JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Changes of Tocopherols, Tocotrienols, γ-Oryzanol, and γ-Aminobutyric Acid Levels in the Germinated Brown Rice of Pigmented and Nonpigmented Cultivars

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ABSTRACT: This study examined the changes of tocopherols (Toc), tocotrienols (T3), γ-oryzanol (GO), and γ-aminobutyric acid (GABA) contents in germinated brown rice (GBR) of pigmented and nonpigmented cultivars under different germination conditions. Results showed that the Toc and T3 contents in GBR were significantly different between treatments in both rice cultivars. The pigmented GBR possessed higher total vitamin E, total Toc, total T3, and GO contents than the nonpigmented GBR; however, its level of GABA was lower. The order of the three highest vitamin E homologues in pigmented and nonpigmented GBR was γ-T3 > γ-Toc > α-Toc and α-Toc > γ-T3 > α-T3, respectively; β-Toc, β-T3, δ-Toc, and δ-T3 were present in only small amounts (≤1.0 mg/kg) in GBR of both cultivars. Although both cultivars showed an increase in GABA contents with increasing germination time, the GABA content in nonpigmented GBR was higher.

KEYWORDS: *germinated brown rice, tocopherols, tocotrienols, γ-oryzanol, γ-aminobutyric acid*

INTRODUCTION

Rice (*Oryza sativa* L.) is the main staple food in Asia and accounts for about 50% of the total global grain production.[1] Brown rice is known to contain a complex mixture of biologically active phytochemicals such as tocopherols (Toc), tocotrienols (T3), γ-oryzanol (GO), and γ-aminobutyric acid (GABA), which together with other bioactive phytochemicals are believed to exert important roles in protection against various degenerative diseases.[2,3] Among the many bioactive phytochemicals in brown rice, GO is rarely found in common crops and vegetables,[4,5] and tocotrienols are present only in monocot species such as *O. sativa*,[6] whereas GABA is present in most plant tissues, especially in germinated seeds.[7] Although brown rice possesses numerous health benefits, it is not considered to be a suitable table rice due to its poor texture, low digestibility, and not-easy-to-cook characteristic.[8]

Germination is known to be an effective strategy to improve cereal quality through softening the kernel structure; it also enhances the contents of nutrient compounds in cereal seeds. Recently, consumption of germinated brown rice (GBR) has become increasingly popular in Asia. It is achieved by soaking the brown rice in water to induce slight germination. During the process of germination, the activated biochemical activities break down the large molecular substances, such as starch, nonstarch polysaccharide, and proteins, to small molecular compounds; this process can also generate bioactive components such as ascorbic acid, Toc, T3, GABA, and phenolic compounds.[9] Germination can also improve the texture of brown rice and enables the nutrients of the grains to be more easily digested and absorbed.[10,11]

Both Toc and T3 belong to the family of vitamin E (also known as tocochromanols or tocols), with each of them comprising four homologues (α, β, γ, and δ). Traditionally, α-Toc is believed to possess the highest biological activity;[12] however, recent studies showed that other vitamin E homologues possessed similar or better antioxidant, anticancer, anti-inflammatory, immunomodulatory, neuroprotective, and hepatoprotective activities than α-Toc.[2,3] GO possesses antioxidant, anti-inflammatory, antitumor, antidiabetes, and cholesterol-lowering properties,[13,14] whereas GABA is beneficial for antistress, antianxiety, lowering blood pressure, and inhibiting colon carcinoma cell migration and leukemia cell proliferation activities.[15,16] Because of the diverse therapeutic benefits, these compounds have received increasing interest from the scientific community and consumers.

Although numerous studies have been reported on the nutritional properties and health benefits of GBR, its Toc, T3, and GO contents and the presence of these bioactive phytochemicals in pigmented GBR have never been investigated. Therefore, this study aimed to examine the effects of germination conditions on the contents of Toc, T3, GO, and GABA in GBR of pigmented and nonpigmented cultivars.

MATERIALS AND METHODS

Materials. Standards of tocopherols (α-, β-, γ-, and δ-Toc of purity > 98%), γ-oryzanol (GO; purity > 98%), γ-aminobutyric acid (GABA; purity > 98%), and GABase were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Tocotrienols (α-, β-, γ-, and δ-T3 of purity > 98%) were obtained from Davos Life Science Pte. Ltd. (Helios, Singapore). All other chemicals used were of analytical grade.

Rice Samples. The rice samples Tainung 71 (nonpigmented) and black glutinous (pigmented) rice were obtained from De Chang Rice Factory from Tainan (Taiwan) and Taitung Farmers' Association (Taitung, Taiwan), respectively, at the same time period of the year. They were chosen to represent the pigmented and nonpigmented commercially available rice varieties in Taiwan. The reasons for selecting these rice varieties were that they are easily accessible by consumers and are commonly available in the local supermarkets and

Received: August 20, 2013
Revised: December 5, 2013
Accepted: December 6, 2013
Published: December 6, 2013

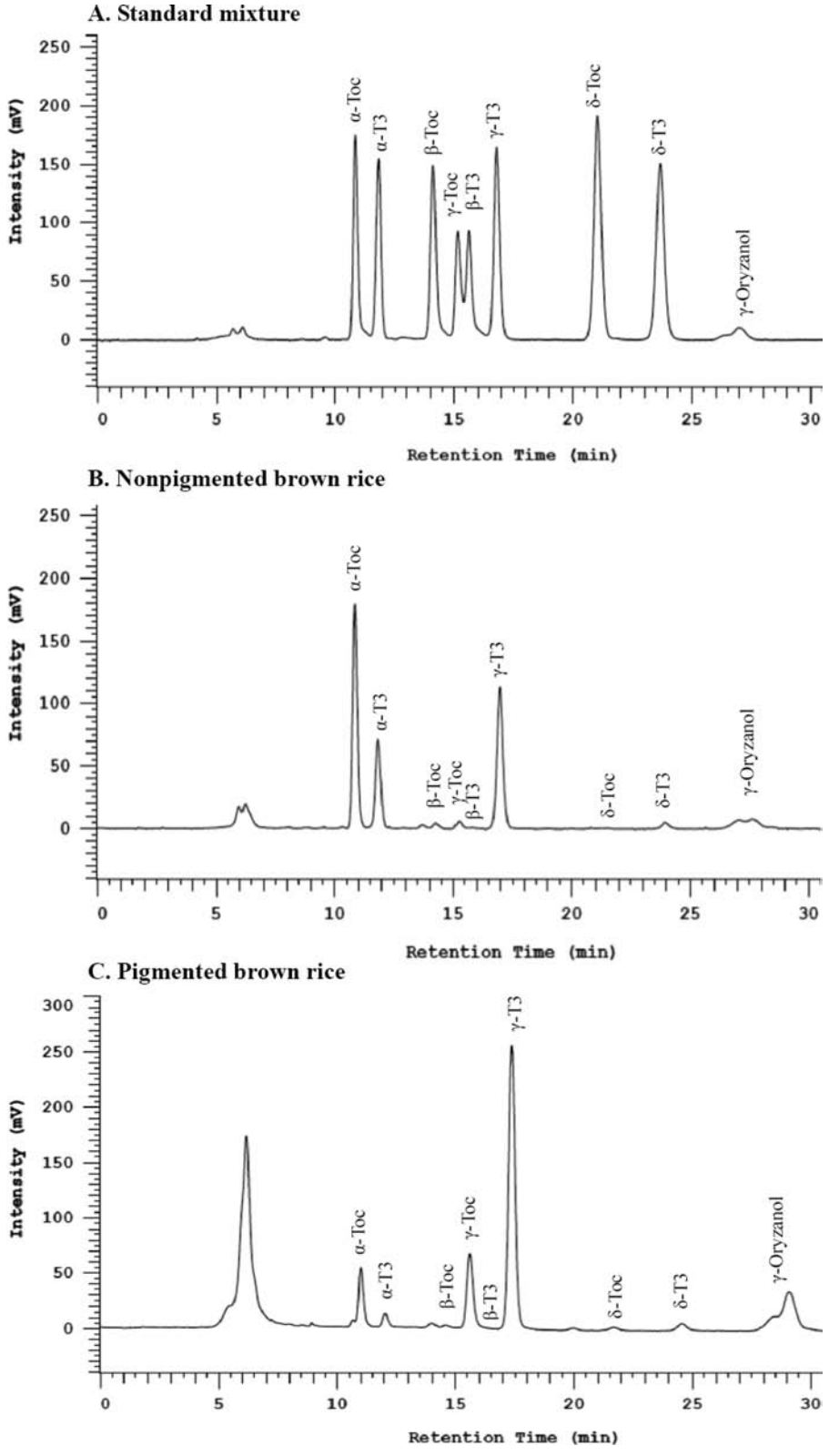

Figure 1. Chromatograms of a standard mixture (A) and nonpigmented (B) and pigmented brown rice (C). *α*-Toc, *α*-tocopherol; *β*-Toc, *β*-tocopherol; *γ*-Toc, *γ*-tocopherol; *δ*-Toc, *δ*-tocopherol; *α*-T3, *α*-tocotrienol; *β*-T3, *β*-tocotrienol; *γ*-T3, *γ*-tocotrienol; *δ*-T3, *δ*-tocotrienol.

stores. All experiments were conducted as completely randomized designs.

Determination of Percentage of Germination Rate. The germination rate of brown rice grains was determined according to the method described by the International Rules for Seed Testing.[17] In brief, the rice grains were considered as a germinated seed when the young radicle or primary root (white root emerging from the lower end of the rice seed) was visible. The percentage of germination rate was determined after the number of germinated grains had been obtained.

Determination of the Optimum Soaking Temperature. According to procedures described by Thakur and Gupta,[18] 10 g of

brown rice grains was washed with distilled water to remove dust particles, followed by soaking with 40 mL of distilled water at 25, 30, 35, and 40 °C for 24 h. The rice grains were then placed on wet filter paper (100% relative humidity) in a 9 cm covered Petri dish and left to germinate at 30 °C for 24 h, followed by examination of the germination rate to obtain the optimum soaking temperature.

Determination of Water Absorption of Brown Rice Grains during Soaking. The water absorption of brown rice grains was analyzed according to procedures described by Thakur and Gupta[18] with minor modifications. In brief, 10 g of brown rice grains was washed twice with distilled water to remove dust particles, followed by placing into a 50 mL plastic container and then soaking with 40 mL of distilled water; the container was left in a water bath at the optimum soaking temperature. The soaking time was 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or 5 h. After each soaking time, 5 g of the soaked rice grains was taken and weighed (after surface water was removed). The samples were dried in an oven at 105 °C until the sample weight was constant. The moisture content was calculated from the initial and final sample weights. The saturated water absorption time was determined by plotting a graph between moisture content and soaking time. The percentage of water absorption was calculated as [weight of rice after soaking at time t − weight of rice after soaking at time $(t - 0.5)$]/weight of rice without soaking × 100%.

Determination of the Optimum Germination Conditions. To investigate the effect of germination temperature and time on Toc, T3, GO, and GABA contents in GBR, different germination times (8, 12, 14, 16, and 20 h) at various temperatures (30, 35, and 40 °C) were tested. For each treatment, 5 g of brown rice grains was taken and washed with distilled water; they were then soaked in 20 mL of distilled water in a 50 mL plastic cylinder box, followed by placing in a water bath at 30 °C for 3 h for nonpigmented rice and at 25 °C for 4 h for pigmented rice. After soaking, water was drained by placing the grains in a cylinder sieve. The grains and sieve were then placed within a wet filter paper in a 9 cm covered Petri dish and kept in an incubator at 30, 35, or 40 °C to complete the germination process time of each treatment. The germinated grains were dried with lyophilization (freeze-drying) systems. The dried samples were collected in an airtight plastic bag and stored at −20 °C until analysis.

Preparation of Rice Sample Extract. Germinated brown rice of each treatment was ground to powdered form and then subjected to hexane extraction for 15 min at 60 °C in an electrical shaker at room temperature.[19] The extract was filtered through a 0.45 μm membrane filter, followed by collection of the filtrate and storage at −20 °C until analysis.

Analysis of Tocopherols, Tocotrienols, and γ-Oryzanol. The analysis was carried out according to a previously described method.[19] Briefly, the HPLC system consisted of a Hitachi L-2130 pump and a Hitachi L-2485 fluorescence detector (Hitachi, Japan) set at an excitation wavelength of 290 nm and an emission wavelength of 330 nm. Chromatographic separation was performed by a normal phase Inertsil SIL 100A (5 μm, 4.6 × 250 mm) column coupled with an Inertsil SIL 100A (5 μm, 4 × 10 mm) guard column (GL Sciences Inc., Tokyo, Japan) and a mobile phase composed of hexane/isopropanol/ethyl acetate/acetic acid (97.6:0.8:0.8:0.8, v/v/v/v) operating at room temperature. The flow rate of isocratic elution varied from 0.7 to 1.5 mL/min.

A typical chromatogram of eight vitamin E homologues (α-, β-, γ-, and δ-Toc and α-, β-, γ-, and δ-T3) and GO in a standard mixture solution and GBR samples is shown in Figure 1. All compounds were confirmed by chromatographic comparisons with their respective authentic standards. Toc, T3, and GO in rice samples were identified by retention time and quantified by the calibration curve of external standards. The calibration curves were linear in the range studied, showing correlation coefficients (r^2) ≥0.99.

Analysis of γ-Aminobutyric Acid. GABA concentration was determined according to the method described by Zhang and Bown[20] with minor modifications. Rice samples were ground in liquid nitrogen to a fine powder, of which 0.5 g was taken and well mixed with 5 mL of methanol by vortexing for 1 min and then centrifuged to obtain the supernatant; the same procedures were repeated twice. The supernatants collected were combined and made up to a volume of 10 mL, of which 100 μL was taken and freeze-dried, and then 100 μL of distilled water was added to dissolve the extract. The reaction was carried out with 55 μL of sample, 15 μL of 4 mM $NADP^+$, 5 μL (2 U/mL) of GABase [dissolved in 0.1 M potassium phosphate buffer (pH 7.2) containing 12.5% glycerol and 5 mM 2-mercaptoethanol], and 20 μL of 0.5 M potassium pyrophosphate buffer (pH 8.6) for 60 min, and further reaction was conducted with 5 μL of 20 mM α-ketoglutarate. The absorbance at 340 nm was monitored before and after the addition of α-ketoglutarate for 20 min at room temperature using a spectrophotometer.

Statistical Analysis. Data were presented as the mean ± standard deviation from three independent analyses. Values were evaluated by one-way ANOVA, followed by Duncan's multiple-range tests using the Statistical Analysis System (SAS Institute, Cary, NC, USA). Difference was considered significant when the P value was <0.05.

RESULTS

Germination Rate. The germination rate of nonpigmented and pigmented brown rice under different germination temperatures is shown in Table 1. Results showed that for the nonpigmented brown rice, the germination rate reached optimum after soaking at 30 °C for 3 h and declined at higher temperature, whereas the pigmented brown rice showed the optimum germination rate after soaking at 25 °C for 4 h, followed by a reduction in the percentage of germination with increasing temperature. Regardless of rice cultivar, the range between the highest and lowest germination rates at the tested temperature was about 10%.

Table 1. Germination Rates of Nonpigmented and Pigmented Brown Rice under Different Soaking Temperatures

	germination rate[a] (%)	
temp (°C)	nonpigmented	pigmented
25	87.9 ± 8.1ab	90.7 ± 2.5a
30	90.0 ± 3.7a	86.0 ± 1.7b
35	85.8 ± 4.8ab	86.3 ± 0.6b
40	75.1 ± 11.6b	77.0 ± 0.4c

[a]Values are the mean ± SD (n = 3). Means followed by different letters in the same column are significantly different.

Water Absorption of Brown Rice Grains. Results showed that the moisture content rapidly increased during the first half hour of soaking in both pigmented and nonpigmented brown rice (Figure 2), followed by a gradual decrease in the amount of water absorption up to 5 h, whereby the grains became saturated at the moisture content of 38.7%. These results indicate that after 1 h of soaking, the moisture content of either nonpigmented or pigmented brown rice has reached a moisture level sufficient for the germination of the grains. The pigmented brown rice was noted to absorb a significantly higher amount of water than the nonpigmented brown rice at 1−2.5 h.

Effects of Germination Conditions on Total Vitamin E and γ-Oryzanol Contents. Results showed that regardless of rice cultivar, total vitamin E content in GBR was higher than that in non-GBR (total vitamin E = 25.2 ± 1.1 mg/kg for nonpigmented brown rice and 29.4 ± 0.8 mg/kg for pigmented brown rice; Tables 2 and 3); however, the levels of GO in all GBR of nonpigmented but not pigmented cultivars were lower than that of the non-GBR of the same cultivar (Table 4). GO in GBR of pigmented cultivar was higher than that of non-GBR,

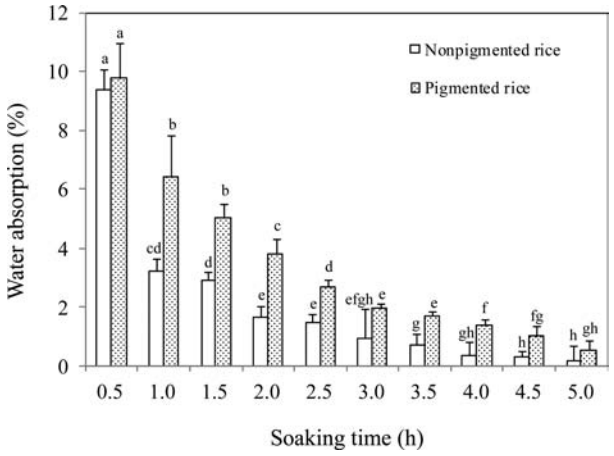

Figure 2. Percentage of water absorption of nonpigmented and pigmented brown rice at different soaking times. Values are the mean ± SD (n = 3). Different letters above the bars indicate significant difference.

with the exception at low germination temperature (30 °C) and at 8, 16, and 20 h of germination time. In the nonpigmented brown rice, the germination temperature of 40 °C for 8 h produced a significantly higher total vitamin E content than other germination conditions; however, at this temperature, further increase in germination time caused a decline in total vitamin E content. The total vitamin E content was also noted to increase at 30 °C with increasing germination time (from 8 to 20 h). By comparison of the GBR of the two cultivars, the pigmented GBR showed higher total vitamin E, total Toc, and total T3 contents than nonpigmented GBR, of which the levels were also noted to be relatively constant under the different tested germination temperatures and time periods.

In contrast to pigmented GBR, an increase in GO was noted in nonpigmented GBR at a germination temperature of 30 °C and a germination time from 8 to 20 h (Table 4); it reached the optimal level at a germination temperature of 35 °C for 8 h, followed by a decline in the concentration with increasing germination temperature. Results also showed that under similar germination conditions, the GO content in pigmented GBR was at least 3-fold higher than the nonpigmented GBR, and its changes between different germination temperatures and germination times were minimal, with the exception at germination temperature of 30 °C for 20 h, where the GO was the lowest as compared with other germination conditions.

Effects of Germination Conditions on the Contents of Tocopherol and Tocotrienol Homologues. Regardless of rice cultivar, the contents of total Toc and total T3 present in GBR were higher than that of non-GBR (for nonpigmented brown rice, the total Toc and total T3 contents were 11.4 ± 0.6 and 13.8 ± 0.5 mg/kg, respectively; for pigmented brown rice, the total Toc and total T3 contents were 11.4 ± 0.9 and 18.0 ± 0.2 mg/kg, respectively). Under the present germination conditions, the pigmented GBR appears to contain a higher T3 level than the nonpigmented GBR (Tables 2 and 3). In the nonpigmented GBR, a trend similar to that of total vitamin E content was noted in the levels of total Toc, but not the total T3, where the levels peaked at a germination time of 8 h at all tested germination temperatures (30, 35, and 40 °C), followed by a decline with increasing germination time (Table 2); optimal total T3 levels were achieved at a germination time of 12 h at 30, 35, and 40 °C. In the pigmented GBR, the changes in total Toc at all tested germination conditions were minimal (Table 3); however, an increase in total T3 was noted with increasing germination temperature and time.

The main vitamin E homologues present in GBR of nonpigmented cultivar were α-Toc (10.5−15.7 mg/kg), followed by γ-T3 (8.3−11.1 mg/kg) and α-T3 (5.0−8.3 mg/kg) (Table 5), whereas in GBR of pigmented cultivar the order of the highest three was γ-T3 (20.8−24.8 mg/kg) > γ-Toc (13.2−14.6 mg/kg) > α-Toc (4.5−6.3 mg/kg) (Table 6). Regardless of rice cultivar, β-Toc, β-T3, δ-Toc, and δ-T3 were present in only small amounts (≤1.0 mg/kg).

Compared with other germination conditions, germination at 35 °C for all time periods and at 40 °C for a shorter germination duration (8 and 12 h) were found to produce higher α-Toc, γ-T3, and α-T3 contents in the nonpigmented GBR. However, under similar germination conditions, the changes in the contents of the main vitamin E homologues (γ-T3, γ-Toc, and α-Toc) in GBR of pigmented cultivar were less pronounced.

Table 2. Effects of Different Germination Conditions on the Concentrations of Total Vitamin E, Total Tocopherols (Toc), and Total Tocotrienols (T3) in Nonpigmented Brown Rice

	concentration[a] (mg/kg, DW) at germination time of			
germination temp (°C)	8 h	12 h	16 h	20 h
		Total Vitamin E		
30	26.5 ± 2.6bB	30.2 ± 2.5bB	28.5 ± 0.8bB	35.7 ± 1.1aA
35	37.3 ± 2.5aA	36.4 ± 1.6aA	37.0 ± 1.3aA	36.9 ± 0.8aA
40	39.4 ± 1.9aA	36.3 ± 2.1aA	32.0 ± 5.5abAB	31.9 ± 0.7bB
		Total Toc		
30	12.3 ± 1.0bB	13.6 ± 0.8bB	13.5 ± 0.5bB	16.7 ± 0.9aA
35	20.3 ± 3.3aA	16.5 ± 0.7aB	16.8 ± 0.5aB	17.1 ± 0.1aAB
40	18.2 ± 1.5aA	17.7 ± 1.1aA	15.5 ± 3.1abAB	14.8 ± 0.6bB
		Total T3		
30	14.2 ± 1.6cC	26.6 ± 1.0aA	15.0 ± 0.3bC	19.0 ± 0.3aB
35	16.9 ± 1.2bC	24.2 ± 0.9bA	20.1 ± 1.0aB	19.8 ± 0.7aB
40	21.2 ± 0.5aB	27.9 ± 1.4aA	16.5 ± 2.5bC	17.1 ± 0.2bC

[a]The total vitamin E, total Toc, and total T3 contents in nongerminated brown rice of nonpigmented cultivar were 25.2 ± 1.1, 11.4 ± 0.6, and 13.8 ± 0.5 mg/kg, respectively. Values are the mean ± SD (n = 3). Means with different capital letters indicate significant difference within the row; means with different lower case letters indicate significant difference within the column of the same tocols.

Table 3. Effects of Different Germination Conditions on the Concentrations of Total Vitamin E, Total Tocopherols (Toc), and Total Tocotrienols (T3) in Pigmented Brown Rice

germination temp (°C)	concentration[a] (mg/kg, DW) at germination time of 8 h	12 h	16 h	20 h
		Total Vitamin E		
30	42.4 ± 0.9bB	46.7 ± 1.2aA	43.9 ± 1.2bAB	45.7 ± 3.4aA
35	43.6 ± 2.7bA	43.3 ± 1.0bA	44.1 ± 3.5abA	45.8 ± 5.3aA
40	47.4 ± 1.3aA	49.1 ± 2.6aA	47.2 ± 0.5aA	48.9 ± 1.8aA
		Total Toc		
30	18.6 ± 0.6bB	20.0 ± 0.2aA	19.4 ± 0.3bA	19.6 ± 0.8aAB
35	19.2 ± 0.9abA	19.1 ± 0.5bA	18.2 ± 1.6bA	19.1 ± 2.2aA
40	20.8 ± 0.5aA	21.2 ± 1.3aA	20.3 ± 0.2aA	20.6 ± 0.4aA
		Total T3		
30	23.8 ± 0.3bB	26.6 ± 1.0aA	24.5 ± 1.1bB	26.1 ± 2.7aAB
35	24.4 ± 1.8abA	24.2 ± 0.9bA	25.9 ± 2.0abA	26.7 ± 3.1aA
40	26.6 ± 0.8aA	27.9 ± 1.4aA	26.9 ± 0.6aA	28.3 ± 1.4aA

[a]The total vitamin E, total Toc, and total T3 contents in nongerminated brown rice of pigmented cultivar were 29.4 ± 0.8, 11.4 ± 0.9, and 18.0 ± 0.2 mg/kg, respectively. Values are the mean ± SD (n = 3). Means with different capital letters indicate significant difference within the row; means with different lower case letters indicate significant difference within the column of the same tocols.

Table 4. Effects of Different Germination Conditions on γ-Oryzanol Concentrations in Nonpigmented and Pigmented Brown Rice

germination temp (°C)	concentration[a] (mg/kg, DW) at germination time of 8 h	12 h	16 h	20 h
		Nonpigmented		
30	66.0 ± 1.4bB	66.9 ± 0.4bB	177 ± 21aA	181 ± 3.7aA
35	193 ± 23aA	125 ± 6.3aC	95.1 ± 7.5bD	140 ± 8.1bB
40	152 ± 22aA	75.9 ± 1.4bB	63.9 ± 2.4cC	79.7 ± 9.5cB
		Pigmented		
30	580 ± 23bB	659 ± 32aA	536 ± 59cBC	489 ± 49bC
35	601 ± 81abB	650 ± 46aAB	730 ± 42aA	670 ± 81aAB
40	629 ± 23aB	680 ± 31aA	652 ± 38bAB	704 ± 54aA

[a]The γ-oryzanol contents in nongerminated brown rice of nonpigmented and pigmented cultivars were 242 ± 44 and 607 ± 57 mg/kg, respectively. Values are the mean ± SD (n = 3). Means with different capital letters indicate significant difference within the row; means with different lower case letters indicate significant difference within the column of the same cultivar.

Table 5. Concentrations of Vitamin E Homologues among Different Germination Temperatures and Times in Nonpigmented Brown Rice

germination conditions (°C/h)	concentration[a] (mg/kg, DW) α-Toc	α-T3	β-Toc	β-T3	γ-Toc	γ-T3	δ-Toc	δ-T3
control	10.0 ± 0.5d	5.78 ± 0.30e	0.29 ± 0.15c	0.08 ± 0.05c	1.08 ± 0.11c	7.46 ± 0.19e	ND	0.47 ± 0.04d
30/8	10.5 ± 0.7cd	4.96 ± 0.55e	0.61 ± 0.13ab	0.16 ± 0.11bc	1.25 ± 0.18bc	8.32 ± 0.78de	ND	0.71 ± 0.19abc
30/12	11.3 ± 0.7c	6.14 ± 0.99cde	0.69 ± 0.15ab	0.13 ± 0.12bc	1.63 ± 0.31b	9.66 ± 1.11abc	0.02 ± 0.02a	0.71 ± 0.18abc
30/16	11.4 ± 0.4c	5.86 ± 0.15d	0.75 ± 0.07a	0.11 ± 0.01c	1.31 ± 0.05c	8.25 ± 0.15cd	0.01 ± 0.02a	0.81 ± 0.06ab
30/20	14.4 ± 0.6a	7.81 ± 0.17b	0.73 ± 0.16ab	0.14 ± 0.02c	1.57 ± 0.14b	10.1 ± 0.3b	0.01 ± 0.02a	0.89 ± 0.04a
35/8	14.9 ± 0.7a	6.90 ± 1.07bc	0.71 ± 0.13ab	ND	4.65 ± 3.39a	9.46 ± 0.18b	0.02 ± 0.02a	0.59 ± 0.08c
35/12	14.2 ± 0.9ab	8.14 ± 0.72ab	0.65 ± 0.06ab	0.13 ± 0.10bc	1.67 ± 0.13b	10.8 ± 0.5ab	ND	0.82 ± 0.15ab
35/16	14.5 ± 0.3a	8.16 ± 0.60ab	0.58 ± 0.09b	0.08 ± 0.11bc	1.74 ± 0.44b	11.1 ± 0.6a	ND	0.75 ± 0.08b
35/20	15.0 ± 0.1a	8.15 ± 0.35a	0.70 ± 0.06a	0.21 ± 0.04b	1.40 ± 0.07b	10.6 ± 0.3ab	0.03 ± 0.02a	0.82 ± 0.03ab
40/8	15.7 ± 0.8a	8.31 ± 0.13a	0.73 ± 0.09a	0.40 ± 0.20a	1.78 ± 0.59b	10.8 ± 0.4a	0.02 ± 0.02a	0.62 ± 0.05c
40/12	15.4 ± 0.5a	7.30 ± 0.50ab	0.79 ± 0.18ab	0.08 ± 0.07c	1.54 ± 0.53bc	10.4 ± 0.6ab	0.05 ± 0.04a	0.77 ± 0.07b
40/16	13.2 ± 1.8ab	6.05 ± 1.46c	0.74 ± 0.05a	0.09 ± 0.07c	1.48 ± 0.29bc	9.54 ± 1.26abcd	0.01 ± 0.02a	0.84 ± 0.09ab
40/20	12.9 ± 0.8b	6.36 ± 0.13c	0.71 ± 0.05a	0.24 ± 0.04b	1.16 ± 0.06c	9.64 ± 0.12c	0.02 ± 0.01a	0.86 ± 0.01a

[a]Values are the mean ± SD (n = 3). Means with different letters indicate significant difference within the column. ND, not detected.

Effects of Germination Conditions on γ-Aminobutyric Acid Contents. GABA contents of GBR obtained from different treatments are reported in Table 7. The results showed that the GABA content of GBR increased with increasing germination time in both nonpigmented and pigmented cultivars; however, the pigmented GBR showed a lower GABA content than the nonpigmented GBR. When compared with non-GBR (25.6 ± 2.9 mg/kg for nonpigmented brown rice and 17.5 ± 2.0 mg/kg for pigmented brown rice), besides GABA contents in pigmented GBR under conditions

Table 6. Concentrations of Vitamin E Homologues among Different Germination Temperatures and Times in Pigmented Brown Rice

germination conditions (°C/h)	concentration[a] (mg/kg, DW)							
	α-Toc	α-T3	β-Toc	β-T3	γ-Toc	γ-T3	δ-Toc	δ-T3
control	2.88 ± 0.01f	1.19 ± 0.25d	0.18 ± 0.11e	ND	8.21 ± 0.79c	16.4 ± 0.4c	0.15 ± 0.06d	0.45 ± 0.06c
30/8	4.48 ± 0.14e	1.89 ± 0.04c	0.56 ± 0.01c	ND	13.2 ± 0.5ab	20.8 ± 0.3b	0.38 ± 0.02bc	1.06 ± 0.01a
30/12	4.81 ± 0.13d	1.99 ± 0.09bc	0.61 ± 0.06bc	ND	14.2 ± 0.1a	23.6 ± 1.0a	0.39 ± 0.05bc	1.05 ± 0.14a
30/16	4.81 ± 0.04d	1.88 ± 0.05c	0.51 ± 0.02d	ND	13.7 ± 0.3ab	21.5 ± 1.0b	0.40 ± 0.03bc	1.10 ± 0.06a
30/20	4.95 ± 0.22d	2.00 ± 0.11bc	0.58 ± 0.03c	ND	13.6 ± 0.6ab	23.1 ± 2.6a	0.38 ± 0.04bc	1.00 ± 0.04a
35/8	4.60 ± 0.34de	1.90 ± 0.26bc	0.68 ± 0.11bc	0.06 ± 0.04a	13.5 ± 0.5ab	21.4 ± 1.6ab	0.39 ± 0.03bc	1.06 ± 0.10a
35/12	4.81 ± 0.12d	1.97 ± 0.01b	0.59 ± 0.07bc	ND	13.3 ± 0.4ab	21.2 ± 0.8ab	0.40 ± 0.04bc	1.03 ± 0.05a
35/16	4.80 ± 0.38de	1.91 ± 0.29bc	0.50 ± 0.09cd	ND	12.5 ± 1.1b	23.0 ± 1.7a	0.39 ± 0.07bc	1.06 ± 0.08a
35/20	5.47 ± 0.57bc	2.28 ± 0.29ab	0.69 ± 0.06b	0.03 ± 0.04a	12.6 ± 1.5ab	23.4 ± 2.8a	0.39 ± 0.04bc	0.95 ± 0.12ab
40/8	5.28 ± 0.14c	2.12 ± 0.08b	0.63 ± 0.03b	ND	14.4 ± 0.3a	23.4 ± 0.8a	0.45 ± 0.02a	1.07 ± 0.05a
40/12	5.56 ± 0.31bc	2.18 ± 0.21b	0.58 ± 0.09bc	0.03 ± 0.04a	14.6 ± 1.0a	24.6 ± 1.2a	0.44 ± 0.02a	1.13 ± 0.06a
40/16	5.79 ± 0.06b	2.17 ± 0.08b	0.63 ± 0.04b	0.08 ± 0.06a	13.5 ± 0.2ab	23.6 ± 0.6a	0.40 ± 0.01b	1.06 ± 0.02a
40/20	6.27 ± 0.13a	2.52 ± 0.05a	0.81 ± 0.01a	0.06 ± 0.09a	13.1 ± 0.4ab	24.8 ± 1.5a	0.36 ± 0.03c	0.94 ± 0.01b

[a]Values are the mean ± SD (n = 3). Means with different letters indicate significant difference within the column. ND, not detected.

Table 7. Effects of Different Germination Conditions on γ-Aminobutyric Acid Concentrations in Nonpigmented and Pigmented Brown Rice

germanation temp (°C)	concentration[a] (mg/kg, DW) at germination time of			
	8 h	12 h	16 h	20 h
Nonpigmented				
30	32.8 ± 0.5bB	28.4 ± 0.3cC	29.5 ± 1.5bC	37.4 ± 1.5bA
35	38.1 ± 4.7aC	40.7 ± 2.6bC	46.1 ± 1.7aB	62.3 ± 5.5aA
40	33.0 ± 4.7abC	46.8 ± 1.5aB	45.2 ± 0.8aB	58.4 ± 1.5aA
Pigmented				
30	10.3 ± 3.1bC	14.8 ± 2.0cC	21.1 ± 0.9bB	27.4 ± 2.8cA
35	22.6 ± 2.5aB	26.4 ± 1.9bB	37.4 ± 3.6aA	39.2 ± 3.4bA
40	26.9 ± 4.2aC	38.1 ± 1.9aB	40.6 ± 4.7aB	48.8 ± 3.6aA

[a]The γ-aminobutyric acid contents in nongerminated brown rice of nonpigmented and pigmented cultivars were 25.6 ± 2.9 and 17.5 ± 2.0 mg/kg, respectively. Values are the mean ± SD (n = 3). Means with different capital letters indicate significant difference within the row; means with different lower case letters indicate significant difference within the column of the same cultivar.

30 °C for 8 and 12 h, the GABA contents in GBR of different treatments were remarkably increased; for instance, both nonpigmented and pigmented GBR showed an increase by >2-fold at germination temperatures of 35 and 40 °C for 20 h. At a germination temperature of 35 °C, the GABA content in GBR at 20 h was about 1.7 and 1.5 times higher than that of 8 and 12 h, respectively.

DISCUSSION

This study revealed that the optimal germination rate was obtained with presoaking temperatures of 30 and 25 °C for nonpigmented and pigmented brown rice, respectively. Grains of both cultivars were able to absorb sufficient water for germination during the first hour of soaking. Regardless of rice cultivar, the contents of total vitamin E, total Toc, total T3, and GABA in GBR were higher than in the non-GBR. The pigmented GBR possessed higher total vitamin E, total Toc, total T3, and GO contents than the nonpigmented GBR; however, its level of GABA was lower. The levels of total vitamin E in pigmented GBR were relatively constant under the different germination conditions. The main vitamin E homologue present in nonpigmented GBR was α-Toc, followed by γ-T3 and α-T3, whereas in pigmented GBR the order of the highest three was γ-T3 > γ-Toc > α-Toc; β-Toc, β-T3, δ-Toc, and δ-T3 were present in only small amounts (≤1.0 mg/kg) in GBR of both cultivars. Both nonpigmented and pigmented GBR showed an increase in GABA contents with increasing germination time, and its levels in nonpigmented GBR were generally higher than in pigmented GBR. These observations suggest that germination conditions not only affect the GABA level but also Toc, T3, and GO contents in both nonpigmented and pigmented GBR.

Studies have shown that germination caused significant changes in chemical compositions, including bioactive compounds and amino acids in GBR.[21,22] Germination promotes the development of hydrolytic enzymes that are inactive in raw seeds.[23] At different germination stages, rice grains undergo different biochemical changes and may have influenced the bioactive component profiles in GBR. Regardless of rice cultivar, the profiles of Toc, T3, GO, and GABA differ between germination conditions. Consistent with other studies,[21,24] the germination process did increase the GABA level. Concentrations of Toc, T3, and GO in GBR appeared to be cultivar and germination duration dependent. The trends observed in different cultivars may also be due to different water uptake rates of rice seeds.[25] Besides germination increasing GO content in certain rice cultivars,[26] this study has shown that Toc and T3 levels were enhanced in the GBR. The major vitamin E homologues in nonpigmented GBR were α-Toc, γ-T3, and α-T3, whereas in pigmented GBR they were γ-T3, γ-Toc, and α-

Toc; this indicates that different germination conditions can result in different levels of these bioactive components, suggesting that some of them may be bound to cellular components in the rice bran and were released during germination.

Although no study has been reported the effect of germination on Toc, T3, and GO contents in brown rice, several germination methods have been applied with the purpose of enhancing GABA content in brown rice; different methods had yielded different amounts of GABA. The age of the grain, grain variety, percentage of germination, and germination temperature and time have been reported to be the major factors affecting GABA synthesis in brown rice during the germination process.[27] The age of grains of nonpigmented (Tainung 71) and pigmented (Black glutinous) cultivars used in this study was <4 months after harvesting, which were considered to be the age effective for yielding high GABA contents; this is because contents of bioactive components in rice grain are generally believed to decrease after 4−6 months of storage.[28] As both pigmented and nonpigmented brown rice had a germination percentage of >96%, implying that there was only 4% of ungerminated grains and that there was no capability to increase GABA through the germination process. Glutamate decarboxylase (GAD, EC 4.1.1.15) is a pyridoxal 5′-phosphate-dependent enzyme, which catalyzes the conversion of α-decarboxylation of L-glutamic acid to GABA and carbon dioxide. In this study, when treatments were compared, the highest GABA content for nonpigmented GBR was at a germination temperature 35 °C for 20 h, and that for pigmented GBR was at a germination temperature 40 °C for 20 h. Zhang et al.[29] reported that the optimum pH of rice germ GAD was between 5.5 and 5.8, whereas the optimum temperature was between 37 and 40 °C. The optimum temperature of the present germination conditions was 35−40 °C, which was close to the optimum temperature of rice germ GAD activation, and the pH was found to be around 5.8. This explains the reason for the rapid increase in GABA content in both nonpigmented and pigmented GBR.

GBR is different from normal brown rice in that it has undergone the process of germination; more specifically, the rice embryo is sprouted under suitable environmental conditions. Furthermore, unlike white rice, GBR is sweeter, has an excellent taste, has better texture, and is easier to cook.[10,11,16] The germination process can also improve the palatable texture of brown rice and the contents of biofunctional substances. Hence, GBR is a better source of functional food than brown rice because it is good in digestion and absorption, and also contains numerous bioactive compounds.

In conclusion, this study has concluded that in addition to quality differences between varieties, the application of the germination process was able to improve the brown rice quality as demonstrated by enhanced Toc, T3, GO, and GABA contents in GBR of both nonpigmented and pigmented cultivars. These results have also suggested that GBR is a good source of functional food.

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Funding

The authors thank the National Science Council of Taiwan for partial funding of this study under Grant NSC100-2815-C-002-147-B.

Notes

The authors declare no competing financial interest.

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