}	21.61 ± 1.62^{f}	1,146^{c}
(8)	24	7	6.87 ± 0.10^{abc}	15.48 ± 1.26^{g}	8.50 ± 0.28^{j}	2,863^{a}
(9)	48	7	6.92 ± 0.09^{ab}	52.71 ± 4.22^{g}	12.28 ± 0.22^{i}	2,758^{a}
(10)	72	7	6.58 ± 0.03^{d}	56.07 ± 7.57^{g}	16.82 ± 0.43^{g}	1,785^{b}
(11)	24	DI^{E}	6.75 ± 0.02^{cd}	58.81 ± 1.82^{g}	14.84 ± 0.18^{h}	2,815^{a}
(12)	48	DI	6.81 ± 0.10^{bc}	349.80 ± 16.66^{e}	25.79 ± 0.67^{e}	1,611^{c}
(13)	72	DI	6.94 ± 0.08^{abc}	485.98 ± 33.04^{d}	38.90 ± 2.82^{c}	358^{d}
GNBR^{C}						
(14)	Control		7.66 ± 0.03^{cd}	15.89 ± 1.44^{h}	2.41 ± 0.66^{f}	1,579^{a}
(15)	24	3	7.73 ± 0.11^{bc}	448.54 ± 9.79^{c}	30.69 ± 0.04^{a}	144^{ef}
(16)	36	3	7.42 ± 0.04^{e}	507.52 ± 20.77^{b}	26.05 ± 0.11^{b}	78^{f}
(17)	48	3	6.97 ± 0.04^{f}	755.42 ± 60.98^{a}	27.95 ± 0.03^{b}	55^{f}
(18)	24	5	7.73 ± 0.03^{bc}	48.39 ± 4.58^{fgh}	14.73 ± 0.07^{c}	325^{cd}
(19)	36	5	7.97 ± 0.06^{a}	63.90 ± 2.24^{ef}	14.45 ± 1.37^{c}	239^{cde}
(20)	48	5	7.77 ± 0.03^{bc}	83.12 ± 3.03^{e}	25.56 ± 3.22^{b}	121^{ef}
(21)	24	7	7.51 ± 0.16^{d}	41.76 ± 3.90^{fgh}	4.25 ± 0.03^{ef}	459^{b}
(22)	36	7	7.62 ± 0.01^{d}	49.21 ± 2.68^{fg}	12.95 ± 0.33^{cd}	367^{bc}
(23)	48	7	7.54 ± 0.13^{d}	63.67 ± 8.85^{ef}	10.95 ± 0.18^{d}	210de
(24)	24	DI^{D}	7.60 ± 0.03^{d}	28.62 ± 3.12^{gh}	6.34 ± 0.46^{e}	216de
(25)	36	DI	7.51 ± 0.03^{e}	34.97 ± 0.42^{fgh}	14.61 ± 0.66^{cd}	124^{ef}
(26)	48	DI	7.89 ± 0.11^{b}	324.64 ± 19.62^{d}	24.81 ± 0.16^{b}	77^{f}

$^{a-h}$ Mean ± standard deviation of duplicate measurements; except for the free GABA content which was determined in triplicate with different superscript letters in each column were significantly different (P<0.05) for each brown rice variety.

^{A} Treatment numbers corresponding to those in the PCA plot in Figure 20.

^{B} The Stirring Number (SN) was based on 14% moisture basis.

^{C} BR = Brown rice (variety KDML105); GNBR = Glutinous brown rice (variety RD6)

[D] DI = distilled water, pH 6.8

1.1.2 Free GABA content

In this study, the free GABA content of the control of both brown rice varieties was 2.11 and 2.41 mg per 100g flour, respectively (Table 6). As the steeping time increased, the free GABA content generally increased significantly (*P*<0.05). The free GABA content of GBRF increased with increasing steeping time for both brown rice varieties when DI was used. A similar result was observed by Ohtsubo *et al.* (2005) who reported that GABA content of brown *Koshihikari* (Japanese) rice increased after steeping for 24 h in distilled water.

The effects of pH of steeping water on the free GABA content are shown in Table 6. The highest amount of free GABA could be accumulated when steeping brown rice grains at pH3 for 48 h for BR (67 mg/100g flour) and pH 3 for 24 h for GNBR (30.69 mg/100g flour). For GBRF prepared from BR, the free GABA content was increased from 32.70 to 67.00 mg/100 g flour when steeping time increased from 24 to 48 h at pH 3.0. This was perhaps due to the fact that GABA synthesis increases rapidly in response to a variety of environmental signals, including acidosis condition (Scott-Taggart *et al.*, 1999).

However, at pH5 and pH7 during 48 to 72 h of steeping time, free GABA content of GBRFs prepared from BR was lower than those obtained under the DI condition. It might be due to the pH change of DI from 6.8 to slightly acidic (pH = 5.4) which was likely caused by the fermentation process. GABA is synthesized from glutamic acid by glutamate decarboxylase (GAD). Previous reports indicated that reduction in cytosolic pH activates GAD activity and GABA accumulation (Bown and Shelp, 1997) by convert glutamate to GABA via GABA shunt (Scott-Taggart *et al.*, 1999). From this study, therefore, steeping brown rice at pH 3.0 will yield a significantly higher amount of free GABA than other steeping conditions, however, at the expense of protein, particularly for the BR varieties.

1.1.3 *Alpha*-amylase activity of GBRFs

Alpha-amylase plays a significant role in seed germination and is required in starch digestion (Muralikrishna and Nirmala 2005). Generally, the activity of *alpha*-amylase does not exist in dry rice seeds, but is rapidly induced and increased as the process of germination takes place. The Stirring Number (SN) test measures changes in physical properties of the starch caused by *alpha*-amylase enzymes. As the enzyme activity increased, the SN decreased. In this study, the *alpha*-amylase activity of both BR and GNBR increased with increasing steeping time (decreasing SN values) (Table 6). This agrees with other reports regarding *alpha*-amylase production during germination in maize (Helland *et al.*, 2002), high amylose rice (Capanzana and Buckle, 1997) and finger millet (Nirmala *et al.*, 2000).

For BR, increasing steeping time from 24 to 48 h significantly increased ($P<0.05$) *alpha*-amylase activity (SN = 62) compared with the control (SN = 2890). The highest *alpha*-amylase activity was observed at pH3 and 72 h of steeping time (SN = 50). For nongerminated GNBR, the SN value of 1579 was considerably lower than the nongerminated BR. As steeping continued from 24 to 36 and to 48 h, *alpha*-amylase activity of all GNBR significantly increased (SN ranged from 459 to 55). The difference in amylase activity of GNBR and BR during steeping was probably due to its granule structure and organization (Witt and Sauter, 1996).

1.1.4 Reducing sugar

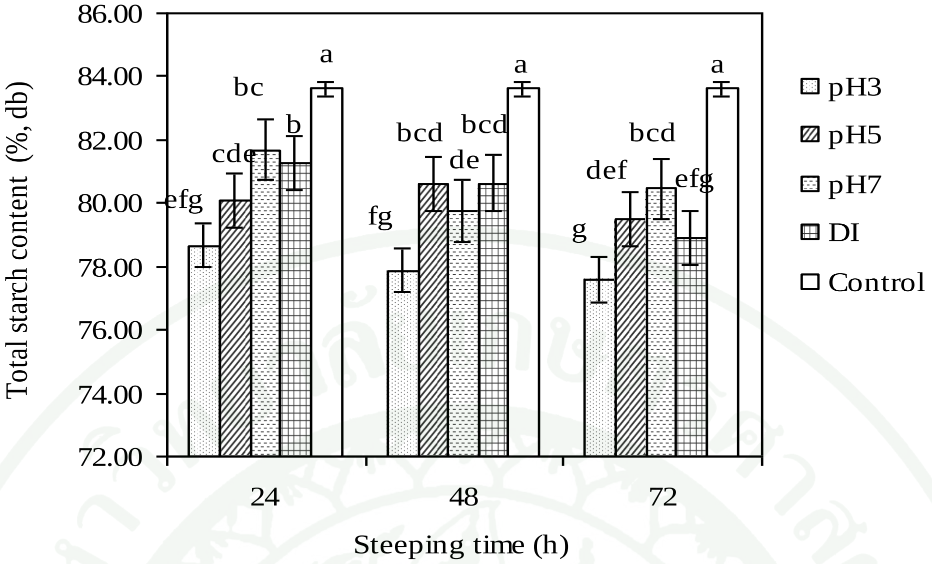
Before germination, cereals contain a small amount of sugars because sugars transported to the seed are generally converted to starch. Breakdown of starch and complex sugars occurs in a saccharification process during germination. In this study, reducing sugar was found to increase with steeping time (Table 6) due to the hydrolysis of starch. Without exception, after 24 h of steeping time, the reducing sugar of both BR and GNBR at all pHs significantly increased compared to those of the respective control.

The maximum content was observed at pH 3 for 72 h (3,130.63 mg/100g flour) for BR, and pH 3 for 48 h (755.42 mg/100g GBRF) for GNBR. These observed increases were perhaps due to amylase activity, resulting in the formation of sugars (Muralikrishna and Nirmala, 2005). A similar observation was made by Colmenares de Ruiz and Bressani (1990) who reported that reducing sugar of amaranth grain increased with germination time. Other experiments have also indicated that germination markedly increased the sugar content of sprouting grains, such as finger millet (Nirmala *et al.*, 2000) and brown rice (Ohstubo *et al.*, 2005).

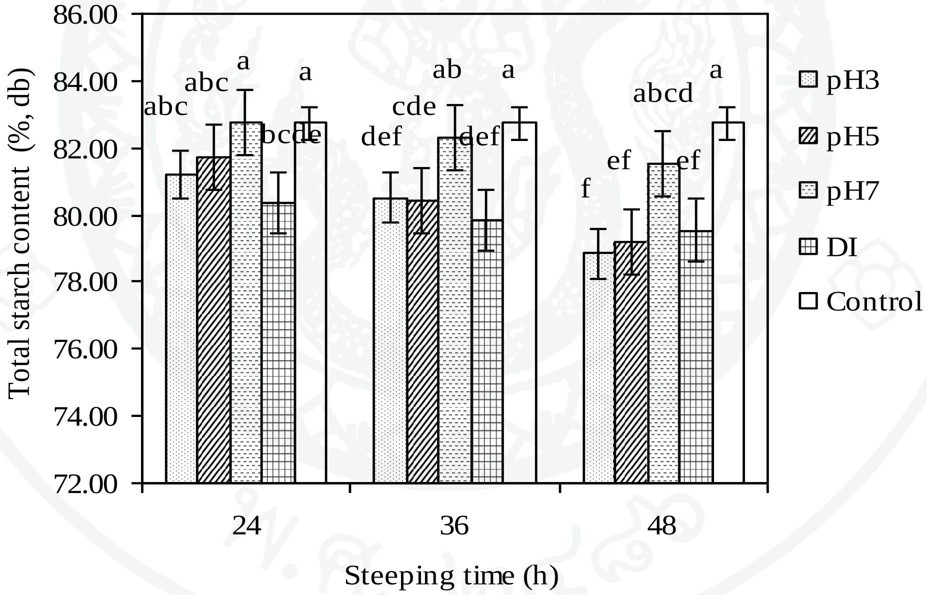
The results also showed that steeping brown rice in low pH especially pH3 increased the reducing sugar content of germinated flours from both varieties. Regardless of the acidic pH, at neutral pH (pH7) and DI condition, the reducing sugar of germinated flour also increased as the steeping time increased which clearly indicated that enzymes were activated during the germination process. It might be the reason explained the increasing of reducing sugar of germinated flours obtained from those conditions.

1.1.5 Total starch content

The starch breakdown has also been found due to an increase in the amylase activity in respiration metabolism during germination (Koller *et al.*, 1962). Accordingly, the action of enzymes during steeping reduced the total starch content of both BR and GNBR (Figure 1). The significant reduction of the total starch content from BR was observed among all conditions compared to that of the control after 24 h of steeping time. The most drastic change was noticed in BR at pH3 where the total starch content decreased from 83.61 to 77.59 % (db) during 72 h of steeping time. For GNBR, under the steeping conditions at pH3, pH5 and pH7 during 24 h, there was no significant difference in the total starch content (ranging from 81.20 - 82.77%, db) compared to that of the control (82.73%, db). A significance decrease in the total starch content of GNBR was found during 48 h of steeping time at pH3, pH 5 and DI, with the lowest at pH3 (78.84 %, db) (Figure 17).



(a) BR



(b) GNBR

Figure 17 Effects of germination conditions on total starch content of (a) BR steeped at pH3, pH5, pH7 and DI (distilled water) for 24, 48 and 72 h, and (b) GNBR steeped at pH3, pH5, pH7 and DI for 24, 36 and 48 h at 35 ^{o}C compared to those of the control (nongerminated). Bars with different letters were significantly different (P < 0.05).

1.1.5 Pasting profiles

Pasting characteristics are one of the important practical properties of flour prepared from germinated brown rice. According to the SN values measured by RVA, *alpha*-amylase activity is quantified in terms of the reduction in viscosity of flour paste resulting from the action of the enzyme (Raschke *et al.*, 1995). In this study, steeping conditions remarkably affected pasting profiles of GBRF compared to those of the control (Figure 18). No significant differences in pasting temperatures between GBRF prepared from BR (72.6 ^{o}C – 73.6 ^{o}C) and its control (74.0 ^{o}C) and between GBRF prepared from GNBR (67.2 ^{o}C – 69.0 ^{o}C) and its control (66.9 ^{o}C) were observed (as Appendix A, Appendix Tables A1 and A2). For other characteristic values of RVA viscogram, there were significant differences (P<0.05) among GBRF samples prepared under varying steeping times and pHs.

As the steeping time increased, the values of trough, breakdown, and final viscosity of both GBRF prepared from BR and GNBR decreased.
The pasting property of GBRF that was most affected by germination was the peak viscosity. This was probably due to the degradation of starch by enzyme activity during the steeping process. The peak viscosity of GBRF prepared from BR (7.42-228.22 RVU) and GNBR (4.42-58.67 RVU) was significantly lower than that of their controls (255.46 and 190.17 RVU, respectively) (as Appendix A, Appendix Tables A1 and A2).

Additionally, the peak viscosity of GBRF obtained at pH3 of both brown rice varieties (49.04 and 27.46 RVU for BR and GNBR, respectively) exhibited a drastic decrease after 24 h of steeping time compared to the control (Figure 19). GBRF obtained from BR at pH3 and 72 h of steeping time showed the lowest peak viscosity compared to those of other conditions of BR. The pasting profiles of the GBRF prepared from BR steeping with the DI water were similar to those at pH5 and pH7, except that when the steeping time was beyond 48 h, the peak viscosity was lower due to higher enzyme activity and pH change of DI (from 6.8 to

5.4) as mentioned previously. In addition, the viscosity drop of GBRF from GNBR occurred rapidly after 24 h of steeping time for all conditions.

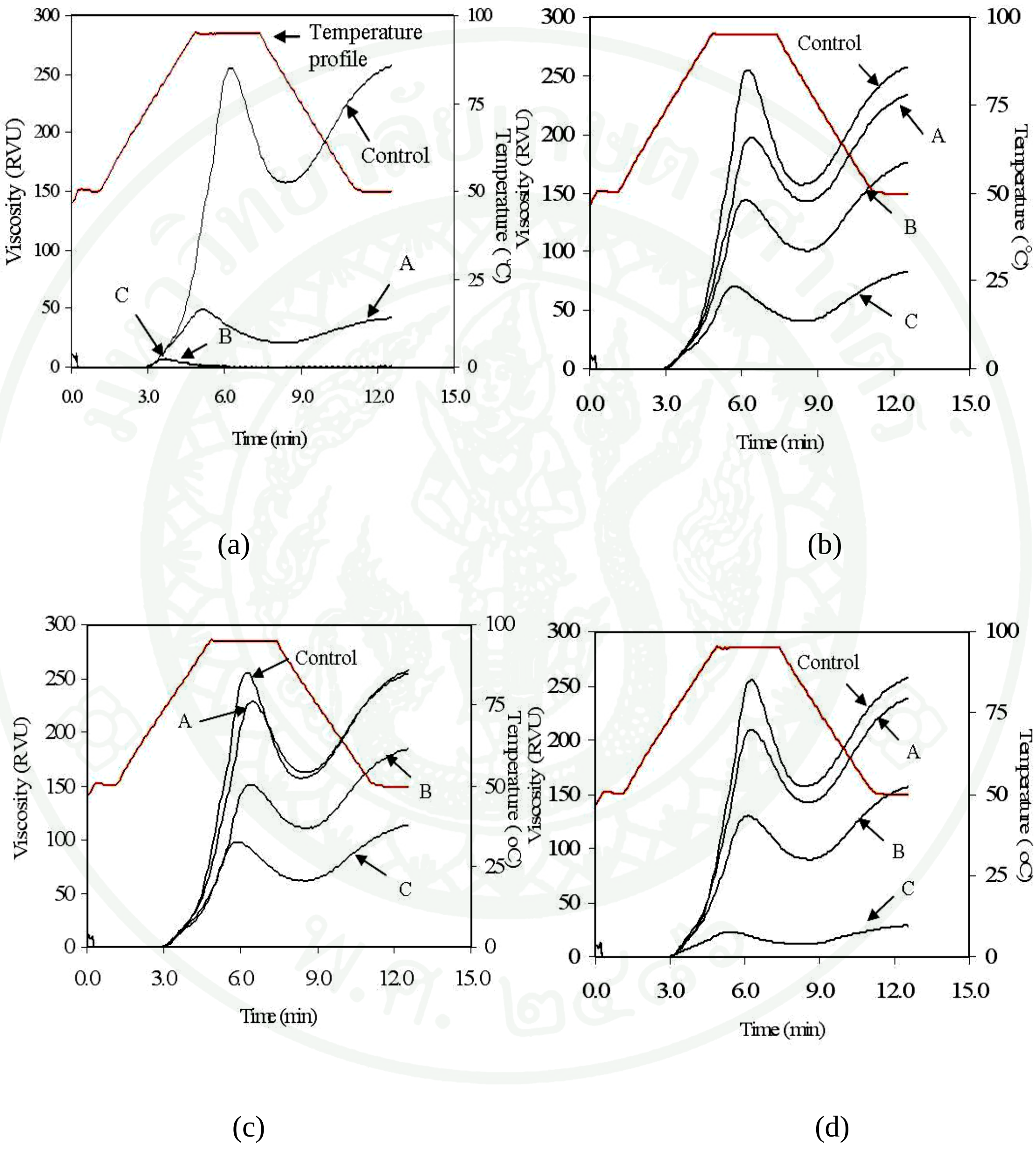


Figure 18 Pasting profiles of germinated brown rice flour prepared from brown rice (KDML105) at various steeping pHs: (a) pH3, (b) pH5, (c) pH7, and (d) DI (pH 6.8) for 24 (A), 48 (B) and 72 h (C) at 35 °C compared to that of the control.

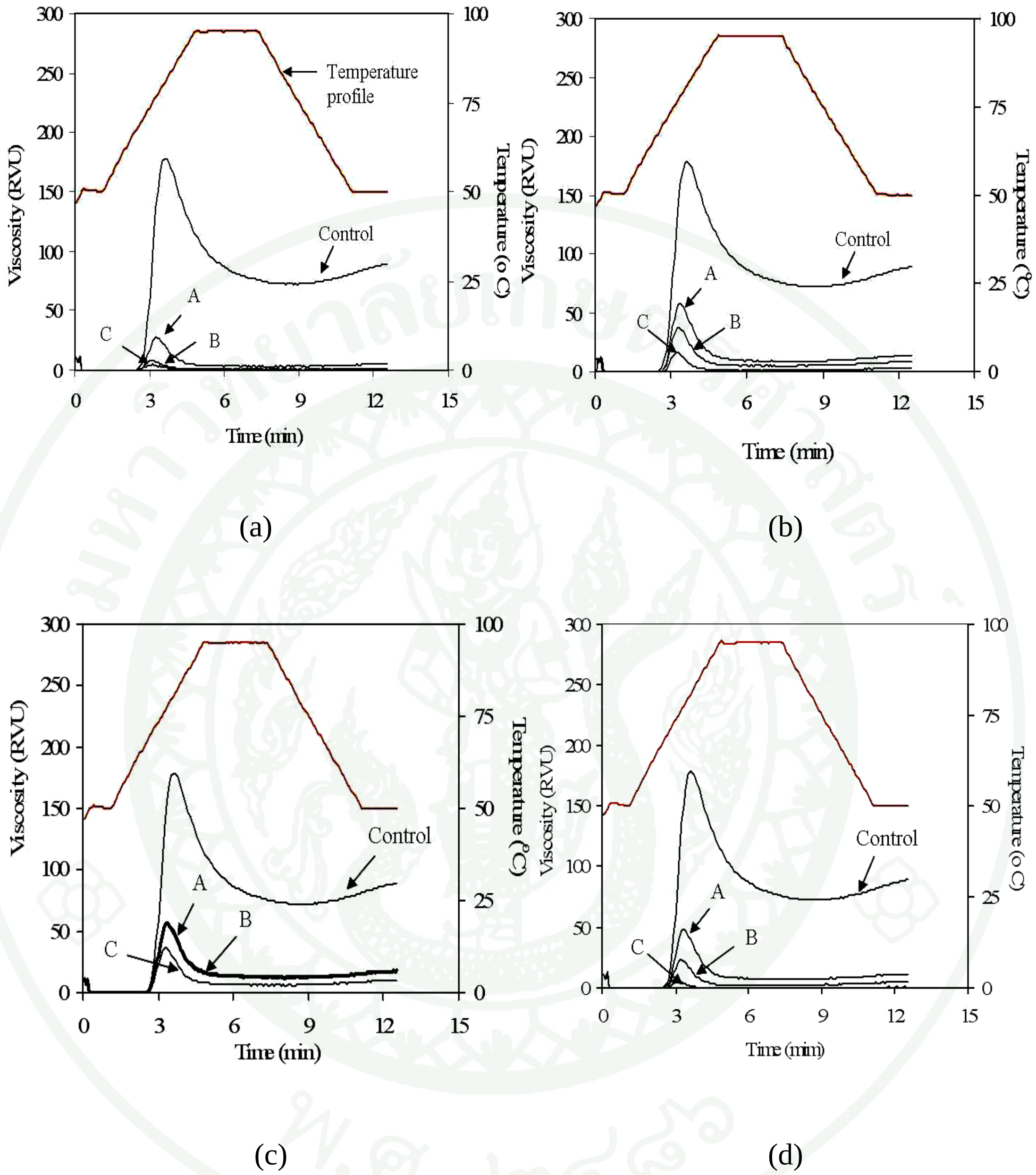


Figure 19 Pasting profiles of germinated brown rice flour prepared from glutinous brown rice (RD6, GNBR) at various steeping pHs: (a) pH3, (b) pH5, (c) pH7 and (d) DI (pH 6.8) for 24 (A), 48 (B) and 72 h (C) at 35 °C compared to that of the control.

The results of pasting properties showed negative relationship between viscosity and enzyme activity. It was revealed that with a higher extent of *alpha*-amylase activity, lower paste viscosities were observed. This was probably due to starch granule degradation by *alpha*-amylase which consequently led to a reduction in the viscosity of the paste. Our observations agreed with those reported by Helland *et al.* (2002) who observed that viscosity of the germinated maize flour decreased as the steeping time increased. However, the effect of the acid used as the steeping water on the pasting properties was preliminary observed (as Appendix A, Appendix Tables A3 and A4 and Appendix Figures A1 and A2). Thus, the reduction of viscosity was affected by both actions of *alpha*-amylase as well as weak acid.

Collectively, the results from this study showed that each pH level brought about changes in selected physicochemical properties. As the steeping time increased, the *alpha*-amylase activity increased, which probably caused the increase in reducing sugar content of GBRF prepared from both BR and GNBR.
The increasing reducing sugar coincided with the increasing free GABA content and the decreasing total starch content and pasting viscosity. On the other hand,
at the same level of steeping time, steeping brown rice at pH3 yielded the highest amount of free GABA and the lowest peak viscosity for both brown rice varieties.

1.2 Principal component analysis (PCA)

The PCA plots provide visualization among all characteristics of GBRF compared to the control of each brown rice variety under different steeping conditions. The results of PCA with a varimax rotation based on the physicochemical properties are shown in Figures 20 and 21. The results showed that the first two components were explained by 89.82% of the total variance (56.79 and 33.03%, respectively). The first principal component (PC1) was closely related to pasting profiles and *alpha*-amylase activity, while the second principal component (PC2) was associated with free GABA, reducing sugar, protein and starch content (Figure 20).

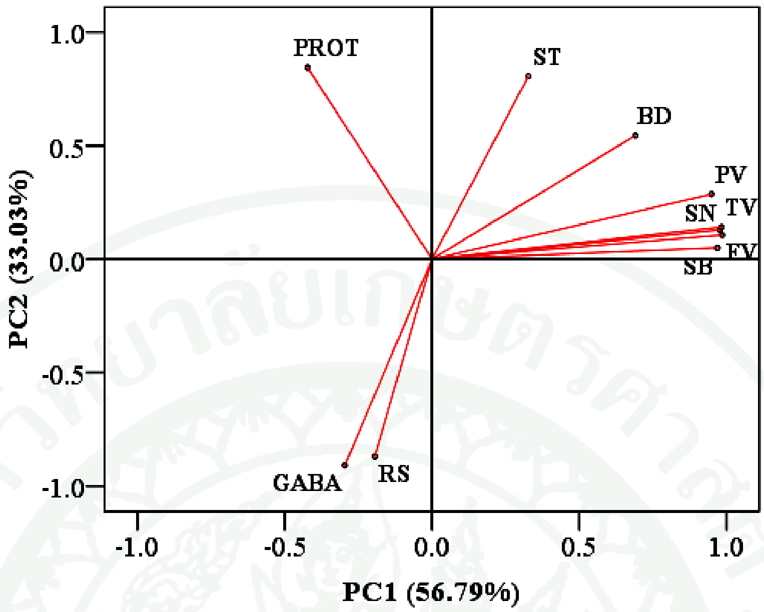


Figure 20 Principal component analysis (PCA): a loading plot of the first principal component (PC1) and the second principal component (PC2) describing the variation among the different properties of GBRF. Abbreviations: PROT: Protein; ST: total starch; PV: Peak viscosity; TV: Trough; BD: Breakdown; SB: Set back viscosity; FV: Final viscosity; SN: Stirring number; GABA: Free gamma-aminobutyric acid; RS: Reducing sugar.

These physicochemical properties revealed that BR was noticeably different from GNBR under various germination conditions. Both GBRFs were significantly different from their controls as shown in Figure 5. The PCA plot revealed that the free GABA and reducing sugar contents were negatively related to the pasting profiles (Figure 20). The differences among GBRFs during steeping, especially the one prepared at pH 3, were due to their higher content of free GABA and reducing sugar with lower pasting viscosities and starch content (Figures 20 and 21).

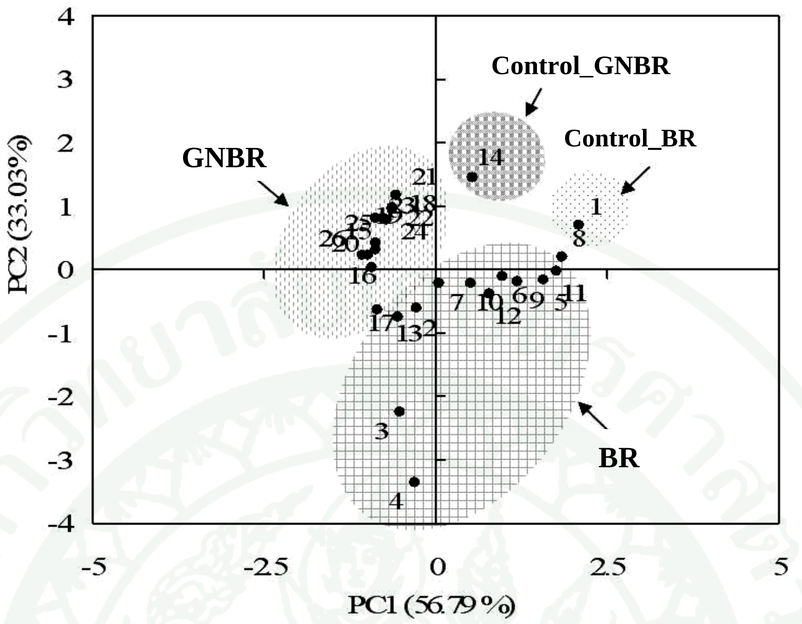


Figure 21 Principal component analysis (PCA): a PC score plot of the first principal component (PC1) and the second principal component (PC2) visualizing among GBRF samples. Abbreviations: Control_BR: nongerminated brown rice; Control_GNBR: nongerminated glutinous brown rice; BR: brown rice; GNBR: glutinous brown rice. The numbers 1-26 were brown rice flours obtained from different conditions as described in Table 6.

The modification of these quality characteristics by germination may offer alternative rice flour that is more nutritious to the food industry. Therefore, food applications of germinated brown rice flour need further investigation.

2. Effects of germination conditions on selected quality of composite wheat-germinated brown rice flour and bread formulations

2.1 Effects of germination conditions on *alpha*-amylase activity and pasting properties of composite flour

From previous study, the results clearly indicated that GBRF obtained from germination conditions at pH 3 or 6.8 steeping for 24 or 48 h contained a higher GABA concentration (14.8–67.0 mg/100g flour) and exhibited lower peak-viscosity and set-back. They were selected and blended with wheat flour with the ratio of wheat:GBRF at 70%:30% to obtain composite flours including Wheat-GBRF.

Variations in the quality of the control and composite wheat-GBRF at a ratio of 70:30, in terms of *alpha*-amylase activity (the SN value) and pasting profiles were presented in Table 7. *Alpha*-amylase activity is rapidly induced and increased as the process of germination takes place (Muralikrishna and Nirmala, 2005). The stirring number (SN) measures changes in physical properties of the starch caused by *alpha*-amylase enzymes. As the enzyme activity increases, the SN decreases. Results indicated that increasing steeping time increased *alpha*-amylase activity, resulting in a lower SN value (Table 7).

Among the wheat-GBRF composite flours, the one with GBRF, prepared at pH 3 and 48 h of steeping time, had the lowest SN (696) value. Among all composite flours, the wheat-BRF had the highest SN value (2188), indicating the lowest *alpha*-amylase activity. Low *alpha*-amylase activity is related to poorer quality of end-products (Mares and Mrva 2008) as described by the terms: dry, crumbly and high loaf density of bread (Hallen *et al.,* 2004).

Table 7 Effects of nongerminated brown rice flour (BRF) and/or germinated brown rice flour (GBRF) on *alpha*-amylase activity and pasting properties of composite flours

Flour[A], pH/Steeping time (h)	SN[B]	Pasting Temperature (°C)	Viscosity (RVU)[C]				
			Peak	Trough	Breakdown	Final	Setback from trough
Wheat	1976±93^{b}	68.45	136.46±1.01^{b}	92.53±0.11^{b}	43.93±0.88^{c}	170.02±0.71^{c}	77.49±0.59^{e}
Wheat-BRF	2188±33^{a}	69.40	150.17±0.18^{a}	102.89±0.00^{a}	47.27±0.18^{a}	221.59±0.47^{a}	118.70±0.47^{a}
Wheat-GBRF, 3/24	1497±51^{d}	69.30	103.90±0.59^{d}	58.26±0.24^{d}	45.64±0.35^{bc}	140.47±0.77^{d}	82.21±1.00^{d}
Wheat-GBRF, 3/48	696±17^{f}	69.30	56.99±0.15^{f}	16.01±0.18^{f}	40.98±0.03^{d}	49.82±0.23^{f}	33.81±0.06^{f}
Wheat-GBRF, 6.8/24	1826±85^{c}	69.40	132.45±0.88^{c}	85.09±0.00^{c}	47.35±0.89^{ab}	199.90±0.65^{b}	114.81±0.65^{b}
Wheat-GBRF, 6.8/48	1255± 2^{e}	67.80	94.62±0.35^{e}	50.07±0.12^{e}	44.55±0.24^{c}	136.88±0.53^{e}	86.80±0.65^{c}

$^{a\text{-}f}$ Mean ± standard deviation of duplicate measurements for *alpha*-amylase activity and triplicate measurement for viscosity with different letters in each column were significantly different (P<0.05).

[A]The ratio of wheat-BRF or wheat-GBRF was 70:30.

[B]The Stirring Number (SN), indicating the *alpha*-amylase activity, was based on 14% moisture basis.

[C]Peak was defined as maximum viscosity; Trough was defined as minimum viscosity after peak; Breakdown was defined as the difference between peak and trough; Final viscosity was defined as viscosity achieved at the end of the test; Setback from trough was defined as the difference between final viscosity and trough.

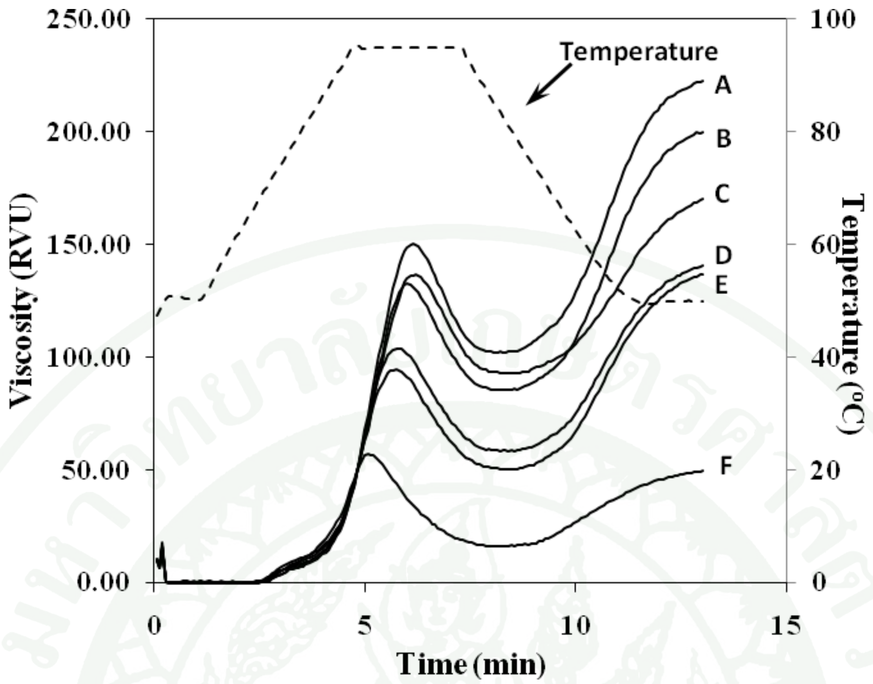


Figure 22 Pasting profiles of composited flours: Wheat-BRF (A), Wheat-GBRF, 6.8/24 (B), Wheat (Control, C), Wheat-GBRF, 3/24 (D), Wheat-GBRF, 6.8/48 (E), and Wheat-GBRF, 3/48 (F). The ratio of Wheat-BRF and Wheat-GBRF was 70:30. BRF = nongerminated brown rice flour; GBRF = germinated brown rice flour. The germination condition was noted as pH/steeping time (h).

Pasting profiles of composite flours were considerably different (P<0.05) from the control (Table 7). The peak viscosity of all flours showed a similar trend with their SN values, that is, the composite flour with a higher *alpha*-amylase activity had lower SN and peak viscosity values. The amylase activity in germinated flour was reported to cause a breakdown of starch (Hallen *et al*., 2004; Muralikrishna and Nirmala 2005). The wheat-GBRF had a lower peak viscosity (ranging from 56.99-132.45 RVU) than the control (136.46 RVU) and wheat-BRF (150.17 RVU). A similar trend was observed for the trough viscosity of all flours. The setback viscosity (SV) of the wheat-GBRF was significantly lower than that of the wheat-BRF or wheat flour, with the lowest SV observed for the one with GBRF prepared at pH 3 and 48 h of steeping time. However, the activity of enzyme during steeping time was not the only factor causing the reduction of pasting viscosity. Other factors include the effects of acidic steeping water (Charoenthaikij *et al*., 2009) as well as the reduced gluten content due to the substitution of wheat flour with up to 30% GBRF.

2.2 Effects of germination conditions on the quality of bread containing composite flours

2.2.1 Physical properties of bread

The physical properties of bread made with wheat or composite flours are presented in Figure 23 and Tables 8 and 9.

2.2.1.1 The surface of bread crumb

Substitution of wheat flour with GBRF at 30% resulted in more uneven pore distribution in bread crumb compared to the control (Figure 23).

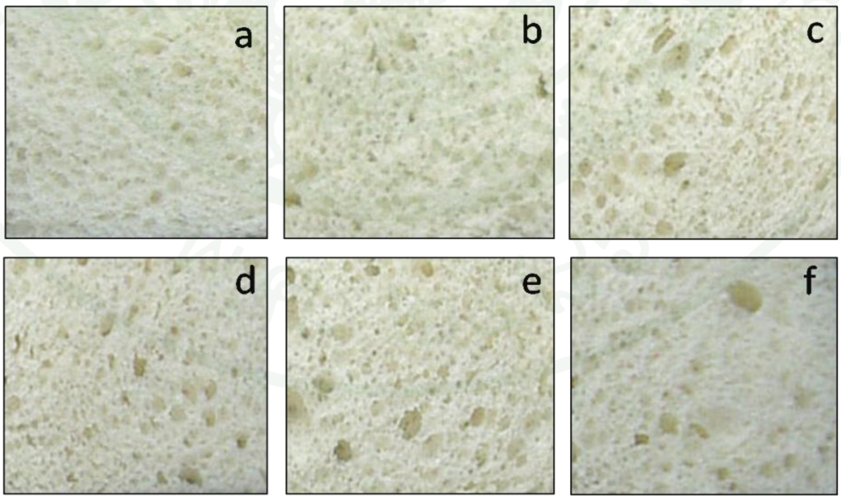


Figure 23 Cross-sectional crumb of wheat bread (a) and bread partially substituted with 30% nongerminated brown rice flour (BRF) (b) or 30% germinated brown rice flour (GBRF) prepared from steeping brown rice at 35 ^{o}C at pH 6.8 for 24 (c) and 48 (d) h or pH 3.0 for 24 (e) and 48 (f) h.

The presence of amylases increases the level of fermentable and reducing sugars in flour and dough, thus promoting yeast fermentation (Goesaert *et al*., 2006). Therefore, higher amylase activity and lower protein content of the wheat-GBRF may result in higher gas production but lower gas retention of bread dough compared to the control.

2.2.1.2 Aw of bread crumb

Water activity (a_w) of all bread crumb from bread containing composite wheat-germinated flour was in the range of 0.896 – 0.904 (Table 8).

2.2.1.3 Specific volume and expansion ratio

Bread formulations, containing 30% BRF or 30% GBRF, had lower specific volume (Table 9 and Figure 24) and lower expansion ratio but greater hardness (P<0.05) than those of the control (Table 9). The decreasing specific volume of bread due to a decreased gluten content by the substitution of wheat flour up to 30% with germinated brown rice flour which is gluten free; this resulted in the escape of gases during a proofing and baking process (Kadan *et al.*, 2001). Watanabe *et al.* (2004) reported that substituting wheat flour with 30% pre-germinated brown *Koshihikari* rice flour resulted in bread with lower specific volume compared to the control wheat bread. Moreover, it is known that bread with lower specific volume which indicated more compacted of cell structure results in firmer crumb (Sluimer, 2005). This may explain the greater hardness of the bread prepared from the composite flour containing 30% BRF or 30% GBRF (Table 9).

Table 8 Water activity (a_w) and color profiles of bread formulations partially substituted with 30% nongerminated brown rice flour (BRF) or 30% germinated brown rice flour (GBRF)

Flour[A], pH/Steeping time (h)	a_w [ns]	L*	a*	b*	C*	h*
Wheat	0.883± 0.009	67.48±0.85[a]	-0.33± 0.09[c]	9.03±0.63[d]	9.04±0.63[d]	92.12±0.59[a]
Wheat-BRF	0.900± 0.003	68.08±1.76[a]	-0.11± 0.05[a]	12.51±0.82[b]	12.51±0.82[b]	90.57±0.50[c]
Wheat-GBRF, 3/24	0.891± 0.007	62.13±1.76[c]	-0.20± 0.04[b]	13.04±0.84[ab]	12.84±0.46[b]	90.66±0.59[bc]
Wheat-GBRF, 3/48	0.899± 0.001	64.09±1.27[b]	-0.22± 0.03[b]	13.65±1.24[a]	13.87±1.04[a]	90.76±0.77[bc]
Wheat-GBRF, 6.8/24	0.904± 0.001	68.91±1.40[a]	-0.24± 0.09[b]	11.37±0.89[c]	11.38±0.89[c]	91.19±0.64[b]
Wheat-GBRF, 6.8/48	0.896± 0.001	63.44±0.78[b]	-0.32± 0.05[c]	13.19±1.19[ab]	12.86±0.96[b]	90.97±0.76[bc]

[a-d] Mean ± standard deviation of duplicate measurements for a_w and triplicate measurements for color profiles with different letters in each column were significantly different (P<0.05).

[A] See Table 5 for detailed bread formulations.

[ns] not significantly different ($P \geq 0.05$).

Table 9 Specific volume, expansion ratio and textural characteristics of bread formulations partially substituted with 30% nongerminated brown rice flour (BRF) or 30% germinated brown rice flour (GBRF)

Formulation[A], pH/Steeping time (h)	Specific volume (mL/g)	Expansion ratio (%)	Hardness (N)	Springiness[ns] (mm)	Cohesiveness[ns]
Wheat	4.2±0.3^{a}	100.0 ± 0.0^{a}	5.90±0.30^{c}	8.28±0.22	0.35±0.01
Wheat-BRF	3.2±0.2^{b}	77.0 ± 4.0^{b}	9.26±0.84^{a}	8.31±0.70	0.33±0.33
Wheat-GBRF, 3/24	2.9±0.1^{bc}	69.8 ± 3.2^{b}	7.91±0.65^{b}	7.63±0.36	0.50±0.15
Wheat-GBRF, 3/48	2.8±0.2^{c}	67.5 ± 4.7^{b}	7.64±0.25^{b}	7.66±1.01	0.29±0.02
Wheat-GBRF, 6.8/24	3.1±0.2^{bc}	74.3 ± 5.6^{b}	8.71±0.58^{a}	8.15±0.39	0.31±0.03
Wheat-GBRF, 6.8/48	3.0±0.2^{bc}	71.5 ± 5.8^{b}	7.78±0.61^{b}	8.03±1.06	0.30±0.03

$^{a-c}$ Mean ± standard deviation of duplicate measurements for specific volume and expansion ratio and triplicate measurements for texture profiles with different letters in each column were significantly different (P<0.05).

[A] See Table 5 for detailed bread formulations.

[ns] not significantly different ($P \geq 0.05$).

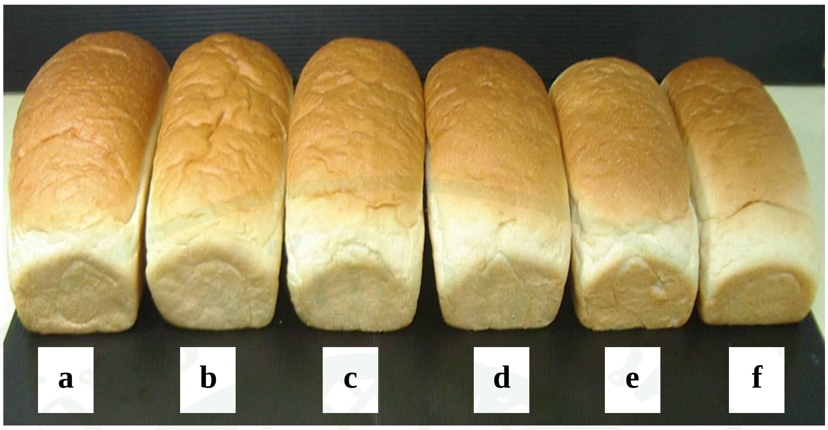


Figure 24 Loaves of wheat bread partially substituted with 30% nongerminated brown rice flour (BRF) (b) or 30% germinated brown rice flour (GBRF) prepared from steeping brown rice at 35^{o}C at pH 6.8 for 24 (c) and 48 (d) h or pH 3.0 for 24 (e) and 48 (f) h compared to the control (a).

2.2.1.4 Color of bread crumb

The crumb color of breads containing 30% BRF or 30% GBRF was white to slightly yellowish. Substituting wheat flour with GBRF (except the one at pH 6.8 and 24 h of steeping time) decreased lightness (L*) of bread compared with that of the wheat and wheat-BRF breads. All crumbs of the wheat-GBRF breads were slightly more yellowish (b*) than the wheat bread (Table 8). This occurrence may have been attributed to reducing sugar in those GBRF flours generated during germination (Charoenthaikij *et al.*, 2009), which reacted with free amino acid causing brown pigments formation.

The darkening of bread crumb due to the addition of germinated flour was also reported for bread containing 5 – 20% germinated cowpea flour (Hallen *et al*., 2004). The a* value of bread containing BRF or GBRF ranged from -0.32 to -0.11. C* (11.38 to 13.87) and h* (90.57 to 91.19) values of bread containing BRF or GBRF were significantly different from those of the control (9.04

and 92.12, respectively). Nevertheless, based on our visual observation, the crumb color of all breads was not considerably different.

2.2.1.5 Texture of bread crumb

There were no significant differences in springiness and cohesiveness among all breads formulations (Table 5). Substitution of wheat flour with 30% GBRF (except for the one prepared at pH 6.8 and 24 h steeping time) yielded breads with softer texture than bread containing 30% BRF. Mares and Mrva (2008) stated that low *alpha*-amylase activity (that is, a high SN value) is related to poorer quality of end-products [dry, crumbly and high loaf density of bread (Hallen *et al.*, 2004)]. The slightly harder texture of the wheat-BRF crumb may have been due to the high SN value (2188) compared those prepared from the wheat-GBRF (696-1497) (Table 7).

In addition, a high amount of *alpha*-amylase commonly may results in bread with a wet sticky crumb due to excessive liquefaction and dextrinization (Hallen *et al*., 2004). Even though the composite flour had low SN value (696) (Table 7), implying the high amylase activity, it was not high enough to produce undesirable bread based on the consumer acceptable data (Table 10).

2.2.2 Sensory evaluation

According to the results (Table 10), overall aroma (6.1 to 6.6), and overall flavor (6.4 to 7.0) scores were not significantly different among six bread formulations. The softness liking scores of the bread containing 30% BRF (6.6, like slightly) or 30% GBRF from all germination conditions (6.4-6.7, like slightly) were slightly lower than that of the control (7.3, like moderately). This may be explained by their greater hardness, lower specific volume, and lower expansion ratio values (Table 9). Overall liking scores of all breads containing 30% BRF or 30% GBRF were not significantly different (P<0.05), ranging from 6.4 to 6.8 (like slightly) but were lower than the control (7.2, like moderately).

Table 10 Preliminary consumer acceptance of bread formulations partially substituted with 30% nongerminated brown rice flour (BRF) or 30% germinated brown rice flour (GBRF)

Formulation^{A}, pH/Steeping time (h)	Appearance	Overall aroma^{ns}	Softness	Overall flavor^{ns}	Overall liking
Wheat	7.5 ± 0.9^{a}	6.6 ± 1.3	7.3 ± 1.0^{a}	7.0 ± 1.2	7.2 ± 1.1^{a}
Wheat-BRF	6.3 ± 1.2^{b}	6.3 ± 1.6	6.6 ± 1.2^{b}	6.4 ± 1.5	6.4 ± 1.4^{b}
Wheat-GBRF, 3/24	6.7 ± 1.3^{ab}	6.6 ± 1.3	6.7 ± 1.3^{b}	6.6 ± 1.1	6.8 ± 1.0^{b}
Wheat-GBRF, 3/48	6.3 ± 1.5^{b}	6.1 ± 1.5	6.7 ± 1.3^{b}	6.6 ± 1.2	6.5 ± 1.1^{b}
Wheat-GBRF, 6.8/24	6.5 ± 1.3^{b}	6.4 ± 1.4	6.5 ± 1.3^{b}	6.6 ± 1.1	6.5 ± 1.2^{b}
Wheat-GBRF, 6.8/48	6.5 ± 1.3^{b}	6.2 ± 1.4	6.4 ± 1.2^{b}	6.6 ± 1.2	6.6 ± 1.2^{b}

$^{a\text{-}b}$ Mean ± standard deviation of 60 consumer responses with different letters in each column were significantly different (P<0.05).

^{ns} not significantly different ($P \geq 0.05$).

2.2.3 Principal component analysis (PCA)

The PCA was applied to provide visualization among all characteristics of bread containing 30% BRF or 30% GBRFs compared to the control. The PCA biplot with a varimax rotation based on the physical properties and sensory acceptability of all bread formulations is shown in Figure 25. The PCA biplot indicated that the first two components were explained by 80.42% of the total variance (64.42 and 16.00%, respectively), and germination conditions had considerable effect on those properties of bread formulations.

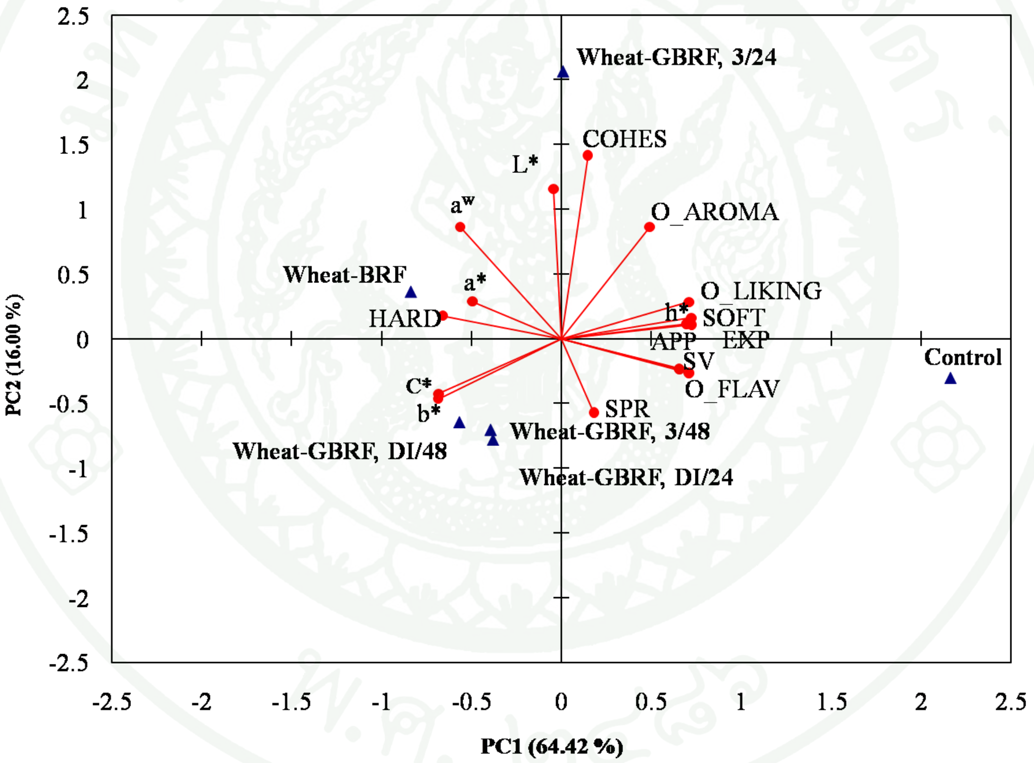


Figure 25 A biplot of the principal component (PC) 1 and 2 visualizing between all bread formulations and physical and sensory acceptability. BRF = non-germinated brown rice flour; GBRF = germinated brown rice flour; DI = distilled water (pH 6.8); SV = Specific volume; EXP = Expansion ratio; HARD = Hardness; COHES = Cohesiveness; SPR = Springiness; APP = Appearance; SOFT = Softness; O_AROMA = Overall aroma; O_FLAV = Overall flavor; O_liking = Overall liking.

Breads containing 30% GBRFs were different from the control. Bread containing 30% BRF was different from those containing 30% GBRFs, particularly in terms of hardness. In addition, the PCA biplot clearly revealed that bread containing 30% GBRF, prepared at pH 3 for 24 h steeping time, was different and positioned far from other wheat-GBRF breads (Figure 25). Thus, GBRF obtained at pH 3 for 24 h was selected to use for the further study.

However, the quality of bread containing GBRF is still lower than the control. Therefore, to improve the texture of bread containing GBRF, the addition of GGNBRF and improvers such as emulsifiers and hydrocolloids (Gujral *et al.*, 2004) were studied.

3. Effects of incorporation of germinated brown rice flour (normal and glutinous germinated brown rice) affecting the selected properties of composite wheat-germinated brown rice flour and its application in bread

3.1 Effects of composite wheat:GBRF:GGNBRF ratios on *alpha*-amylase activity and pasting profiles of composite flours

In this study, GGNBRF was incorporated in bread formulation with the objective of an improvement of the texture. The results from preliminary study indicated that this flour could be substituted only about 10% because the addition of GGNBRF at more than 10% yielded bread with glutinous texture (soft but sticky) which was unacceptable by consumer.

The changes in the quality of wheat flour (control) and wheat flour:GBRF:GGNBRF (germinated flour from steeping at pH3) at 70:30:0, 60:30:10, 60:40:0, 50:40:10 in terms of *alpha*-amylase activity and pasting profiles were presented in Table 11. The results showed that the substitution of GBRF and GGNBRF had a significant impact on the *alpha*-amylase activity of composite flours (P<0.05). Among the composite flours, the one containing wheat:GBRF:GGNBRF at 50:40:10 ratios, containing highest amount of germinated flour, obtained the lowest SN value (1008) indicated the highest *alpha*-amylase activity compared to the control (SN=1976) and others composite flours (SN = 1043–1384). The similar result was observed by Hallen *et al*. (2004) who indicated that the partially substitution wheat flour with germinated cowpea flour (5% to 20%) had considerably higher *alpha*-amylase activity than the control. However, as mentioned previously, the reduction of gluten according to the substitution of wheat flour with germinated flour and the effect of the acid solution used as the steeping water may also response for the reduction of pasting viscosity.

Table 11 Effects of germinated brown rice flour (GBRF) and/or germinated glutinous brown rice flour (GGNBRF) on *alpha*-amylase activity and pasting properties of composite flours

Composite flour			SN[A]	Pasting Temperature (°C)	Viscosity (RVU)[B]				
Wheat flour	GBRF	GGNBR			Peak	Trough	Breakdown	Final	Setback
100	0	0	1976±93^{a}	68.45	136.46±1.01^{a}	92.53±0.11^{a}	43.93±0.88^{c}	170.02±0.71^{a}	77.49±0.59^{b}
70	30	0	1497± 5^{b}	69.30	103.90±0.59^{b}	58.26±0.24^{b}	45.64±0.35^{b}	140.47±0.77^{b}	82.21±1.00^{a}
60	30	10	1043±10^{d}	70.25	70.42±1.24^{d}	32.06±2.07^{d}	38.37±0.83^{d}	83.92±1.90^{d}	51.87±0.18^{d}
60	40	0	1384±12^{c}	70.10	97.97±0.83^{c}	47.81±0.35^{c}	50.16±0.47^{a}	119.03±0.47^{c}	71.22±0.83^{c}
50	40	10	1008± 9^{d}	68.95	68.54±0.35^{d}	31.89±0.18^{d}	36.66±0.53^{e}	81.29±0.41^{e}	49.40±0.24^{e}

$^{a-e}$Mean ± standard deviation of duplicate measurements for *alpha*-amylase activity and triplicate measurement for viscosity with different letters in each column were significantly different (P<0.05).

[A]The Stirring Number (SN), indicating the *alpha*-amylase activity, was based on 14% moisture basis.

[B]Peak was defined as maximum viscosity; Trough was defined as minimum viscosity after peak; Breakdown was defined as the difference between peak and trough; Final viscosity was defined as viscosity achieved at the end of the test; Setback from trough was defined as the difference between final viscosity and trough.

As ratios of substitution increased, the *alpha*-amylase activity also increased among composite flours containing 30, 40, and 50% of germinated flours. Additionally, for the substitution of wheat flour with germinated flours at 40%, wheat:GBRF:GGNBRF at 60:30:10 and 60:40:0 ratios, the higher *alpha*-amylase activity (the lower SN value) of composite flour containing GGNBRF was observed (SN = 1043 and 1384, respectively).

Pasting characteristics data of composite flours were summarized in Table 11 and Figure 26. The results showed that the substitution of GBRF and GGNBR at different ratios to the wheat flour significantly affected the pasting properties of the composite flours ($P<0.05$).

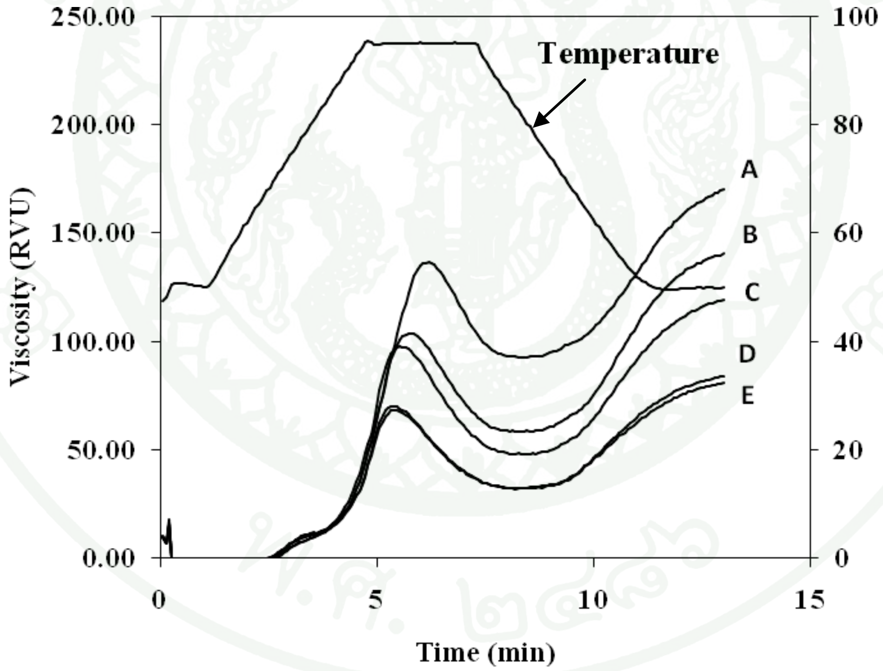


Figure 26 Pasting profiles of wheat flour (Control, A) and composite flours: Wheat: GBRF:GGNBRF = 70:30:0 (B), Wheat:GBRF:GGNBRF = 60:40:0 (C), Wheat: GBRF:GGNBRF = 60:30:10 (D), Wheat:GBRF:GGNBRF = 50:40:10 (E). GBRF = germinated brown rice flour; GGNBRF = germinated glutinous brown rice flour.

Possible reasons for this modification may attribute to the amylase activity in germinated flour caused a breakdown of starch (Hallen *et al*., 2004; Muralikrishna and Nirmala, 2005), differences in the amylose/amylopectin ratios of starches (Hung *et al*., 2007), and the reduction of gluten content according to the substitution of wheat flour with germinated flour resulted in different pasting properties of the composite flours.

The addition of GBRF and GGNBRF significantly decreased the peak, trough, and final viscosity of composite flours compared to the control which may result from lower total starch content (Hung *et al*., 2007) of germinated flours as affected by amylase activity as well as the reduction in the gluten as mentioned above. The composite flours containing wheat:GBRF:GGNBRF at 60:30:10 and 50:40:10 ratios demonstrated the lowest peak viscosity (70.42 and 68.54 RVU, respectively) (Table 11 and Figure 26). Peng *et al*. (2009) reported the similar trend that the incorporation of waxy wheat flour into wheat flour resulted in the decreasing in peak viscosity.

Moreover, the increasing of breakdown was observed for composite flour containing wheat:GBRF:GGNBRF at 60:40:0 ratios (50.16 RVU). This result agreed with Hayakawa *et al*. (2004) who demonstrated that the peak viscosity and setback of waxy wheat flour was much lower than those of non-waxy wheat flour. The low amylose content flour especially of waxy flour was generally reported to have higher resistance to retrogradation (Hung *et al*., 2006) which may consider being responsible for a lower setback characteristic.

In comparison, at the 40% substitution, the set back of composite flour containing GGNBRF (51.87 RVU) were lower than that of one containing GBRF (71.22 RVU). The composite flour with wheat:GBRF:GGNBRF at 50:40:10 ratios revealed the lowest set back (49.40 RVU) compared to the others (51.87 RVU– 82.21 RVU). The reduction in the pasting properties of composite flours associated with their *alpha*-amylase activities as observed by the previous study.

3.2 The feasibility of using composite flours in bread formulations

The presence of too high *alpha*-amylase commonly results in bread with an inferior quality as described by the terms of wet sticky crumb due to the excessive liquefaction and dextrinization (Hallen *et al*., 2004). This inferior quality was not observed in bread containing composite flour with high amylase activity (low SN value). According to the preliminary consumer acceptability results (Table 12), overall aroma (6.9 to 7.0), overall flavor (6.6 to 7.2), and overall liking (6.8 to 7.2) scores were not significantly different among five bread formulations (Table 12).

However, in this study the preference on the appearance of the breads made with composite flours (6.4 to 6.9; like slightly) was significant lower than the control (7.5; like moderately). This may be attributed to the fact that the substitution of germinated flour in bread formulation yielded a porous crumb structure which was observed from the previous study.

The results from this preliminary sensory study indicated that the substitution of GBRF and/or GGNBRF for wheat flour at 30 – 50% of total flour were possible to produce acceptable bread without adversely affecting sensory acceptance. Nevertheless, based on the physicochemical and sensory properties, the composite flour containing wheat:GBRF:GGNBRF at 60:30:10 was selected for the further study.

Table 12 Preliminary consumer acceptance of bread formulations partially substituted with germinated brown rice flour (GBRF) and/or germinated glutinous brown rice flour (GGNBRF)

Composite flour			Appearance	Overall aroma^{ns}	Softness^{ns}	Overall flavor^{ns}	Overall liking^{ns}
Wheat flour	GBRF	GGNBR					
100	0	0	7.5 ± 0.9^{a}	6.9 ± 1.3	7.3 ± 1.0	7.0 ± 1.2	7.2 ± 1.1
70	30	0	6.8 ± 0.9^{bc}	7.0 ± 0.9	7.0 ± 0.9	6.6 ± 0.9	6.9 ± 0.8
60	30	10	6.9 ± 0.9^{b}	6.8 ± 1.1	7.2 ± 1.1	6.7 ± 1.1	7.0 ± 0.8
60	40	0	6.7 ± 1.1^{bc}	6.8 ± 1.2	7.2 ± 1.0	7.2 ± 0.8	6.9 ± 1.0
50	40	10	6.4 ± 1.0^{c}	7.0 ± 1.0	7.1 ± 1.2	6.9 ± 0.9	6.8 ± 0.8

$^{a-c}$ Mean ± standard deviation of 30 consumer responses with different letters in each column were significantly different (P<0.05).

^{ns} not significantly different (P≥0.05).

GBRF = Germinated brown rice flour; GGNBRF = Germinated glutinous brown rice flour

4. Functionality of selected bread improvers on the quality of dough and wheat-germinated brown rice bread

Monoglycerides including DATEM are widely used as anti-staling agents according to their ability to complex with amylose, reduce interfacial tension, and increase aeration (Orthoefer, 2008). Meanwhile, hydrocolloids are expected not only to increase water retention in bread because of their strong water binding abilities (Gallagher *et al*., 2004) but also decrease firmness and starch retrogradation (Collar *et al*., 1999). In this study, then, Fibrotec™ as a hydrocolloid and DATEM as an emulsifier were selected according to those properties and evaluated the functionality on the dough and bread containing composite flours.

4.1 Effect of selected improvers on rheological properties of composite flours

Combinations of Fibrotec™ (0.0, 0.5, and 1.0%, w/w) and DATEM (0.0, 1.0, and 2.0%, w/w) were used to improve the quality of bread containing wheat-germinated flour (wheat:GBRF:GGNBRF at 60:30:10) in this study. The results showed that the rheological properties of composite flours with selected improvers were summarized in Table 13. Without improvers, water absorption (WA) of composite flour (65.5%) was significant higher than that of the control (64.2%), corresponding to a maximum consistency of 500 BU. The results also demonstrated that the improvers had minor effect on the WA capacity of all composite flours (64.7% to 66.3%).

Among composite flours containing Fibrotec™ alone, increasing in the concentration of Fibrotec™ do not affected the WA of all flours. In absence of Fibrotec™, as the concentration of DATEM increased, the WA of flours was in the range of 65.5% to 64.7%. This result agrees with the previous finding reporting that some emulsifiers such as DATEM and monoglyceride (MG) do not have any effect on the water absorption of wheat dough (Stampfli and Nersten, 1995; Gomez *et al*., 2004).

Dough development time (DDT) is the required time for dough development to reach 500 BU of dough consistency. In this study, DDT of all composite flours (DDT = 1.5 – 2.0) was practically not affected by the incorporation of improvers in bread formulation compared to the control (DDT = 2.0) (Table 13).

Table 13 Effect of Fibrotec™ and DATEM on the bread dough rheology determined by Brabender farinograph

Sample	Fibrotec™ (%,w/w)	DATEM (%,w/w)	Water absorption (%)	Mixing Tolerance Index (BU)	Stability (min)
1	Control	-	64.2 ± 0.0^{e}	22.5 ± 3.5^{c}	17.5 ± 0.0^{a}
2 (A)	0.0	0.0	65.5 ± 0.0^{c}	45.0 ± 7.1^{a}	7.0 ± 0.0^{c}
3 (B)	0.0	1.0	64.9 ± 0.1^{d}	55.0 ± 7.1^{a}	6.3 ± 0.4^{c}
4 (C)	0.0	2.0	64.7 ± 0.1^{d}	45.0 ± 7.1^{ab}	6.8 ± 0.4^{c}
5 (D)	0.5	0.0	65.8 ± 0.0^{bc}	50.0 ± 0.0^{a}	7.9 ± 0.2^{bc}
6 (E)	0.5	1.0	66.0 ± 0.1^{ab}	50.0 ± 0.0^{a}	5.8 ± 1.9^{c}
7 (F)	0.5	2.0	66.0 ± 0.0^{ab}	47.5 ± 3.5^{ab}	5.3 ± 0.4^{c}
8 (G)	1.0	0.0	66.0 ± 0.0^{ab}	30.0 ± 0.0^{bc}	10.5 ± 0.4^{b}
9 (H)	1.0	1.0	66.3 ± 0.2^{a}	37.5 ± 3.5^{abc}	6.9 ± 2.3^{c}
10 (I)	1.0	2.0	66.3 ± 0.0^{a}	42.5 ± 3.5^{ab}	5.8 ± 0.0^{c}

$^{a-e}$Mean ± standard deviation of duplicate measurements with different letters in each column were significantly different (*P*<0.05).
Fibrotec^{TM} = Microcrystalline cellulose colloidal grade with carboxymethyl cellulose; DATEM = Diacetyl tartaric acid ester of monoglycerides. Wheat flour served as the control.

The mixing tolerance index, an indication of the flour strength with lower value, was affected by the selected improvers. The addition of improvers had a significant impact on rheological properties of bread containing wheat-germinated brown rice flour compared to their counterpart without improvers. The presence of improvers tended to increase the mixing tolerance index (MTI) (45 – 55 BU) of flours from all conditions compared to that of the control (22.5 BU), with an exception of flour containing 1.0 % Fibrotec^{TM} at 0% and 1.0% DATEM (MTI = 30 – 37.5 BU) (Table 13). The most remarkable effect on MTI was observed with the addition of 1.0% Fibrotec^{TM} without DATEM (30 BU). The ability of improvers such as anionic emulsifier such as SSL to form complexes with the proteins called gluten-starch lipid complexes resulted in a strengthening effect on the bread dough (Kamel and Hoover, 1992). DATEM was reported to have an effective increasing the dough stability to support the kneading (Armero and Collar, 1996). However, it was not observed in the addition of DATEM at 2.0% (MTI = 50 BU) in bread containing wheat-germinated brown rice flour. The investigated effect was agreed with Rosell *et al*. (2001) in the case of wheat flour fortified with the addition of some improvers such as HPMC and κ-carrageenan exhibited higher MTI (lower stability) than did the control.

The stability of dough, an indication of the flour strength, was clearly affected by the addition of improvers. Dough with higher stability value was suggested to be stronger dough (Rosell *et al*., 2001). Doughs of bread containing improvers (ranged from 5.3 min to 10.5 min) obtained a reduction in stability than did the control (17.5 min). The *alpha*-amylase containing in germinated brown rice flour may induce dextrinization of starch granules reducing the ability of damaged starch to immobilize water. The increasing of water available to interact with other components of dough caused a decreasing in dough consistency (Collar *et al*., 1999).
On the contrary, the greatest effect on the stability of doughs among bread containing composite flour was produced by the addition of Fibrotec^{TM} at 1.0% without DATEM (10.5 min) with related to lower value of mixing tolerance index (MTI = 35) than other ratios of improvers.

4.2 Effect of selected improvers on physical characteristics of bread quality

4.2.1 The surface of bread

In comparison with the control, bread containing composite flours had inferior quality (Figure 27, sample A) which may result from the alteration of dough properties. The addition of improvers resulted in the improvement of bread crumb of wheat-germinated brown rice bread. This may attribute to the functional property of DATEM in increasing aeration (Orthoefer, 2008) and the positive influence on the fermentation and strong CO_2 retention (Stampfli and Nersten, 1995).

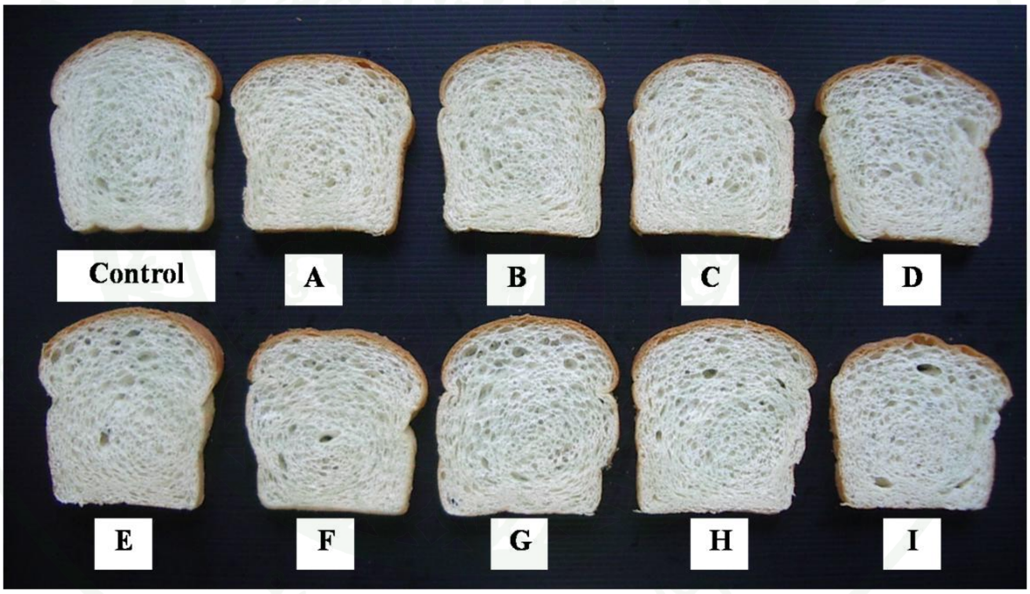


Figure 27 Cross-sectional bread crumb of bread containing different concentrations of improvers compared to the control. Samples A-I were different bread formulations as presented in Table 13. Wheat bread served as the control.

4.2.2 Specific volume and expansion ratio

The cross-sectional bread crumb indicated that improvers helped to alleviate the quality of bread crumb of bread containing composite flours compared with their counterpart without improvers (Figure 27). However, some combination had a negative effect on the appearance of bread crumb, particularly the bread containing 1.0% Fibrotec™ and 2.0% DATEM (Figure 27, sample I).

The bread made from composite flour without improvers obtained lower specific volume (3.6) than did the control (4.4) which was explained as a percentage of the expansion ratio (lower value). An improvement in the specific volume ranging from 3.7-4.0 was observed when adding improvers to bread containing composite flour compared to bread without improvers (3.6). This improvement of specific volume might be due to the stability enhancement of the protein network conferred by improvers (Gomez *et al*., 2004).

DATEM alone was likely to increase the specific volume of bread containing composite flour (Table 14 and Figure 27; samples B and C). A parallel trend was examined for bread containing 0.5 % of Fibrotec™ alone (Figure 27, samples E). Thus, Fibrotech™ or DATEM at 0.5% and 1.0%, respectively was sufficient to improve specific volume of bread and no further modification was observed by increasing the improvers concentration.

4.2.3 Color of bread crumb

The color profiles of all bread formulations were evaluated compared to the control. Among breads containing composite flour, improvers had slight effects on their color characteristics (Table 14). However, the results noticeably indicated that lightness of bread containing composite flour with and without improvers (L^* ranging from 36.24 – 40.02) were lower than that of the control ($L^* = 67.48$).

Table 14 Effect of Fibrotec™ and DATEM on the specific volume, expansion ration, and color profiles of bread formulations

Sample	Fibrotec™ (%, w/w)	DATEM (%, w/w)	Specific volume (mL/g)	Expansion Ratio (%)	L*	a*	b*	C*	h*
1	Control	-	4.4±0.0^{a}	100.0±0.0^{a}	67.48±0.85^{a}	-0.33±0.09^{a}	9.03±0.63^{a}	9.04±0.63^{a}	92.12±0.59^{d}
2	0.0	0.0	3.6±0.1^{c}	81.4±1.1^{c}	39.71±0.78^{bcd}	-0.48±0.03^{b}	4.22±0.69^{cd}	4.26±0.69^{de}	97.58±1.99^{c}
3	0.0	1.0	3.9±0.2^{bc}	88.1±4.5^{bc}	40.02±2.81^{b}	-0.72±0.09^{c}	4.23±0.52^{cd}	4.33±0.50^{de}	99.57±1.87^{b}
4	0.0	2.0	3.8±0.1^{bc}	85.8±2.7^{bc}	38.42±3.00^{bcde}	-0.73±0.08^{c}	4.77±0.48^{b}	4.88±0.41^{b}	98.15±1.84^{bc}
5	0.5	0.0	3.9±0.0^{bc}	88.9±0.5^{bc}	36.46±2.04^{e}	-0.72±0.08^{c}	4.13±0.56^{de}	4.19±0.55^{de}	99.73±1.81^{b}
6	0.5	1.0	4.0±0.1^{ab}	90.8±1.6^{b}	36.24±2.56^{e}	-0.67±0.11^{c}	4.31±0.45^{cd}	4.28±0.54^{de}	99.20±2.06^{bc}
7	0.5	2.0	3.7±0.1^{bc}	84.2±2.3^{bc}	38.37±3.24^{bcde}	-0.68±0.07^{c}	4.29±0.47^{cd}	4.38±0.46^{cd}	100.02±2.46^{ab}
8	1.0	0.0	3.8±0.1^{bc}	88.1±1.3^{bc}	37.57±2.97^{de}	-0.74±0.06^{c}	3.72±0.25^{e}	3.89±0.38^{e}	101.74±3.61^{a}
9	1.0	1.0	3.7±0.1^{bc}	85.0±1.6^{bc}	37.48±3.20^{cde}	-0.69±0.07^{c}	4.22±0.37^{cd}	4.28±0.37^{de}	99.02±1.89^{bc}
10	1.0	2.0	3.7±0.1^{bc}	84.7±3.1^{bc}	39.98±2.22^{bc}	-0.70±0.06^{c}	4.67±0.51^{bc}	4.79±0.46^{bc}	98.40±1.84^{bc}

$^{a-e}$Mean ± standard deviation of duplicate measurements with different letters in each column were significantly different (P<0.05).

Wheat bread served as the control.

4.2.4 Texture of bread crumb

In this study, the texture analysis was used to measure the texture characteristics of bread after baking for 24 h. The improvers had significant effects on texture profiles of bread crumb, especially crumb hardness as shown in Table 15. Bread containing composite flour without improvers obtained greater crumb hardness (7.23 N) than did the control (6.28 N). The crumb hardness of bread containing composite flour with some combinations of improvers (Table 15; sample no. 4, 6, and 8 with hardness ranging from 5.04 N to 7.05 N) was lower than the one without improvers (7.23 N).

Table 15 Effect of Fibrotec™ and DATEM on the texture profiles analysis of bread formulations

Sample	Fibrotec™ (%, w/w)	DATEM (%, w/w)	Hardness (N)	Springiness (mm)	Cohesiveness
1	Control	-	6.28 ± 0.39^{d}	8.72 ± 0.24^{a}	0.346 ± 0.021^{ab}
2	0.0	0.0	7.23 ± 0.42^{b}	8.23 ± 0.11^{bc}	0.333 ± 0.023^{bc}
3	0.0	1.0	7.05 ± 0.57^{bc}	8.12 ± 0.15^{bcd}	0.316 ± 0.010^{c}
4	0.0	2.0	5.15 ± 0.31^{e}	7.86 ± 0.15^{ef}	0.350 ± 0.022^{ab}
5	0.5	0.0	7.30 ± 0.92^{b}	8.30 ± 0.05^{b}	0.350 ± 0.020^{ab}
6	0.5	1.0	6.57 ± 0.36^{cd}	7.91 ± 0.26^{de}	0.329 ± 0.015^{bc}
7	0.5	2.0	7.32 ± 0.32^{b}	7.99 ± 0.07^{de}	0.332 ± 0.022^{bc}
8	1.0	0.0	5.04 ± 0.33^{e}	8.05 ± 0.21^{cde}	0.361 ± 0.017^{a}
9	1.0	1.0	7.29 ± 0.28^{ab}	8.09 ± 0.19^{cd}	0.337 ± 0.023^{abc}
10	1.0	2.0	9.13 ± 0.46^{a}	7.68 ± 0.05^{f}	0.332 ± 0.013^{bc}

$^{a-f}$ Mean ± standard deviation of duplicate measurements with different letters in each column were significantly different (*P*<0.05).
Wheat bread served as the control.

The results indicated that either Fibrotec^{TM} or DATEM when used alone could improve the hardness of bread containing composite flour, especially at the highest concentration (1.0% or 2.0%, respectively). The softening effect should be attributed to the water retention capacity of a three-dimensional network structure of microcrystalline cellulose (Iijima and Takeo, 2000). However, the strongest negative effect on hardness was exhibited when both Fibrotec^{TM} and DATEM were used together at the highest concentration (9.13 N).

The small effect of improvers on the cohesiveness and springiness of bread crumb had been demonstrated. The springiness of all bread formulations containing composite flour with improvers ranged from 7.68 to 8.30 mm and was significantly lower than that of the control (8.72 mm). In addition, bread containing mixed improvers at the highest concentration exhibited the lowest springiness (7.68 mm). This might be attributed to the compact crumb with lower specific volume (3.73 mL/g) of this bread formulation (Sample I). The cohesiveness of all breads containing improvers was in the range of 0.316 to 0.361.

4.2.5 Sensory evaluation

The consumer acceptance of bread formulations was preliminary conducted as demonstrated in Table 16. The results showed that the incorporation of improvers in bread formulations significantly affected sensory attributes of bread with an exception of overall aroma ($P<0.05$). The appearance liking score of all bread formulations containing composite flour ranged from 6.4 to 7.0 (like slightly to like moderately) which was a little bit lower than did the control (7.5, like moderately). However, the acceptability in softness was not correlated to the hardness from the instrumental measurement.

Table 16 Effect of Fibrotec™ and DATEM on the consumer acceptability of bread formulations

Sample	Fibrotec™ (%)	DATEM (%)	Appearance	Overall aroma^{ns}	Softness	Overall flavor	Overall liking
1	Control		7.5 ± 0.9^{a}	6.9 ± 1.3	7.3 ± 1.0^{a}	7.1 ± 1.3^{a}	7.4 ± 1.1^{a}
2	0.0	0.0	7.0 ± 1.0^{ab}	6.7 ± 1.2	6.5 ± 1.1^{bc}	7.0 ± 1.0^{ab}	6.9 ± 0.9^{ab}
3	0.0	1.0	6.8 ± 1.3^{b}	6.5 ± 1.1	6.8 ± 0.9^{ab}	6.4 ± 1.1^{bc}	6.4 ± 1.1^{bc}
4	0.0	2.0	7.0 ± 1.1^{ab}	6.4 ± 1.3	7.1 ± 1.1^{ab}	6.5 ± 1.1^{abc}	6.4 ± 1.1^{bc}
5	0.5	0.0	6.8 ± 1.2^{b}	6.6 ± 1.3	6.6 ± 1.4^{bc}	6.8 ± 1.3^{ab}	6.8 ± 1.3^{bc}
6	0.5	1.0	7.0 ± 1.2^{ab}	6.7 ± 1.2	7.2 ± 1.1^{ab}	6.7 ± 1.3^{ab}	6.7 ± 1.4^{bc}
7	0.5	2.0	6.9 ± 1.2^{ab}	6.5 ± 1.0	7.1 ± 0.8^{ab}	6.4 ± 1.0^{bc}	6.1 ± 1.4^{cd}
8	1.0	0.0	6.9 ± 1.0^{ab}	6.5 ± 1.2	6.7 ± 1.3^{abc}	7.0 ± 1.1^{ab}	6.7 ± 1.1^{bc}
9	1.0	1.0	6.4 ± 1.2^{b}	6.3 ± 1.2	6.1 ± 1.6^{c}	6.4 ± 1.1^{bc}	6.1 ± 1.2^{cd}
10	1.0	2.0	6.7 ± 1.3^{b}	6.3 ± 1.2	6.7 ± 1.1^{abc}	6.0 ± 1.2^{c}	5.7 ± 1.4^{d}

$^{a-d}$ Mean ± standard deviation of triplicate measurements with different letters in each column were significantly different (P<0.05).

The overall liking of bread containing composite flour with improvers was slightly lower than did the control. Conversely, bread containing improvers at the highest concentration obtained the lowest liking score of overall flavor and overall liking (6.0 and 5.7, respectively) compared to all bread formulations.

Based on the results of this part, composite flour bread with Fibrotec^{TM} and DATEM at 0.5% and 1.0%, respectively were selected for the further study with reasons of the improvement of aeration, high specific volume, soft texture, and acceptance in terms of all sensory attributes including appearance, overall aroma, softness, overall flavor, and overall liking.

5. Determination of the quality of developed bread from composite wheat-germinated brown rice flour

After obtaining the final formulation of composite bread, the quality of main developed bread was determined and described as follows.

5.1 Proximate and mineral composition of developed bread

The selected proximate and mineral compositions based on dry basis of fresh wheat bread and fresh composite flour bread were similar such as crude fat, crude fiber, and ash content, except for the protein and iron content (Table 17).

Table 17 Selected proximate compositions, mineral content, and free GABA of bread containing composite flour.

Component	Wheat bread	Composite-flour bread
Protein (%, db)	14.05±0.26	11.30±0.34
Crude Fat (%, db)	9.94±0.22	9.40±0.14
Crude Fiber (%, db)	0.39±0.13	0.49±0.06
Ash (%, db)	1.30±0.08	1.40±0.02
Carbohydrate (%, db)[A]	74.32±0.26	77.41±0.25
Copper (ppm)	3.15±0.30	2.91±0.01
Calcium (%)	0.05±0.01	0.04±0.00
Iron (ppm)	48.05±5.21	29.30±1.35
Magnesium (%)	0.40±0.00	0.23±0.22
Manganese(ppm)	5.38±0.25	5.96±0.03
Phosphorus(ppm)	0.12±0.01	0.15±0.00
Potassium (%)	0.13±0.00	0.13±0.00
Sodium (%)	0.25±0.00	0.24±0.00
Sulphur (%)	0.09±0.00	0.08±0.00
Zinc (ppm)	14.80±2.41	13.23±1.13
Free GABA	ND*	5.68±0.11

[A]Carbohydrate content obtained from the calculation of 100 % - (% crude protein + % crude fat + %crude fiber + % ash, based on dry basis).
*ND = Not determined, db = dry basis

The wheat bread had higher protein (14.05%) and iron (48.05 ppm) content compared to those (11.3% and 29.3 ppm) of the fresh composite flour bread. This was due to the fact that wheat flour contained higher protein (16.93%) and iron (83.96 ppm) content than the germinated flour (GBRF: 7.67% protein and 42.97 ppm of iron; GGBRF: 8.73% protein and 44.47 ppm of iron). In addition, bread from composite wheat-germinated flour contained 77.41% carbohydrate (db).

5.2 Free GABA content of the developed bread

Germinated flours used as ingredient in this experiment were obtained from rice harvested in 2009 (free GABA content of 9.61 and 10.25 mg/100 g flour for GBRF and GGNBRF, respectively) which had lower free GABA content than the one from 2008 (free GABA content of 32.70 and 30.69 mg/100 g flour for GBRF and GGNBRF, respectively). Based on the estimation, bread from the composite flour containing 30% GBRF and 10% GGNBRF (from rice harvested in 2009) should obtain total free GABA content of 3.91 mg/100 g flour. However, the results indicated that free GABA content of fresh developed bread from composite wheat-germinated flour remained as of 5.68 mg/100 g dry sample (Table 17). It could approximate that the free GABA content of developed bread with 40% of germinated flour would be about 2.27 mg/100 g germinated flour.

Thus, the free GABA content in bread sample had been lost about 41.95% which might be mainly due to the application of high temperature (180 ^{o}C, for 25 min) during baking process. Moreover, if we consume one serving of bread (2 pieces of bread) which is about 54 with 37.63% moisture content, we would acquire 1.91 mg/serving of free GABA.

5.3 Microbiological quality of bread

The microbiological quality of bread containing composite wheat-germinated brown rice flour was determined for microbial safety. The total plate count (TPC) and yeast & mold count of all breads were less than 2.0 log CFU/g, which was considered safe for consumption (Deibel and Swanson 2001).

5.4 Estimated cost of bread containing composite wheat-germinated brown rice flour

Based on the estimation, the cost of bread containing composite wheat-GBRF including cost of raw materials plus other variable costs such as man power and the electricity expense (exclude the packaging cost) would be approximately 31 baht/270 g (as the estimation in Appendix D, Appendix Tables D1, D2, and D3). Thus, the price of composite flour bread would be a little bit higher (1.5-2 times) than the price of existing bread product in Thai market such as whole wheat bread or mixed whole cereal grain bread which has the price of 20 baht/250 g (the price as of April, 2010). However, the price of composite flour bread could be competed with the premium product available in the market which has price of 50-60 baht/300-400 g.

6. The consumer acceptance test

The consumer acceptance test of the fresh developed bread from composite flour containing wheat:GBRF:GGNBRF (60:30:10) with Fibrotec^{TM} and DATEM at 0.5% and 1.0% w/w, respectively was carried out both in Thailand and in the US.

Consumers participating in Thailand (*n* = 114) were 30% male and 70% female; between 18 and 55 years of age whereas in the US. (*n* = 116) were 46.5% male and 53.5% female; between 18 and 65 years of age. The results demonstrated that the fresh developed bread from composite flour was acceptable by both Thai and the US. consumers (Tables 18 and 19).

Table 18 Mean consumer acceptability scores from Thai consumers and purchase intent of fresh breads containing wheat or composite flour

Attribute	Wheat bread	Composite-flour bread
Appearance	6.2 ± 1.4^{a}	6.2 ± 1.4^{a}
Color	6.9 ± 1.2^{a}	6.5 ± 1.3^{b}
Overall Aroma	7.2 ± 1.1^{a}	6.5 ± 1.6^{b}
Moistness	6.5 ± 1.3^{a}	6.3 ± 1.3^{a}
Smoothness	6.4 ± 1.3^{a}	5.9 ± 1.5^{a}
Softness	6.7 ± 1.2^{a}	6.4 ± 1.4^{a}
Overall flavor	6.8 ± 1.1^{a}	6.4 ± 1.3^{b}
Overall liking	6.7 ± 1.0^{a}	6.3 ± 1.3^{b}
Purchase intent (%) [B]		
Before	82.5	75.4
After	-	95.6*

[a, b]Mean ± standard deviation based on a 9-point hedonic scale in the same row followed by different letters are significantly different (P<0.05).
[B]Purchase intent was obtained from both before and after consumers had gained information about health benefits of germinated brown rice flour.
*Indicated significant differences of purchase intent (before vs. after) based on the McNemar's test (P<0.05).

For Thai consumers, the acceptability scores for 8 sensory attributes ranged from 6.2 to 7.2 and from 5.9 to 6.5 for fresh wheat bread and fresh composite-flour bread, respectively (Table 18). The overall liking score (6.3) for the fresh composite-flour bread was lower than that (6.7) of fresh wheat bread; this was likely due to the lower score of overall aroma and smoothness of the developed bread (Table 18).
In addition, the similar results obtained from the US. consumers were determined. The acceptability scores for 8 sensory attributes ranged from 7.1 to 7.6 and from 7.0 to 7.3, respectively, for fresh wheat bread and fresh composite-flour bread (Table 18). The overall liking score (7.1) for fresh composite-flour bread was also slightly lower than that (7.6) of fresh wheat bread; this was likely due to the lower liking score of appearance and texture-related attributes were noticed (Table 19).

Table 19 Mean consumer acceptability scores from US. consumers and purchase intent of fresh breads containing wheat or composite flour [A]

Attribute	Wheat bread	Composite-flour bread
Appearance	7.6 ± 1.2^{a}	7.1 ± 1.3^{b}
Color	7.4 ± 1.2^{a}	7.2 ± 1.2^{ab}
Overall Aroma	7.1 ± 1.5^{a}	7.0 ± 1.7^{a}
Moistness	7.5 ± 1.2^{a}	7.3 ± 1.1^{a}
Smoothness	7.4 ± 1.1^{a}	7.0 ± 1.3^{b}
Softness	7.5 ± 1.1^{a}	7.0 ± 1.3^{b}
Overall flavor	7.5 ± 1.3^{a}	7.1 ± 1.3^{a}
Overall liking	7.6 ± 1.1^{a}	7.1 ± 1.3^{b}
Purchase intent (%)		
Before	91.4	76.7
After	-	88.8^{*}

[A]Mean $\pm$ standard deviation based on a 9-point hedonic scale in the same row followed by different letters are significantly different ($P<0.05$).
*Indicated significant differences of purchase intent (before vs. after) based on the McNemar's test ($P<0.05$).

The results remarkably demonstrated that the US. consumer rated higher score for all sensory attributes than Thai consumers which perhaps due to the US. consumers associated with a large number of bread varieties available in their markets especially whole grain type rather than Thai consumers who have less familiar with different types of bread. In addition, the way of consumption of both consumers was different. The US. consumers generally consume bread as a staple food but Thai consumers consume bread as a snack. The results also indicated that Thai consumers did not prefer this bread as a result of lower liking score of texture-related attributed, especially smoothness (5.9, like slightly).

Moreover, consumers preferred to purchase the fresh wheat bread (82.5% and 91.4% for Thai and the US. consumers, respectively) than the fresh composite-flour bread (75.4% and 76.7% for Thai and the US. consumers, respectively) (Tables 18 and 19). However, after providing the health benefit information of germinated brown rice to consumers, the purchase intent significantly increased based on the McNemar's test (P<0.05) for both consumers, particularly of Thai consumers (increased from 75.4 % to 95.6%) (Table 18).

7. The stability of bread from wheat-germinated brown rice flour during storage times

Physicochemical properties and consumer acceptability of fresh wheat bread (day 0) and composite-flour breads stored for 0, 3, and 5 days at 25 ^{o}C and 70% RH are shown in Tables 20 and 21.

7.1 Physicochemical properties

7.1.1 Water activity and moisture content

Among composite-flour breads, water activity of crumb slightly decreased from 0.947 to 0.932 after 5 days of storage (Table 20). Moisture content (34.66%) of fresh wheat bread was lower than that (37.63%) of fresh composite-flour bread. After 5 days of storage, moisture content of crumb of composite-flour breads significantly decreased from 37.63% to 35.93%, indicating that water migrated from the inner crumb to the crust (Goesaert *et al.,* 2009a) and might perhaps due to the evaporation of water from bread to the packaging.

7.1.2 Color of bread crumb

Crumb color differences between fresh wheat bread and fresh composite-flour bread were instrumentally observed (Table 20), with the latter having higher chroma, lower hue angle and lower lightness which was likely attributed to browning pigment formation caused by reducing sugar present in germinated flour. The reducing sugar had been reported to be obtained in germinated flour of 400 – 600 mg/100 g flour (Charoenthaikij *et al.,* 2009). The noticeable color of the fresh bread containing composite flour was off-white rather than the control. There were no differences in yellowness of crumb of composite-flour breads, but their redness slightly increased while color lightness increased with storage time. Although some significant color differences were instrumentally observed, differences based on visual observation may not be that different.

Table 20 Effect of storage time on moisture content, water activity (a_w), and color of bread containing composite flour

Product [A]	Moisture (%)	a_w	L*	a*	b*	C*	h*
Control	34.66±0.14^{c}	0.940±0.011^{ab}	65.07±0.93^{a}	-1.72±0.17^{c}	13.70±0.69^{b}	13.79±1.13^{b}	97.23±0.98^{a}
A	37.63±0.65^{a}	0.947±0.002^{a}	63.14±0.92^{b}	-1.53±0.06^{b}	14.95±0.67^{a}	15.03±0.66^{a}	95.85±0.35^{b}
B	36.78±0.64^{a}	0.939±0.003^{a}	65.74±0.21^{a}	-1.51±0.09^{b}	14.14±0.54^{a}	14.22±0.54^{ab}	95.88±0.63^{b}
C	35.93±0.23^{b}	0.932±0.006^{b}	66.12±1.39^{a}	-1.22±0.12^{a}	14.94±0.82^{a}	14.99±0.80^{a}	94.71±0.66^{c}

$^{a-c}$ Mean ± standard deviation of duplicate measurements for moisture content and a_w and triplicate measurements for color profiles with different letters in each column were significantly different (P<0.05).

[A]Products A, B, and C were bread containing composite flour stored for 0, 3, and 5 day(s), respectively. Wheat bread served as the control.

7.1.3 Texture of bread crumb

Crumb hardness, cohesiveness and springiness can be used to indicate bread freshness. Substituting wheat flour with germinated brown rice flour significantly affected the textural quality of bread (Table 21). Regardless of storage time, the composite-flour bread crumb was harder with less cohesiveness and springiness compared to the fresh wheat bread. Storage time significantly affected the textural properties of composite-flour breads; crumb hardness increased while both cohesiveness and springiness decreased with storage time.

Table 21 Effects of storage time on texture profiles of bread containing composite flour

Product [A]	Hardness	Cohesiveness	Springiness
Control	3.38±0.83[d]	0.65±0.05[a]	0.94±0.01[a]
A	4.16±0.43[c]	0.54±0.01[b]	0.84±0.01[b]
B	6.94±0.73[b]	0.38±0.03[c]	0.79±0.05[c]
C	10.37±0.92[a]	0.28±0.03[d]	0.74±0.08[d]

[a-d] Mean ± standard deviation of duplicate measurements with different letters in each column were significantly different (P<0.05).
[A] Product A, B, and C were breads containing composite flour stored for 0, 3, and 5 days, respectively.

The crumb hardness of composite-flour breads increased from 4.16 N to 10.37 N after 5 days of storage (Table 21). This increase may be attributed to development of a network formed by recrystallization of amylopectin (Goesaert *et al.*, 2009a) as a result of starch retrogradation evidenced in Table 22. This effect is reported to be more pronounced in rice bread (Gujral *et al.*, 2003) than regular wheat bread. Kadan *et al.* (2001) and Watanabe *et al.* (2004) also reported that rice bread crumb had a significant higher firming rate than wheat bread associated with the higher hardness and enthalpy (ΔH) energy.

During storage, the molecular change in bread such as starch retrogradation/recrystallization and changes in water migration contribute to crumb quality deterioration (Gray and BeMiller 2003; Goesaert *et al.*, 2009b), including firming of bread crumb as observed in this study.

7.1.4 Thermal properties

One important process involved in bread staling is starch retrogradation, which was determined using DSC in this study (Table 22). There were no significant ($P \geq 0.05$) differences in T_o and T_p between fresh wheat bread and fresh composite-flour bread. Low endothermic signals were observed between 40 and 60 ^oC which were likely due to melting of the very low levels of recrystallized amylopectin in the sample (Goesaert *et al.*, 2009a).

T_p, T_c and ΔH of composite-flour bread slightly increased as the storage time increased (Table 22). The retrogradation enthalpy (ΔH) of the fresh composite-flour bread and the control was 0.53 and 0.30 J/g dry sample, respectively. The results showed that after 3-day storage, ΔH of the composite-flour bread increased (from 0.53 to 0.90). Gujral *et al.* (2003) reported that rice bread crumb showed a very fast amylopectin retrogradation. Our previous study (Charoenthaikij *et al.*, 2009) found that germinated brown rice flour contained some *alpha*-amylase enzyme, which has been reported to reduce amylopectin retrogradation and firming of wheat bread (Champenois *et al.*, 1999) and rice bread (Gujral *et al.*, 2003). However, this effect was not observed in this current study. The highest retrogradation enthalpy (ΔH = 1.16 J/g) was observed in crumb of the control after 5-day storage.

Table 22 Effect of storage time on thermal properties of bread crumb containing composite wheat-germinated brown rice flour

Product	Storage time (day)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g, dry sample)
Wheat bread	0	42.31±0.62^{c}	46.51±0.30^{b}	52.29±0.32^{c}	0.30±0.01^{d}
	3	48.57±0.22^{a}	53.13±0.00^{a}	60.01±0.02^{a}	0.43±0.02^{d}
	5	47.61±0.02^{ab}	53.25±0.54^{a}	60.46±0.19^{a}	1.16±0.02^{a}
Composite -	0	40.38±1.61^{c}	45.22±1.29^{b}	50.53±0.83^{d}	0.53±0.01^{cd}
flour bread	3	46.09±1.10^{b}	52.54±0.00^{a}	58.26±0.90^{b}	0.90±0.19^{bc}
	5	47.34±0.65^{ab}	53.88±0.00^{a}	57.47±0.59^{b}	0.88±0.25^{ab}

$^{a-c}$ Mean ± standard deviation of duplicate measurements with different letters in each column were significantly different (*P*<0.05).
T_o defined as onset temperature; T_p defined as peak temperature; T_c defined as conclusion temperature; ΔH defined as enthalpy energy.

7.2 Microbiological quality of bread

Prior to the consumer acceptance test, all breads were tested for microbial safety. The total plate count (TPC) and yeast & mold count of all breads were less than 2.0 log CFU/g, which was considered safe for consumption (Deibel and Swanson 2001).

7.3 Consumer acceptance (US. consumers)

7.3.1 Product discriminating sensory attributes

To determine further which attributes were responsible for group differences, DDA was performed in this study. The texture-related attributes (moistness, smoothness and softness) were discriminating attributes with canonical correlations of 0.84-0.87, whereas appearance, color and overall aroma least contributed to group differences (Table 24); this result substantiated that of ANOVA (Table 23).

Table 23 Mean consumer acceptability scores from US. consumer and purchase intent of breads containing wheat or composite wheat-germinated brown rice flour

Attribute	Control^{B}	Stored composite bread		
		0-day (A)	3-day (B)	5-day (C)
Appearance	7.6 ± 1.2^{a}	7.1 ± 1.3^{b}	6.7 ± 1.6^{c}	6.6 ± 1.5^{c}
Color	7.4 ± 1.2^{a}	7.2 ± 1.2^{ab}	6.8 ± 1.4^{c}	7.0 ± 1.3^{bc}
Overall aroma	7.1 ± 1.5^{a}	7.0 ± 1.7^{a}	6.7 ± 1.6^{a}	6.7 ± 1.5^{a}
Moistness	7.5 ± 1.2^{a}	7.3 ± 1.1^{a}	4.8 ± 1.8^{b}	4.6 ± 1.9^{a}
Smoothness	7.4 ± 1.1^{a}	7.0 ± 1.3^{b}	4.9 ± 1.7^{c}	4.4 ± 1.8^{c}
Softness	7.5 ± 1.1^{a}	7.0 ± 1.3^{b}	4.9 ± 1.9^{c}	4.5 ± 1.9^{c}
Overall flavor	7.5 ± 1.3^{a}	7.1 ± 1.3^{a}	5.4 ± 1.8^{b}	5.3 ± 1.9^{b}
Overall liking	7.6 ± 1.1^{a}	7.1 ± 1.3^{a}	5.1 ± 1.8^{c}	4.9 ± 1.8^{c}
Purchase intent (%)				
Before	91.4	76.7	24.1	19.8
After	-	88.8^{*}	51.7^{*}	50.0^{*}

$^{a\text{-}c}$Mean ± standard deviation from 116 responses based on a 9-point hedonic scale. Mean values in the same row followed by different letters were significantly different (P<0.05). Wheat bread (Day 0) served as the control.
*Indicated significant differences of purchase intent (before vs. after) based on the McNemar's test (P<0.05).

Table 24 Canonical structure matrix of sensory attributes describing group differences among 4 breads containing wheat or composite flour [A]

Attribute	Can1	Can2	Can3
Appearance	0.262	0.607	0.312
Color	0.195	0.221	0.665
Overall Aroma	0.119	0.114	0.242
Moistness	0.872*	-0.228	0.184
Smoothness	0.861*	0.091	-0.061
Softness	0.841*	0.269	0.143
Overall flavor	0.604*	0.123	0.348
Overall liking	0.774*	0.194	0.413
Cumulative variance explained (%)	96.0	98.8	100.0

[A] Composite wheat-germinated brown rice flour composed of wheat:GBRF:GGBRF at 60:30:10 ratio. Based on the pooled within group variances. Can1, 2 and 3 refer to the 1st, 2nd, and 3rd canonical discriminant functions, respectively.
*Indicated attributes that accounted for the group differences in the 1st dimension. Canonical correlation of 0.6 was arbitrarily used as a cut-off point.

7.3.2 Analysis of diagnostic JAR attributes by the penalty and SNR analyses

Based on Table 23, only the JAR including moistness JAR, smoothness JAR, and softness JAR responses for discriminating sensory attributes were analyzed. During storage, the percentage of consumers considering moistness, smoothness, and softness perception to be “not enough or non JAR” increased (Table 25). The mean drop and penalty analyses of products B and C (3-day and 5-day stored composite-flour bread) were performed to gain an understanding of the product attributes needed to be adjusted. For product A, there were less than 20% of the non-JAR responses, thus it was excluded from the analysis.

The calculated total penalty of products B and C ranged from 1.62 to 2.28. The high penalty value indicates a large change in the hedonic ratings (Cavitt *et al.*, 2005). For products B and C, the penalty for moistness (2.28) was higher than smoothness (1.72 and 1.67, respectively) and softness (1.62 and 1.77, respectively). The results indicated that bread after 5-day storage was considered by consumers to be “not moist enough”, “not smooth enough” and “not soft enough,” having a mean drop of 2.91, 2.42, and 2.51, respectively, on a 9-point hedonic scale (Table 24).

The signal-to-noise-ratio (SNR) analysis was further performed on the JAR responses for these three discriminating sensory attributes. There was a positive relationship between overall liking and the SNR values (Figure 28). The higher the SNR value the closer the product to be JAR (Gacula *et al*., 2007). It can be clearly seen that both fresh wheat bread and fresh composite-flour bread (A) had high SNR values for these three discriminating attributes. In contrast, products B and C obtained lower SNR values for moistness (0.55 and 0.48), smoothness (1.11 and 1.13), and softness (1.75 and 1.03), that were associated with lower overall liking scores ranging from 4.8 to 5.1. From Tables 23, 24, and 25 and Figure 28, it is clear that texture-related attributes should be the focus for further improvement or refinement of the composite-flour bread formulation.

Table 25 Consumer penalty analysis of Just-About-Right (JAR) diagnostic attributes: Percentage of consumers and mean drop for liking score of each JAR category of breads containing composite flour

Product[A]	Moistness JAR		Smoothness JAR		Softness JAR		Total penalty [C]		
	Not enough	Too much	Not enough	Too much	Not enough	Too much	Moistness JAR	Smoothness JAR	Softness JAR
A	-	-	-	-	-	-	-	-	-
B	76.5* (2.98)[B]	-	67.6 (2.54)	-	58.4 (2.78)	-	2.28	1.72	1.62
C	78.4 (2.91)	-	69.0 (2.42)	-	70.5 (2.51)	-	2.28	1.67	1.77

[A]Products A, B, and C were bread containing composite flour stored for 0, 3, and 5 day(s), respectively. Wheat bread served as the control.

[B]The number in the parentheses is the mean drop calculated as [mean liking score for the JAR response – mean liking score for the non-JAR response]. *Indicated the percentage of consumers who found each product to be “Not enough” for moistness, smoothness, and softness JAR.

[C]Total penalty = Mean drop x percentage of non-JAR consumer responses. A total penalty > 0.05 is high and > 0.25 is noteworthy (Cadot *et al.*, 2010).

(-) Indicated less than 20% of consumers’ selected corresponding JAR category.

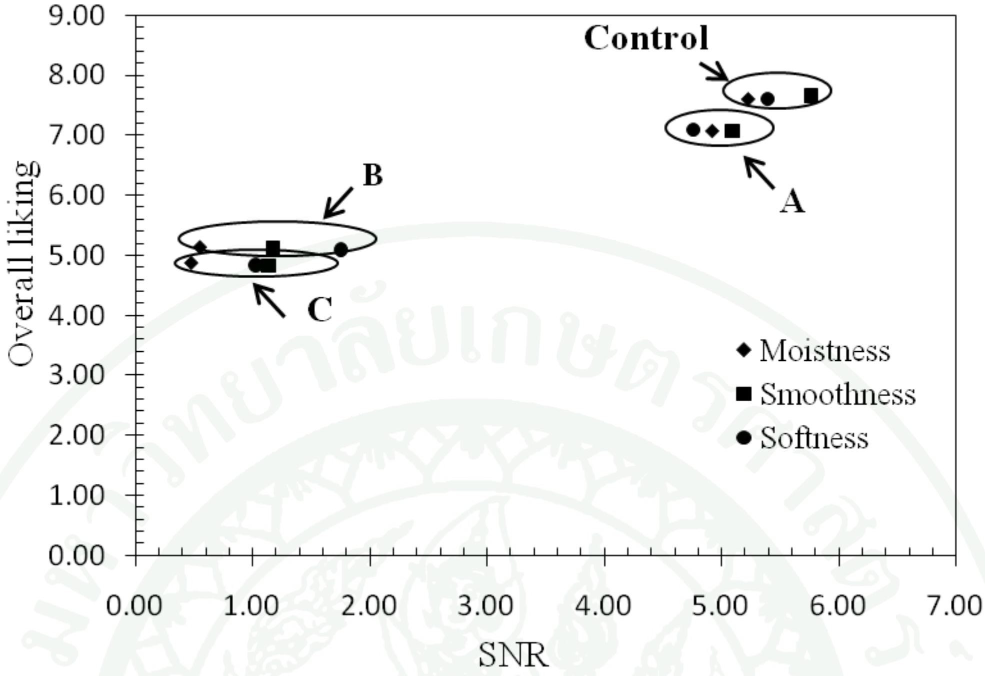


Figure 28 Signal-to-noise ratio (SNR) of moistness, smoothness, and softness against overall liking of 4 bread products. Products A, B, and C were bread containing composite wheat-germinated brown rice flour stored for 0, 3, and 5 day(s).

7.3.3 Consumers' purchase intent analyses

Consumers preferred to purchase the fresh wheat bread (91.4%) than the fresh composite-flour bread (A, 76.7%) (Table 23). Purchase intent of composite-flour breads (B and C) after storage decreased to less than 25%. After providing the health benefit information of germinated brown rice to consumers, the purchase intent of products B and C significantly increased based on the McNemar's test ($P<0.05$); this increase was at least 200%, i.e., from 24.1 to 51.7% and 19.8 to 50%, respectively (Table 23).

There were many attributes influenced the purchase intent of consumers. Thus, by the extraction method of the principal component analysis, two new variables called F1 and F2 (Table 26) were extracted from 8 attributes and 3 JAR. The results showed that the first two components were explained by 76.07 % of the total variance (60.44 and 15.63%, respectively). The first component (Factor 1, F1) related to the texture-related attributes, overall flavor, and overall liking. The second component (Factor 2, F2) related to surface properties (appearance and color) and aroma (Table 26).

Table 26 Factor loading and the percentage of variance explained of new components based on variables obtained from the consumer acceptance test (US. consumers)

Attribute	Component	
	1 (F1)	2 (F2)
Appearance	0.556	**0.665**
Color	0.515	**0.707**
Overall Aroma	0.415	**0.665**
Moistness	**0.907**	-0.100
JAR_Moistness*	**0.779**	-0.330
Smoothness	**0.910**	-0.133
JAR_Smoothness*	**0.804**	-0.295
Softness	**0.924**	-0.086
JAR_Softness*	**0.796**	-0.322
Overall flavor	**0.826**	0.058
Overall liking	**0.909**	-0.038
Variance explained (%)	60.44	15.63
Cumulative variance	76.07	

* Only JAR not enough and JAR (just-about-right) data were used for the analysis.

To identify attributes that influenced purchase intent, the LRA was performed using those new variables (F1 and F2). Among all 4 samples (Control, A, B, and C), both F1 and F2 influenced purchase intent of consumers, with the highest odds ratio (13.960) observed for F1 (Table 27). It means that the chance that the product would be purchased will be 13.960 times higher than not-purchased with a 1-unit increase of liking score (based on 9-piont hedonic scale) of attributes related in F1 (texture-related attributes, overall flavor, and overall liking). The odds ratio of F2 was 2.152, indicating the probability of the product being purchased would be 2.473 times higher than not-purchased if a 1-unit of appearance, color, and aroma (F2) score of product increased.

Table 27 Parameter estimates, probability, and odds ratio estimates for predicting purchase intent of bread made of wheat flour or composite flour [A]

Variables	Coefficient	$Pr > \chi^2$	Odds ratio
F1	2.636	0.000	13.960
F2	0.767	0.000	2.152
Constant	-0.101	0.522	-

[A] Based on the logistic regression analysis, using 2 new variables extracted from 8 sensory acceptability and 3 JAR attributes. The analysis of maximum likelihood estimates was used to obtain parameter estimates. Significance of parameter estimates was based on the Wald $\chi 2$ value at $P < 0.05$.

CONCLUSION AND RECOMMENDATION

Conclusion

The significant physicochemical property changes of brown rice flour and glutinous brown rice flour were observed under different germination conditions. Increasing steeping time (from 24 to 72 h) or decreasing the pH of steeping water (in the range of pH3 to pH7) caused an increase in reducing sugar, free GABA and *alpha*-amylase activity, but a decrease in total starch content and viscosities of GBRF obtained from both brown rice varieties.

The germination conditions of brown rice had significant effects on the physicochemical properties of composite wheat-geminated brown rice flours and the bread quality. Breads containing 30% GBRF from all germination conditions exhibited lower specific volume but greater hardness than the wheat control bread. However, these breads were acceptable to consumers with the overall liking score of 6.4-6.7. This study demonstrated that GBRF, especially the one prepared at pH3 and 24 h steeping time, could be used to substitute wheat flour at 30% in bread formulation without compromising sensory quality.

The substitution of wheat flour with different ratios of GBRF and/or GGNBRF from steeping at pH3 for 24 h had significant effect on the physicochemical properties of composite flours. At 40% substitution, composite flours containing GGNBRF exhibited dramatic changes of their pasting properties. A higher rate of substitution contributed to lower viscosity of composite flours as a result of increased *alpha*-amylase activity, reduction of gluten as the substitution of germinated flour, and the acid solution used as the steeping water. This study demonstrated feasibility of incorporating up to 40% germinated brown rice flour in a wheat bread formulation.

Dough and bread quality were significantly affected by the selected bread improvers. The Fibrotec^{TM} and DATEM improvers had no effect on the WA of dough containing composite flours. The supplementing composite bread with improvers significantly improved the specific volume of breads when compared with bread without improvers, but still lower than that of the control. Bread formulations containing improvers were not significantly different in overall liking from the one without improvers but less than the control. This study demonstrated that to improve the quality of bread containing germinated flours, Fibrotech^{TM} and DATEM should be used at 0.5 and 1.0 % (w/w), respectively.

The developed bread contained free GABA of 5.68 mg/100 g dry sample or 1.91 mg/serving (2 slices of bread about 54 g). The presence of germinated brown rice flour and storage time had a significant effect on selected physicochemical properties and sensory acceptability of wheat-germinated brown rice breads. At least 75% of Thai and US. consumers would purchase the fresh composite-flour bread if commercially available with overall liking score of 6.3 (like slightly) and 7.1 (like moderately) for Thai and the US. consumers, respectively. In addition, based on the LRA and SNR analysis, the texture-related attributes (moistness, smoothness, and softness) and flavor should be the focus for further refinement of the composite-flour bread formulation. The health benefit information provided to consumers significantly increased positive purchase intent of this product.

Recommendation

This type of bread may be sold as frozen bread which would have a longer shelf life, or may be supplied as a foodservice product which would be made-to-order or made fresh daily as currently practiced in some major grocery stores. Thus, further studies on investigation of the quality of frozen bread are necessary.

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Appendices

Appendix A

Pasting profiles of germinated brown rice flour

Appendix Table A1 Pasting characteristics of germinated brown rice flour prepared from brown rice (BR, KDML105) as affected by various germination conditions

Germination condition		Pasting	Viscosity (RVU)				
Steeping time (h)	pH of steeping water	temperature ^{ns} (°C)	Peak	Trough	Breakdown	Final Viscosity	Setback from trough
Control^{B}		74.00 ± 2.31	255.46 ±11.90^{a}	157.09 ± 1.22^{a}	98.38 ±10.98^{a}	257.50 ±12.79^{a}	100.42 ±11.85^{a}
24	3	73.23 ± 0.53	49.04 ± 1.00^{ef}	20.46 ± 1.24^{h}	28.59 ± 0.23^{cd}	41.67 ± 0.94^{f}	21.21 ± 0.30^{e}
48	3	72.78 ± 0.80	8.12 ± 0.38^{g}	0.00 ± 0.00^{i}	7.97 ± 0.05^{e}	0.68 ± 0.25^{g}	0.68 ± 0.06^{f}
72	3	73.08 ± 0.45	7.42 ± 0.12^{g}	0.00 ± 0.00^{i}	7.75 ± 0.11^{de}	1.13 ± 0.06^{g}	1.13 ± 0.06^{f}
24	5	73.03 ± 0.98	196.92 ± 3.99^{b}	142.36 ± 1.23^{a}	51.84 ± 0.09^{b}	233.75 ± 1.74^{a}	91.39 ± 1.90^{ab}
48	5	73.35 ± 0.57	144.17 ± 2.35^{c}	100.67 ± 1.06^{d}	43.50 ± 3.42^{c}	175.84 ± 1.29^{bc}	75.17 ± 0.23^{c}
72	5	72.60 ± 0.43	70.31 ± 0.86^{de}	40.81 ± 0.81^{g}	30.34 ± 0.67^{cd}	82.83 ± 1.36^{e}	42.03 ± 0.71^{d}
24	7	72.78 ± 0.75	228.22 ± 2.50^{b}	162.00 ± 3.18^{a}	66.22 ± 2.82^{b}	254.06 ± 2.12^{a}	92.05 ± 1.07^{b}
48	7	73.65 ± 1.27	156.79 ± 6.07^{c}	114.04 ± 2.77^{c}	42.75 ± 3.30^{c}	189.59 ± 5.78^{b}	75.55 ± 3.01^{c}
72	7	73.60 ± 0.00	97.88 ± 0.29^{d}	61.46 ± 0.06^{f}	36.42 ± 0.35^{c}	113.46 ± 0.30^{d}	52.00 ± 0.35^{d}
24	DI	73.36 ± 0.44	208.11 ± 2.08^{b}	138.83 ± 6.47^{b}	69.36 ± 3.98^{a}	240.15 ± 7.49^{a}	97.89 ± 2.88^{ab}
48	DI	73.07 ± 0.46	129.61 ± 1.49^{c}	87.97 ± 3.71^{e}	40.21 ± 0.65^{c}	154.89 ± 4.14^{c}	66.92 ± 0.80^{c}
72	DI	73.05 ± 0.64	23.21 ± 0.06^{fg}	11.55 ± 0.53^{h}	11.67 ± 0.59^{de}	28.63 ± 0.42^{f}	17.08 ± 0.11^{e}

$^{a-i}$Mean ± standard deviation with different letters in each column were significantly different ($P < 0.05$).
Trough was defined as minimum viscosity after peak; Breakdown was defined as the difference between peak and trough; Setback from trough was defined as the difference between final viscosity and trough.
^{B}Non germinated BR served as the control. ^{ns} not significantly different ($P \geq 0.05$).

Appendix Table A2 Pasting characteristics of germinated brown rice flour prepared from glutinous brown rice (GGNBR, RD6) as affected by various germination conditions

Germination condition		Pasting temperature ^{ns} (^{o}C)	Viscosity (RVU)				
Steeping time (h)	pH of steeping water		Peak	Trough	Breakdown	Final Viscosity	Setback from trough
Control^{B}		66.93 ± 0.41	190.17 ±10.79 ^{a}	79.19 ± 7.94 ^{a}	110.98 ± 3.06 ^{a}	96.77 ± 8.19 ^{a}	17.59 ± 0.38 ^{a}
24	3	68.08 ± 0.04	27.46 ± 0.53 ^{cd}	2.88 ± 0.29 ^{cd}	24.59 ± 0.23 ^{d}	4.96 ± 0.30 ^{def}	2.09 ± 0.01 ^{ef}
36	3	68.48 ± 0.53	8.63 ± 0.06 ^{ef}	0.27 ± 0.08 ^{d}	8.42 ± 0.12 ^{f}	1.13 ± 0.06 ^{f}	0.86 ± 0.15 ^{gh}
48	3	68.98 ± 0.92	4.42 ± 0.40 ^{f}	0.00 ± 0.00 ^{d}	4.48 ± 0.48 ^{f}	0.47 ± 0.09 ^{f}	0.47 ± 0.09 ^{h}
24	5	67.18 ± 0.31	58.67 ± 5.07 ^{b}	12.60 ± 1.67 ^{b}	47.79 ± 4.86 ^{b}	18.73 ± 1.96 ^{b}	6.13 ± 0.31 ^{b}
36	5	68.08 ± 1.10	37.30 ± 4.77 ^{c}	4.63 ± 0.64 ^{cd}	33.13 ± 3.47 ^{c}	8.74 ± 0.97 ^{cde}	4.11 ± 0.33 ^{cd}
48	5	68.05 ± 0.00	16.88 ± 0.06 ^{de}	0.79 ± 0.06 ^{d}	16.09 ± 0.12 ^{e}	2.92 ± 0.23 ^{ef}	2.13 ± 0.29 ^{ef}
24	7	67.33 ± 0.04	56.59 ± 0.83 ^{b}	11.92 ± 0.23 ^{b}	44.67 ± 0.59 ^{b}	17.88 ± 0.06 ^{b}	5.96 ± 0.17 ^{b}
36	7	67.70 ± 0.57	55.67 ± 0.12 ^{b}	11.54 ± 0.41 ^{b}	44.13 ± 0.53 ^{b}	17.25 ± 0.24 ^{b}	5.71 ± 0.65 ^{b}
48	7	68.40 ± 0.57	37.21 ± 0.53 ^{c}	6.09 ± 0.12 ^{bcd}	31.13 ± 0.64 ^{c}	9.75 ± 0.82 ^{cd}	3.67 ± 0.70 ^{d}
24	DI	67.67 ± 0.40	50.04 ± 7.86 ^{b}	8.21 ± 2.25 ^{bc}	41.83 ± 5.87 ^{b}	12.99 ± 3.07 ^{bc}	4.78 ± 0.86 ^{c}
36	DI	68.45 ± 0.11	23.00 ± 1.41 ^{d}	2.00 ± 0.00 ^{d}	21.00 ± 1.41 ^{de}	4.59 ± 0.12 ^{def}	2.59 ± 0.12 ^{e}
48	DI	68.88 ± 0.11	7.88 ± 0.53 ^{ef}	0.25 ± 0.00 ^{d}	7.63 ± 0.53 ^{f}	1.63 ± 0.18 ^{f}	1.38 ± 0.18 ^{fg}

$^{a-h}$Mean ± standard deviation with different letters in each column were significantly different (P<0.05).

Trough was defined as minimum viscosity after peak; Breakdown was defined as the difference between peak and trough; Setback from trough was defined as the difference between final viscosity and trough.

^{B}Non germinated GNBR served as the control. ^{ns} not significantly different ($P \geq 0.05$).

Appendix Table A3 The effect of steeping water on pasting profiles of normal rice flour (KDML 105)

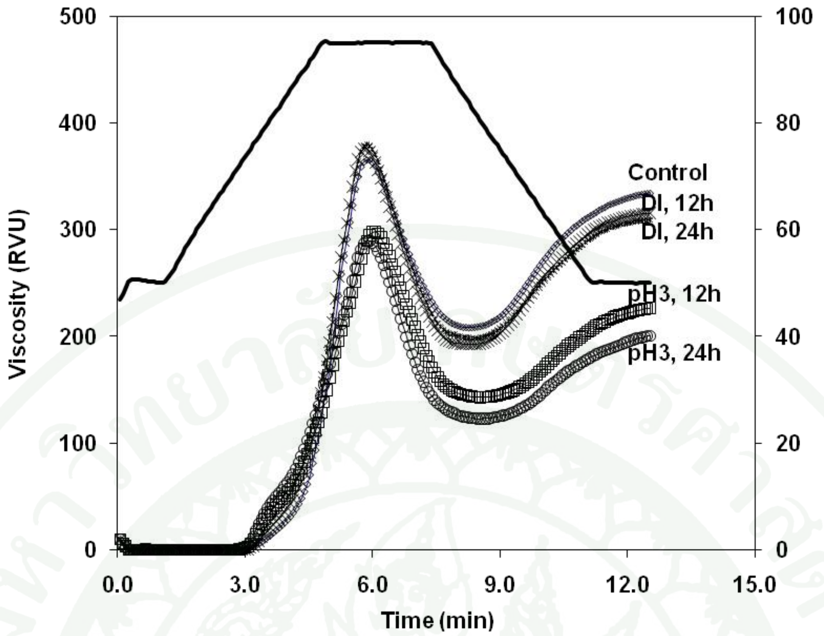
Germination condition		Viscosity (RVU)					
Steeping time (h)	pH of steeping water	Pasting temp (^{o}C)	Peak	Trough	Breakdown	Final	Setback from through
12	3	72.79±0.08^{c}	299.27±5.36^{c}	142.63±4.45^{d}	156.65±5.97^{d}	226.44±4.74^{c}	83.81±0.86^{c}
24	3	72.63±0.35^{c}	289.81±3.13^{d}	122.71±2.01^{e}	167.11±2.96^{c}	200.48±2.48^{d}	77.77±0.59^{c}
12	DI	73.93±0.43^{b}	377.83±3.55^{a}	191.43±3.07^{c}	186.40±5.46^{a}	314.87±2.22^{b}	123.43±2.14^{a}
24	DI	73.41±0.38^{b}	380.34±0.40^{a}	198.31±3.00^{b}	182.02±2.77^{b}	309.04±4.72^{b}	110.73±2.94^{b}
-	-	74.62±0.46^{a}	366.03±3.25^{b}	207.92±7.29^{a}	158.11±4.85^{d}	334.28±3.02^{a}	126.36±5.20^{a}

$^{a-e}$Mean ± standard deviation followed by different letters in the same column were significantly different (P<0.05). (-) non-germinated brown rice (control).

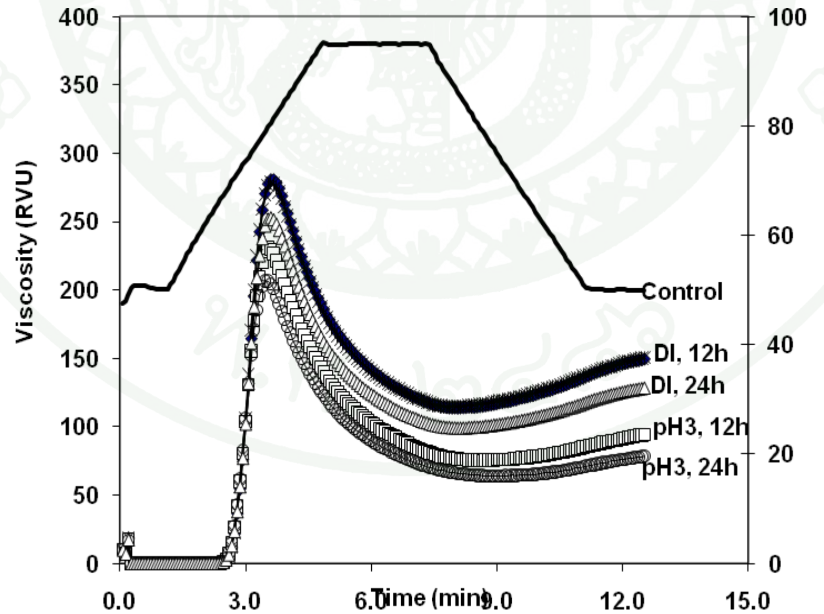
Appendix Table A4 The effect of steeping water on pasting profiles of glutinous rice flour (RD6)

Germination condition		Viscosity (RVU)					
Steeping time (h)	pH of steeping water	Pasting temp (^{o}C)	Peak	Trough	Breakdown	Final	Setback from through
12	3	72.79±0.08^{c}	299.27±5.36^{c}	142.63±4.45^{d}	156.65±5.97^{d}	226.44±4.74^{c}	83.81±0.86^{c}
24	3	72.63±0.35^{c}	289.81±3.13^{d}	122.71±2.01^{e}	167.11±2.96^{c}	200.48±2.48^{d}	77.77±0.59^{c}
12	DI	73.93±0.43^{b}	377.83±3.55^{a}	191.43±3.07^{c}	186.40±5.46^{a}	314.87±2.22^{b}	123.43±2.14^{a}
24	DI	73.41±0.38^{b}	380.34±0.40^{a}	198.31±3.00^{b}	182.02±2.77^{b}	309.04±4.72^{b}	110.73±2.94^{b}
-	-	74.62±0.46^{a}	366.03±3.25^{b}	207.92±7.29^{a}	158.11±4.85^{d}	334.28±3.02^{a}	126.36±5.20^{a}

$^{a-e}$Mean ± standard deviation followed by different letters in the same column were significantly different (P<0.05). (-) non-germinated brown rice (control).



Appendix Figure A1 The effect of steeping water on pasting properties of normal rice flour (KDML 105)



Appendix Figure A2 The effect of steeping water on pasting properties of glutinous rice flour (RD6)

Appendix B

Questionnaire and consumer acceptance test

Research Consent Form for Consumer Research (for the US. consumers)

I, _________________________, agree to participate in the research entitled "Consumer Acceptability of Bread" which is being conducted by Dr. Witoon Prinyawiwatkul, Professor of the Department of Food Science at Louisiana State University, Agricultural Center, phone number (225) 578-5188.

I understand that participation is entirely voluntary and whether or not I participate will not affect how I am treated on my job. I can withdraw my consent at any time without penalty or loss of benefits to which I am otherwise entitled and have the results of the participation returned to me, removed from the experimental records, or destroyed. One hundred and twenty consumers will participate in this research. For this particular research, about 20 minute participation will be required for each consumer.

The following points have been explained to me:

1. In any case, it is my responsibility to report prior participation to the investigator any food allergies I may have.

2. The reason for the research is to gather information on consumer acceptability of bread. The benefit that I may expect from it is a satisfaction that I have contributed to development of more nutritious bread formulations.

3. The procedures are as follows: Four coded samples will be placed in front of me, and I will evaluate them by normal standard methods and indicate my evaluation on score sheets. All procedures are standard methods as published by the American Society for Testing and Materials and the Sensory Evaluation Division of the Institute of Food Technologists.

4. Participation entails minimal risk: The only risk which can be envisioned is that of an allergic reaction to commercial wheat flour, rice, milk, sugar, butter, egg, and commonly used ingredients. However, because it is known to me beforehand that the food to be tested contains common food ingredients, the situation can normally be avoided.

5. The results of this study will not be released in any individual identifiable form without my prior consent unless required by law.

6. The investigator will answer any further questions about the research, either now or during the course of the project.

The study has been discussed with me, and all of my questions have been answered. I understand that additional questions regarding the study should be directed to the investigator listed above. In addition, I understand the research at Louisiana State University, Agricultural Center, which involves human participation, is carried out under the oversight of the Institutional Review Board. Questions or problems regarding these activities should be addressed to Dr. David Morrison (225)578-8236. I agree with the terms above and acknowledge.

I have been given a copy of the consent form.

____________________________ ____________________________

Signature of Investigator Signature of Participant

Witness: ____________________ Date: ______________________________

Sample code ……...

What is your **GENDER**? **( ) MALE** **( ) FEMALE**

1. How would you rate the **OVERALL APPEARANCE** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
( )	( )	( )	( )	( )	( )	( )	( )	( )

2. How would you rate the **CRUMB COLOR** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
( )	( )	( )	( )	( )	( )	( )	( )	( )

3. How would you rate the **OVERALL AROMA or ODOR** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
( )	( )	( )	( )	( )	( )	( )	( )	( )

4. How would you rate the **CRUMB MOISTNESS** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
( )	( )	( )	( )	( )	( )	( )	( )	( )

5. Please rate the **CRUMB MOISTNESS** of this product based on your preference.

   ( ) Not moist enough **( ) Just about right** **( ) too moist**

6. How would you rate the **CRUMB SOFTNESS** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
( )	( )	( )	( )	( )	( )	( )	( )	( )

7. Please rate the **CRUMB SOFTNESS** of this product based on your preference.

   ( ) Not soft enough **( ) Just about right** **( ) too soft**

8. How would you rate the **SWEETNESS** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
( )	( )	( )	( )	( )	( )	( )	( )	( )

9. Please rate the **SWEETNESS** of this product based on your preference.

   ( ) Not sweet enough **( ) Just about right** **( ) too sweet**

10. How would you rate the **OVERALL FLAVOR** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
( )	( )	( )	( )	( )	( )	( )	( )	( )

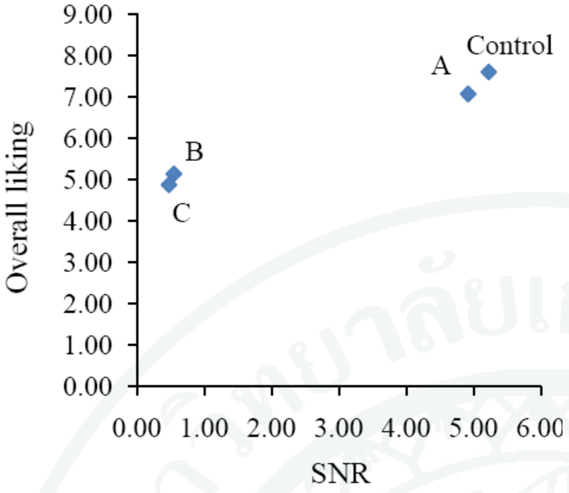
11. How would you rate the **OVERALL LIKING** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
( )	( )	( )	( )	( )	( )	( )	( )	( )

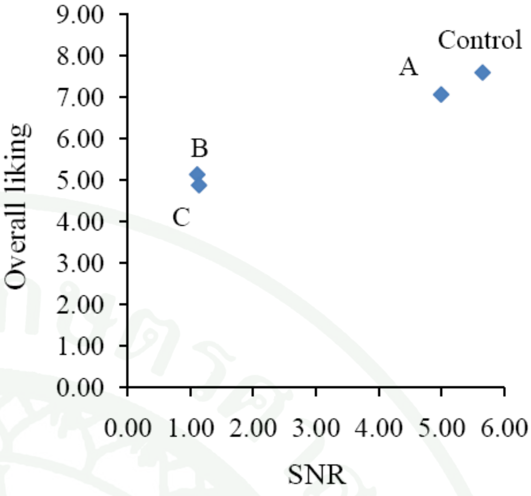
12. Would you **PURCHASE** this product if it were **commercially available**?

    ( ) YES **( ) NO**

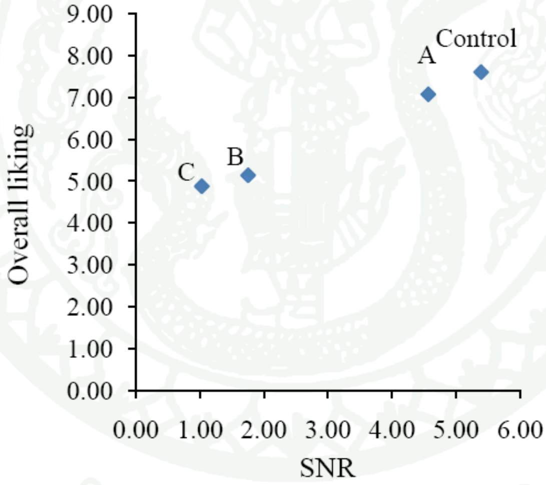
14. Would you **PURCHASE** this product if it contains **germinated brown rice flour** which contains a functional component that may help decrease blood pressure and alleviate chronic neurological disorder? **( ) YES** **( ) NO**



(a) Moistness JAR



(b) Smoothness JAR



(c) Softness JAR

Appendix Figure B1 Signal-to-noise ratio (SNR) of moistness, smoothness, and softness against overall liking of 4 bread products. a) moistness JAR; b) smoothness JAR; c) softness JAR. Products A, B, and C were bread containing composite flours stored for 0, 3, and 5 day(s).

Appendix C

Physicochemical measurements

1. Measurement of *alpha*-amylase activity with the Rapid Visco Analyser (RVA) AACC method 22-08 (AACC, 2000)

Procedure

1.1 Instrument preparation

Switch on instrument and allow at least 30 min to warm up. Switch on computer, if required, and run control software. Configure the various models to run SN test as described in manual or as follows:

Series 4: Select SN profile from instrument keypad, or connect to computer and run control software.

Idle tolerance and speed settings are normally preconfigured. Idle temperature is 95^{o}C and the idle tolerance is 1^{o}C. Where control software is used, enter a file name and select run option. The instrument disperses sample by rotating paddle at 960 rpm for first 10 sec of the test, after which viscosity is sensed using a constant paddle rotation speed of 160 rpm.

1.2 Determination

a) Weigh 4.00 g whole meal or 3.50 g flour (14% moisture basis) into a weighing vessel before transfer into test canister.

b) Dispense 25.0 ml water (14% moisture basis) into a canister.

c) Transfer sample onto water surface in canister. Place paddle into canister and vigorously jog blade through sample up and down 10 times. If any lumps remain on water surface or adhere to paddle, repeat jogging action.

d) Place paddle into canister and insert paddle and canister assembly firmly into paddle coupling so that paddle is properly centered. Initiate measurement cycle by depressing motor tower of instrument. Do not allow sample to stand in the water for more than 1 min before commencing test. Test will proceed and terminate automatically. Discard canister after use.

e) Record SN shown on display of RVA at end of test (total time of 3 min), or displayed as final viscosity on computer.

2. Measurement of reducing sugar (Somogyi, 1951)

Apparatus

1. Analytical balance
2. Thermosatted water bath set at 80 – 85 ^{o}C
3. Vortex mixer
4. Spectrophotometer set at 520 nm
5. Bench centrifuge, required speed 3000 rpm
6. Filter paper, Whatman no. 1, or equivalent
7. Stop clock

Reagents

1. Alkaline copper reagent

a.	di-sodium hydrogen orthophosphate dodecahydrate ($Na_2HPO_4.12H_2O$)			71 g	
b.	Potassium sodium (+) tartrate	(Rochelle Salt)		40 g	
c.	Sodium hydroxide	(NaOH)	1 N	100 ml	(4 g)
d.	Copper (II) sulphate	($CuSO_4.5H_2O$)	10%	80 ml	(8 g)
e.	Sodium sulphate	(Na_2SO_4)		180 g	

The $Na_2HPO_4.12H_2O$ and Rochelle salt are dissolved in about 250 ml of water, then the dissolved NaOH pallet is introduced with stirring, and this is followed by the addition of the $CuSO_4.5H_2O$. The sodium sulfate is dissolved in about 500 ml of hot water and boiled to expel air. After cooling, the two solutions are united and diluted to volume in a 1000 ml volumetric flask. The solvent should be prepared few days or a week before using according to solvent stability. The filtration is required before use.

2. Nelson reagent

a.	Ammonium molydate $[(NH_4)_6Mo_7O_{24}.4H_2O)]$	53.2 g
b.	96% Sulfuric acid (H_2SO_4)	21.0 ml
c.	di-Sodium hydrogen arsenate heptahydrate 12% ($NaHAsO_4.7H_2O$)	50.0 ml (6g)

All reagents are dissolved in distilled water one by one, respectively and adjusted to volume in 1000 ml volumetric flask. The solvent should be prepared few days or a week before using according to solvent stability. The filtration is required before use.

Sample preparation

Sugar was extracted from 100 mg of samples by 5mL of 80% ethyl alcohol, and incubate tube at 80–85°C for 5 min. Mix contents on vortex stirrer and add another 5 ml of 80% aqueous ethanol. Centrifuge the tube for 10 min at 1000 × *g* (about 3,000 rpm) on bench centrifuge (AACC, 2000). Made up to volume (10 ml).

Procedure

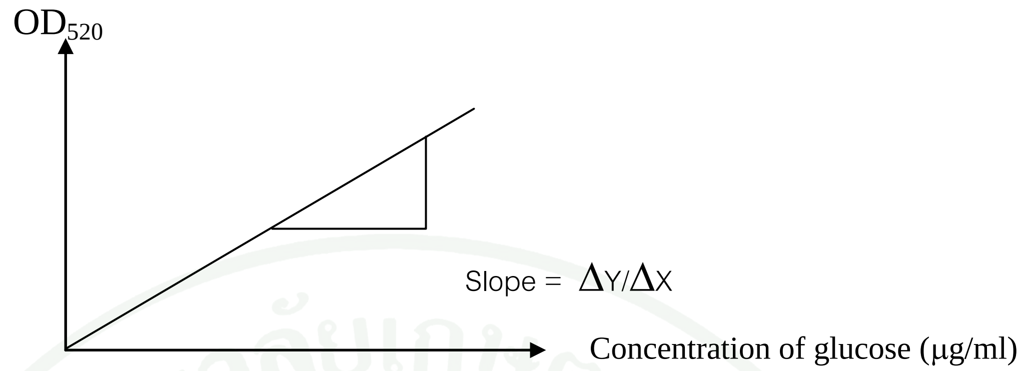
1. Pipette 1 ml extracted solution into a test tube.
2. Add 1ml alkaline copper reagent, mix, and immerse test tube in vigorously boiling water bath. Surface of liquid in test tube should be 3–4 cm below surface of boiling water. (Delay between filtering of extract and treatment in boiling water bath should not exceed 10–15 min. Further delay may cause error due to sucrose hydrolysis in acid solution). Let test tube remain in boiling water bath exactly 15 min.
3. Cool test tube in ice bath, add 1 ml of Nelson reagent, mix thoroughly, let it stand for 30 min at room temperature.
4. Add 5 ml of distilled water, mix and read color absorbance at 520 nm immediately.

Standard curve

1. Prepare standard solutions of glucose 200 µg/ml (0.02 g of dry glucose/ 100 ml).
2. Determine quantities needed and prepare working solutions of standard glucose solution for the standard curve as the following table by the same procedure as in steps 1–4 of procedure above.

Appendix Table C1 Preparation of standard glucose solution with different concentration

Concentration of glucose (µg/ml)	Distilled water (ml)	Standard solution of glucose 200 µg/ml (ml)
0	10	0
40	8	2
80	6	4
120	4	6
160	2	8
200	0	10



3. Measurement of rheological properties of flour using farinograph method AACC method 54-21(AACC, 2000)

Apparatus

Barbender Farinograph, with mixing bowls for 300 g flour.

Procedure

1. Turn on thermostat and circulating pump at least 1 hr prior to use the instrument.

2. Determine moisture content of flour (Keep flour samples in moisture-proof containers. Accurate moisture values are very important.).

3. Place in bowl 300 ± 0.1 g flour (14% moisture basis).

4. Fill the buret with water at room temperature, making sure that tip is full and automatic zero adjustment of buret is functioning properly.

5. Add water to volume nearly that of expected absorption of flour. When dough begins to form, with the mixer running, scrape down sides of bowl with plastic scraper, starting on right side, front, and working counterclockwise. Cover with glass plate. If it appears that mixing curve will level off at value larger than 500 BU, cautiously add more water. This will be used to estimate next attempt. After water is added, again cover bowl with glass plate to prevent evaporation.

6. First titration attempt rarely produces curve that has maximum resistance centered on 500-BU line; therefore, in subsequent titration, adjust absorption either up or down until this is achieved to within 20 BU. The higher the absorption, the lower the maximum resistance. Titration producing wider variation affects scoring of curve. As a guide to correcting preliminary titration values, it can be reckoned that the difference between each horizontal line (20 BU) corresponds approximately to 0.6–0.8% absorption (1.8–2.4 ml water), depending on flour. When correct absorption is achieved, curve at maximum dough development is centered on 500-BU line.

7. For final titration, add all water within 25 sec after opening buret stopcock. Permit machine to run until adequate curve is available for evaluation as desired

8. Report absorption values to nearest 0.1%. Calculate absorption on 14% moisture basis determined with large bowl, by means of following equation: Absorption percent = $(x + y – 300)/3$, where x = ml water to produce curve with maximum consistency centered on 500-BU line, and y = g flour used, equivalent to 300 g, 14% moisture basis.

Interpretation

Values other than absorption are frequently derived from farinograph curves. Among those that have been proposed are the following:

1. Dough development time. This is interval, to nearest 0.5 min, from first addition of water to that point in maximum consistency range immediately before first indication of weakening. This value has also been referred to as “peak” or “peak time.” For flours having curve that is nearly flat for several minutes, peak time may be determined by taking mean between midpoint of flat portion of top of curve and highest point of arc of bottom of curve. Occasionally two peaks may be observed; the second should be taken for determination of dough development time.

2. Tolerance index. This value is difference in BU from top of curve at peak to top of curve measured at 5 min after peak is reached.

3. Stability. This is defined as time difference, to closest 0.5 min, between point where top of curve first intersects 500-BU line (arrival time) and point where top of curve leaves 500-BU line (departure time). If curve is not centered exactly on 500 line at maximum resistance but rather, for example, at 490 or 510 level, line must be drawn at 490 or 510 level parallel to 500 line.

Appendix D
Cost estimation

Appendix Table D1 Cost estimation of GBRF and GGNBRF

Raw material[A]	Process	Price (baht/kg)	Yield (%)	Cost (baht/kg)	Grand total cost (baht)
KDML105	Paddy → Brown rice	24.0	66	36.4	
	Brown rice → GBRF	36.4	90	40.4	
GBRF (Cost of rice + buffer cost = 40.4 + 6.0*)					46.4
RD6	Paddy → Brown rice	25.0	66	37.9	
	Brown rice → GBRF	37.9	90	42.1	
GGNBR (Cost of rice + buffer cost = 42.1 + 6.0*)					48.1

[A]Raw material price as of April, 2010.

[*]Buffer as citrate buffer was used at rice:water of 1:5, thus using 1kg of brown rice needed about 15 L (including the water used in changing twice during steeping time) of water (assumed as 15 kg). Citrate buffer (0.4 baht/kg) were prepared from citric acid and sodium citrate which cost of 65 baht/kg. Thus, total cost of buffer per 1 kg of rice was 6.0 baht (obtained from 0.4 baht/kg x 15 kg).

Appendix Table D2 Raw material cost estimation of bread containing composite wheat-GBRF based on 300 g flour

Ingredient	Price (baht/unit)[A]	Formulation (g)	Cost (baht)
GGNBRF	48.1/kg	30.00	1.44
GBRF	46.4/kg	90.00	4.18
Wheat flour	44.0/kg	180.00	7.92
Egg [B]	103.0/kg	30.00	3.09
Sugar	23.0/kg	34.00	0.78
Butter [C]	77.0/loaf	60.00	20.35
Milk powder	207.5/kg	12.00	2.49
Datem (1%, flour basis)	400.0/kg	3.00	1.20
Fibrotec™ (0.5%, flour basis)	500.0/kg	1.50	0.75
Drinking water [D]	1.0/L	162.00	0.16
		Grand total (baht)	42.37

[A] Price of raw materials as of April, 2010

[B] Based on 50 g/one whole egg (the price of 1 dozen of egg was 62 baht)

[C] One loaf of butter was about 227 g

[D] Estimated that 1mL of drinking water equivalent to 1g

This formulation could get 2 loaves of bread with 270 g each. Thus, the f of raw material for one loaf of composite-flour bread was about 21.19 baht (= 42.37/2).

Appendix Table D3 Another selected variable cost of bread containing composite wheat-GBRF per day

Expenditure	Price (baht/day)
Electricity [A]	31
Man power (1 person) [B]	206
Grand total (baht/day)	237

[A] Total working hour was estimated at 8 h per day. Four batches of bread could be produced per day with the bread making machine.

[B]Electricity obtained from 3 machines as follows;

a) two breadmaking machines (450 W each) operated for 0.5 h each batch,

b) one commercial oven (2.2 kW) operated for 1 h each batch

b) one commercial incubator comprised of a heater (200 W) and a fan (10 W) with humidifier (40 W) which would operate per day (8 h of working hour) for 1.5 h for the heater and the humidifier and 8 h for the fan (data provided by the supplier).

[B] As the lowest wage of Bangkok (as of May, 2010).

[D] 1 unit = 1 kWh

Calculation

Total electric energy (kWh) = energy usage of 2 bread making machines + energy usage of a commercial oven + energy usage of a commercial incubator with humidifier

= (450 W/1000)x(0.5h x 4 batches x 2 machines) + 2.2kW x (1.0 h x 4 batches x 1 machines) + {[(200 W/1000) x 1.5h] + [(40 W/1000) x 1.5h] + [(10 W/1000) x 8h]}

= 12.36

Cost of the electricity (baht/day) = 12.36 kWh x 2.50* (baht/unit)

= 30.9 or about 31 baht

* Reference from the provincial electricity authority as of May 26, 2010

If 24 loaves (4 batches/day with 6 loaves each) of bread were produced per day then the cost/loaf of bread was calculated as follows;

Cost (baht/loaf) = Total cost of the selected variable cost (as Appendix Table D3)/total amount of bread produced per day

= 237 baht/24 loaves

= 9.88

Therefore, the total cost of bread containing wheat-GBRF exclude the packaging cost would be about 30.07 baht/loaf.

Total cost (baht/loaf) = Cost of raw materials + Cost of another variable cost

= 21.19 + 9.88

= 31.07

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