

Analysis of Chemical Components Involved in Germination Process of Rice Variety Jhapra

M. A. Islam^{1*} and J. X. Becerra²

¹Faculty of Agriculture, Tokyo University of Agriculture and Technology, Japan

²Laboratory of Chemical Ecology, Department of Entomology, University of Arizona, Tucson, AZ 85721, USA

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Abstract

Jhapra, a local rice variety of Bangladesh, is produced in large quantity as interim crop between Boro and Amon seasons. This variety is mainly cultivated in Bogra district situated in the northern part of Bangladesh. Germination of brown rice was investigated to determine the optimum condition that would maximize γ -aminobutyric acid (GABA) content. Brown rice was soaked in various solutions such as phosphate buffer p^H 7, citrate buffer p^H 5 and p^H 3 and distilled water at room temperature (30±2⁰C) for 5 hours. The same method of germination was followed for 12, 24, 36 and 48 hours. Results indicated that the highest GABA content (44.53 mg 100 g⁻¹) was found in local brown rice variety which soaked in citrate buffer p^H 3 and germinated for 36 hours. The GABA content in germinated brown rice had increased 16.74 times as compared with brown rice (2.66 mg 100 g⁻¹). The nutritional components namely, protein, fat, ash, total dietary fiber, total free sugar and bioactive components like γ -oryzanol, ferulic acid and phytate in germinated brown rice were also analyzed. Compared to un-germinated 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. In contrast, germinated brown rice contained phytate content lower than brown rice.

Keywords: Jhapra; Germinated brown rice; γ -aminobutyric acid; γ -oryzanol, Ferulic acid; Phytate.

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1. Introduction

Rice grain is the seed of the monocot plant *Oryza sativa* of the grass family known as Gramineae. As a cereal grain, it is the most popular cereal worldwide, serving as a staple food for 39 countries and nearly half of the world population [1]. Globally rice accounts

* Corresponding author: islam162@yahoo.com

for 22% of total energy intake [2]. It is also the main staple food crop for the people of Bangladesh. The demand of rice is constantly rising in Bangladesh with nearly 2.3 million people being added each year to its population of about 150 million. Increasing rice production must be achieved at a faster rate than in most other countries in a situation where the cultivated land area is not expanding.

Yet, rice cultivation plays important role on Bangladesh economy and agriculture, accounting for nearly 18% of the Gross Domestic Product (GDP) and providing about 70 percent of an average citizen's total calorie intake. The total rice production area is about 10 million ha and accounts for 75% of the total area of agricultural crops and 93% of the total area planted to cereals. Therefore, the rice sector is the most important provider of rural employment.

Bangladesh has more than a thousand varieties of rice of which about 100 are well-known and acceptable varieties in terms of quality and texture of grain. Jhapra is one of the brown rice varieties, which is soft with approximately 15% of the amylase content. As a brown rice variety, it contains more nutritional components such as dietary fibers, phytic acid, vitamin E and γ -aminobutyric acid (GABA) than the ordinary milled rice. Those biofunctional components exist mainly in the germ and bran layers most of which are removed by polishing or milling. Unfortunately, brown rice takes long time to cook and cooked brown rice is harder to chew and not as tasty as white rice [3].

Germination process involved in incorporating those events that commence with the uptake of water by a quiescent dry seed and terminate with elongation of the embryonic axis [4]. This phenomenon is an incredible event. It signals the birth of a new life. At the time of germination, 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, the available nutrients are prepared for the growth of sprout. Increased nutrients in germinated brown rice included γ -aminobutyric acid, dietary fiber, inositols, ferulic acid, phytic acid, tocotrienols, magnesium, potassium, zinc, γ -oryzanol and polyendopeptidase inhibitor. Additionally, germinated brown rice contained free bound minerals, making them absorbable in our bodies and also tasty and tender [5].

GABA plays an important role in biochemical reaction during early stage of germination [41]. GABA in rice grains is synthesized from glutamic acid by glutamate decarboxylase (GAD). The activity of GAD showed high correlation with the germination ratio [6]. Gama-Oryzanol is a mixture of sterol esters of ferulic acid. It has been suggested to have potential functionality such as antioxidant activity [7], reduction of serum cholesterol [8], reduction of cholesterol absorption, decrease of early atherosclerosis, inhibition on platelet aggregation and inhibition of tumor promotion [9-10]. Therefore, this study is concerned with biochemical activities of local brown rice variety.

During germination, nutrients in the brown rice change drastically. Nutrients that increased in content during germination included γ -amirobutyric acid (GABA), dietary fiber, inositols, ferulic acid, phytic acid, tocotrienols, magnesium, potassium, zinc, γ -oryzanol, and polyendopeptidase inhibitor [42]. According to Kenichi, germinated brown rice contained total ferulic acid 126%, total dietary fiber 145%, soluble dietary fiber 120% and insoluble dietary fiber 150% that were more as compared with the brown

rice [43]. Ferulic acid has the capability to prevent the build up of superoxide, controlling the aggregation of blood platelets [5] and cholesterol-lowering properties as well as for their antioxidant capacity [12]. Phytate or phytic acid (myoinositol hexaphosphate) is the major storage form of phosphate in plant seed and grains. With its well designed molecular structure that was charged with six phosphate groups extending from central inositol ring is a potential chelator of iron and many minerals [13]. Phytic acid have some anticancer and antioxidant functions and prevents coronary diseases It is able to prevent the build up of superoxide, as well as to boost the immune system. Recently it is found that colon cancer, liver cancer, lung cancer, skin cancer etc. can be prevented. It is also able to prevent anemia and cardiac infarctions and diabetes [5].

Recent report [45] concluded that the concentration of crude protein, total free amino acids, α -tocopherol, γ -oryzanol, thiamine, niacin and pyridoxine in the germinated rough rice and germinated rice extracted powder were significantly higher than those of the germinated brown rice and un-germinated rice. There were no significant differences in the levels of crude fat, carbohydrate and ash between germinated rough rice and germinated brown rice. The amino acid contents of the germinated rice products were also investigated and differences were found among the samples. The most significant changes, in γ -aminobutyric acid, glycine, lysine and leucine were observed in the germinated rough rice and the germinated rice extracted powder. All these facts prompted us to investigate the biochemical changes during germination of local brown rice variety.

Various requirements must obviously be satisfied before germination started. 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 [14]. The nutritional and bio-active compounds of the germinated brown rice are influenced by the nature of the raw material, variety, steeping and germination conditions. However, there was no report on biochemical changes of germinated brown rice of Bangladeshi varieties. Therefore, this study was aimed to rectify this gap of knowledge by studying optimum germination conditions for brown rice and evaluating their bio-active compounds.

2. Materials and Methods

Jhapra is one of the principal rice cultivar in Bogra district of Bangladesh. This variety was cultivated at the field laboratory of Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur in 2009. To obtain brown rice samples, the paddies were milled by a home-scale miller and packed under vacuum in plastic bags. Packed samples were brought to the laboratory of chemical ecology, University of Arizona for further studies. The samples were kept in cold room (4⁰C) throughout the experiment.

2.1. Optimum germination conditions for brown rice

Washed brown rice was steeped in various soaking solutions, using grain-to-solution ratio of 1:2 (w/v) for 5 hours at room temperature (30±2⁰C). Citrate buffer of 0.1 M of p^H 3.0 and 5.0, 0.1 M phosphate buffer p^H 7.0 and distilled water was used for the control. After

5 hours, the soaking solutions were drained off and the rice grains were wrapped with cheesecloth to maintain moisture and left in the dark room for 24 hours for germination. The germinated brown rice was dried to <13% moisture content using a tray dryer at 50°C and analyzed GABA content. The soaking solution which gave the highest concentration of GABA had been selected for further study.

To determine optimum germination period, 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 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 had been selected for subsequent studies.

2.2. Chemical analysis

All the samples were analyzed for moisture, protein, lipid, ash and dietary fiber contents by the AOAC [18] method and sugar content was measured using the method described by Ohtsubo *et al.* [15].

2.3. Bio-active compound analysis

GABA content was determined by the method as described by Varayanond *et al.* [16] 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 hours. Thereafter, 200 µL of 3% sulfosalicylic acid was added and the mixture centrifuged at $4,500 \times g$ for 10 min. 50 µL of the supernatants were added to 50 µL of 100 mM NaHCO₃ and 50 µL of 4 mM 4-dimethylaminoazobenzene-4-sulfonyl chloride acetonitrile solutions. The mixtures were heated to 70°C for 10 min for derivatization. After the derivatization, the samples were added to 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, 4.6×150 mm, 2 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.

Gama-Oryzanol content was determined as described by Chen *et al.* [17] 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 g 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: acetonitrile: 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.

Total ferulic acid content was determined by using the method of Ohtsubo *et al.* [15]. 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 with 50 ml of ethyl acetate, for 5 min. The extraction technique was repeated three times. Thereafter, ethyl acetate layer evaporated and the sample was re-dissolved in methyl alcohol and H₂O (1:1) and injected into a HPLC. All samples were filtered through a 0.45 μ m pore size syringe-driven filter before injection. A 5 μ L aliquot of sample solution was separated using an Agilent HPLC system equipped with a diode array detector on a 4.6 \times 150 mm, 5 μ m, and Agilent Eclipse XDB-C18 analytical column. The mobile phase was the mixture of 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 320nm. Pure ferulic acid used as standard for calibration.

Phytate was analyzed by the standard method of AOAC [18]. Rice sample (2.0 g) was extracted with 40 mL of 2.4% HCl by shaking vigorously for 3 hours at room temperature before filtering. The filtrate was mixed with 1 mL of Na₂EDTA/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 solutions and wet-digested with a mixture of concentrated HNO₃-H₂SO₄ to release phosphate, which was measured colorimetrically with a spectrophotometer at the wavelength 640 nm. The amount of phytate in the original sample was calculated as hexaphosphate equivalent.

2.4. 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 was assessed by paired t-test with a level of significance of 0.05.

3. Results and Discussion

3.1. Optimum germination conditions for brown rice

GABA contents of germinated brown rice in various soaking solutions were shown in Table 1. The soaking solution which gave the highest of GABA content was citrate buffer pH 3. The similar result was reported for brown rice variety by Khao Dawk Mali 105 [19]. In addition, Sunte *et al.* [20] stated that optimum germination of brown rice occurred after soaking in buffer solution at pH 5. In contrast, Watchraparpaiboon *et al.* [21] found that

brown rice soaked in water at p^H 6 that showed highest GABA content. It was apparent that GABA in germinated brown rice increased when rice was soaked in acid solution. The synthesis of GABA was rapidly stimulated by a variety of stress conditions including hypoxia. The advantages of this process would be the concomitant H^+ consumption, which ameliorates the cytosolic acidification associated with hypoxia or other stresses [22]. Similarly, the synthesis of GABA through glutamate decarboxylase in reduced oxygen supply occurred by the effect of decreasing cytoplasmic p^H in carrot cell suspension [23].

Table 1. GABA contents of germinated brown rice at various soaking solutions.

Soaking solution	GABA content * (mg 100 g ⁻¹)
Citrate buffer, p^H 3.0	8.36 ± 0.06 d
Citrate buffer, p^H 5.0	5.79 ± 0.10 b
Phosphate buffer, p^H 7.0	4.58 ± 0.31 a
Distilled water	6.36 ± 0.06 c

* The different letter in each column means significant differences at $p < 0.05$ level.

GABA content of brown rice germinated for different lengths of time was shown in Table 2. The highest GABA content was obtained when brown rice germinated for 36 hours. Oxygen content of vessel (plastic box) decreased throughout the germination process, after 36 hours oxygen content reached 16%. Soaking after 3 hours, germination with gaseous treatment (no exchange of air) at 35°C for 21 hours showed highest GABA content than the conventional germination method [24]. The results indicated that glutamic acid might be synthesized by the glutamate synthase (GOGAT) in glutamine synthetase (GS) cycle. The GS/GOGAT cycle plays an important role in anaerobic accumulation of GABA and alanine [25].

Table 2. GABA contents of brown rice germinated for various times.

Germinating time (h)	GABA content * (mg 100 g ⁻¹)
12	9.12 ± 0.42 a
24	17.51 ± 0.77 b
36	44.53 ± 1.93 d
48	39.04 ± 1.54 c

* The different letter in each column means significant differences at $p < 0.05$ level.

Therefore, optimum conditions to produce the highest GABA content in the brown rice are: i) soaking in citrate buffer solution at p^H 3 for 5 hours, and ii) germination for 36 hours.

3.2. Chemical compositions of germinated brown rice

Chemical composition of germinated brown rice is shown in Table 3. The protein, fat and dietary fiber content increased significantly after germination due to the biosynthesis of new compounds during germination. These results showed similar findings reported for soybeans by Kim *et al.* [26] and germinated brown rice by Ohtsubo *et al.* [15] and Lee *et al.* [27].

Table 3. Chemical compositions of un-germinated and germinated brown rice*.

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

* The different letter in each column means significant differences at $p < 0.05$ level.

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 to the increase in small dextrin and fermentable sugar [15, 28]. This change produced a special sweet flavor in germinated brown rice [5]. Another report [46] stated that germinated brown rice had 6.8, 2.5, 1.5, and 3.7 g/100g for protein, lipid, ash and total dietary fiber, respectively. These results are at variance with the findings of this study. The variation may be due to the geographical position, varietal difference and environmental factors etc.

3.3. Bio-active component in germinated brown rice

GABA contents in un-germinated and germinated brown rice are shown in Table 4. The results indicated that germination process induced increased GABA content. The GABA content increased 16.7 folds, after germination. These results indicating that introducing a germination process were successful in terms of increasing this bio-active compound in brown rice. In germinated cereal grains, hydrolytic enzymes were activated and decomposed 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 [5]. Mazzucotelli *et al.* [40] reported that, in stress condition, GABA content might be changed.

Table 4. Bio-active chemicals of un-germinated and germinated brown rice*.

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

* The different letter in each column means significant differences at $p < 0.05$ level.

Initially, γ-oryzanol was thought to be a single compound, but it is now known to be a mixture of at least 10 phytosteryl 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 [29-30]. The γ-oryzanol content in un-germinated and germinated brown rice is shown in Table 4. The various forms were quantified based upon the peak of 24-methylene cycloartanyl ferulate which was the major oryzanol component. After germination, γ-oryzanol content was at 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 hours [15]. Similar report by Shallan *et al.* [48] stated that γ-oryzanol did not change before and after germination in brown rice varieties. However, a reverse trend was observed for γ-oryzanol content in herbal germinated brown rice. When brown rice was soaked in pandanus solution for 6 hours and germinated in the dark for 24 hours, γ-oryzanol content was seen to increase. On the other hand, when brown rice was soaked in lemon grass solution for 6 hours and germinated in the dark for 24 hours, γ-oryzanol content decreased [31]. Additionally, 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 [32]. 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 were bound to polysaccharides containing glucose, arabinose, xylose, galactose, rhamnose, and mannose residues in the cell wall [11]. Ferulic acid content in un-germinated and germinated brown rice is shown in Table 4. The results indicated that germination induced 1.43 fold increase in ferulic acid content. Ferulic acid in germinated brown rice has the capability to prevent the build up of superoxide [5].

Phytic acid and myo-inositol 1, 2, 3, 4, 5, 6 -hexakisphosphate (IsnP6) have long been known as a form of stored phosphorus in seeds. Approximately 70% of total phosphorus in seeds co-exists with phytic acid and its content typically accounts for 1% or more of seed dry weight [33]. Phytic acid is hydrolyzed enzymatically by phytases or broken down

chemically into other inositol phosphates 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 [34].

Table 4 shows the phytate content of brown rice and germinated brown rice. The phytate content was significantly higher in un-germinated brown rice. The phytate content in germinated brown rice was decreased 0.71 fold, as compared with un-germinated brown rice. The present results agreed with the reports of brown rice, corn, and oats [35-37]. Previously Wei *et al.* [47] noted that the phytate content in brown rice varieties ranged between 780-1110 mg/100g. In contrast, our present result showed a lower amount (609.17±4.48). Germination ability is mainly based on the action of enzymes whereas soaking is necessary for a combination of diffusion and enzymatic action [38]. Soaking of intact grains, as a first step of germination, decreased the phytic acid to 14-28% due to the activity of endogenous phytase and diffusion of phytic acid into the soaking medium [35] which was similar with pearl millet, legume and soybean [39]. Diffusion of phytic acid was 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 p^H of the medium [35-36, 39]. Liang *et al.* [35] also observed that soaking in acidic buffer was more effective to remove phytic acid 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 [35-36, 38]. As for comparison among the bioactive chemicals between germinated and un-germinated local brown rice variety, GABA content was found to increase quickly after germination but phytate content decreased. Ferulic acid content showed statistically significant difference after germination. In nutritional point of view most of the sugar content increased in germinating brown rice variety.

When a brown rice grain starts to sprout, enzymes that have been inactive, start to make many changes within the grain. These enzymes convert the nutrients as the stored forms that are accessible to nourish the sprout. Due to the enzymatic activities nutrient content changes remarkably in the brown rice. Exact soaking, exact sprouting and exact drying methods give this rice exceptional nutrients, flavor, stability and digestibility.

4. Conclusion

This study showed that, as the soaking time increased, GABA content increased. The tendency of increase varied with the p^H of the soaking solution. The GABA content was the highest in citrate buffer p^H 3 that was germinated for 36 hours. Therefore, germination was an important technique for enhancing GABA content in brown Bangladeshi local rice variety. Available nutrients and bio-active compounds in the brown rice greatly increased

after germination. The physico-chemical properties of germinated brown rice flour were not different than 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.

The process of germination allowed tiny sprouts at the end of the grain that enhanced the nutritional values of the local brown rice variety. Phytic acid is an inhibitor of nutrient absorption and can be found in all grains, beans and seeds. Neutralizing of the phytic acid allowed more absorption of many nutrients that was found in sprouted brown rice. In the present investigation, GABA level was tested in the germinated brown rice. This study showed the increasing of GABA level in the germinated brown rice by sprouting. This finding may help in the study of nutritional aspects of Bangladeshi local brown rice variety.

The present study was carried out to evaluate the different bio-active chemicals and their comparisons during germination of a local rice variety. Results revealed the information of chemical attributes and their functions in Bangladeshi local brown rice variety. Those chemicals may be helpful for identifying the chemopreventive properties and antioxidant activities in future study. This report emphasizes the biochemical phenomenon during germination of a local brown rice variety. Finally, it can be concluded that, local brown rice variety possesses good biochemical properties, more likely due to the nutritional components that increased in content following the process of germination such as phytic acid, ferulic acid, and dietary fiber.

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