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Original Article

Increasing the bio-active compounds contents by optimizing the germination conditions of Southern Thai Brown Rice

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Abstract

Three Thailand rice varieties (cv. Niaw Dam Peuak Dam, Sangyod Phatthalung and Chiang Phatthalung) were germinated and their γ-aminobutyric acid (GABA) content was monitored. Optimum germination conditions to maximize GABA content in brown rice were determined. The brown rice was first soaked in various solutions (buffer solution pH 2, 3, 5, and 7 and distilled water) at room temperature (30±2°C) for 5 hours, followed by germinating in either open or closed vessel for 12, 24, 36, and 48 hours. Results indicated that the highest GABA content was obtained when the rice was soaked in a citrate buffer with pH 3 and germinated in closed vessel for 36 hours, Sangyod Phatthalung and Chiang Phatthalung, and for 48 hours, Niaw Dam Peuak Dam. Compared to regular brown rice, the GABA content in germinated brown rice increased 9.43-16.74 times. Germination also increased the ferulic acid 1.12-1.43 times and significantly decreased the phytate content while the γ-oryzanol content was in the same level for both un-germinated and germinated brown rice.

Keywords: germinated brown rice, γ-aminobutyric acid, ferulic acid, phytate, γ-oryzanol

1. Introduction

Rice is one of the most important cereal grains in the world, apart from wheat and corn. The total rice paddy production area is about 154 million hectare and the annual production of rice is about 594 million tons. Rice accounts for over 22% of global energy intake, and its production and consumption is concentrated in Asia (Kainuma, 2004). In Thailand, rice is of special importance as it is the main food for 64.24 million people. Thailand consumes about 55% of its rice production while the remaining 45% is exported to the world market (Vanichanont, 2004). Thailand exports many kinds of rice including white rice derived from varieties of rice, aromatic rice, parboiled rice, and glutinous rice. In 2008, the Thai Office of Agriculture Economics reported that the approximate total rice paddy area and production of rice were 9.19 million hectares and 23 million tons, respectively, including southern Thailand with a total rice paddy area and production of rice of 0.31 million hectares and 0.75 million tons, respectively. Rice productions areas in southern Thailand are the Songkhla Lake Basin covering three provinces, Nakhon Si Thammarat, Songkhla, and Phattalung.

In the past, several traditional rice varieties could be produced in the area under different conditions. The traditional rice varieties in southern Thailand comprise of more than 4,000 varieties. Changes in farming practices resulted in

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growing modern rice cultivars instead of the traditional ones. Most traditional rice varieties have been collected for conservation whereas some of them have already been lost. Consequently, there are concepts of direct use of traditional rice by improving the cultivars suitability for specific planting areas and by studying the nutrition of traditional rice varieties for their utilization in food, pharmaceutical, and cosmetic products in order to increase their added value.

Niaw Dam Peuak Dam, Sangyod Phatthalung, and Chiang Phatthalung are the traditional rice varieties planted in southern Thailand. The brown rice grains are different in pigment color such as black, purple, or red. The black and red varieties are planted mainly in South East Asia, Italy, Greece, U.S.A., China, and Japan, where people have long consumed pigmented rice. However, in Thailand, the total consumption of pigmented rice is very low due to the hard texture. In pigmented rice, there is a natural colorant, called anthocyanin. A commonly found anthocyanin in red rice is acetylated procyanidins, which is reported to posses a free radical scavenging activity (Oki *et al.*, 2002). However, information about traditional rice is limited. Brown rice contains more nutritional components, such as dietary fibers, phytic acid, vitamin E, and vitamin B, than the ordinary milled rice. These bio-functional components exist mainly in the germ and bran layers most of which are removed by polishing or milling (Champagne *et al.*, 2004). Compared to Khao Dawk Mali 105, which is the most famous and accepted rice variety in the world in terms of quality of flavor and texture, Sangyod Phatthalung contains higher amounts of protein, while Chiang Phatthalung contains a higher amount of fiber (Pongsawatmanit *et al.*, 2003). Since traditional rice receives little attention in academic and commercials aspects, it is interesting to study its properties in more details. Unfortunately, brown rice takes longer to cook and cooked brown rice is harder to chew and not as tasty as white rice. In germinated cereal grains, hydrolytic enzymes are activated and they decompose starch, non-starch polysaccharides and proteins, which leads to an increase in the oligosaccharides, and amino acids (Maung et al., 1995). The decomposition of the high molecular weight polymers during germination leads to the generation of bio-functional substances and the improvement of the organoleptic qualities due to the softening of texture and the increase in the flavor. Germinated brown rice offers considerable benefits, which include an increase in γ-aminobutyric acid (GABA), dietary fiber, inositols, ferulic acid, phytic acid, tocotrienols, magnesium, potassium, zinc, γ-oryzanol, and prolylendopeptidase inhibitor. Additionally, the germination of brown rice frees its bound minerals, making them more absorbable by the body and the rice tendered and tastier (Kayahara, 2004).

GABA in rice grains is synthesized from glutamic acid by glutamate decarboxylase (GAD), and the activity of GAD shows a high correlation with the germination ratio (Bautista *et al.*, 1964). GABA is a neurotransmitter in the brain and the spinal cord of mammals. It can lower hypertension, promote the sleepiness and has the benefit for human health (Okada *et al.*, 2000). γ-Oryzanol is composed of esters of *trans*-ferulic acid with phytosterols such as cycloartenylferulate, sitosterylferulate, 24-methylenecycloartanylferulate, and campesterylferulate. It has been suggested to have potential functionality such as antioxidant activity (Xu and Godber, 1999), reduction of serum cholesterol (Sasaki *et al.*, 1990), reduction of cholesterol absorption and decrease of early atherosclerosis (Rong *et al.*, 1997), inhibition on platelet aggregation (Seetharamaiah *et al.*, 1990), and inhibition of tumor promotion (Yasukawa *et al.*, 1998). Ferulic acid is the major phenolic compound in rice (Tian *et al.*, 2005). Ferulic acid has the capability to prevent the build-up of superoxide, controlling the aggregation of blood platelets (Kayahara, 2004) and cholesterol-lowering properties as well as for their antioxidant capacity (Nystrom *et al.*, 2007). Phytate or phytic acid (myoinositol hexaphosphate) is the major storage form of phosphate in plant seed and grains. With its well design molecular structure that charged with six phosphate groups extending from central inositol ring, it is a potential chelator of iron and many minerals (Allen and Ahluwalia, 1997).

However, there has been no report on germinated brown rice of Southern Thailand varieties. This study aimed to minimize this gap of knowledge by studying optimum germination conditions of brown rice of three cultivars and evaluating their bio-active compounds. The application of the germinations condition will be promising for the development of novel GABA-rich products and the promoting of the consumption of traditional rice.

2. Materials and methods

2.1 Materials

Three indica rice (*Oryza sativa*) cultivars with different amylose content, obtained from the Rice Research Center located in Phattalung, Thailand, i.e. Niaw Dam Peuak Dam (2.17%), Sangyod Phatthalung (14.69%), and Chiang Phatthalung (21.72%), were used in this study. The amylose content was determined by the method of Shanthy *et al.* (1980) and Sombhagya and Bhattacharya (1979). The paddy was harvested at 28-30 days after flowering. To obtain brown rice samples, the paddies were milled by a home-scale miller and packed under vacuum in plastic bags. The samples were kept in cold room ($\leq$ 4°C) throughout the experiment for approximately a year.

2.2 Hydration characteristics of brown rice during soaking

Brown rice was washed with distilled water to rinse out any contaminants. Each of the rice samples was soaked in distilled water at room temperature (30±2°C) for 24 hours. At various time intervals during soaking, the rice samples were analyzed for moisture (AOAC, 2000a) and GABA contents (Varanyanond *et al.*, 2005).

2.3 Study on the optimum germination conditions for brown rice

Washed brown rice was steeped in soaking solutions of various buffer solutions: 0.1 M glycine-hydrochloric acid buffer pH 2.0, 0.1 M citrate buffer pH 3.0 and pH 5, 0.1 M phosphate buffer pH 7.0 and distilled water, using grain-to-solution ratio of 1:2 w/v, for 5 hours at room temperature (30±2°C, RH 80-85%). After 5 hours, the soaking solutions were drained off and the rice grains were wrapped with cheesecloth to maintain moisture and left in the incubator (30±2°C, RH 80-85%) for 24 hours to germinate. The germinated brown rice was dried to <13% moisture content using a tray dryer at 50°C and analyzed for GABA content. The soaking solution, which gave the highest concentration of GABA, was selected for further study.

To determine optimum germination times, the brown rice samples were steeped in the selected soaking solution as described above. The steeped rice grains were then wrapped with cheesecloth and left in either an open or close vessel. In an open vessel, the rice grains were left in a plastic box and covered with punctured lids, where allowed the air to circulate within (amount of oxygen in the system was constant), and in close vessel, the rice grain were left in a plastic box with an air-lid, which excluded the air (amount of oxygen in the system declined). The rice grains were germinated for 12, 24, 36, or 48 hours. After germination, the brown rice samples were taken out and dried to <13% moisture content using a tray dryer at 50°C. The dried germinated brown rice samples were analyzed for GABA content. Germination times that gave the highest concentration of GABA were selected for subsequent studies.

2.4 Determination of bio-active content in germinated brown rice

γ-Aminobutyric acid (GABA)

GABA content was determined by the method of Varanyanond *et al.* (2005) with slight modifications. One-fifth to one-half gram (0.2-0.5 g) of ground germinated brown rice samples were weighed in plastic tubes and 1.8 mL of deionized water was added and the slurries were shaken at room temperature for 1.5 hours. There after, 200 mL of 3% (by volume) sulfosalicylic acid was added and the mixtures were centrifuged at 4500xg for 10 min. To 50 mL of the supernatants were added 50 mL of 100 mM $NaHCO_3$ and 50 mL of 4 mM 4-dimethylaminoazobenzene-4-sulfonyl chloride acetonitrile solutions. The mixtures were heated to 70°C for 10 min to effect derivatization. After the derivatization, the samples were added 250 mL of absolute ethanol and 250 mL of 25 mM phosphate buffer (pH 6.8). The samples were then filtered and 5 mL of the filtrate were injected into Agilent HPLC (1200 Series, Japan), with Supelcosil LC-DABS column, 4.6x 150 mm, 3 mm (Supelco, Bellefonte, PA). The HPLC was equipped with an UV-Vis photodiode array detector set at 465 nm wavelength. The mobile phases were 25 mM acetate buffer and acetonitrile (65:35) operated at the flow rate of 0.5 mL/min, and 55°C. Pure GABA was used as standard for calibration.

γ-Oryzanol

γ-Oryzanol content was determined by the method of Chen and Bergman (2005) with slight modification. Rice samples (0.05 g) were extracted in 3 mL of methanol HPLC grade. The mixtures were shaken using a vortex for 1 min. After the extraction, the samples were centrifuged for 10 min at 825xg. The supernatants were collected by filtering and the residues were extracted two more times and 50 mL of the samples were injected into the Agilent HPLC (1200 Series, Japan), with Alltech Econosphere C18 column, 4.6x250 mm, 5 mm. The HPLC was equipped with an UV-Vis photodiode array detector set at 330 nm wavelength. The mobile phases were methanol : acetonitile : dichloromethane : acetic acid (50:44:3:3) operated at ambient temperature, and the flow rate of 1 mL/min. γ-Oryzanol was used as standard for calibration. The four components of γ-oryzanol were indentified as cycloartenyl ferulate, 24-methylene cycloartanyl ferulate, campesteryl ferulate and sitosteryl ferulate at retention time 9.24, 10.02, 10.79, and 12.16 minutes (Figure 1).

Ferulic acid

Total ferulic acid content was determined using the method of Ohtsubo *et al.* (2005). A 0.5 g of rice was extracted with 50 mL of 1 M NaOH for 3 hours at 40°C and neutralized by 26 mL of 2 M HCl. The sample was extracted three times with 50 mL of ethyl acetate, each time for 5 min. Thereafter, the ethyl acetate layer was evaporated and the sample was re-dissolved in methyl alcohol and H_2O (1:1). All samples were filtered though a 0.45 mm pore size syringe-driven filter before injection. A 5 mL aliquot of sample solution was injected into the Agilent HPLC system equipped with a diode array detector on a 4.6x150 mm, 5 mm, and Agilent Eclipse XDB-C18 analytical column. The mobile phases were acetic acid (2.5% by volume) and acetonitrile (88:12) at a flow rate of 0.5 mL/min. Column temperature was set at 40°C and ferulic acid was detected at the wavelength of 320 nm. Pure ferulic acid used as standard for calibration and indentified at retention time 5.69 minutes (Figure 2).

Phytate

Phytate was analyzed by the standard method of AOAC (2000b). Rice (2.0 g) was extracted with 40 mL of 2.4% (by volume) HCl by shaking vigorously for 3 hours at room temperature before filtering. The filtrate was mixed with 1 mL Na_2EDTA/NaOH solution and diluted to 25 mL with deionized water, then poured into an anion-exchange column (Dowex 1x8, 200-400 mesh, chloride form, Fluka, Germany). Phytate solution was eluted with 0.7 M NaCl solution and wet-digested with a mixture of concentrated HNO_3-H_2SO_4 to release phosphate, which is measured colorimetrically with a spectrophotometer at the wavelength 640 nm. The amount of phytate in the original sample was calculated as hexa-

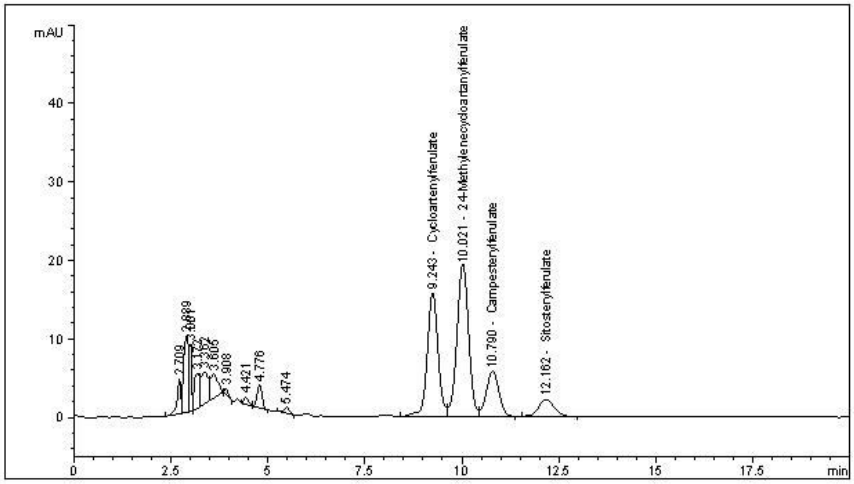

Figure 1. Chromatogram of γ-oryzanol in the analytical reverse-phase HPLC.

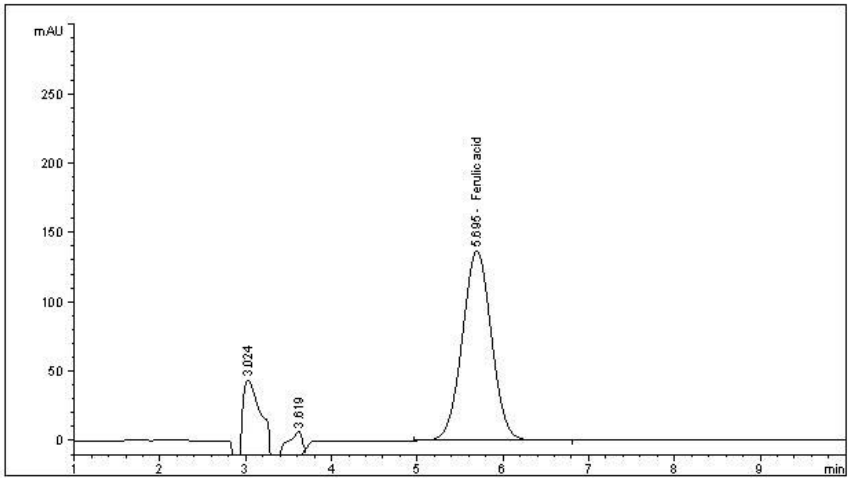

Figure 2. Chromatogram of ferulic acid in the analytical reverse-phase HPLC.

phosphate equivalent.

2.5 Statistical analysis

All experiments were carried out using three freshly prepared germinated samples and three replicates of each sample were analyzed. The results were statistically analyzed using one way analysis of variance (ANOVA). Means were compared by Duncan multiple range test with mean square error at 5% probability. Differences between un-germinated and germinated brown rice were assessed by paired t-test with a level of significance of 0.05.

3. Results

3.1 Hydration characteristics of brown rice during soaking

All three rice varieties exhibited similar water uptake behavior. At the early stage of soaking, water uptake rapidly increased due to the absorption into the embryo of the kernel (Bello *et al.*, 2004; Wijngaard *et al.*, 2005). Subsequently, rice kernels absorbed water slowly and came to an equilibrium or saturation point. During this stage water diffused slowly into an endosperm of the kernel. After soaking for 24 hours the moisture content of Niaw Dam Peuak Dam, Sangyod Phatthalung, and Chiang Phatthalung reached 42.88, 37.64, and 32.50%, respectively (Figure 3). The moisture content after 5 hours changed non-significantly and the uptake rate was extremely slow. Normally, germinated brown rice contained 30-35% of moisture and high moisture levels (35-50%) promoted microbial growth (Komatsuzaki *et al.*, 2007). Adequate hydration was achieved when the brown rice was soaked for a period sufficient to ensure proper germination (until the germ swelled) and to attain a desired moisture level for subsequent gelatinization of the starch. Soaking the three varieties of brown rice grains for 5 hours resulted in a moisture value of 30-40%, similar to that of normal brown rice; therefore, this was used for further study.

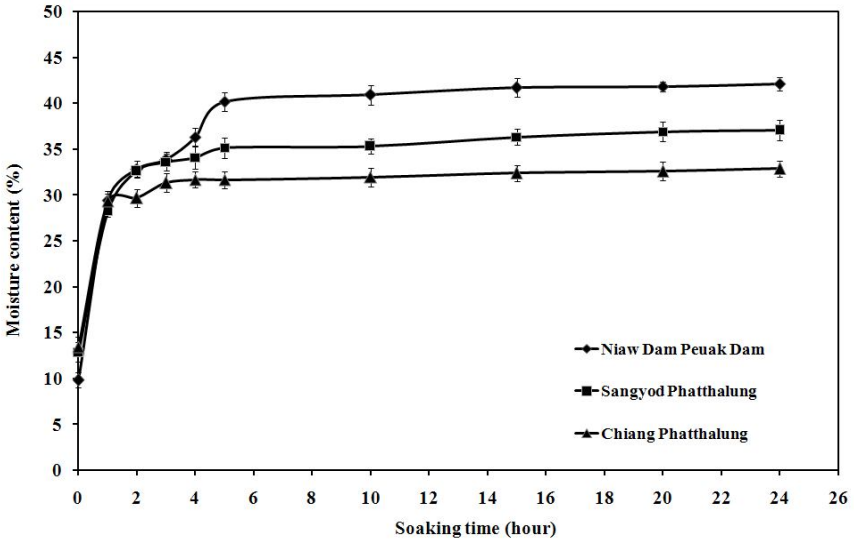

Figure 3. Changes in moisture content in the three varieties of rice during soaking at room temperature.

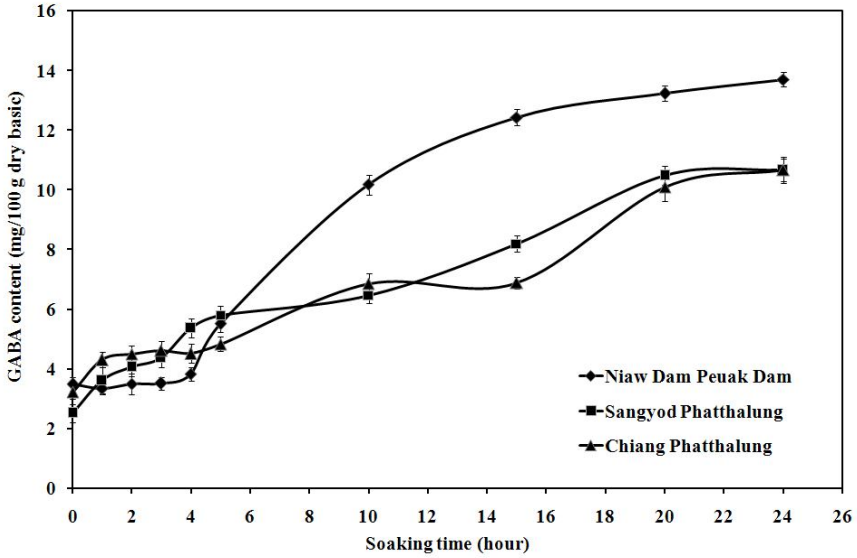

Figure 4. Changes in GABA content in the three varieties of rice during soaking at room temperature.

Niaw Dam Peuak Dam was found to have greater amount of water uptake than Sangyod Phatthalung and Chiang Phatthalung due to the greater amount of amylopectin. These results agree with research results reported of Indica rice var. Kor-Kor 6 (94% amylopectin) and Khao Dawk Mali 105 (14% amylose) (Benjamasuttikul and Naivikul, 2007).

Change in the GABA content of brown rice during soaking is shown in Figure 4. After 24 hours, the GABA contents of Niaw Dam Peuak Dam, Sangyod Phatthalung, and Chiang Phatthalung were 18.70, 10.67, and 10.66 mg/100 g, respectively. Results indicating that GABA content in brown rice greatly increased during soaking in the water. Theses results agree with reports of Japonica rice var. Koshihikari and Haiminori (Saikusa *et al.*, 1994a; Komatsuzaki *et al.*, 2007) and germ of Thai rice var. Pathum Thani 1, Khao Dawk Mali 105, Chai Nat 1 and Suphan Buri 1 (Varanyanon *et al.*, 2005).

3.2 Optimum germination conditions for brown rice

GABA content of germinated brown rice in various soaking solutions is shown in Table 1. The highest value was found in samples soaked in citrate buffer at pH 3, with Niaw Dam Peuak Dam having the highest amount at 14.48 mg/100 g dry basis. GABA content of brown rice germinated for different lengths of time are shown in Table 2**.** The highest GABA content was obtained when germinated in a closed

Table 1. GABA content in the three varieties of germinated brown rice at various soaking solutions.

Soaking solution	GABA content (mg/100 g dry basis)		
	Niaw Dam Peuak Dam	Sangyod Phatthalung	Chiang Phatthalung
Glycine-HCl buffer pH 2.0	11.28±0.08^{b}	7.02±0.05^{d}	8.79±0.16^{c}
Citrate buffer pH 3.0	14.48±0.23^{e}	8.36±0.06^{e}	14.01±0.11^{d}
Citrate buffer pH 5.0	9.13±0.10^{a}	5.79±0.10^{b}	6.78±0.07^{a}
Phosphate buffer pH 7.0	12.13±0.09^{c}	4.58±0.31^{a}	8.510.34^{bc}
Distilled water	9.47±0.38^{a}	6.36±0.06^{c}	8.73±0.15^{c}

$^{a-e}$ Same letters under the same column indicate no significant difference ($p>0.05$).

Table 2. GABA content in the three brown rice varieties germinated in either open or closed vessel for various times.

Rice	Germination method	Germinating time (hours)			
		12	24	36	48
Niaw Dam Peuak Dam	Open	10.34±0.29^{Aa}	14.48±0.23^{Ab}	20.92±0.86^{Bc}	24.66±1.10^{Ad}
	Close	8.49±1.02^{Aa}	15.32±0.41^{Ab}	18.82±0.74^{Ac}	40.72±0.29^{Bd}
Sangyod Phatthalung	Open	6.86±0.24^{Aa}	8.36±0.06^{Ab}	26.88±0.71^{Ad}	22.82±1.01^{Ac}
	Close	9.12±0.42^{Ba}	17.51±0.77^{Bb}	44.53±1.93^{Bd}	39.04±1.54^{Bc}
Chiang Phatthalung	Open	8.36±0.36^{Aa}	14.01±0.11^{Ab}	22.98±0.51^{Ac}	25.81±1.00^{Bd}
	Close	16.91±0.78^{Ba}	24.88±1.21^{Bc}	29.25±0.77^{Bd}	20.70±0.14^{Ab}

$^{A-B}$ Same letters under the same column for each rice variety indicate no significant difference using a paired t-test ($p>0.05$). $^{a-d}$ Same letters under the same row for each rice variety with different germination time indicate no significant difference ($p>0.05$).

vessel for 36 hours, for Sangyod Phatthalung and Chiang Phatthalung, and for 48 hours, Niaw Dam Peuak Dam. Therefore, optimum conditions for producing the highest GABA content in the three varieties of brown rice were: soaking in citrate buffer at pH 3 for 5 hours, and germinating in closed vessel for 36 hours, Sangyod Phatthalung and Chiang Phatthalung, and for 48 hours for Niaw Dam Peuak Dam.

3.3 Bio-active content in germinated brown rice

GABA content in un-germinated and germinated brown rice is shown in Table 3. Among the un-germinated brown rice samples, Niaw Dam Peuak Dam had the highest GABA content. After germination, the GABA content in Niaw Dam Peuak Dam, Sangyod Phatthalung, and Chiang Phatthalung increased 11.28, 16.74, and 9.43 times, respectively. In particular GABA increased greatly in the Sangyod Phatthalung variety.

γ-Oryzanol is a mixture of ferulic acids esterified with normal sterols or triterpene alcohols. The sterol components of γ-oryzanol are primarily campesterol and sitosterol, and the triterpene alcohol components are cycloartenol and 24-methylene cycloartanol. Ten fractions of γ-oryzanol isomers from crude rice bran have been successfully identified and isolated using reverse-phase HPLC. The four components of γ-oryzanol were identified as cycloartenylferulate, 24-methylenecycloartanylferulate, campesterylferulate and sitosterylferulate, which were major components (Xu and Godber, 1999). The γ-oryzanol contents in un-germinated and germinated brown rice are shown in Table 4. Results indicated that Niaw Dam Peuak Dam (black-glutinous rice) had the highest γ-oryzanol content compared to Sangyod Phatthalung and Chiang Phatthalung (non-glutinous rice) according to the reported values of Manuswarakul *et al.* (2003). After germination, the g-oryzanol content showed the same level for both un-germinated and germinated brown rice ($p>0.05$).

Ferulic acid is the major phenolic compound in rice and exists in the form of free, soluble conjugated and soluble bound. Most of these compounds are bound to polysaccharides containing glucose, arabinose, xylose, galactose, rhamnose, and mannose residues in the cell wall (Tian *et al.*, 2005). Ferulic acid content in un-germinated and germinated brown rice is shown in Table 5. The ferulic acid content of Niaw Dam Peuak Dam, Sangyod Phatthalung, and Chiang Phatthalung increased 1.12, 1.43, and 1.37 times, respectively, after germi-

Table 3. GABA content in un-germinated and germinated brown rice.

Rice	GABA content (mg/100g dry basis)	
	Un-germinated brown rice	Germinated brown rice
Niaw Dam Peuak Dam	3.61±0.06[a]	40.72±0.29[b] (11.28)
Sangyod Phatthalung	2.66±0.11[a]	44.53±1.93[b] (16.74)
Chiang Phatthalung	3.09±0.05[a]	29.25±0.77[b](9.47)

[a-b] The same letters under the same row indicate no significant difference using a paired t-test ($p>0.05$). Figure in parentheses indicate time increased over un-germinated brown rice.

Table 4. γ-Oryzanol content in un-germinated and germinated brown rice.

Rice		γ-oryzanol content (mg/100g dry basis)			
		Cycloartenyl ferulate	24-Methylene cycloartanylferulate	Campesteryl ferulate	Sitosteryl ferulate
Niaw Dam Peuak Dam	UG	77.79±0.83[ns]	92.39±0.42[ns]	133.02±1.90[ns]	204.08±2.66[ns]
	GBR	73.03±3.04[ns]	91.82±1.11[ns]	130.69±1.49[ns]	201.57±4.64[ns]
Sangyod Phatthalung	UG	26.25±1.12[ns]	64.16±1.10[ns]	101.41±1.89[ns]	135.09±3.16[ns]
	GBR	22.46±0.45[ns]	63.61±2.40[ns]	96.17±3.54[ns]	129.07±2.84[ns]
Chiang Phatthalung	UG	31.67±1.49[ns]	71.27±0.65[ns]	81.32±1.13[ns]	81.59±0.69[ns]
	GBR	26.58±2.44[ns]	67.84±1.40[ns]	77.21±1.49[ns]	75.86±3.51[ns]

UG: Un-germinated brown rice, GBR: Germinated brown rice. [ns] denote not significant differences which comparing UG *vs* GBR among same compound for each rice variety using a paired t-test ($p>0.05$).

Table 5. Ferulic acid content in un-germinated and germinated brown rice.

Rice	Ferulic acid content (mg/100g dry basis)	
	Un-germinated brown rice	Germinated brown rice
Niaw Dam Peuak Dam	26.03±1.08[a]	29.23±1.15[b](1.12)
Sangyod Phatthalung	21.75±0.64[a]	31.02±1.02[b](1.43)
Chiang Phatthalung	23.02±0.67[a]	31.50±0.43[b](1.37)

[a-b] The same letters under the same row indicate no significant difference using a paired t-test ($p>0.05$). Figure in parentheses indicate time increased over un-germinated brown rice.

nation. The results indicated that germination induced an increase in the ferulic acid content similar with results reported by Ohtsubo *et al.* (2005).

Phytic acid, myo-inositol 1, 2, 3, 4, 5, 6-hexakisphosphate (InsP6), has long been known as a form of stored phosphorus in seeds. Approximately 70% of total phosphorus in seeds coexists with phytic acid and its content typically accounts for 1% or more of seed dry weight (Liu *et al.*, 2005). Phytate is a mixed cation salt of phytic acid, which acts as a store of inositol, phosphate, K, Mg, Ca, Mn, Fe, and Zn for use by the seedling. These are released to the developing seedlings by the action of phytase enzymes (Lott *et al.*, 2000). The phytate content in un-germinated and germinated brown rice is shown in Table 6. The phytate content of brown rice are varied from 860.77 to 883.91 mg/100g. After germination, the phytate content of Niaw Dam Peuak Dam, Sangyod Phatthalung, and Chiang Phatthalung decreased 0.73, 0.71, and 0.72 times, respectively.

4. Discussion

Increasing GABA content in brown rice during soaking in water was due to the synthesis of glutamic acid by glutamate decarboxylase (GAD). In addition, the amino acid

Table 6. Phytate content in un-germinated and germinated brown rice.

Rice	Phytate content (mg/100g dry basis)	
	Un-germinated brown rice	Germinated brown rice
Niaw Dam Peuak Dam	862.86±20.85[b]	629.98±23.13[a] (0.73)
Sangyod Phatthalung	860.77±7.55[b]	609.17±4.48[a] (0.71)
Chiang Phatthalung	883.91±29.89[b]	633.25±41.02[a] (0.72)

[a-b] The same letters under the same row indicate no significant difference using a paired t-test ($p>0.05$). Figure in parentheses indicate time decreased over un-germinated brown rice.

in brown rice being used as storage proteins, which are decomposed by water absorption, changed into transportable amides and supplied to the growing parts of the rice seedling. The result in Figure 3 showed a similar trend after 5 hours, which is in accordance with the reported one of brown rice var. Khao Dawk Mali 105 and Kor-Kor 6 (Benjamasuttikul and Naivikul, 2007). During soaking, three brown rice varieties absorbed water slowly and came close to the saturation point. The soaking loss indicated the amount of particles leaching out of the rice grain during soaking. Soaking loss was mainly due to three factors: the displacement of residual dust, the leaching of soluble materials and the metabolic activity of the grain releasing CO_2 and small amounts of ethanol (Wijngaard *et al.*, 2005). The leaching particles included polysaccharide, protein and other water-soluble component. The appropriated amount of water uptake of the brown rice during soaking directly affected quality of the germinated brown rice. Different cultivars had different characteristics of water absorption. The moisture contents of Niaw Dam Peuak Dam, Sangyod Phatthalung and Chiang Phatthalung after soaking for 5 hours were 40.15, 35.16, and 31.63%, respectively (Figure 3). Komatsuzaki *et al.* (2007) reported that the germinated brown rice contained 30-35 % moisture, and the moisture content of brown rice var. Haiminori as 36.9% at 3 hours soaking. Hirunpong and Tungjaroenchai (2008) also reported the moisture up take of brown rice var. Khao Dawk Mali 105, Kor-Khor 23 and Chai Nat 1 during soaking at 35°C were 29.01, 29.64 and 31.04% at time 2, 3, and 3 hours, respectively, was the optimal soaking time. The GABA contents of three cultivars were 76, 77, and 186 mg/100 g of germ, as a result of 24 hours germination. In this study, the brown rice was soaked for 5 hours, which attained the saturation point, was the optimal soaking time. After soaking for 5 hours, the GABA content of brown rice ranged from 4.83-5.80 mg/100 g (Figure 4). These values were higher than that of raw rice grain. This result indicates that soaking contributes to the increase in GABA content. Similar results have been reported of rice grains (Saikusa *et al.*, 1994a,b), where water soaking increased the contents of most amino acids and GABA. As reported by Komatsuzaki *et al.* (2007), the increase in GABA content during water soaking may be due to the activation of glutamate decarboxylase (GAD), which converts glutamate to GABA. Soaking could lead to hypoxia due to the limited availability of oxygen for the grain (Dewar *et al.*, 1997) and GABA content may increase rapidly in plant tissues in response to hypoxia (Crawford *et al.*, 1994). Bautista *et al.* (1964) reported that GAD activity was a more reliable index for the viability of rice. This variation in GABA among different rice cultivars might be governed by varying GAD accumulation. Consequently, the soaking prior to germination may enhance the residual GABA content by activating GAD from the hypoxia condition. Different cultivars and moisture contents of rice therefore affected both germination and GABA production.

Brown rice soaked in citrate buffer pH 3 had the highest GABA content. A similar result was reported by Charoenthaikij *et al.* (2007) who found that the highest amount of GABA could be accumulated when soaking brown rice var. Khao Dawk Mali 105 in citrate buffer pH 3. However, Sunte et al. (2007) found that brown rice soaked in buffer solution has a pH 5 while Watchraparpaiboon *et al.* (2007) found brown rice soaked in water at pH 6 had the highest GABA content. It is apparent, that GABA in germinated brown rice increased when the rice was soaked in acid solution. The synthesis of GABA is rapidly stimulated by a variety of stress conditions including hypoxia. The advantage of this process would be the concomitant H^+ consumption, which ameliorates the cytosolic acidification associated with hypoxia or other stresses (Crawford *et al.*, 1994). Similarly, the synthesis of GABA through glutamate decarboxylase in reduced oxygen supply occurred by the effect of decreasing cytoplasmic pH in carrot cell suspension (Carroll *et al.*, 1994).

The GABA content in brown rice germinated in the closed vessel was higher than the open vessel. Similarly, Komatsuzaki *et al.* (2007) have reported that the GABA content in brown rice var. Haiinori after soaking for 3 hours, and germinated with gaseous treatment (no exchange of air) at 35°C for 21 hours, was higher than prepared after the conventional germination method. The results might indicate that the glutamic acid was synthesized by the glutamate synthase (GOGAT) glutamine synthetase (GS) cycle. The GS/GOGAT cycle plays an important role in anaerobic accumulation of GABA and alanine (Aurisano *et al.*, 1995).

GABA content in germinated brown rice increased

9.43-16.74 times, as compared to the un-germinated samples, with similar reports for Indica rice var. Khao Dawk Mali 105 (Charoenthaikij *et al.*, 2007; Sunte *et al.*, 2007; Watchraparpaiboon *et al.*, 2007). In germinated cereal grains, hydrolytic enzymes are activated and decompose starch, non-starch polysaccharides, and amino acids. The decomposition of high molecular weight polymers during germination leads to the generation of bio-active compounds, and to improvements in organoleptic qualities due to the softening of texture and increase of flavor in cereal grains.

The γ-oryzanol content was in the same level for the brown rice than for the germinated brown rice. These results agree with research report of Japonica rice var. Koshihikari soaked in water at 30°C for 72 hours (Ohtsubo *et al.*, 2005). However, a reverse trend was observed for γ-oryzanol content in herbal germinated brown rice. When brown rice soaked in pandanus solution for 6 hours and germinated in the dark for 24 hours the γ-oryzanol content increased. On the other hand, when brown rice soaked in lemon grass solution for 6 hours and germinated in the dark for 24 hours the γ-oryzanol content decreased (Chutipanya, 2006). Additional, Jiamyangyeun (2006) found that the red brown rice (Munpoo) and brown rice var. Khao Dawk Mali 105 soaked in water for 6 hours and germinated in the dark for 24 hours showed the highest γ-oryzanol content, which increased 1.3-1.5 times more than compared to un-germinated brown rice.

Phytate content in germinated brown rice decreased 0.71-0.73 times, as compared with un-germinated brown rice; that agrees well with reports of brown rice, corn, and oats (Larsson *et al.*, 1995; Fageer *et al.*, 2004; Liang *et al.*, 2008). Decreases caused by germination are mainly based on the action of enzymes while, in soaking, a combination of diffusion and enzymatic action is expected (Henderson and Ankrah, 1985; Mahgoub and Elhag, 1998).

Soaking of intact grains, as a first step of germination, was decreased of 14-28% of phytic acid due to the activity of endogenous phytase and diffusion of phytic acid into the soaking medium (Liang *et al.*, 2008). Similar results were reported of pearl millet, legume, and soybean (Lestienne *et al.*, 2005a,b,c).

Diffusion of phytic acid was reportedly influenced by the nature of the phytate, which may be in the form of salts with different minerals, such as potassium, calcium, or magnesium, and the pH of the medium (Henderson and Ankrah, 1985; Mahgoub and Elhag, 1998). Liang *et al*. (2009) also observed that soaking in acidic buffer was more effective to remove phytic from brown rice and rice bran than in demineralized water, presumably because of the higher solubility of phytate in acidic conditions.

Furthermore, the reduction of phytic acid increased with germination time, which agrees with previous studies reporting that the activity and/or production of phytase increased during germination (Henderson and Ankrah, 1985; Larsson and Sandberg, 1995; Moong-ngarm, 2005; Liang *et al.*, 2008). The stepwise hydrolysis of phytate to phosphate and inositol occurs by the action of phytase. Phosphatase hydrolyzes a broad spectrum of phosphate esters, while phytase is a phytate-specific phosphatase (Greiner *et al.*, 1998). The phosphatase is not capable of degrading phytate (Konietzny *et al.*, 1995; Greiner *et al.*, 2000). Two types of phytase have been identified, which initiate the hydrolysis of phytate at either the 3- or 6-position of the inositol ring (Konietzny *et al.*, 1995; Greiner *et al.*, 2001). The phytases from microorganisms, such as *Aspergillus niger*, is considered as 3-phytases (EC 3.1.3.8), while plant phytases as 6-phytases (EC 3.1.3.26) (Turk *et al.*, 1996; Greiner *et al.*, 1998, 2000).

The principal function of phytase in seeds or grains is to produce inorganic phosphate from phytate during germination. The inorganic phosphate thus produced is then utilized for the purpose of plant growth. Previous authors have studied various plant phytases from maize, barley, spelt, canola seed, and rye (Houde *et al.*, 1990; Laboure *et al.*, 1993; Konietzny *et al.*, 1995; Greiner *et al.*, 1998, 2000). It appears that phytase activity usually increases on germination and the types of phytase may be plant-dependent.

Conclusions

This study shows that, as the soaking time increased, GABA content increased. The increase varied with the pH of the soaking solution, and the GABA content was highest in a citrate buffer with pH 3. The hypoxia-induced GABA accumulation appeared to be a process for brown rice. Therefore, germination was an important technique for enhancing the GABA content in brown rice. Germinated brown rice may be used as a nutritional ingredient in functional food products such as beverages or confectioneries.

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