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Effects of Different Pretreatments on the GABA Content of Germinated Brown Rice

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Abstract: Brown rice germination increases γ-aminobutyric acid (GABA) levels and enhances its antioxidant activity. In this study, Kaohsiung 145 brown rice was used as the raw material, and soaked in various solutions for 6 h before being processed with either high-pressure processing (HPP) or ultrasonic treatment to increase the GABA content. The GABA and antioxidant components of brown rice were analyzed after 42 h of germination and subsequent air-drying to obtain a moisture content of 14%. The results showed that non-germinated brown rice had GABA at 7.10 mg/100 g and treatment with various soaking solutions (0.1% $CaCl_2$, 0.1% Glu, 0.2% $CaCl_2$, and 0.2% Glu) increased GABA contents. Specifically, 0.1% $CaCl_2$ and 0.1% Glu exhibited higher GABA content, at 42.51 and 44.64 mg/100 g. Furthermore, the GABA content increased synergistically when combined with HPP (100 MPa, 10 min) and ultrasonic (20 min) treatments after soaking. The results showed that the GABA contents in germinated rice were the greatest after ultrasonic treatment, followed by HPP treatment, and the least with only soaking treatment. The treatments with 0.1% $CaCl_2$ and 0.2% Glu combined with ultrasonic processing for 20 min resulted in the highest GABA content at 102.38 and 110.88 mg/100 g, respectively. Finally, 0.1% $CaCl_2$ with ultrasonic treatment for 20 min was chosen, as it demonstrated a considerable improvement in total polyphenols content and DPPH scavenging abilities, as seen by improved scores in subsequent taste evaluations.

Keywords: germination; brown rice; soaking; GABA

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1. Introduction

Brown rice is regarded as a healthier choice than polished white rice because it retains more germ and bran layers during processing, maintaining dietary fiber, vitamins, plant sterols, and bioactive components such as GABA [1]. However, brown rice is rarely consumed as a staple food because of its dark appearance and hard texture. The germination of brown rice can be used to improve its taste and further enhance its nutritional value and health functions [2]. In addition, brown rice germination can improve water absorption, cooking gelatinization quality, and hence, brown rice texture. Kim et al. [3] reported that the water absorption stage of brown rice took 8 h, and moisture content increased from 15.9% to 36.8% after 48 h of germination. The sprouting ratio increased to 97% at 32 h.

Brown rice germination can enhance the nutritional value and bioactive components, including total polyphenols, and flavonoids, to increase antioxidant activity [4,5]. Both γ-oryzanol and γ-aminobutyric acid (GABA) can be improved by germination and fermentation [6,7]. GABA is widely present in both animals and plants, where it serves critical functions. In plants, GABA not only regulates the internal environment to cope with biotic/abiotic stimuli, but it also serves as a signaling molecule. When plants are stimulated externally, GABA can perform a variety of tasks, including regulating the carbon/nitrogen (C/N) ratio [8], plant defense [9], and phenolic compound accumulation [10]. In animals, GABA serves a variety of physiological functions, including reducing blood stress [11], improving sleep [12], and relieving stress [13]. Consequently, functional foods rich in GABA, such as tea, beverages, and dairy products, have gained popularity in many countries [14].

GABA metabolism occurs in the cytoplasm and is primarily divided into two pathways: GABA shunt and polyamine degradation. The GABA shunt is the primary synthetic pathway, producing 70% of GABA under anaerobic conditions [15]. The GABA shunt involves the irreversible decarboxylation of cytoplasmic L-glutamic acid at the α position by glutamate decarboxylase (GAD, EC 4.1.1.15), resulting in GABA. Inside plant cells, Ca^{2+} can bind to its receptor protein, calmodulin (CaM), initiating the response. As the Ca^{2+} concentration increases, active enzymes known as Ca^{2+}/CaM complex promote production. Baum et al. [16] postulated that GAD, which is involved in GABA production, is a binding protein (CaM) that can be activated by Ca^{2+} and CaM, hence increasing GAD activity and thus metabolizing more GABA content.

High-pressure processing (HPP), also known as high hydrostatic pressure processing or ultra-high-pressure processing, is an emerging non-thermal food preservation technique. The primary purposes of HPP are microbial inactivation, effectively eliminating hazardous pathogens or spoilage microorganisms present in food, and altering the characteristics of food. HPP can change the texture, appearance, and flavor of food. Moreover, HPP can cause cold gelatinizing effects, imparting unique properties to food and improving food quality [17]. Xia et al. [18] investigated the effect of HPP on GABA in germinated brown rice and found that treating germinated brown rice at 300 MPa for 10 min increased the GABA content by 45.16%. The HPP conducted at 100 MPa increased the GABA content in germinated brown rice, which can be attributed to the stimulation of glutamate metabolism, as glutamate is a precursor of GABA [19–21]. Therefore, combining germination and HPP treatment can accelerate GABA and glutamate metabolism pathways, resulting in increased GABA content.

Ultrasonication, an emerging processing technique, is used in the food industry and biotechnology processes such as filtering, extracting, and degassing [22]. The formation of cavities (cavitation) in liquids, followed by the rise and collapse of air bubbles, results in cavitation microstreaming, which causes pressure and velocity changes in the fluid and generates fine jets [23]. These actions can be used to enhance the yield of bioactive compounds (including primary and secondary metabolites) in plant-based foods. Yang et al. [24] studied the use of ultrasonic treatment to improve the taste and nutrition of soybeans, finding that soaking followed by (40 kHz, 300 W) for 30 min improved the GABA content in soybean sprouts by 43.4%. The findings revealed that ultrasonication improved water absorption, total phenolic accumulation, energy metabolism, lipid metabolism, and protein hydrolysis in brown rice, hence affecting GABA content. Ding et al. [25] used ultrasonication on red rice at various stages of germination and found that ultrasonication for 5 min after 66 h of germination resulted in a GABA content of 75.82 mg/100 g, which was 69.2% higher than that of red rice germinated for 72 h, implying that ultrasonic treatment could be a novel method to stimulate seed germination and accumulate bioactive compounds such as GABA and riboflavin (vitamin B_2).

The objective of this study was to use organically grown Kaohsiung 145 brown rice as raw material, soak it in various soaking solutions for 6 h, and then apply HPP and ultrasonic treatment to continue germination at 25 °C for 36 h. The germinated brown rice was dried by 35 °C cold air drying and analyzed for GABA content, phenolic compounds, and antioxidant capacity.

2. Materials and Methods

2.1. Chemicals

Calcium chloride, L-2-aminopentanedioic acid, 4-aminobutanoic acid, 3,4,5-trihydroxybenzoic acid (gallic acid), sodium carbonate, trichloroalumane, o-phthalaldehyde (OPA), 2-sulfanylethan-1-ol, boric acid, N,N-diethylethanamine, ethanoic acid, and oxolane were purchased from Nippon Soda (Osaka, Japan). Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one), 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazine (DPPH), ascorbic acid ((5R)-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxyfuran-2(5H)-one), Folin & Ciocalteus phenol reagent, BHA (2-tert-butyl-4-methoxyphenol), and sodium acetate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile was obtained from

Avantor (Radnor, PA, USA), and methanol was purchased from Spectrum Chemical (New Brunswick, NJ, USA). All other reagents used were of analytical or reagent grade.

2.2. Preparation of Germinated Brown Rice with Various Soaking Solutions

Brown rice grains (Kaohsiung 145) were obtained from Colim Spring Rice (first rice growing season of 2022, Yilan, Taiwan). In polypropylene (PP) boxes, distilled water was poured, and approximately 500 g of brown rice was weighed to achieve a rice-to-water ratio of 1:1. The brown rice was then soaked in 0.1%/0.2% calcium chloride and 0.1%/0.2% L-glutamic acid solutions separately for 6 h at 25 °C. After soaking, the excess moisture was drained, and the brown rice was vacuum-sealed using nylon/linear low-density polyethylene vacuum bags (NY/LLDPE).

2.3. Physical Pretreatments of HPP or Ultrasonic in Germinated Brown Rice

Following the methodology outlined by Wu et al. [26], after germinated brown rice was soaked for 6 h, the HPP (600 MPa/6.2 L, Kuntec International Co., Ltd., Yunlin, Taiwan) was carried out at 100 MPa for 10 min. In addition, following the methodology outlined by Hung and Chen [27], the soaked brown rice could also undergo ultrasonic (DC-600H, Delta Ultrasonic Co., Ltd., New Taipei, Taiwan) treatment at a power intensity of 400 W for 20 min. Then, the brown rice was drained of excess moisture and spread evenly in the plastic PP box. It was then incubated at 25 °C for 36 h for germination (total duration of 42 h).

2.4. Cold Air-Drying Germinated Brown Rice

The 1 kg of germinated brown rice was placed in a 35 °C cold air-drying machine (YK-112 RS, Zhudian Hsing Industries Co., Ltd., Taichung, Taiwan) until its moisture content decreased to below 14% on a wet basis. After drying, the germinated brown rice was allowed to equilibrate at room temperature for 2 h before being packed into Ziplock bags and stored in a 4 °C refrigerator for subsequent experimental analysis. The weight and surface temperature changes of the germinated brown rice during drying were recorded to plot the heating and drying curves.

2.5. Moisture Content Determination

The method specified in AACC [28] was followed. Five grams of germinated brown rice wet sample weight (W_i) was placed in a pre-weighed aluminum dish and placed in a 105 °C oven (Channel DCM 45, Kehua Co., Ltd., Yilan, Taiwan) until constant weight (W_o) was achieved. Three replicates were performed for each treatment, and the moisture content on a wet basis (w.b.) was calculated using the following formula.

$$\text{Moisture content } (\%) = \frac{W_i - W_o}{W_i} \times 100\% \quad (1)$$

2.6. Color Determination

A colorimeter (Sph900, ColorLite Gmbh, Katlenburg-Lindau, Germany) was used to measure the L*, a*, and b* values of samples subjected to various processing methods. Each sample was measured 6 times, and the average values were taken to compare the color differences between different treatments. Here, L* represents brightness, black (0) to white (100); a* represents redness (+) to greenness (−); and b* represents yellowness (+) to blueness (−).

The measurement range of Delta E (△E) is between 0 and 100, where values closer to 0 indicate a smaller color difference, while 100 indicates complete distortion. L*, a*, and b*, respectively, represent the brightness, red–green color, and yellow–blue color of the

control group of ungerminated brown rice; L_1*, a_1*, and b_1* represent the values in the experimental group of germinated rice.

$$\triangle E = \sqrt{(L^* - L_1^*)^2 + (a^* - a_1^*)^2 + (b^* - b_1^*)^2} \quad (2)$$

2.7. Water Absorption Percentage Determination

Take 100 g (W_i) of dried brown rice and soak it in room temperature water for 0–6 h. Drain off the water and weigh the rice every hour. Observe the weight change (W_f) due to water absorption during the soaking period, and calculate the percentage increase in weight of the soaked brown rice.

$$\text{Water absorption } (\%) = \frac{W_f - W_i}{W_i} \times 100\% \quad (3)$$

2.8. γ-Aminobutyric Acid (GABA) Content Determination

Following the methodology outlined by Le et al. [29], dried germinated brown rice powder (5 g) was mixed with 25 mL of reverse osmosis (RO) water and subjected to ultrasonic extraction for 2 h at room temperature using a solid-to-liquid ratio of 1:5. Subsequently, the sample was centrifuged at 6000 rpm (3870× *g*) for 15 min (CB-2060, Hsiangtai Precision Machine Co., Ltd., New Taipei, Taiwan), and the supernatant was collected. The centrifugation step was repeated at 10,000 rpm (8050× *g*) for 10 min (MCD-259, Hsiangtai Precision Machine Co., Ltd., New Taipei, Taiwan) to obtain the supernatant for further analysis. The supernatant (2 mL) was transferred to a 2 mL Eppendorf tube and reacted with 10 μL of derivatization reagent (OPA) for 2 min. The derivatizing agent (OPA) was prepared by dissolving in acetonitrile and adding 2-mercaptoethanol and boric acid buffer. The reaction mixture was then drawn into a syringe, filtered through a 0.22 μm PVDF membrane, and injected into an HPLC (Waters, MA, USA) sample vial for γ-aminobutyric acid (GABA) quantification. The obtained sample concentrations were interpolated into the standard curve of GABA (y = 33,714x − 6592.8; R^2 = 0.997) over a concentration range of 0, 2.5, 5, and 10 mg/L, with a limit of detection (LOD) of 0.88 mg/L and a limit of quantification (LOQ) of 2.65 mg/L, to convert them into GABA concentrations.

The HPLC system conditions were as follows: Phase A, 8.0 g of crystalline sodium acetate was weighed and diluted to 1000 mL with water, then 220 μL of triethylamine was added, stirred, and the pH was adjusted to 7.2 using 5% acetic acid, followed by the addition of 5 mL of tetrahydrofuran; Phase B, 8.0 g of crystalline sodium acetate was weighed and diluted to 1000 mL with water, the pH was adjusted to 7.2 using 2% acetic acid, then the sodium acetate solution was mixed with acetonitrile and methanol in a volume ratio of 1:2:2; the HPLC mobile phase was shown in the following Table 1; the separation column employed was Ascentis® C18 (25 cm × 4.6 mm, 5 μm, Supelco, Bellefonte, PA, USA); the detection wavelength was UV 338 nm, flow rate was 1 mL/min, and column temperature was 25 °C; the injection volume was 10 μL; and GABA retention time was 22 min. Data processing was performed using the QChrom data processing system (Scientific Information Service Co., Ltd., New Taipei, Taiwan).

Table 1. HPLC mobile phase gradient of GABA.

Time (min)	Phase A (%)	Phase B (%)	Flow Rate (mL/min)
0	92	8	1.0
20	60	40	1.0
28	92	8	1.0

2.9. Total Polyphenols Content Determination

Referring to the method outlined by Lin and Tang [30] for the quantification of total polyphenols, dried germinated brown rice powder (2.5 g) was accurately weighed and mixed with 50 mL of 95% ethanol. The mixture was subjected to ultrasonic extraction for

20 min at room temperature, using a solid-to-liquid ratio of 1:20. Subsequently, the sample was centrifuged at 6000 rpm (3870× *g*) for 15 min (CB-2060, Hsiangtai Precision Machine Co., Ltd., New Taipei, Taiwan), and the supernatant was collected for further analysis. For the subsequent analysis, 0.2 mL of the supernatant was mixed with 1 mL of Folin & Ciocalteu's phenol reagent and 0.8 mL of 7.5% sodium carbonate (Na_2CO_3) solution. The mixture was then allowed to react in the dark at room temperature for 30 min. Absorbance was measured at a wavelength of 765 nm using a spectrophotometer (CT-2700, Jiancheng Technology Co., Ltd., Taipei, Taiwan). The obtained absorbance values were then compared to a standard curve of gallic acid ($y = 0.0084x - 0.0889$; $R^2 = 0.9925$) for the quantification of gallic acid equivalents in the sample.

2.10. Flavonoids Content Determination

Following the method described by Lin and Tang [30] for the quantification of flavonoids, dried germinated brown rice powder was extracted as in session 2.9 for TPC determination. For the TFC analysis, 0.2 mL of the supernatant was mixed with 1 mL of 2% $AlCl_3 \cdot 6H_2O$-MeOH solution and thoroughly mixed. The mixture was then allowed to react in the dark at room temperature for 10 min. Absorbance was measured at a wavelength of 430 nm using a spectrophotometer (CT-2700, Jiancheng Technology Co., Ltd., Taipei, Taiwan). The obtained absorbance values were then compared to a standard curve of quercetin ($y = 0.0292x - 0.0051$; $R^2 = 0.9999$) for the quantification of quercetin equivalents in the sample.

2.11. DPPH Radical Scavenging Activity Determination

Based on the method outlined by Lin et al. [31] for evaluating the scavenging activity of DPPH radicals, the following steps were followed.

The supernatant of the extraction described in Section 2.9 was then concentrated under reduced pressure at 50 °C and re-dissolved in 95% ethanol to achieve a concentration of 20 mg/mL for subsequent analysis.

DPPH assay: A total of 2 mL of the prepared 20 mg/mL sample solution was combined with 2 mL of 0.2 mM DPPH-MeOH solution. The mixture was allowed to react in darkness at room temperature for 30 min. After incubation, the absorbance of the reaction mixture was measured at a wavelength of 517 nm with a spectrophotometer (CT-2700, Jiancheng Technology Co., Ltd., Taipei, Taiwan). The scavenging activity of DPPH radicals was calculated using a formula. Vitamin C-RO and BHA-MeOH at a concentration of 20 mg/mL were employed as control groups.

This method facilitates the assessment of the scavenging activity of DPPH radicals in germinated brown rice samples.

$$\text{Scavenging DPPH free radicals} = \frac{ABS_{blank} - ABS_{sample}}{ABS_{blank}} \times 100\% \quad (4)$$

2.12. Analysis of Taste Value

The germinated brown rice was sent to the East District Branch of the Agriculture and Food Agency, Yilan County, for taste analyzer analysis. The principle of the taste analyzer (TM-3500, Shizuoka Seiki Co., Ltd., Fukuroi-shi, Japan) involves using near-infrared transmission to measure the moisture content, protein content, amylose content, free fatty acids, and taste value of the control brown rice and experimental sprouted brown rice at continuous wavelengths ranging from 500 nm to 1010 nm.

2.13. Statistical Analysis

The experimental results were expressed as mean ± standard deviation. Statistical analysis was performed using Statistical Analysis System Enterprise Guide (SAS EG, Cary, NC, USA) version 9.4 software. One-way analysis of variance (ANOVA) and Duncan's

Multiple Range Test (DMRT) were employed for significant difference analysis, comparing the means of each group at a significance level of 5% ($p < 0.05$).

3. Results and Discussion

3.1. Effect of Soaking on Brown Rice Germination

It was ensured that the brown rice had sufficient moisture for germination after water absorption. Figure 1 shows the water absorption curve of organic brown rice (Kaohsiung No. 145) at room temperature. After 1 h of soaking, there was a significant rise in water absorption, with a ratio of 16.5%. After 6 h of soaking, the water absorption curve flattened out, with a water absorption ratio of 27.1%, and the moisture content of brown rice was approximately 35%, which was conducive to microbial growth and may lead to deterioration and unpleasant odors. Therefore, drying was used to terminate the germination process and reduce moisture content for preservation and analysis.

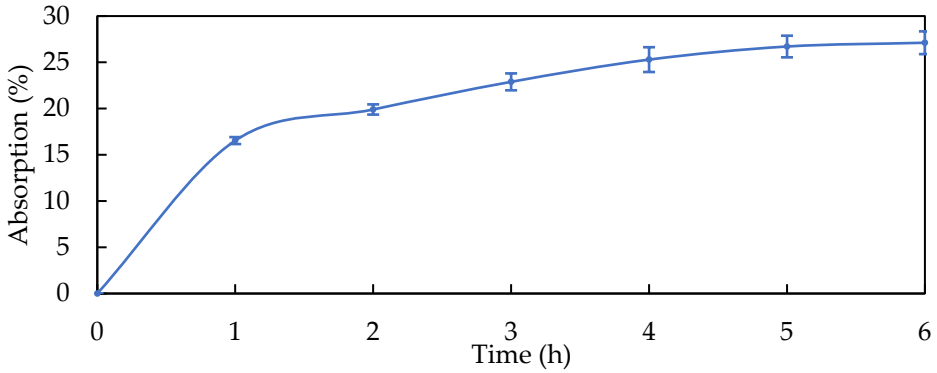

Figure 1. The water absorption curve of brown rice during soaking at room temperature. Data were expressed as mean and error bars of ± S.D. ($n = 3$).

Figure 2 exhibits photos of germinated brown rice at different germination stages. At 36 h, sprouts develop, and then they reach a length of about 0.2 cm by 42 h, almost half the length of the rice grain. For the subsequent drying operation, this experiment selected a germination time of 42 h. Yen et al. [32] mentioned that if the sprout length of germinated brown rice exceeds 0.3 cm, it tends to fall off during drying, causing undesirable appearance issues. The sprout length of germinated rice was controlled to keep it below 0.3 cm.

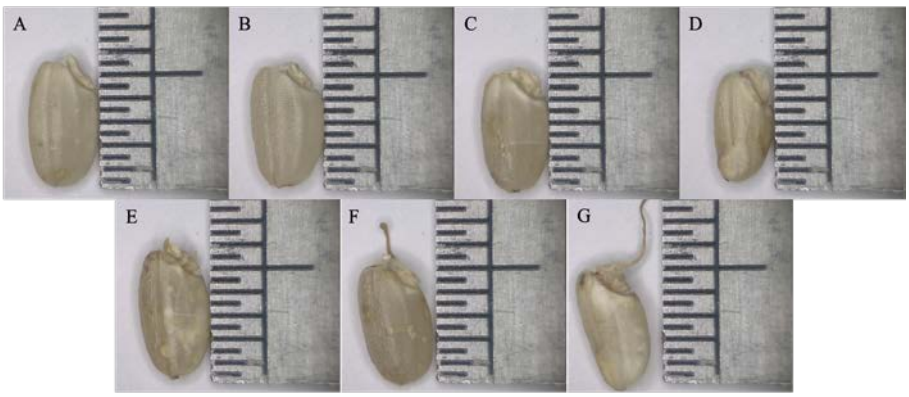

Figure 2. Pictures of germinated brown rice at different germination times: (**A**) 0 h, (**B**) 12 h, (**C**) 24 h, (**D**) 30 h, (**E**) 36 h, (**F**) 42 h, and (**G**) 48 h.

The study examined the GABA content of Taichung Sen 10 brown rice at various germination times and found that brown rice germinated at 25 °C for 42 h also had the highest GABA content, which was three times greater than that of non-germinated brown rice [32].

Figure 3 shows the drying and temperature curves of 1 kg of germinated brown rice placed in a 35 °C cold air dryer. Germinated brown rice had a temperature of about 30 °C. At the end of germination, germinated brown rice had a moisture content of 0.53 g water/g dry material (dry basis, d.b.). After 8 h of drying, the moisture content was reduced to less than 0.18 g water/g DM, allowing for easier preservation and analysis. The moisture content of germinated rice should be kept below 15% (wet basis, approximately 0.18 g water/g DM on a dry basis) to prevent spoilage and ensure long-term storage stability [33].

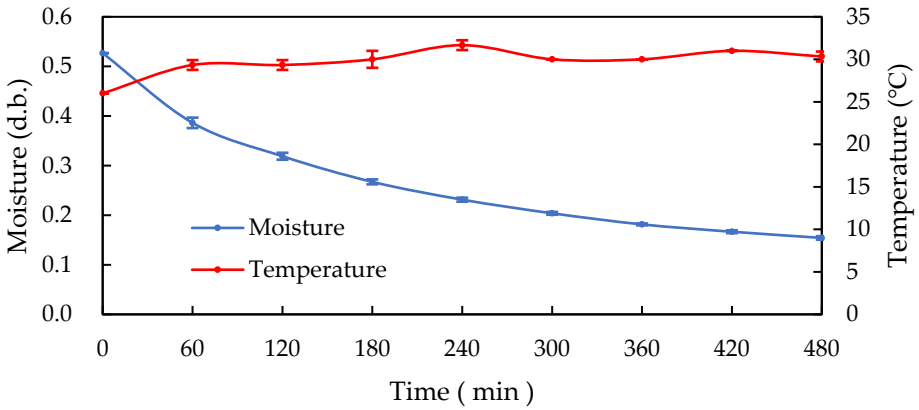

Figure 3. Drying and temperature curves of 1 kg germinated brown rice during 35 °C cold air drying. Data were expressed as mean and error bars of ± S.D. (n = 3).

3.2. Effect of Various Soaking Solutions on GABA and Antioxidants of Germinated Brown Rice

According to previous studies, soaking treatment can increase GABA and bioactive compound content [34]. Table 2 indicates the GABA content of germinated brown rice after various soaking solutions. When ungerminated, the Kaohsiung No. 145 brown rice contained only 7.10 mg/100 g of GABA. Following 42 h of germination treatment, the GABA content increased to 22.30 mg/100 g. Moreover, soaking treatments with 0.1% $CaCl_2$ and 0.1% glutamate (Glu) solutions further increased GABA content to 42.51 and 44.64 mg/100 g, respectively. However, when the soaking solution concentration was increased to 0.2%, GABA content decreased with no significant difference compared to the 42 h water germination, implying that the osmotic pressure of the solution may affect the activity of GABA decarboxylase, thereby influencing the biosynthesis of GABA.

Table 2. Effects of various soaking solutions on GABA content of germinated brown rice.

Germination Treatment	GABA (mg/100 g)
Ungermination	7.10 ± 2.53 ^{c}
Water	22.33 ± 7.87 ^{b}
Soaking	
0.1% $CaCl_2$	42.51 ± 11.37 ^{a}
0.1% Glu	44.64 ± 2.95 ^{a}
0.2% $CaCl_2$	30.09 ± 9.51 ^{b}
0.2% Glu	27.40 ± 7.89 ^{b}

Data were expressed as mean and error bars of ± S.D. (n = 3). Means with different superscript letters in the same column are significantly different ($p < 0.05$).

Wu et al. [35] investigated the effect of various $CaCl_2$ concentrations on GABA content after germination in glutinous brown rice. The results revealed that soaking in 2.0% $CaCl_2$ for 24 h increased the GABA content by 1.76 times compared to the control group. However, increasing the concentration led to a decrease in GABA content, indicating that high $CaCl_2$ concentrations may inhibit the activity of glutamate decarboxylase and the metabolism pathway of GABA transaminase [36]. Wu et al. [26] studied the effect of 0.1% and 0.2% soaking solutions ($CaCl_2$ and glutamic acid) on germinated brown rice from the Taichung

Sen 10 variety. This variety had undetectable GABA content while ungerminated, although soaking in 0.2% $CaCl_2$ exhibited higher GABA content compared to the results of this experiment, indicating rice variety differences.

Table 3 shows an analysis of the antioxidant components and DPPH radical scavenging capacity of germinated brown rice in various soaking solutions. The total phenolic content of germinated brown rice ranged from 0.85 to 0.93 mg gallic acid equivalent/g, with no significant differences observed following water germination and soaking in various solutions. Similarly, the flavonoid content ranged from 3.68 to 4.41 μg quercetin equivalent/g, with no significant differences found. The DPPH radical scavenging capacity decreased from 87% to 84% after germination but increased to around 90% following soaking in various solutions. Wu et al. [5] found a positive correlation between the antioxidant ability of phenolic compounds and their content during the brown rice germination process.

Table 3. Analyses of antioxidant active components and DPPH free radical scavenging ability in germinated brown rice by various soaking solutions.

Germination Treatment	Total Polyphenols (mg Gallic Acid Equivalent/g)	Flavonoids (μg Quercetin Equivalent/g)	Scavenging DPPH Free Radicals (%)
Ungermination	0.91 ± 0.01^{ab}	3.68 ± 0.00^{a}	87.01 ± 0.11^{d}
Water	0.84 ± 0.01^{b}	4.41 ± 0.00^{a}	84.61 ± 2.61^{e}
Soaking			
0.1% $CaCl_2$	0.90 ± 0.03^{ab}	4.17 ± 0.42^{a}	89.98 ± 0.24^{abc}
0.1% Glu	0.85 ± 0.02^{b}	3.68 ± 0.00^{a}	89.39 ± 0.17^{bc}
0.2% $CaCl_2$	0.90 ± 0.07^{ab}	4.17 ± 0.85^{a}	88.94 ± 1.83^{c}
0.2% Glu	0.93 ± 0.01^{a}	3.68 ± 0.00^{a}	88.69 ± 0.25^{cd}
Vitamin C			91.72 ± 0.00^{a}
BHA			91.06 ± 0.00^{ab}

Data were expressed as mean and error bars of ± S.D. (n = 3). Means with different superscript letters in the same column are significantly different ($p < 0.05$). The concentrations of the sample, vitamin C, and BHA for the DPPH assay were 20 mg/mL.

3.3. Effect of HPP and Ultrasonic Treatment on GABA and Antioxidants of Germinated Brown Rice

Table 4 shows the effect of various soaking solutions combined with HPP or ultrasonic treatment on the GABA content of germinated brown rice. GABA contents in the HPP group reached 83.10 mg/100 g after HPP treatment (100 MPa, holding 10 min) with 0.1% $CaCl_2$ soaking solution. Similarly, when treated with 0.1% Glu soaking solution, the GABA content was 64.29 mg/100 g. However, increasing the soaking solution concentration to 0.2% $CaCl_2$ and Glu, reduced the GABA content to around 62 mg/100 g. Therefore, 0.1% $CaCl_2$ combined with HPP processing was more effective in increasing GABA content. In the ultrasonic group, soaking with 0.1% $CaCl_2$ for 20 min resulted in a higher GABA content of 102.38 mg/100 g. In general, the ultrasonic group obtained higher GABA contents than the HPP group, and these two physical treatments after soaking various solutions enhanced GABA contents in germinated brown rice.

Sun et al. [14] integrated mechanisms of HPP to promote GABA, including (1) changing enzyme conformation; (2) promoting H^+ release in plants; (3) disrupting cell structure, accelerating mass transfer between substrate and cells, and promoting enzyme reactions; and (4) altering the structure of Ca^{2+} channels, promoting calcium ion release [36]. Wu et al. [35] investigated the effect of ultrasonic treatment combined with $CaCl_2$ soaking on brown rice to stimulate brown rice for the first 9 h before germination.

Although 0.2% Glu contained more GABA content, there was no significant difference between them, and $CaCl_2$ is cheaper than that of L-Glu, making $CaCl_2$ a more cost-effective option for increasing GABA content. When comparing the equipment cost and convenient operation of HPP and ultrasonic treatments, ultrasonic treatment was significantly less expensive to operate [37]. Considering production costs, subsequent research recommended

0.1% $CaCl_2$ paired with ultrasonic treatment at a power intensity of 400 W for 20 min for germinated brown rice.

Table 5 shows an analysis of antioxidant activity components and DPPH radical scavenging capacity in germinated brown rice after HPP and ultrasonic treatment. While HPP resulted in a total phenolic content of 0.89–1.00 mg gallic acid equivalent/g, ultrasonic treatment significantly increased the content to 1.06–1.08 mg gallic acid equivalent/g. The flavonoid content remained similar at 3.94–5.15 µg quercetin equivalent/g after both HPP and ultrasonic treatments. The DPPH radical scavenging capacity improved to 93% after ultrasonic treatment, indicating that ultrasonic processing enhances the antioxidant capacity of germinated brown rice.

Table 4. Effects of various soaking solutions with HPP or ultrasonic treatments on GABA content of germinated brown rice.

Germination Treatment	GABA (mg/100 g)
Ungermination	7.10 ± 2.53^{f}
Water	22.33 ± 7.87^{e}
Soaking/HPP (100 MPa, 10 min)	
Water	67.17 ± 3.99^{d}
0.1% $CaCl_2$	83.10 ± 1.65^{c}
0.1% Glu	64.29 ± 2.38^{d}
0.2% $CaCl_2$	62.58 ± 3.34^{d}
0.2% Glu	62.62 ± 7.91^{d}
Soaking/ultrasonic treatment (400 W, 20 min)	
Water	84.85 ± 1.45^{c}
0.1% $CaCl_2$	102.38 ± 6.53^{ab}
0.1% Glu	96.73 ± 11.28^{b}
0.2% $CaCl_2$	81.24 ± 5.48^{c}
0.2% Glu	110.88 ± 6.02^{a}

Data were expressed as mean and error bars of ± S.D. (n = 3). Means with different superscript letters in the same column are significantly different ($p < 0.05$).

Table 5. Effects of HPP and ultrasonic treatment on the content of antioxidants in germinated brown rice.

Germination Treatment	Total Polyphenols (mg Gallic Acid Equivalent/g)	Flavonoids (µg Quercetin Equivalent/g)	Scavenging DPPH Free Radicals (%)
Ungermination	0.91 ± 0.01^{c}	3.68 ± 0.00^{c}	87.01 ± 0.11^{d}
Water	0.84 ± 0.01^{d}	4.41 ± 0.00^{abc}	84.61 ± 2.61^{e}
Soaking/HPP (100 MPa, 10 min)			
Water	0.90 ± 0.02^{c}	3.92 ± 0.42^{c}	86.62 ± 1.23^{d}
0.1% $CaCl_2$	0.94 ± 0.01^{c}	5.15 ± 1.27^{a}	89.58 ± 0.71^{c}
0.1% Glu	0.89 ± 0.04^{c}	3.68 ± 0.00^{c}	86.83 ± 1.48^{d}
0.2% $CaCl_2$	0.93 ± 0.02^{c}	3.92 ± 0.74^{c}	89.14 ± 0.05^{c}
0.2% Glu	1.00 ± 0.06^{b}	4.90 ± 0.42^{ab}	86.33 ± 0.44^{d}
Soaking/ultrasonic treatment (400 W, 20 min)			
Water	1.06 ± 0.02^{a}	3.92 ± 0.42^{c}	92.50 ± 0.51^{ab}
0.1% $CaCl_2$	1.06 ± 0.01^{a}	3.94 ± 0.00^{c}	93.10 ± 0.10^{ab}
0.1% Glu	1.08 ± 0.03^{a}	3.92 ± 0.42^{c}	93.21 ± 0.09^{a}
0.2% $CaCl_2$	1.08 ± 0.02^{a}	4.15 ± 0.46^{bc}	93.08 ± 0.08^{a}
0.2% Glu	1.06 ± 0.01^{a}	4.41 ± 0.00^{abc}	93.00 ± 0.21^{a}
Vitamin C			91.72 ± 0.00^{ab}
BHA			91.06 ± 0.00^{b}

Data were expressed as mean and error bars of ± S.D. (n = 3). Means with different superscript letters in the same column are significantly different ($p < 0.05$). The concentrations of the sample, vitamin C, and BHA for the DPPH assay were 20 mg/mL.

3.4. Effect of Pretreatments on the Quality of Germinated Brown Rice

To understand whether the quality of germinated brown rice changes under different treatments. Table 6 shows the color appearance of germinated brown rice after soaking, and combined with HPP and ultrasonic treatment. The L* value remained around 55.65 for all treatments, while the a* and b* values showed slight increases after germination. The △E of brown rice and various soaked and germinated brown rice was 1.1~1.98, while the △E of HPP treatment slightly increased to 3.24~4.07, and the △E of ultrasonic treatment increased to 2.18~3.48. However, there were minor variations in color appearance between different pretreatments and ungerminated brown rice.

Table 6. Color of germinated brown rice by different pretreatments.

Germination Treatment	L*	a*	b*	△E
Ungermination	55.33 ± 1.34 ^{bcd}	2.19 ± 0.21 ^{d}	16.92 ± 1.15 ^{d}	0
Water	55.65 ± 0.13 ^{bcd}	2.58 ± 0.45 ^{cd}	18.83 ± 2.15 ^{bc}	1.98
Soaking				
0.1% $CaCl_2$	56.16 ± 0.73 ^{bcd}	3.01 ± 0.32 ^{abcd}	17.62 ± 1.22 ^{cd}	1.36
0.1% Glu	54.55 ± 1.97 ^{cd}	2.93 ± 0.34 ^{abcd}	16.65 ± 0.42 ^{d}	1.11
0.2% $CaCl_2$	54.62 ± 1.16 ^{cd}	2.86 ± 0.45 ^{bcd}	17.35 ± 0.67 ^{cd}	1.07
0.2% Glu	54.21 ± 0.79 ^{d}	3.22 ± 0.32 ^{abc}	17.34 ± 0.52 ^{cd}	1.58
Soaking/HPP (100 MPa, 10 min)				
Water	56.77 ± 1.21 ^{abc}	3.69 ± 1.22 ^{ab}	18.20 ± 1.22 ^{bcd}	2.44
0.1% $CaCl_2$	58.92 ± 1.70 ^{a}	2.47 ± 0.33 ^{cd}	17.72 ± 1.26 ^{cd}	3.69
0.1% Glu	56.18 ± 0.94 ^{bcd}	3.91 ± 0.30 ^{a}	19.53 ± 0.56 ^{ab}	3.24
0.2% $CaCl_2$	55.88 ± 1.04 ^{bcd}	3.70 ± 0.66 ^{ab}	20.66 ± 0.77 ^{a}	4.07
0.2% Glu	57.12 ± 0.79 ^{ab}	3.71 ± 0.79 ^{ab}	19.79 ± 0.63 ^{ab}	3.71
Soaking/ultrasonic treatment (400 W, 20 min)				
Water	55.81 ± 1.43 ^{bcd}	3.12 ± 0.58 ^{abcd}	17.67 ± 0.24 ^{cd}	1.29
0.1% $CaCl_2$	56.12 ± 1.45 ^{bcd}	3.06 ± 0.55 ^{abcd}	19.00 ± 0.37 ^{abc}	2.39
0.1% Glu	55.92 ± 0.16 ^{bcd}	3.22 ± 0.48 ^{abc}	18.85 ± 0.66 ^{bc}	2.27
0.2% $CaCl_2$	57.31 ± 2.17 ^{ab}	3.21 ± 0.17 ^{abc}	19.59 ± 1.06 ^{ab}	3.48
0.2% Glu	55.96 ± 0.76 ^{bcd}	2.60 ± 0.05 ^{cd}	18.97 ± 0.12 ^{abc}	2.18

Data were expressed as mean and error bars of ± S.D. (n = 6). Means with different superscript letters in the same column are significantly different ($p < 0.05$).

Because the sensory evaluation of rice requires a large amount of people and time, the taste score values can be used to quickly determine taste quality. The taste analyzer utilizes near-infrared reflectance measurements of key rice ingredients like amylose starch, protein, water, and fatty acids to convert them into a taste value [38]. Table 7 shows the results of the taste evaluation of germinated brown rice using a taste analyzer. The protein content ranged from 5.7 to 7.9, while the amylose content ranged from 18.8% to 17.2%. The fatty acid content varied from 12 to 21 mg/100 mg KOH after various pretreatments. The pretreatment of germinated brown rice affected the composition changes and taste value, the ultrasonic treatment resulted in higher taste values. The group of 0.1% $CaCl_2$ with ultrasonic treatment germinated brown rice has lower protein content and the highest taste value.

Amylose content significantly influences rice quality, with low levels typically associated with higher taste quality [38]. Shi et al. [39] conducted a taste examination of seven different rice varieties to investigate the effect of nitrogen fertilizer usage during rice cultivation on taste scores, and higher taste scores were found to be negatively correlated with protein content.

Table 7. Analysis of the taste profile of germinated brown rice under different soaking treatments.

Germination Treatment	Moisture (%)	Protein (%)	Amylose (%)	Fatty Acid (mg/100 mg KOH)	Taste Value
Ungermination	14.5	6.9	18.8	19	78
Water	14.8	7.9	18.2	14	70
0.1% $CaCl_2$	14.9	7.8	18.8	16	69
0.1% $CaCl_2$ + HPP	14.5	7.8	18.3	12	71
0.1% $CaCl_2$ + US	13.2	5.7	17.2	21	87

Data were expressed as mean (n = 3).

4. Conclusions

In this study, germinated brown rice was treated with various soaking solutions (0.1% $CaCl_2$, 0.1% Glu, 0.2% $CaCl_2$, and 0.2% Glu). The results showed that higher concentrations (0.2%) of the soaking solutions decreased the GABA content. Subsequently, combination treatments with HPP or ultrasonic treatment were applied. Ultrasonic processing for 20 min resulted in higher GABA contents, reaching 84.85~110.88 mg/100 g, compared to HPP (100 MPa, 10 min), which reached 62.58~83.10 mg/100 g. Additionally, the combination of 0.1% $CaCl_2$ pretreatment followed by ultrasonic treatment significantly increased total polyphenols, flavonoids, and DPPH radical scavenging activity, and enhanced taste value. Considering the equipment cost, ultrasonic treatment is more economical and convenient than HPP, making it a preferable method for enhancing the nutritional quality of germinated brown rice.

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