

Effect of Soaking Temperature and Time on GABA and Total Phenolic Content of Germinated Brown Rice (Phitsanulok 2)

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Abstract

Rice grains riched in gamma-amino butyric acid (GABA) are increasingly popular, particularly in the health food market. It has a major inhibitory effect on neurotransmitter function, cancer cell and also reduces blood pressure. GABA contents in rice were investigated during germination. GABA levels in rice are influenced by many factors including the duration of seed incubating, soaking temperature, soaking time or in pre-germinated brown rice. The objective of this study was to investigate the effect of soaking temperature and time conditions on GABA content of brown rice germination. Phitsanulok 2 rice was germinated following a central composited design with different soaking temperature durations (20-40°C) and time (60-300 min) for 10 treatments, then incubating at 25°C for 24 hr. Physical quality, pH of soaking water, protein, GABA and total phenolic compound (TPC) content were analyzed. The results showed that GABA content and TPC increased from 6.71 mg/100g, 13.68 mgGAE/100g of dry sample (raw material) to 18.74 mg/100g, 33.95 mgGAE/100g of dry sample, respectively. Regression model showed that an increase of soaking temperature and time affected on an increase of GABA content ($p \leq 0.05$) as well as TPC. On the other hand, an increase of soaking temperature and time significantly decrease pH of soaking water ($p \leq 0.05$). The optimum conditions regarding soaking temperature and time were 33°C for 300 min, which provided the highest GABA content (18.74 mg/100g), TPC (33.95 mg/100g) and pH of soaking water (5.7). This indicated that soaking conditions for germinated Phitsanulok 2 rice could increase the nutrition value of rice as functional food product.

Keywords: brown rice, germination, GABA, total phenolic compound, Phitsanulok 2 rice

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1. Introduction

Rice is among the most important staple foods of the world. It has been reported that as much as 75% of the daily calorie intake of people in some Asian countries is derived from rice [1]. In many developing countries, rice is the main source of calories and proteins. A rice grain is made up of an outside husk layer, a bran layer, and an endosperm. When the husk is removed, the rice is called brown rice [2]. It is a functional food and an excellent source of nutrients such as protein, dietary fiber, fats, minerals, vitamin E, vitamin B, and gamma-amino butyric acid (GABA) in comparison with ordinary milled rice [3]. The functional properties of brown rice can be improved by the process of germination. General interest in consumption of germinated rice has increased because of increase in people's awareness of its health benefits, mainly the amplified levels of gamma-amino butyric acid, and improvement in the texture of cooked rice [4]. Germinated brown rice is made from brown rice which has been germinated by soaking in water. The germ produces substances from the physiological action and enzymes to improve nutrition and the texture of brown rice. Endosperm and seed coat hydrolyzed by alpha-amylase into sugars which provide the energy required for the growth of roots. Storage proteins decompose into amides, and proteins can break down into amino acids, especially glutamic acid which can change into GABA [5]. GABA is a neurotransmitter in the brain and spinal cord of mammals. It can promote sleepiness, prevent headaches and depression, help in the reduction of hypertension, and inhibit the development of cancer cells [6]. Several studies have reported with regard to the production of germinated brown rice that the accumulation of GABA during germination deepened depending on the variety of rice, the time, and the temperature of soaking water as well as the pH [7]. There are a number of reports on producing germinated brown rice. But reports on germinated Phitsanulok2 rice are few and far between. This is because production of the Phitsanulok2 rice goes over capacity, gives high yield, and is marred by rare diseases. Hard texture and fewer amounts of nutrients in this rice are the reasons for less consumption. The aim of this research was to improve the nutrition of the Phitsanulok2 rice by study effect of soaking temperature and time on the properties of germinated brown rice to provide the high quantities of GABA and TPC.

2. Materials and Methods

2.1 Raw material

Phitsanulok2 rice was used for the experiment. The paddy samples were harvested from the Phitsanulok Rice Research Center, Phitsanulok, located in the central region of Thailand. Before conducting the experiment, rough rice was stored in aluminum foil bags. The Phitsanulok2 rice paddy (moisture content 11%) samples were husked to brown rice in a laboratory rice mill at the Lanna Rice Research Center, Chiang Mai University, Thailand, and then analyzed for moisture content [8], protein [8], TPC [9], and GABA [10].

2.2 Rice germination experiment

The experiment was performed by soaking 500 g of brown rice in 1,000 g of water using a water bath and allowing the rice to germinate in an incubator at 25°C for 24 hr. The germinated brown rice was then dried in an oven at 50°C (moisture content $\leq 10\%$). This study was based on the central composite design (CCD) and the experiment was carried out as 10 treatments with 3 replications using different soaking temperatures (20-40°C) and soaking times (60-300 min.) [11]. The 10 treatments are shown in Table 1.

Table 1. Treatments with different soaking temperatures (20-40°C) and soaking times (60–300 min.), using CCD design ($2^2\text{CCD} + 4 \alpha + 2\text{cp}$)

Treatment	Temperature *factor code	Soaking Time *factor code	Temperature (°C)	Soaking Time (min.)
1	-1	-1	20	60
2	-1	+1	20	300
3	+1	-1	40	60
4	+1	+1	40	300
5	- α	0	15	180
6	+ α	0	44	180
7	0	- α	30	11
8	0	+ α	30	350
9	0	0	30	180
10	0	0	30	180

*Meaning of factor codes:

(-1) = minimum level of soaking temperature/soaking time for germination of rice

(+1) = maximum level of soaking temperature/soaking time for germination of rice

(0) = mid point level of soaking temperature/soaking time for germination of rice

(- α) = soaking temperature/soaking time for germination of rice less than mid points 1.414 times

(+ α) = soaking temperature/soaking time for germination of rice higher than mid points 1.414 times

cp = mid point level of soaking temperature/soaking time for germination of rice

2.3 Determination of GABA content

The derivative sample was determined by high performance liquid chromatography HPLC (Shimadzu: class vp-5 LC-10 Aup). Ultra C18 column (250×46 mm; 5 μm) was used to obtain the chromatograms. A quantity of 50 mM of sodium dihydrogen orthophosphate was used, with the following profile: 0–8.5 min., 35%B; 8.51–12.00 min., 70%B; 12.01–14.00 min., 35%B. The flow rate was 1 mL/min., and the column temperature was 37°C; the injection rate was 10 $\mu\text{L}/\text{min}$. [10].

2.4 Determination of total phenolic content (TPC)

The 1.0000 of germinated rice was shaken with 10 mL of methanol. A volume of 200 μL was mixed with 800 μL of 7.5% sodium carbonate and 1,000 μL of Folin-Ciocalteu's reagent. The reaction mixture was left for 30 min. in dark room temperature. The absorbance was recorded at 765 nm. The results were expressed as milligrams of Gallic acid equivalent (GAE) program of dry material [9].

2.5 Determination of pH, total nitrogen and rice yield.

The pH value of the water was taken using a pH meter (portable pH meter: Starter 300 OHAUS, USA). The protein content was determined according to the Kjeldhal method. The rice yield was calculated by yield (%) = $(W1 \times 100) / W2$, where W1 = weight of germinated brown rice and W2= weight of brown rice.

2.6 Statistical analysis

The experiments were carried out by following a central composite design (CCD) using a Design Expert version 6.0.2 (Stat-Ease Inc., USA) with two factors. The first factor was

soaking temperature (20-40°C) and the second factor was soaking time (60-300 min.). The experimental data were subjected to statistical evaluation using analysis of variance (ANOVA) for a completely random design. Duncan's new multiple range tests were used to determine the difference between the means, and the significance was defined at $p \leq 0.05$.

3. Results and Discussion

3.1 Physical and chemical composition of Phitsanulok2 brown rice

The chosen Phitsanulok2 rice was non-aromatic and mostly cultivated in the center and lower north region of Thailand. The Phitsanulok2 brown rice contains high amylose (24-35%), low protein (5-7%), and harder texture than other types of rice [12]. The major composition of brown rice (raw material) was analyzed. The GABA content was 6.71 mg/100 g, TPC was 13.68 mg GAE/g, protein was 6.23%, and moisture content was 12.71%.

3.2 Effect of temperature and soaking time on germinated rice

The study findings with regard to the soaking temperature (20-40°C) and the time (60-300 min.) on the pH of the soaking water, GABA, TPC, protein, and the %yield are shown in Table 2 and Table 3. Different soaking temperature and time are significantly affected ($p < 0.05$) on pH, GABA, and TPC while protein, moisture content, and % yield were not significantly different ($p > 0.05$). The regression models are shown in Table 4. Due to the soaking process, there was soaking loss, mainly from the displacement of residual dust, the leaching of soluble materials, and the metabolic activity of the grain releasing CO₂ and small amounts of ethanol [13]. The leaching particles included polysaccharides, proteins, and other water-soluble components. The appropriate amount of water uptake of the brown rice during soaking directly affected the quality of the germinated brown rice. Different cultivars had different characteristics as regards water absorption [14]. The different conditions of soaking temperature and time had an effect on the pH of the soaking water.

Table 2. pH of soaking water, gamma-amino butyric content, and total phenolic content of germinated brown rice.

Soaking Temperature (°C)	Soaking time (min.)	pH	GABA ¹ Content (mg/100g dry sample)	TPC ² Content (mg GAE/ g dry sample)
20	60	6.28±0.08 ^a	9.92±0.29 ^{de}	17.98±0.97 ^d
20	300	6.06±0.07 ^{abc}	10.01±0.10 ^{de}	17.83±0.32 ^d
40	60	5.98±0.11 ^{def}	10.49±0.66 ^d	29.88±0.97 ^{abc}
40	300	5.71±0.05 ^{ab}	18.67±0.74 ^a	32.51±0.62 ^a
15	180	6.15±0.04 ^f	9.54±0.46 ^{de}	16.48±0.45 ^d
44	180	5.74±0.04 ^a	18.20±1.60 ^{ab}	33.77±0.86 ^a
30	11	6.42±0.13 ^{cde}	8.95±0.19 ^e	13.94±0.74 ^d
30	350	5.94±0.09 ^{bcd}	17.66±1.11 ^b	30.70±1.29 ^{ab}
30	180	5.93±0.06 ^{cde}	16.22±0.57 ^c	27.29±0.11 ^{bc}
30	180	5.95±0.04 ^{ef}	15.55±0.41 ^c	25.76±0.97 ^c

The values are mean ± standard deviation (n=3). ^{a-f}Means within each column followed by different letters are significantly different ($p \leq 0.05$) using Duncan's new Multiple Range Test (DMRT).

¹Gamma-aminobutyric acid (mg/100 g dry sample), ²Total phenolic content (mg GAE/g dry

sample)

The regression model in Table 4 and Figure 1a shows that an increase in the soaking temperature and time made the pH of the soaking water decrease. This observation is the same as the result obtained in the study of soaking red jasmine brown rice, which found that an increase in the temperature from 30°C to 40°C brought about a decrease in the pH. A study on Hom Mali 105 rice found that brown rice soaked in buffer solution at pH 5 had the highest GABA [15], while a study on Chainat 1 rice found that brown rice soaked in water at pH 6 had the highest GABA [16]. It is apparent that GABA in germinated brown rice increased when the rice was soaked in an acid solution. In conclusion, the soaking water having the optimum pH will increase the quantity of GABA content [17].

Table 3. Protein, moisture content, and %yield of germinated brown rice

Soaking Temperature (°C)	Soaking time (min.)	Protein ¹ (%) ns	MC ² (%) ns	Yield ³ (%) ns
20	60	6.53±0.11	8.76±0.04	84.3±0.94
20	300	6.41±0.07	8.61±0.02	81.8±0.51
40	60	6.67±0.09	8.29±0.07	85.6±0.81
40	300	6.78±0.12	8.74±0.04	75.5±1.09
15	180	6.39±0.13	8.09±0.08	86.5±0.67
44	180	6.88±0.10	8.82±0.11	74.4±1.11
30	11	6.28±0.17	8.39±0.05	87.7±1.23
30	350	6.70±0.09	8.52±0.10	79.1±0.87
30	180	6.74±0.10	8.39±0.08	81.6±0.92
30	180	6.69±0.09	8.28±0.06	79.0±1.32

The values are mean ± standard deviation (n=3).^{ns} Non significant difference using The Duncan's new Multiple Range Test (DMRT).

¹Protein, % dry sample, ²Moisture content, % dry sample, ³Yield, % dry sample

This suggested that soaking process increased acidity. This is compliant with the findings of a study on probiotic fermentation: an increase in the soaking temperature and time decreased the pH of the soaking water because the optimum pH had an effect on bacterial fermentation [18]. The chemical composition and the properties of the rice were observed to change. Moreover, complex proteins were found to change to simple proteins and amino acids. A temperature of 40°C was found to be the best condition for the growth of bacteria (lactic acid bacteria) [19]. Moreover, the effect of acid pH direct manifesting itself in dissolving of the seed coat which involves stimulating conditions for development of some species of fungi whose action causes perforation of the seed coat while soaking [11]. Soaking temperature and time also have an effect on the content of GABA. The regression model presented in Table 4 and Figure 1b indicates that an increase in the soaking temperature and time increased GABA. This result indicates that soaking contributes to the increase in the GABA content. Similar results have been reported for brown rice [20] and soaked rice germ [11]. Upon soaking the rice embryo for 1 hr., it was observed that the mapping metabolic transcript levels rapidly changed the metabolism and also increased the phosphates, tricarboxylic acid cycle (TCA), and GABA [21]. Changes in the metabolic process have an effect on the carbohydrate, amino acid, and cell wall metabolism of rice. The slightly acidic condition of H⁺ stimulates the glutamate decarboxylase enzyme and it catalyzes the decarboxylation of L-glutamic acid to carbon dioxide and GABA, which causes the glutamic acid to decrease and GABA to increase [22].

Table 4. Regression equation model of soaking temperature and time which affected physical content and chemical content.

Physical and chemical content	Regression equation model	p-value	Adj.R ²
pH	$6.02 - 0.16 (\text{temp}) - 0.14 (\text{time})$	0.003	0.81
GABA content	$15.88 + 2.69 (\text{temp}) + 2.58 (\text{time}) - 1.33 (\text{temp})^2 - 1.61 (\text{time})^2 + 2.04 (\text{temp}*\text{time})$	0.011	0.81
Phenolic content	$26.52 + 6.55 (\text{temp}) + 3.45 (\text{time}) - 0.42 (\text{temp})^2 - 1.80 (\text{time})^2 + 1.06 (\text{temp}*\text{time})$	0.044	0.89

Moreover, soaking could lead suspension cells to become adapted to water stress. Such stress may also contribute to the accumulation of GABA by reducing the oxidation of succinic semi aldehyde to succinate [23]. In the study, it was observed that at 40°C and 5 hr. of soaking with the pH of water at 5.7, the GABA content was the highest at 5.7 mg/100 g. The best condition as regards soaking temperature, time, and pH of water to aid the germination of red jasmine brown rice has been found to be 40°C, 180 min., and 5 [15].

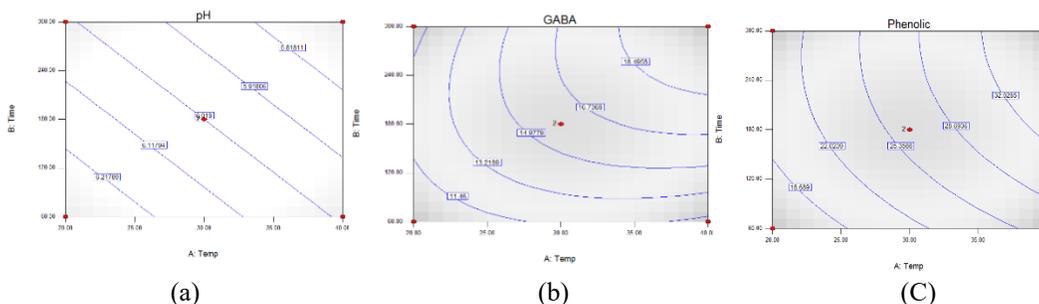


Figure 1. The contour plot of (a) pH of soaking water, (b) GABA content, (c) TPC content of germinated brown rice with different soaking temperature and time.

3.3 Optimization of brown rice

Germination improved the TPC and the antioxidant activity. Many factors had an effect on TPC such as extraction method, variety, growth condition, and germination time. In the study, it was observed that the TPC significantly changed on changing the condition of soaking temperature and time (Table 4 and Figure 1c). An increase in the soaking temperature and time increased the TPC. This observation is the same as the finding of the study on Thai waxy paddy rice RD6: during the germination process, the antioxidant capacity increased continuously with the germination time [19]. The germination process increased the TPC because the cell wall of the rice was changing [24]. During the production of Hang rice, the phenolic compounds were found to be affected by heat, and the steep water might have partially removed the soluble phenolic compounds from the grain [25]. The antioxidant capacity of germinated paddy rice is directly related to the TPC. Insoluble phenolic content is a major contributor to antioxidant capacity, which was found to be about 71% in rice [26]. During germination, biomolecules such as lipid and protein increase. Soaking water acts as a hydrolytic enzyme to digest the protein and the lipid rice bran, causing the protein and the lipid contents to increase. The soaking method has an effect on the protein in rice bran because soaking increases the permeability into the rice seed [27].

The objective of the experiment was to optimize the appropriate condition of soaking temperature and time for the production of germinated Phitsanulok 2 brown rice to provide high

quantities of GABA and TPC. The different conditions of soaking temperature and time affected the yield quantities of GABA and TPC as well as the predicted value of pH (soaking water) (Table4). The optimization of the soaking temperature and the time was achieved by setting criteria to obtain the highest yield of GABA and TPC (Figure 2). The analysis showed that the optimization of using 33°C of soaking temperature for 300 min. was the best condition.

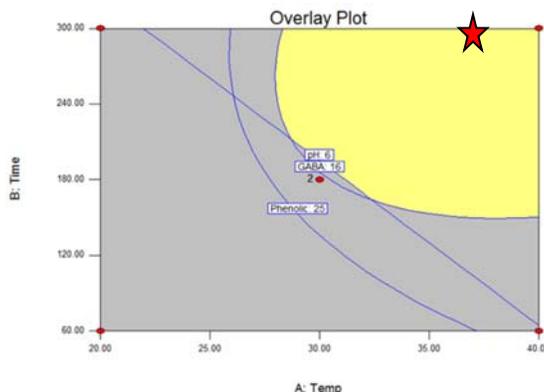


Figure 2. Overlay plot used for optimization of soaking temperature and time.

4. Conclusions

This study shows that, as the soaking time and temperature increased, GABA content and total phenolic content increased. While pH value of soaking water decreased. The GABA content and total phenolic content were highest at 33°C of soaking temperature for 300 min. Therefore, germination was an important techniques for enhancing GABA content in brown rice. Available nutrients and bioactive compounds in the brown rice greatly increase after germination. Germinated brown rice may be used as a nutritional ingredient in functional food products such as beverages or confectioneries. The food industry can be used optimum condition to germinate Phitsanulok 2 rice or apply with another species of rice by focus on the relationship between two factors, soaking time and temperature.

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