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The study of soaking and germination times to improve the nutritional quality of white and black glutinous rice

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Abstract

This study examined the effect of soaking and sprouting times on germination rates, biochemical (protein, lipid, and carbohydrate), and phytochemical contents gamma-amino butyric acid (GABA), gamma-oryzanol, tocopherols, and B vitamins in dehulled white and black glutinous rice (N97 and DH6). The dehulled white and black glutinous rice (N97 and DH6) were soaked in distilled water for 2, 4, 6, and 8 h at 30 °C. Then, they were germinated for 12, 24, 36 h at room temperature under an aerobic condition (RH%=83-85%). The results showed that the germination rates were positively correlated to the soaking time and the germination time. Specifically, at the soaking time of 6 h and the germination time of 24 h, the germination rate of dehulled white and black glutinous rice cultivars (N97 and DH6) achieved maximum values (97.98% and 95.19%, respectively). The results also showed that the biochemical and phytochemical contents of the germinated rice were substantially improved at 6 h of soaking and 24 h of sprouting. The extended soaking (8 h) and germination periods (36 h) caused the degradation of the biochemical (i.e., protein, lipid, carbohydrate) and phytochemical contents (i.e., GABA, tocopherol, gamma- oryzanol, and vitamins), which resulted in the low nutritional values of the germinated rice. The results of this study are expected to offer a possible solution to the improvement of the germinated rice quality associated with the soaking and germination conditions.

Keywords: White and black glutinous rice, Soaking, Germination, Biochemical, Phytochemical content

1. Introduction

Rice (*Oryza sativa*) is one of important cereal grains in the world today. It is used as the main staple food of over half of the world's population [1,2]. Rice is generally classified into glutinous and non-glutinous categories based on the types of amylose and amylopectin found in starch endosperm. Glutinous rice is composed mainly of amylopectin and very low amylose contents (below 5%) [3-5]. It also contains B vitamins, gamma-amino butyric acid (GABA), gamma-oryzanol, and tocopherols [6,7]. Glutinous rice is a common rice cultivars that is cultivated mainly in Southeast and East Asia including Vietnam [1,5]. According to Sattaka et al. 2017, Vietnamese glutinous cultivars are divided into two cultivars, they are pigmented and non-pigmented glutinous rice. Pigmented glutinous rice (DH6) and non-pigmented glutinous rice (N97) are the most popular rice varieties because of their pleasant aromas and textures [1,8]. They are used as the staple food not only in every meal but also on special occasions in Vietnam [8]. However, some previous studies showed that the glutinous rice grains is still low in phytochemical contents [9] and digestibility [10]. Germination is known as an effective method to improve the nutritional values and texture properties of rice [11,12]. The germination can be divided into two steps: soaking and sprouting. Soaking and sprouting lead to the breakdown of some compoments of grains into simpler compounds [11,12]. These simple compounds enhance the texture, flavor, aroma, and taste of brown

rice. In addition, germination also enhanced hydrolytic enzymes synthesis, which helped in producing phytochemical contents such as gamma-oryzanol, GABA, and vitamin B [13-15]. In [13], the germination time for 12, 24, 36 to 48 h at 30 °C were studied to improve the GABA, gamma-oryzanol and phytate contents. The results showed that the GABA and gamma-oryzanol increased, whereas the phytate decreased due to extended germination time. In [12], the effects of germination process on the germination rate and the amount of GABA content in the germinated Jasmine rice of Thailand was studied. The extended soaking time from 4 h to 24 h insignificantly increased the germination rate, and the increased soaking temperature from 30 °C to 40 °C had no effect on the germination rate. The GABA content (11.67 mg/100 g) was significantly higher than that of brown Jasmine rice (1.08 mg/100 g). Nowadays, many studies have examined the effects of different germination conditions on brown rice. However, there exists no research on the effect of soaking and germination process on the germination rate and the quality of the dehulled white and black glutinous rice. Thus, the objective of this study is to investigate the effect of soaking and sprouting times on the germination rates, biochemical (lipid, protein, and carbohydrate content), and phytochemical compositions (GABA, gamma-oryzanol, tocopherols, and vitamin B content) of white and black glutinous rice. The soaking time and germination time were varied between 2.0, 4.0, 6.0, and 8.0 h; and 12, 24, and 36 h, respectively.

2. Materials and methods

2.1 Materials and chemicals

The grains of white and black glutinous rice (N97 and DH6) were provided from Minh Tam Phat factory in An Giang province, Vietnam. All samples were de-hulled using dehusking machine (Satake, Japan), and separated by rice grader (Ogawa Seiki.Co.Ltd, Japan). The samples were then vacuumed and stored in cold room (4-6 °C) before use it for further experiment.

Standards of tocopherols, gamma-oryzanol (purity > 99%), gamma-aminobutyric acid (GABA; purity > 98%), and vitamin B (thiamine, riboflavin, and niacin) were from Sigma-Aldrich (Darmstadt, Germany). All the other chemicals were of analytical grade and were used without any further purification.

2.2 Preparation of germinated rice

The samples (N97 and DH6) were steeped in distilled water (sample: water ratio=1:2) for 2, 4, 6, and 8 h at room temperature (30-32 °C). Then the steeping water was drained off and the steeped rice seeds were incubated in plastic basket. After that, the plastic basket was covered by double layers of cotton cloth. The steeped rice was germinated for the specified durations. The germination times were varied from 12, 24, to 36 h with 30 °C, the temperature recommended for germination of rice [14]. During germination, the relative humidity was maintained from 83 to 85% using seed germination chamber GR-36L (Geneva Scientific, Fontana, USA). The germination rates of the germinated rice were determined according to [9]. The percentage of germination rate was calculated after the number of germinated rice had been obtained. After that, the germinated white and black glutinous rice were dried at 50 °C, to about 10-12% dry basic (db) of moisture content before grounding for further analysis.

2.3 Determination of GABA content

Extraction and analysis of GABA by High-Performance Liquid Chromatography (HPLC) following [15] method with some modifications. The germinated rice powders were extracted with 4% acetic acid in 80% ethanol solution by shaking at 150 rpm for 120 min, and centrifuged at 6000 rpm for 10 min. The supernatants were then collected by filtering through 0.2 μL filter. The obtained supernatants were evaporated at 55 °C by a vacuum evaporator (Rotavapor R-210, BUCHI) to acquire the concentrated extracts. The concentrated extracts were analyzed by (HPLC), using Agilent 1200 series HPLC (SHIMADZU, Japan), C18 column. The mobile phase consisted of ammonium acetate buffer 25 mM and acetonitrile with the ratio of 55:45 (v/v). The flow rate was 1 mL/min with a 10 μL injection volume. The UV detector and the column temperature were operated at 465 nm and 23±1 °C. GABA standard was used to construct the standard curve and calculate the concentration of GABA.

2.4 Determination of α-tocopherol content in germinated rice

The tocopherol content (α-, β-, γ-, δ-) of rice bran oils were evaluated by reversed-phased HPLC (Agilent 1200 series equipped with Hypersil Octadecylsilan (ODs) column (250×4.0 mm, 5.0 μm, Phenomenex, USA)) using a modification of [16]. The standards and extracts were separated chromatographically on Hypersil ODS column. The solvent mixture of acetonitrile and methanol was used in the mobile phase under a gradient

condition. The gradient solvent was performed as methanol (5% v/v) and acetonitrile (95% v/v) for 3 min, methanol (100% v/v) and acetonitrile (0% v/v) for 30 min. A UV/VIS diode array detector (DAD) with wavelengths of 290 and 330 nm was equipped for the sample detection. The overall flow rate was set a 1.0 mL/min and the injection volume was 20 µL.

2.5 Determination of gamma-oryzanol

Gamma-oryzanol was determined by using reversed-phased HPLC following [17]. Germinated rice powder (0.5 g) was weighed and put into test tube. After that 10 mL ethyl acetate was added, and the test tube was covered by aluminum foil. The mixture was shaken by vortex for 10 min before centrifuged at 10,000 rpm for 10 min. The supernatant was filtered through 0.2 µL filter. The liquid was then analyzed by HPLC equipment equipped with a Poroshell 120 EC-C18 column (3.0 mm × 150 mm, 2.7 µm). Ratios of acetone and acetonitrile were 40:60 for 5 min; 50:50 for 10 min, and 60:40 for 5 min, the flow rate was 1.5 mL/min, and the column temperature was maintained at 35 °C. The standard was used to construct the standard curve using for calculation the concentration of gamma-oryzanol.

2.6 Determination of vitamin B

1 g of samples were mixed with 5 mL trichloroacetic acid (TCA). The solution was then mixed vigorously using a Vortex for 2 min and centrifuged for 10 min at 10,000 rpm. Then, the protein was precipitated and filled through a 0.45 mm membrane (Millex HV filter, Darmstadt, Germany). The liquid containing the B vitamins was collected and stored at 5 °C for further HPLC analysis. Standard stock solutions (thiamine, riboflavin, and niacin) were prepared as reported previously [18]. The samples and standard solutions were analyzed by using a reversed phase- (RP-) HPLC column (Agilent Technologies, Inc.; Santa Clara, CA, USA) equipped with a pump (LPG300), poroshell 120 EC-C18 (250 × 4.6 mm i.d., 5 µm). The mobile phase using 30/60 v/v, 50:50 v/v, and 40:60 v/v methanol to phosphoric acid (0.023 M H_3PO_4) were carried out for 5 min each, with the flow rate of 0.5 mL/min. The ultraviolet (UV) detector was recorded at 254 nm at room temperature. The vitamin B content was calculated by comparison to the standard curve.

2.7 Other analysis

The moisture were determined following [19] with some modifications. 5 g samples were filled into moisture can and dried in the hot air oven at 105 °C for 16 h when the samples reached a constant weight. Determination of protein, lipid, and carbohydrate in non-germinated rice and germinated rice were carried out according to the methods of AOAC [20].

2.8 Statistical analysis

All the statistical analyses were carried out in triplicate, and the means ± standard deviations (SD) were calculated by using Statgraphic Centrution XV (Statsoft Inc., Umeå, Sweden). The one-way analysis of variance (ANOVA) was carried out to determine differences among the group means, given the 95% confidence level.

3. Results and discussion

3.1 Chemical composition

The proximate composition of white and black glutinous rice (N97 and DH6) is shown in Table 1. The moisture content of white and black glutinous rice varied between 12.15% db–12.56% db, which is suitable for storage of processed rice. The biochemical content (crude protein, lipid, and carbohydrate) were significantly different between both cultivars. The crude protein content of black glutinous rice was 6.90 mg/100 g, and higher than white glutinous rice (4.90 mg/100 g). The lipid and carbohydrate contents of white and black glutinous rice (N97 and DH6) were from 2.12 to 2.49 mg/100 g and 77.90 to 78.97 mg/100 g, and near about the desired range (2–4 mg/100 g and 77-79 mg/100 g) [21].

Our experiment results also showed that regardless of the rice cultivar, the content of the phytochemical compounds were differed (Table 1). The gamma-oryzanol, β- and δ-tocopherol content of the black glutinous rice (DH6) were higher than that of the white glutinous rice (N97). Meanwhile, the levels of B vitamins, GABA, α-, and γ-tocopherol of the black glutinous rice (DH6) are comparable to white glutinous rice (N97). The differences could be the effect of environmental, fertilizer application, and genetic factors [22].

Table 1 Initial chemical ingredient of white and black glutinous rice varieties (N97 and DH6).

Rice variety	Moisture (%)	Lipid (mg/100 g, DW)	Crude protein (mg/100 g, DW)				Carbohydrate (mg/100 g, DW)		
White glutinous rice (N97)	12.15^{a}±0.94	2.48^{a}±0.10	4.90^{b}±0.21				78.92^{a}±1.45		
Black glutinous rice (DH6)	12.56^{a}±0.91	2.10^{b}±0.12	6.90^{a}±0.18				77.90^{a}±1.65		
	Gamma-oryzanol (mg/100 g, DW)	GABA (mg/100 g, DW)	Tocopherol (mg/100 g, DW)				Vitamin B (mg/100 g, DW)		
			α-	β-	γ-	δ-	Thiamin	Riboflavin	Niacin
White glutinous rice (N97)	174.39^{b} ±2.56	36.09^{b} ±1.01	1.03^{a} ±0.05	0.14^{b} ±0.03	0.52^{a} ±0.02	0.15^{b} ±0.00	0.21^{a} ±0.01	7.10^{a} ±0.02	0.071^{a} ±0.00
Black glutinous rice (DH6)	202.12^{a} ±2.98	37.12^{a} ±0.62	0.94^{a} ±0.06	0.36^{a} ±0.01	0.56^{a} ±0.03	0.25^{a} ±0.00	0.25^{a} ±0.01	7.15^{a} ±0.01	0.087^{a} ±0.00

Means followed by different superscript letters in the same column denote a significant difference at $p<0.05$ as analyzed using Duncan's multiple range test. The values are the means of the three replications ± the standard deviation (SD).

3.2 Effect of soaking time and sprouting time on rice germination rate

The moisture content of soaked rice seed is one of the important critical factors for determining the germination rate. As shown in Table 2, the initial moisture content of the two rice cultivars (N97 and DH6) was 12.15% and 12.56% db, respectively and in the range of optimum grains storage [4]. The moisture content of two rice cultivars increased rapidly from 12.15 to 39.98% and 12.56 to 38.94% in the first 6 h soaking. After 8 h of soaking, the moisture content of white and black glutinous rice (N97 and DH6) increased slightly and reached at 41.12% and 39.14%. The variation in the moisture content for different soaking time could be the results of the breakdown of cell wall of white and black glutinous rice, thereby absorbing water rapidly. A similar result was reported for brown rice [14]. In Table 2, the germination rate and the moisture content were possibility correlated. Longer soaking time enhanced the moisture content of soaked rice and resulted in the high germination rate. The maximum germination rate of both rice cultivars (N97 and DH6) was 97.91% and 95.19%, repsectively after 6 h of soaking and 24 h of sprouting. The finding is comparable to [23], who reported that an extended soaking and sprouting time enhanced germination rate. However, the germination rate tends to decrease when the soaking time extended to 8 h and sprouting time is extended to 36 h. Further study is needed to investigate the germination rate of 8 h soaking and 36 h germination.

Table 2 Effects of different soaking and germination times on the moisture content and the germination rate.

Soaking time (h)	Moisture content (%)	Germination time (h)		
		12	24	36
White glutinous rice (N97)				
2	18.25^{c}±0.35	78.21^{cC}±1.02	85.31^{cB}±1.04	89.15^{cA}±1.06
4	31.45^{b}±0.91	82.56^{bC}±1.01	91.91^{bB}±1.09	94.76^{bA}±1.03
6	39.98^{a}±0.98	89.15^{aB}±1.04	97.91^{aA}±1.04	97.87^{aA}±1.06
8	41.12^{a}±1.01	89.21^{aB}±1.03	97.78^{aA}±1.05	97.04^{aA}±1.05
Black glutinous rice (DH6)				
2	17.54^{c}±1.05	78.91^{cC}±1.20	84.57^{cB}±1.11	87.87^{cA}±1.10
4	30.13^{b}±1.10	83.45^{bB}±1.19	92.14^{bA}±1.12	93.59^{bA}±1.09
6	38.94^{a}±1.10	85.67^{aB}±1.08	95.15^{aA}±1.09	95.03^{aA}±1.08
8	39.14^{a}±1.09	86.12^{aB}±1.11	95.11^{aA}±1.05	95.01^{aA}±1.10

Means with different superscript letters (upper case in rows and lower case in columns) indicate statistically a significant difference at $p<0.05$. The values are mean of three replications ±SD. g, respectively. Means with different superscript letters (upper case in rows and lower case in columns) indicate statistically a significant difference at $p<0.05$. The values are mean of three replications ± SD.

Table 3 Effects of different soaking and germination times on the lipid, protein, and carbohydrate of white glutinous rice (N97).

	Content (mg/100 g, DW) at germination time of			
Soaking time (h)	0	12	24	36
Crude protein content				
2	4.90^{aB} ± 0.08	5.12^{abAB} ±0.10	5.45^{abA}±0.09	4.22^{aC} ± 0.12
4	5.04^{aC} ± 0.11	5.35^{aB} ± 0.11	5.77^{aA} ± 0.10	4.18^{aD} ± 0.11
6	5.04^{aC} ± 0.09	5.38^{aB} ± 0.12	5.83^{aA} ± 0.09	4.12^{aD} ± 0.10
8	5.05^{aC} ± 0.15	5.40^{aB} ± 0.13	5.78^{aA} ± 0.09	4.15^{aD} ± 0.09
Lipid content				
2	2.49^{abB}± 0.10	2.51^{bB} ± 0.05	2.74^{bA} ± 0.06	2.00^{abC}±0.08
4	2.54^{aB} ± 0.08	2.68^{abB} ± 0.03	3.09^{aA} ± 0.06	2.08^{aC} ± 0.08
6	2.58^{aC} ± 0.09	2.79^{aB} ± 0.02	3.09^{aA} ± 0.07	2.12^{aD} ± 0.09
8	2.58^{aC} ± 0.10	2.79^{aB} ± 0.03	3.09^{aA} ± 0.07	2.11^{aD} ± 0.09
Carbohydrate content				
2	78.95^{aA} ±1.23	79.91^{aA} ± 1.21	78.57^{aA} ± 1.21	75.07^{aB} ± 1.23
4	79.05^{aA} ±1.16	79.45^{aA} ± 1.32	78.95^{aA} ± 1.32	74.76^{aB} ± 1.32
6	79.21^{aA} ±1.21	78.97^{aA} ± 1.45	79.21^{aA} ± 1.45	74.01^{aB} ± 1.34
8	79.18^{aA} ±1.34	79.12^{aA} ± 1.34	78.96^{aA} ± 1.45	74.01^{aB} ± 1.23

The total protein, lipid, and carbohydrate contents in non-germinated white glutinous cultivar were 4.90 ± 0.1, 2.48 ± 0.6, and 78.80 ± 0.5 g/100 g, respectively. Means with different superscript letters (upper case in rows and lower case in columns) indicate statistically a significant difference at $p<0.05$. The values are mean of three replications ± SD.

Results in Tables 3-4 showed that germination caused significant changes in crude protein, carbohydrate, and lipid content, whereas soaking treatment did not affect to those components. The crude protein and lipid content of white and black glutinous rice (N97 and DH6) reached maximum values (5.83 g/100 g and 3.09 g/100 g; and 7.82 g/100g and 3.91 g/100 g, respectively) at 24-h germination. After 36 h, these components were about 20-27% and 23.00-30.42% lower than that of 24 h. Meanwhile, there was no significant difference in the levels of carbohydrate when germination occurred from 12 h to 24 h. The highest carbohydrate content of rice sprouts (N97 and DH6) was 79.21 g/100 g and 78.01 g/100 g, respectively found at soaking time of 6 h and germination time of 24 h. As the germination time increased to 36 h, the carbohydrate contents of spruots (N97 and DH6) decreased to 74.01 g/100 g and 75.14 g/100 g. The reduction of the biochemical compounds could be explained by the activated hydrolytic enzymes, which decomposed carbohydrate, lipid, and amino acids to produce the necessary energy for the biochemical and physicochemical modifications. As reported by [24], starch, protein, and fat transformed into a simple matter provides nutrients to nourish the embryo for germ grows and sprouts. Those authors also reported that when soaking and sprouting the seeds for long time, the nutritional value of germinated rice was reduced. Hence, the 6 to 8 h soaking and 24 h sprouting resulted in a high germination rate and improved nutrition values.

Table 4 Effects of different soaking and germination times on the lipid, protein, and carbohydrate of black glutinous rice (DH6).

Soaking time (h)	Content (g/100 g, DW) at germination time of			
	0	12	24	36
Crude protein content				
2	$6.90^{aB} \pm 0.10$	$6.96^{bB} \pm 0.11$	$7.33^{bA} \pm 0.10$	$6.11^{cC} \pm 0.11$
4	$6.91^{aB} \pm 0.13$	$7.73^{aA} \pm 0.12$	$7.81^{aA} \pm 0.09$	$6.13^{cC} \pm 0.09$
6	$6.78^{abB} \pm 0.13$	$7.71^{aA} \pm 0.12$	$7.82^{aA} \pm 0.11$	$6.30^{bC} \pm 0.10$
8	$6.76^{abB} \pm 0.14$	$7.69^{aA} \pm 0.15$	$7.62^{aA} \pm 0.13$	$6.55^{aB} \pm 0.09$
Lipid content				
2	$2.11^{aC} \pm 0.08$	$2.91^{aB} \pm 0.09$	$3.17^{cA} \pm 0.10$	$2.55^{bC} \pm 0.08$
4	$2.12^{aC} \pm 0.08$	$3.01^{aB} \pm 0.07$	$3.79^{aA} \pm 0.12$	$2.99^{aB} \pm 0.09$
6	$2.11^{aD} \pm 0.09$	$3.01^{aB} \pm 0.10$	$3.91^{aA} \pm 0.10$	$2.98^{aB} \pm 0.09$
8	$2.19^{aC} \pm 0.09$	$3.02^{aB} \pm 0.11$	$3.87^{aA} \pm 0.08$	$2.05^{cC} \pm 0.08$
Carbohydrate content				
2	$77.95^{aA} \pm 1.27$	$77.15^{aA} \pm 1.29$	$77.98^{aA} \pm 1.30$	$75.54^{aB} \pm 1.31$
4	$77.91^{aA} \pm 1.29$	$77.91^{aA} \pm 1.26$	$77.99^{aA} \pm 1.25$	$75.19^{aB} \pm 1.23$
6	$77.79^{aA} \pm 1.30$	$77.01^{aA} \pm 1.27$	$78.01^{aA} \pm 1.27$	$75.21^{aB} \pm 1.30$
8	$77.92^{aA} \pm 1.26$	$77.05^{aA} \pm 1.29$	$77.91^{aA} \pm 1.34$	$75.14^{aB} \pm 1.28$

The total protein, lipid, and carbohydrate contents in non-germinated black glutinous cultivar were 6.90 ± 0.2, 2.10 ± 0.2, and 77.90± 1.1 g/100 g, respectively. Means with different superscript letters (upper case in rows and lower case in columns) indicate statistically a significant difference at $p<0.05$. The values are mean of three replications ± SD.

3.3 Bioactive components in germinated rice

GABA, gamma-oryzanol, and tocopherol are bioactive compounds, which has a positive effect on human health. Some previous studies showed that the GABA, gamma-oryzanol, and tocopherols accumulated when rice was soaked and germinated [9,11]. In this study, after soaking and incubation, the GABA, gamma-oryzanol, and tocopherol contents in the germinated rice were generally higher than in non-germinated rice. The changes of the GABA content of soaked white and black glutinous rice (N97 and DH6) ranged from 36.11 to 37.72 and 37.15 to 38.56 mg/100 g, respectively. Meanwhile, the GABA content in germinated white and black glutinous rice (N97 and DH6) increased considerably with increasing of the germination time (12 to 24 h) (Tables 5-6). The increase in GABA content could be attributed by the activation of glutamate decarboxylase (GAD) during germination. This converts glutamate to GABA thus enhances the GABA content in the germinated rice. The obtained results agreed with [23], who documented that the extended soaking and sprouting times enhanced the GABA content of germinated rice by activating GAD. However, the GABA content of two rice cultivars (N97 and DH6) in this study decreased to 36.50 and 37.01 mg/100 g after germinated for 36 h.

Table 5 Effects of different soaking and germination times on the concentrations of GABA and gamma-oryzanol in white glutinous rice (N97).

Soaking time (h)	Content (mg/100 g, DW) at germination time of			
	0	12	24	36
Gamma-oryzanol				
2	175.41^{cD} ± 2.16	412.21^{cC}±1.96	514.12^{cA}±2.25	498.16^{cB}±2.04
4	212.35^{bD} ± 2.07	495.14^{bC}±1.95	598.76^{bA}±2.02	562.45^{bB}±2.11
6	221.24^{bC} ± 2.15	556.56^{aB}±2.05	617.01^{aA}±2.03	569.56^{aB}±2.05
8	230.32^{aD} ± 2.05	559.45^{aC}±2.04	615.67^{aA}±2.09	562.56^{bB}±2.15
GABA				
2	36.11^{abB} ±1.21	37.92^{aB}±1.23	39.43^{aA}±1.23	36.84^{aB}±1.28
4	37.72^{aB} ± 1.23	37.80^{aB}±1.21	39.45^{aA}±1.25	36.51^{aB}±1.29
6	37.09^{aB} ± 1.21	37.11^{aB}±1.21	39.97^{aA}±1.29	36.50^{aB}±1.33
8	37.01^{aB} ± 1.21	37.15^{aB}±1.23	39.43^{aA}±1.29	36.50^{aB}±1.23

The total gamma-oryzanol and GABA contents in non-germinated white glutinous rice were 174.39± 2.56 mg/100 g and 36.09 ± 1.01 mg/100 g, respectively. Means with different superscript letters (upper case in rows and lower case in columns) indicate statistically a significant difference at $p<0.05$. The values are mean of three replications ±SD.

Gamma-oryzanol is the major active compound of rice that can be found in rice bran [16]. Gamma-oryzanol has the most potential as a nutraceutical and pharmaceutical [25]. The gamma-oryzanol content in the germinated white and black glutinous rice samples (N97 and DH06) obtained by various soaking and sprouting times is shown in Tables 5-6. As presented in Tables 5-6, the gamma-oryzanol content of soaked white and black glutinous rice at 8 h slightly increased to 230.32 mg/100 g and 232.31 mg/100 g, compared with the nonsoaked rice. Meanwhile, the gamma-oryzanol content significantly increased after germination from 12-36 h. The gamma-oryzanol of the sprouts achieved maximum values at 24-h incubation for germination. A similar observation was reported for germinated rough and brown rice [11]. However, a reverse trend was observed for gamma-oryzanol content in germinated Sangyod Muang Phatthalung rice [26]. Those author reported that gamma-oryzanol content was the same level for both un-germinated and germinated brown rice. Until now, the mechanism of synthesis of gamma-oryzanol in germinated rice is still uncovered. According to our results, the gamma-oryzanol contains four main components including cycloartenyl ferulate (retention time RT=6.240-6.243), 24- methylene cycloartenyl ferulate (retention time RT=7.065-7.265), campesteryl ferulate (retention time RT=7.760-7.765), and β-sitosteryl ferulate (retention time RT=9.006-9.056) (Figure 1). The chemical composition of the gamma-oryzanol standard and two rice cultivars were similar, consistent with [17].

Table 6 Effects of different soaking and germination times on the concentrations of GABA and gamma-oryzanol in black glutinous rice (DH6).

Soaking time (h)	Content (mg/100 g, DW) at germination time of			
	0	12	24	36
Gamma-oryzanol				
2	202.21^{bD} ± 1.93	401.26^{dC}±1.94	665.30^{cA}±2.14	572.01^{bB}±1.64
4	219.13^{aD} ± 2.06	519.56^{cC}±2.11	719.42^{bA}±1.20	638.09^{abB}±1.85
6	222.34^{aD} ± 2.04	581.34^{aC}±1.43	798.49^{aA}±1.77	651.28^{aB}±1.76
8	232.31^{aD} ± 2.15	556.21^{bC}±1.75	798.42^{aA}±1.95	650.39^{aB}±1.56
GABA				
2	37.15^{aB} ± 1.21	39.95^{aA}±1.25	41.09^{aA}±1.25	37.05^{aB}±1.29
4	38.44^{aB} ± 1.22	40.05^{aA}±1.21	41.29^{aA}±1.30	37.59^{aC}±1.31
6	38.56^{aB} ± 1.23	40.03^{aA}±1.30	41.98^{aA}±1.26	37.91^{aB}±1.30
8	38.55^{aAB}±1.31	40.11^{aA}±1.25	41.20^{aA}±1.29	37.01^{aB}±1.30

The total gamma-oryzanol and GABA contents in non-germinated black glutinous rice were 202.12±2.98 mg/100 g and 37.12±1.02 mg/100 g, respectively. Means with different superscript letters (upper case in rows and lower case in columns) indicate statistically a significant difference at $p<0.05$. The values are mean of three replications ± SD.

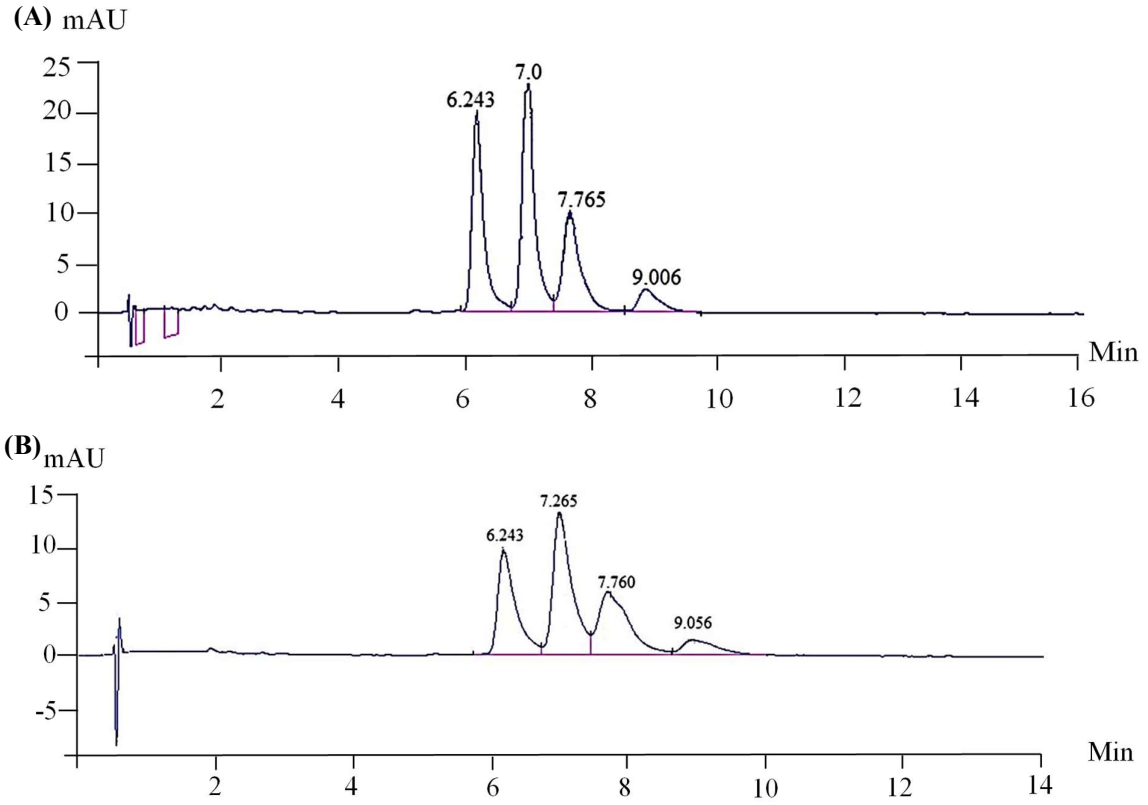

Figure 1 HPLC chromatograms of standard gamma-oryzanol (A) and germinated rice gamma-oryzanol (B): denotes (1) cycloartenylferulate, (2) 24-methylenecy cloartanyl ferulate, (3) campesterylferulate, and (4) β- sitosteryl ferulate.

In Table 7, it can be seen that the major isomers of tocopherol in all the germinated rice extracts was an α-tocopherol, followed by γ-, β-, and δ-tocopherols. The contents of tocopherol isomers in germinated rice were higher than non-germinated rice. These compounds changed depending on the soaking time and the sprouting time. The soaking and germination at a shorter period (2-4 h and 12 h, respectively) were found to produce lower β-tocopherol and δ-tocopherol than longer soaking and germination time (6-8 h and 24-36 h, respectively). Specifically, an increase in tocopherol isomers was noted in the germinated rice at a germination time of 24 and 36 h; it reached the optimal level at a germination time of 24 h. Similar results were reported for rough rice and brown rice [9,27]. In 2014, Ayernor and Ocloo reported that germination promoted the development of hydrolytic enzymes that are inactive in raw seeds, thus enhancing the biochemical changes [28].

3.4 Contents of thiamine, niacin, and riboflavin

The thiamin, niacin, and riboflavin are classified as the water-soluble B vitamins, which are coenzymes factors involving in many metabolic pathways [29]. The contents of thiamin, niacin, and riboflavin of un-germinated and germinated rice were determined by using HPLC and are listed in Tables 8-9. The thiamine, niacin, and riboflavin level in the un-germinated rice was near to those reported by [30], who found that the content of thiamine, niacin and riboflavin of rice was 0.50, 7.32, and 0.071 mg/100 g, respectively. Our results showed that when soaked rice from 2 to 8 h, the levels of thiamin, niacin, and riboflavin significantly reduced and lower than that of un-soaked rice (Tables 8-9). This could be attributed to the longer soaking time led to a leaching out of the water soluble vitamins, and caused the reduction of B vitamins in soaked rice.

In Tables 8-9, it is shown that the contents of three vitamins were significantly increased with increasing germination time from 12 to 24 h. However, the prolonged germination time over 36 h led to the reduction of thiamine and niacin. The reduction was approximately 10-21% for thiamin and 11-15% for niacin compared with the germinated two rice cultivars at 24 h. Meanwhile, the riboflavin contents still remained stable after 36 h. Its finding is similar with [11], who reported that the thiamin and niacin content in germinated rice were significantly reduced with increasing germination time. However, there is not much information in the literature about the effect of germination on the riboflavin contents in glutinous rice.

Table 7 Effects of different soaking and germination times on the concentrations of α-, β-, γ-, δ-tocopherol in white and black glutinous rice (N97 and DH6).

Soaking time (h)	Rice variety	Content of tocopherol (mg/100 g, DW) at germination time of							
		0	12	24	36	0	12	24	36
		α				β			
2	White	1.03^{aC}±0.01	1.21^{bB}±0.03	1.33^{bA}±0.06	1.30^{bA}±0.05	0.14^{aC}±0.01	0.21^{bB}±0.01	0.99^{bA}±0.01	1.04^{cA}±0.03
4	glutinous	1.04^{aC}±0.01	1.34^{aB}±0.03	1.45^{aA}±0.05	1.45^{aA}±0.01	0.13^{aC}±0.02	0.21^{bB}±0.01	1.51^{aA}±0.01	1.53^{bA}±0.05
6	rice (N97)	1.05^{aC}±0.00	1.34^{aB}±0.04	1.45^{aA}±0.02	1.46^{aA}±0.02	0.13^{aC}±0.01	0.25^{aB}±0.01	1.59^{aA}±0.02	1.58^{aA}±0.05
8		1.05^{aB}±0.01	1.36^{aAB}±0.02	1.46^{aA}±0.05	1.45^{aA}±0.04	0.14^{aC}±0.02	0.25^{aB}±0.01	1.57^{aA}±0.02	1.57^{aA}±0.05
2	Black	0.94^{aC}±0.08	1.31^{aB}±0.03	1.41^{abA}±0.02	1.49^{abA}±0.02	0.36^{aB}±0.01	0.38^{bB}±0.02	1.03^{bA}±0.06	1.03^{bA}±0.09
4	glutinous	0.94^{aB}±0.07	1.36^{aB}±0.03	1.54^{aA}±0.04	1.55^{aA}±0.01	0.37^{aC}±0.01	0.48^{aB}±0.04	1.35^{aA}±0.09	1.33^{aA}±0.04
6	rice	0.95^{aC}±0.07	1.36^{aB}±0.02	1.58^{aA}±0.06	1.55^{aA}±0.03	0.37^{aC}±0.01	0.50^{aB}±0.04	1.35^{aA}±0.07	1.35^{aA}±0.05
8	(DH6)	0.95^{aC}±0.05	1.36^{aB}±0.04	1.57^{aA}±0.04	1.58^{aA}±0.02	0.36^{aC}±0.01	0.53^{aB}±0.05	1.32^{aA}±0.05	1.34^{aA}±0.08
		Content of γ- and δ-tocopherol (mg/100 g, DW) at germination time of							
		γ				δ			
2	White	0.52^{aC}±0.01	0.84^{cB}±0.04	0.91^{cB}±0.06	1.33^{bA}±0.05	0.15^{aB}±0.01	0.20^{bA}±0.01	0.21^{abA}±0.01	0.23^{aA}±0.01
4	glutinous	0.52^{aC}±0.01	1.12^{bB}±0.05	1.39^{bA}±0.05	1.36^{aA}±0.06	0.16^{aB}±0.02	0.22^{abA}±0.02	0.21^{abA}±0.02	0.22^{aA}±0.01
6	rice (N97)	0.51^{aD}±0.02	1.23^{aC}±0.05	1.45^{aA}±0.07	1.35^{aB}±0.07	0.15^{aB}±0.00	0.25^{aA}±0.01	0.24^{aA}±0.02	0.22^{aA}±0.02
8		0.52^{aD}±0.03	1.25^{aC}±0.04	1.45^{aA}±0.06	1.36^{aB}±0.06	0.15^{aB}±0.01	0.25^{aA}±0.01	0.25^{aA}±0.01	0.22^{aA}±0.01
2	Black	0.56^{aB}±0.05	1.50^{bA}±0.03	1.50^{aA}±0.02	1.48^{bA}±0.05	0.25^{cC}±0.02	0.29^{cB}±0.02	0.28^{bB}±0.02	0.39^{bA}±0.01
4	glutinous	0.57^{aB}±0.04	1.51^{abA}±0.02	1.51^{aA}±0.03	1.50^{aA}±0.04	0.27^{bC}±0.02	0.35^{bB}±0.01	0.34^{aB}±0.02	0.41^{aA}±0.01
6	rice	0.57^{aC}±0.04	1.56^{aA}±0.02	1.50^{aB}±0.02	1.49^{aB}±0.04	0.31^{aB}±0.01	0.38^{aA}±0.02	0.38^{aA}±0.01	0.40^{aA}±0.02
8	(DH6)	0.56^{aC}±0.06	1.54^{aA}±0.01	1.52^{aA}±0.02	1.49^{aB}±0.04	0.32^{aB}±0.02	0.39^{aA}±0.01	0.38^{aA}±0.02	0.39^{bA}±0.01

The α, β, γ, δ-tocopherol contents in non-germinated white and black glutinous rice cultivar were 1.03 mg/kg, 0.14 mg/kg, 0.52 mg/kg and 0.15 mg/kg; 0.94 mg/kg, 0.36 mg/kg, 0.56 mg/kg, and 0.25 mg/kg, respectively. Values are mean ±standard error. Means with different superscript letters (upper case in rows and lower case in columns) indicate statistically a significant difference at $p<0.05$.

Table 8 Effects of different soaking and germination times on the concentrations of thiamin, niacin, and riboflavin in white glutinous rice (N97).

Soaking time (h)	Content (mg/100 g) at germination time of			
	0	12	24	36
Thiamin				
2	0.21^{aB}±0.01	0.25^{bA}±0.02	0.26^{bA}±0.01	0.25^{aA}±0.02
4	0.19^{bC}±0.02	0.28^{aB}±0.02	0.31^{aA}±0.01	0.26^{aB}±0.01
6	0.18^{bD}±0.01	0.29^{aB}±0.01	0.32^{aA}±0.02	0.25^{aC}±0.01
8	0.11^{cD}±0.01	0.28^{aB}±0.01	0.32^{aA}±0.01	0.25^{aC}±0.01
Niacin				
2	7.09^{aB}±1.11	7.55^{aAB}±0.91	7.91^{abA}±0.99	7.14^{aB}±1.00
4	6.51^{bD}±1.05	7.67^{aB}±1.08	8.16^{aA}±1.01	7.15^{aC}±1.01
6	6.45^{bD}±1.09	7.73^{aB}±1.09	8.13^{aA}±1.01	7.15^{aC}±1.01
8	6.15^{cC}±1.07	7.48^{bB}±1.10	8.01^{abA}±1.00	7.11^{aB}±1.00
Riboflavin				
2	0.071^{aC}±0.001	0.075^{cB}±0.001	0.076^{bB}±0.002	0.079^{bA}±0.001
4	0.063^{bC}±0.001	0.077^{cB}±0.002	0.076^{bB}±0.001	0.080^{bA}±0.001
6	0.062^{bC}±0.001	0.082^{aB}±0.001	0.087^{aA}±0.001	0.090^{aA}±0.002
8	0.051^{aC}±0.001	0.079^{bB}±0.002	0.086^{aA}±0.001	0.089^{aA}±0.001

The thiamin, riboflavine, and niacin contents in non-germinated white glutinous rice cultivar were 0.21, 7.09, and 0.071 mg/kg, respectively. Means with different superscript letters (upper case in rows and lower case in columns) indicate statistically a significant difference at $p<0.05$. The values are mean of three replications ±SD.

Table 9 Effects of different soaking and germination times on the concentrations of thiamin, niacin, and riboflavin in black glutinous rice (DH6).

Soaking time (h)	Content (mg/100 g) at germination time of			
	0	12	24	36
Thiamin				
2	0.25^{aB}±0.01	0.27^{bA}±0.01	0.28^{bA}±0.02	0.25^{bB}±0.02
4	0.16^{bC}±0.02	0.27^{bB}±0.01	0.31^{aA}±0.01	0.28^{aB}±0.01
6	0.15^{bC}±0.02	0.30^{aB}±0.02	0.33^{aA}±0.01	0.29^{aB}±0.01
8	0.09^{cD}±0.01	0.26^{bC}±0.02	0.31^{aA}±0.01	0.28^{aB}±0.02
Niacin				
2	7.15^{aC}±0.99	7.50^{aB}±0.94	7.91^{bA}±0.97	6.82^{aD}±0.99
4	6.56^{bC}±1.00	7.69^{aB}±0.99	8.26^{aA}±1.10	6.98^{aC}±1.02
6	6.50^{bC}±1.01	7.74^{aB}±1.01	8.23^{aA}±0.97	6.97^{aC}±1.00
8	6.10^{cD}±1.00	7.41^{bB}±1.01	8.20^{aA}±1.02	6.89^{aC}±1.01
Riboflavin				
2	0.087^{aC}±0.001	0.14^{bB}±0.002	0.16^{bA}±0.003	0.17^{bA}±0.002
4	0.083^{bC}±0.001	0.17^{aB}±0.003	0.19^{aA}±0.003	0.20^{aA}±0.003
6	0.082^{bC}±0.001	0.16^{aB}±0.003	0.20^{aA}±0.003	0.21^{aA}±0.003
8	0.080^{cC}±0.001	0.17^{aB}±0.003	0.20^{aA}±0.002	0.21^{aA}±0.003

The thiamin, riboflavine, and niacin contents in non-germinated black glutinous rice cultivar were 0.25, 7.15, and 0.087 mg/kg, respectively. Means with different superscript letters (upper case in rows and lower case in columns) indicate statistically a significant difference at $p<0.05$. The values are mean of three replications ±SD.

4. Conclusion

This study investigates the effects of different soaking and germination conditions on the germination rates of rice and its quality. The soaking and germination times were varied between 2.0, 4.0, 6.0, and 8.0 h; and 12, 24, and 36 h, respectively. The germination rates, biochemical and bioactive compounds of the germinated white and black glutinous rice were determined and compared. The results showed that the optimal conditions for the soaking time and germination time were found at 6 h and 24 h. At this condition, the germination rate, the biochemical and phytochemical contents were significantly improved. The results also showed that longer soaking time to 8.0 h and prolonger germination time to 36 h resulted in the low germination percentage and degradation of biochemical content (i.e., protein, lipid, carbohydrate, GABA, tocopherol, gamma-oryzanol, and B vitamins). Therefore, 6 h of soaking and 24 h of sprouting was suitable parameters for enhancing the germination rates and biochemical as well as phytochemical contents in germinated glutinous rice.

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6. Conflict of interests

Authors declare that they have no conflict of interest.

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