Effect of Germinating Processes on Bioactive Component of Sangyod Muang Phatthalung Rice

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Abstract

Sangyod Muang Phatthalung Rice is one of the local rice variety planted in the Southern Thailand, in particular Phattalung province. Germination of brown rice was investigated to determine the optimum condition that would maximize of γ-aminobutyric acid (GABA) content. Brown rice was first soaking in various solutions (phosphate buffer pH 7, citrate buffer pH 5 and pH 3 and distilled water) at room temperature (30±2°C) for 5 h, followed by germinated for 12, 24, 36 and 48 h. Results indicated that the highest GABA content (44.53 mg 100 g^{-1}) was found in brown rice soaked in citrate buffer pH 3 and germinated for 36 h. The GABA content in germinated brown rice had increased 16.74 times in comparison to brown rice (2.66 mg 100 g^{-1}). The nutritional component (protein, fat, ash, total dietary fiber and total free sugar) and bioactive components (γ-oryzanol, ferulic acid and phytate) in germinated brown rice were also analyzed. Compared to brown rice, the germinated brown rice contained more protein, fat, total dietary fiber, total free sugar and ferulic acid while γ-oryzanol content was in the same level for both brown rice and germinated brown rice. On the contrary, germinated brown rice contained phytate content lower than brown rice.

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

Introduction

Rice grain is the seed of the monocot plant *Oryza sativa*, of the grass family (Poaceae). As a cereal grain, it is the most popular cereal worldwide, serving as a stable food for 39 countries and nearly half of the world's population (Juliano, 1993). Globally rice account for 22% of total energy intake (Kainuma, 2004). Rice is the main staple food crop of the Thai people and a major foreign exchange earner. As one of the world major rice suppliers the countries export over million tons of milled rice annually. In 2008, The Thai office of agriculture economics reported that approximate total rice paddy area and production of rice were 9.25 million hectares and 24 million tons.

Rice in Thailand has many varieties, may be more than 5,900 varieties which Khao Dawk Mali 105 is the most famous and acceptable variety on the quality of flavor and texture over the world. In Northern and Northeastern, rice production especially glutinous rice is only sufficient for local consumption. On the other hand, non-glutinous varieties producing in Southern and Central plain are preferred. Sangyod Muang Phatthalung Rice refers to the Sangyod variety of rice, a local early

lowland rice, which is light-sensitive and grown in the annual planting season at the province of Phatthalung, Thailand (Department of Intellectual Property, 2006). Sangyod Muang Phatthalung rice is soft with approximately 14-15% of the amylose content. They are small grain, beautiful red (brown rice) as well as pink and white (coarse rice). In addition, the cooked rice is soft and appetizing. The analysis of the nutrient volume was found that Sangyod rice has high nutritional value, namely, iron, vitamin B and niacin (Department of Health, 2004).

Brown rice contains more nutritional components, such as dietary fibers, phytic acid, vitamin E, vitamin B, and γ-aminobutyric acid (GABA), 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. Unfortunately, brown rice takes longer to cook and cooked brown rice is harder to chew and not as tasty as white rice (Champagne et al., 2004).

Germination is a process involved in incorporating those events that commence with the uptake of water by a quiescent dry seed and terminate with the elongation of the embryonic axis (Bewley and Black, 1994). The phenomenon of germination is an incredible event. It signals the birth of a new life. At the time of germinating, huge amounts of nutrients are prepared for the growth of sprout. The birth of the sprout activated all the dormant enzymes in the rice in order to supply the sprout with the best nutrition. As a result of this, the available nutrients in the rice greatly increase. Increased nutrients in germinated brown rice include γ-aminobutyric acid, dietary fiber, inositols, ferulic acid, phytic acid, tocotrienols, magnesium, potassium, zinc, γ-oryzanol and prolylendopeptidase inhibitor. Additionally, germinated brown rice is that it frees bound minerals, making them absorbable in our bodies and also tasty and tender (Kayahara, 2004).

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). GABA in rice grains is synthesized from glutamic acid by glutamate decarboxylase (GAD), and the activity of GAD shows high correlation with the germination ratio (Bautista et al., 1964). γ-Oryzanol is a mixture of sterol esters of ferulic acid. 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 (Seetharamaial et al., 1990) and inhibition of tumor promotion (Yasukawa et al., 1998). Ferulic acid is the major phenolic compounds 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). Phytic acid has some anticancer and antioxidant functions and prevents coronary disease. It is able to prevent the build up of superoxide, as well as to boost the immune system. It recently found its way into the spotlight because of its ability to prevent colon cancer, liver cancer, lung cancer, skin cancer, etc. It is also able to prevent anemia and cardiac infarction and diabetes (Kayahara, 2004).

Various requirements must obviously be satisfied before germination can occur. 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 (Mayer and Poljakoff-Mayber, 1975). The nutritional and bio-active compound of the germinated brown rice is influenced by the nature of the raw material, variety, steeping and germination conditions. However, there has been no report on germinated brown rice of southern Thailand varieties. This study aimed to rectify this gap of knowledge by studying optimum germination conditions for brown rice and evaluating their bio-active compounds.

Materials and Methods

Material

Sangyod Maung Phatthalung rice which is the principal rice cultivar in Southern Thailand, produced at Rice Research Center located in Phattalung, Thailand in 2008, was used as the sample rice. 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 (4°C) throughout the experiment.

Optimum Germination Conditions for Brown Rice

Washed brown rice was steeped in soaking solutions of various solutions, using grain-to-solution ratio of 1:2 (w/v) for 5 h at room temperature (30±2°C). The buffer solution of the soaking solutions were 0.1 M citrate buffer pH 3.0 and 5.0, 0.1 M phosphate buffer pH 7.0 and distilled water was used for the control. After 5 h, the soaking solutions were drained off and the rice grains were wrapped with cheesecloth to maintain moisture and left in the dark for 24 h 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 plastic box with a lid to germinate for 12, 24, 36 and 48 h. 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.

Chemical Analysis

All the samples were analyzed for moisture, protein, lipid, ash and dietary fiber contents by the AOAC (2000) method, and sugar content by the method described in Ohtsubo et al. (2005).

Bio-active Analysis

GABA content was determined by the method of Varanyanond et al. (2005) with slight modification. One-fifth to one-half gram of ground germinated brown rice samples were weighed in plastic tubes and 1.8 mL of deionized water was added and the slurries shaken at room temperature for 1.5 h. There after, 200 μL of 3% sulfosalicylic acid was added and the mixtures centrifuged at 4,500xg for 10 min. To 50 μL of the supernatants were added 50 μL of 100 mM $NaHCO_3$ and 50 μL of 4 mM 4-dimethy-laminoazobenzene-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 μL of absolute ethanol and 250 μL of 25 mM phosphate buffer (pH 6.8). The samples were then filtered and 5 μL of the filtrate were injected into Agilent HPLC (1200 Series, Japan), with Supelcosil LC-DABS column, 4.6x150 mm, 3 μm (Supelco, Bellefonte, PA). The HPLC was equipped with a UV-Vis photodiode array detector set at 465 nm wavelength. The mobile phase was 25 mM acetate buffer and acetonitrile (65:35) operated at the flow rate of 0.5 mL min^{-1}, and 55°C. Aminobutyric acid was used as standard for calibration.

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 at 825 xg for 10 min. The supernatants were collected by filtering and the residues were extracted two more times and 50 μL of the samples were injected into the Agilent HPLC (1200 Series, Japan), with Alltech Econosphere C18 column, 4.6 × 250 mm, 5 μm. The HPLC was equipped with a 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^{-1} γ-Oryzanol was used as standard for calibration.

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 h 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, ethyl acetate layer was evaporated and the sample was re-dissolved in methyl alcohol and H_2O (1:1) and injected into a HPLC. All samples were filtered though a 0.45 μm pore size syringe-driven filter before injection. A 5 μL aliquot of sample solution was separated using an Agilent HPLC system equipped with a diode array detector on a 4.6 × 150 mm, 5 μm, and Agilent Eclipse XDB-C18 analytical column. The mobile phase was acetic acid (2.5%) and acetonitrile (88:12) at a flow rate of 0.5 mL min^{-1} 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.

Phytate was analyzed by the standard method of AOAC (2000). Rice sample (2.0 g) was extracted with 40 mL of 2.4% HCl by shaking vigorously for 3 h at room temperature before filtering. The filtrate was mixed with 1 mL of Na_2EDTA/NaOH solution and diluted to 25 mL with deionized water, then poured into an anion-exchange column (anion exchange resin, 100-200 mesh, chloride form). 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 hexaphosphate equivalent.

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. Difference between un-germinated and germinated brown rice were assessed by paired t-test with a level of significance of 0.05**.**

Results and Discussion

Optimum Germination Conditions for Brown Rice

GABA content of germinated brown rice in various soaking solutions is shown in Table 1. The soaking solutions which gave the highest of GABA content were citrate buffer pH 3 which similar results were reported of brown rice var. Khao Dawk Mali 105 (Charoenthaikij et al., 2007). However, Sunte et al. (2007) found brown rice soaked in buffer solution pH 5 while Watchraparpaiboon et al. (2007) found brown rice soaked in water at pH 6 had highest of 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).

GABA content of brown rice germinated for different lengths of time are shown in Table 2. The highest GABA content was obtained when germinated for 36 h. Oxygen content of vessel (plastic box) was decline throughout the germination processes, after 36 hour oxygen content reached 16%. Komatsuzaki et al. (2007) have reported that GABA content in brown rice var. Haiinori after soaking for 3 h, and germinated with gaseous treatment (no exchange of air) at 35°C for 21 h had higher than 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).

Therefore, optimum conditions to produce the highest GABA content in the brown rice were: soaking in citrate buffer at pH 3 for 5 h, and germinated for 36 h.

Chemical Compositions of Germinated Brown Rice

Chemical compositions of germinated brown rice are shown in Table 3. The protein, fat and dietary fiber content increased significantly after germination, probably because of the biosynthesis

Table 1 GABA content of germinated brown rice at various soaking solution.

Soaking solution	GABA content[1/] (mg 100 g^{-1})
Citrate buffer, pH 3.0	8.36±0.06d
Citrate buffer, pH 5.0	5.79±0.10b
Phosphate buffer, pH 7.0	4.58±0.31a
Distilled water	6.36±0.06c

[1/] The different letter in each column mean significant differences at $p<0.05$ level.

Table 2 GABA content of brown rice germinated for various times

Germinating time (h)	GABA content[1/] (mg 100 g^{-1})
12	9.12±0.42a
24	17.51±0.77b
36	44.53±1.93d
48	39.04±1.54c

[1/] The different letter in each column mean significant differences at $p<0.05$ level.

of new compounds during germination. Increase in dietary fiber result from the formation of primary cell walls, through an increase in pectic substance in the middle lamella. These results agree with research reported for soybeans (Kim et al., 1993), mung bean (Park et al., 1986), and germinated brown rice (Jung et al., 2005; Lee et al., 2007; Ohtsubo et al., 2005).

Total free sugar content of brown rice increased 2.0 folds as compared to the un-germinated sample. Degradation of starch in grains during germination led the increase in small dextrin and fermentable sugar (Ohtsubo et al., 2005; Wijngaard et al., 2005). This change produces a special sweet flavor in germinated brown rice (Kayahara, 2004).

Bio-Active Component in Germinated Brown Rice

GABA content in un-germinated and germinated brown rice is shown in Table 4. The results indicated that germination process induced increases GABA content. The GABA content increased 16.7 folds, after germination. This indicates that introducing a germination process was successful in terms of increasing this bio-active compound in brown rice. In germinated cereal

Table 3 Chemical composition of un-germinated and germinated brown rice[1/].

Composition (%)	Un-germinated brown rice	Germinated brown rice
Protein	8.93±0.04a	9.20±0.04b
Fat	2.24±0.02a	2.75±0.03b
Ash	1.20±0.01a	1.50±0.07b
Total dietary fiber	4.13±0.28a	5.26±0.28b
Total free sugar	0.50±0.02a	1.00±0.01b

[1/] The different letter in each column mean significant differences at $p<0.05$ level.

Table 4 Bioactive of un-germinated and germinated brown rice[1/].

Composition (mg 100 g^{-1})	Un-germinated brown rice	Germinated brown rice
GABA	2.64±0.11a	44.53±1.93b
γ-Oryzanol	64.16±1.10ns	63.61±2.40ns
Ferulic acid	21.75±0.64a	31.02±1.02b
Phytate	860.77±7.55b	609.17±4.48a

[1/] The different letter in each column mean significant differences at $p<0.05$ level.

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-functional substances, and to improvements in organoleptic qualities due to the softening of texture and increase of flavor in cereal grains (Kayahara, 2004).

Initially, γ-oryzanol was thought to be a single compound, but it's now known to be a mixture of at least 10 phytosterryl ferulates. Cycloartenyl ferulate, 24-methylene cycloartanyl ferulate and campesteryl ferulate have been identified as the major compounds, accounting for 80% of the γ-oryzanol in rice bran oil (Xu et al., 2001; Marero et al., 1991). The γ-oryzanol content in un-germinated and germinate brown rice are shown in Table 4. The various forms were quantified based upon the peak of 24-methylene cycloartanyl ferulate which is the major oryzanol component. After germination, γ-oryzanol content was the same level for both un-germinated and germinated brown rice. These results agree with research report of Japonica rice var. Koshihikari soaked in water at 30°C for 72 h (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 h and germinated in the dark for 24 h, γ-oryzanol content increased. On the other hand, when brown rice soaked in lemon grass solution for 6 h and germinated in the dark for 24 h, γ-oryzanol content decreased (Chutipanya, 2006). Additionally, Jiamyangyeun (2006) found that the red brown rice (Munpoo) and brown rice var. Khao Dawk Mali 105 soaked in water for 6 h and germinated in the dark for 24 h showed the highest γ-oryzanol content which increased 1.3-1.5 times higher than un-germinated brown rice.

Ferulic acid is the major phenolic compounds 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 (Tain et al., 2005). Ferulic acid content in un-germinated and germinated brown rice is shown in Table 4. The results indicated that germination induced 1.43 folds increase in ferulic acid content. Ferulic acid in germinated brown rice has the capability to prevent the build up of superoxide (Kayahara, 2004).

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). Phytic acid is hydrolyzed enzymatically by phytases or broken down chemically into other inositol phosphate such as inositol pentaphosphate (IP5), inositol tetraphosphate (IP4), inositol triphosphate (IP3), and possibly inositol di- and monophosphates during storage, fermentation, germination, food processing, and digestion in the human gut. Only IP6 and IP5 have a negative effect on the bioavailability of minerals; the other hydrolytic products formed have a poor capacity to bind minerals, or the complexes formed are even more soluble (Sandberg et al., 1989). The phytate content of brown rice and germinated brown rice are shown in Table 4. The phytate content was significantly higher in un-germinated brown rice. Phytate content in germinated brown rice decreased 0.71 folds, as compared with un-germinated brown rice; agree well with reports of brown rice, corn, and oats (Liang et al., 2008; Fageer et al., 2004; Larsson et al., 1995). 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) which 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 de-mineralized 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; Liang et al., 2008; Moong-ngarm, 2005).

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 citrate buffer pH 3 and germinated for 36 h. Therefore, germination was an important technique for enhancing GABA content in brown rice. Available nutrients and bioactive compounds in the brown rice greatly increase after germination. The physico-chemical properties of germinated brown rice flour are not different to those of the un-germinated 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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