



Germination conditions for brown rice (*Oryza sativa* L.) and its application in beverage production

Do Viet Phuong^{ORCID}, Nguyen Khac Kiem*^{ORCID}

Industrial University of Ho Chi Minh City^{ORCID}, Ho Chi Minh, Vietnam

e-mail: nguyenkhackiem@iuh.edu.vn

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Abstract:

Sprouted brown rice (*Oryza sativa* L.) has significantly higher levels of bioactive compounds than unsprouted rice, such as phenolics, γ -aminobutyric acid, and vitamins. This study aimed to optimize processing parameters to develop a germinated brown rice beverage combined with green tea that has superior sensory quality, nutritional value, and storage stability. The main parameters under study included soaking time (0–24 h) and germination time (0–4 days). After germination, the rice was subjected to high-temperature treatment at 180°C for different durations (4–16 min). The extraction time using water as the solvent ranged from 5 to 20 min, and the mixing ratios of germinated rice extract and green tea were 4:6, 5:5, and 6:4. Based on the results, the optimal conditions for producing high-quality products were: soaking for 12 h to achieve maximum moisture content, germinating for 3 days to maximize germination rate and polyphenol content, high-temperature treatment for 8 min to achieve the desired color and sensory characteristics, extracting for 15 min, and a 6:4 mixing ratio (germinated rice:green tea) for a balanced and acceptable flavor. The resulting beverage showed stability when stored at 30°C, retaining approximately 70% of the total polyphenol content after 72.68 days, as predicted by the Q_{10} shelf-life model. These findings provide a scientific basis for developing functional beverages derived from sprouted brown rice with enhanced biological properties and extended shelf life.

Keywords: Beverage industry, brown rice, *Oryza sativa* L., GABA, germination, sprouting

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INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important and widely cultivated food crops in Asia. It is also the primary food source for more than half of the global population [1]. The traditional rice processing method typically removes the husk and germ, resulting in a significant loss of vitamins, minerals, fiber, and bioactive compounds [2].

To avoid these losses, rice grains with their husk and germ intact are produced and called brown rice. Brown rice contains higher levels of protein, lipids, vitamins (such as B₁, B₂, E), minerals (iron, calcium, magnesium), and bioactive compounds (phenolics, γ -oryzanol, and ferulic acid) compared to white rice [3, 4]. However, brown rice is rarely used due to its hard texture, long cooking time, and unappealing sensory characteristics [5]. Therefore, the sprouting of brown rice grains has been incorporated into processing to overcome these limitations and enhance the nutritional value and functionality of brown rice, particularly its biological activity.

Germinated brown rice is produced by soaking brown rice grains in water under carefully controlled conditions (25–35°C for 24–72 h) followed by moist incubation to stimulate germination [6]. This process undergoes biochemical changes while activating certain hydrolytic enzymes such as amylase, protease, and lipase. These enzymes help break down complex macromolecules such as proteins, starches, and lipids into simpler forms such as oligosaccharides, amino acids, and free fatty acids [1], while synthesizing new bioactive compounds [7]. Once brown rice seeds have germinated, the seed structure becomes softer, improving digestibility and providing some rare bioactive compounds such as γ -aminobutyric acid, also known as GABA. This is what makes germinated brown rice a valuable functional food [2].

GABA is a non-protein amino acid synthesized from glutamate through the action of glutamate decarboxylase [2]. Its concentration in germinated brown rice can increase tenfold compared to ungerminated brown rice,

reaching 0.1–0.5 mg/g depending on germination conditions [4, 7]. GABA offers numerous physiological benefits, including blood pressure-lowering effects, by inhibiting angiotensin-converting enzyme. Additionally, GABA supports stress reduction and improved sleep. Finally, its neuroprotective effects reduce the risk of Alzheimer's disease and anxiety disorders [1].

Brown rice typically has various pigments (purple, red, black, and brown) due to the presence of bioactive plant compounds such as flavonoids and other phenolic compounds, particularly anthocyanins. During grain development, these important metabolites accumulate in the outer layer, thus forming the color of brown rice [8]. Anthocyanins, which are mainly concentrated in the bran layer, provide brown rice with antioxidant effects. The types of anthocyanins found in brown rice are cyanidin-3-glucoside and peonidin-3-glucoside, with their contents ranging from 150 to 500 mg/100 g dry matter [9].

Anthocyanins in brown rice have attracted considerable attention due to their potential applications in functional foods and nutritional products. These compounds play important roles in the human body, such as reducing blood lipids, lowering blood pressure, reducing the risk of neurodegenerative diseases, regulating immunity, and fighting inflammation [10]. In brown rice, these phenolics are typically bound to cell wall polysaccharides and are released during germination. Their free radical scavenging capacity protects against oxidative stress-related diseases such as cancer, cardiovascular disease, and diabetes [1, 6].

To achieve the highest levels of bioactive compounds and nutrients in germinated brown rice, the germination process must be thoroughly studied, with factors such as time, temperature, light, and oxygen during soaking and germination. Additionally, different types of brown rice yield different results. For example, Lin *et al.* [7], who germinated two Taiwanese rice varieties (TJ-9 and TCI-10) in sealed containers in the dark at 36°C for 72 h, reported a GABA content of up to 0.48 mg/100 g and Trolox equivalent antioxidant activity being 70% higher than in non-germinated brown rice. Similarly, Pal *et al.* [4] compared five Indian rice varieties (PB1, PS44, PB1509, PB1121, PS5) and found that germination increased GABA, essential amino acids (histidine, arginine, methionine), and phenolics, with differences depending on the variety (e.g., PB1121 showed the greatest improvement in protein and foam stability).

In addition to environmental factors, the method of brown rice pre-treatment plays a crucial role in germination. The thin but fairly hard silk coat hinders the seed soaking and germination process and therefore needs to be softened. One of the most researched and widely used methods for removing this silk layer involves using enzymes to break down cell walls and increase the release of bound phenolics and GABA. Chamsai & Wanyo [6] optimized this process by using a combination of enzymes and ultrasonic-assisted pretreatment, which increased GABA and phenolics by 115.74 and 128.53%, respectively. In addition, Junyusen *et al.* [5] found that

autoclave treatment after germination at 115°C for 20 min increased GABA and ferulic acid while enhancing palatability and reducing blood glucose responses in patients with metabolic syndrome.

Overall, previous studies have demonstrated a strong correlation between the germination of brown rice and its antioxidant activity, bioactive compounds, nutrition, and sensory properties. However, there is still a lack of standardization in germination conditions among brown rice varieties, as well as limited applications of germinated brown rice. Although incorporated into bread, tea, and fermented products to improve shelf life and nutritional value [2], its potential in beverage production remains largely untapped. Beverages based on germinated brown rice could be a convenient, non-dairy option rich in GABA and antioxidants, meeting the demand for functional beverages that support mental health, metabolic balance, and immunity [1].

This study aimed to optimize parameters such as soaking time, temperature, light, and humidity for red brown rice grains in order to maximize polyphenols in germinated brown rice. We also explored the potential application of germinated brown rice in beverage production, evaluating the sensory characteristics, nutritional properties, and shelf life of beverages made from germinated brown rice.

STUDY OBJECTS AND METHODS

Materials. The brown rice (*Oryza sativa* L.) used in this study is red brown rice, one of the most nutritious types of brown rice, second only to purple brown rice (Fig. 1). The rice was grown and harvested in the Mekong Delta, Vietnam. After harvesting, the rice grains were preliminarily milled to remove the husk, retaining the bran layer and germ, then dried at 40°C to a moisture content of 12–14% and stored in sealed PE bags at 4°C until use.

The brown rice grains selected for this study were of medium size (5 to 6 mm long), had a reddish-brown husk but pink endosperm, and were shiny, fragrant, and free from pests.

Chemicals and equipment. The chemicals used in the study included gallic acid (Sigma-Aldrich, Singapore); Folin-Ciocalteu's phenol reagent (Merck, Germany); sodium carbonate, methanol, and ethyl acetate (China).

The equipment included a UV-Vis spectrophotometer to measure absorbance (Genesys 10S, Thermo Scientific);



Figure 1 Red brown rice

a Konica Minolta colorimeter, model CR-410 (Konica, Japan); and an aging cabinet, model TK120 (Nuve, Turkey).

Germination method and determination of germination rate. The germination method and determination of the germination rate followed the ISTA (International Seed Testing Association) method¹.

Procedure. Step 1. The rice grains were cleaned by rinsing several times with sterile water.

Step 2. The rice grains were soaked in clean water under standard conditions: water/seed ratio (3:1 v/w), soaking temperature 25°C, soaking time 24 h.

Step 3. The sprouts were incubated under standard conditions: on damp paper, incubation temperature 25°C, incubation time 7 days, humidity 90–95% (mist spray daily), low light or dark, light ventilation twice a day.

Step 4. The germination rate, %, was determined by using the formula (1). For this, the number of germinated seeds was counted following germination. A seed was considered germinated if the root length was ≥ 10 –20 mm and the sprout length was ≥ 5 mm.

$$\text{Germination rate} = \text{Number of germinated seeds} \times \frac{100}{\text{Total number of seeds}} \quad (1)$$

Grain color determination. Principle. Each organic compound absorbs and reflects light from its surface when illuminated by a light source. The Minolta CR410 colorimeter measures the light reflectance spectrum of the sample to calculate the color coordinates L^* , a^* , and b^* in the CIE Lab color space².

In this system, L^* represents the brightness-darkness correlation, with values ranging from 0 (black) to 100 (white); a^* represents the red-green correlation ($+a$ is red, $-a$ is green), and b^* represents the yellow-blue correlation ($+b$ is yellow, $-b$ is blue). The colors in the Lab* coordinate system are highly correlated with human visual perception [11].

Procedure. Step 1. Samples were prepared by weighing 30 g of seeds per sample and spreading them evenly on a white, flat, smooth, and scratch-free surface.

Step 2. The measuring device was calibrated according to the manufacturer's instructions, including checking and replacing (if necessary) the batteries, as well as inspecting the electrical and optical connections. The device was then calibrated using a standard white plate before each new sample measurement.

Step 3. The SCI (Specular Component Included) measurement mode was selected, with D65/10° light source and 50 mm aperture.

Step 4. For measurement, a sample was placed in a fixed position on the sample holder, ensuring that the measurement surface was perpendicular to the direction of light. The measurement was started by pressing the "Start" button or the corresponding start button on the

colorimeter. The measurement results displayed on the screen were recorded or printed out. At least 3–5 random points were measured on the sample to calculate the average $\pm$ SD.

Step 5. The results were read and stored, including L^* (0–100), a^* (–128 to +128), and b^* (–128 to +128).

Formula for calculating color difference:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (2)$$

where $\Delta L^* = L^*_2 - L^*_1$ is the brightness difference; $\Delta a^* = a^*_2 - a^*_1$ is the red-green difference; $\Delta b^* = b^*_2 - b^*_1$ is the yellow-blue difference.

Polyphenol content determination. Sample preparation. After drying, the sprouted rice was ground into a fine powder and sieved through a 0.1–0.5 mm mesh. Then, 1 g was weighed and 8 mL of ethanol (70%) was measured in 24 h. Following centrifugation in the dark at 5,000 rpm, the supernatant was collected as the sample solution [12].

In separate graduated test tubes, 1 mL of gallic acid standard solution, 1 mL of distilled water, and 1 mL of diluted tea extract were added using a pipette (each step performed twice for accuracy). Then, 5 mL of Folin-Ciocalteu's reagent was added to each tube and mixed thoroughly. After 3–8 min, 4 mL of sodium carbonate solution was added to each tube. The tubes were capped, mixed again, and left to stand at room temperature for 60 min. Absorbance was then measured at 765 nm using a UV-Vis spectrophotometer [13]. The content of polyphenols, mg GAE/g, was calculated using Eq. (3).

$$\text{Polyphenol content} = \frac{Cx}{C} \times \frac{1}{a} \times \frac{Vdm}{100 - W} \times K \quad (3)$$

where Cx is the total polyphenol content in the extract calculated from the standard curve, $\mu\text{g/mL}$; a is the weight of the sample soaked in the extract, g; C is the conversion value in ppm, $C = 10^3$; Vdm is the volume of the extract after titration, mL; k is the dilution factor (if any); and W is the sample moisture content, %.

Moisture determination. Moisture content was determined by the constant-weight drying method at 105°C [14].

Sensory evaluation. Principle. We used a standard method for sensory evaluation of food products, applicable to the evaluation of all sensory attributes or individual attributes (appearance, color, odor, taste) of each type of product and commodity (Table 1).

The method was based on evaluating sensory perceptions according to their type and intensity.

Sensory criteria or individual criteria for the product used a 20-point system based on a unified 6-level 5-point scale (from 0 to 5), in which: 5 points is the highest score for the criteria and 0 points is the lowest score for a product that is "damaged" and unusable (Table 2).

Procedure. The samples, as well as the reference samples, were prepared for sensory evaluation. The testing tools were pens and paper. The sensory evaluation panel consisted of 10 members. Each panel member evaluated each criterion individually according to the standard

¹ ISTA. International Rules for Seed Testing. International Seed Testing Association. 2023.

² Minolta K. CR-410 Chroma Meter User Manual. 2024. 156 p.

Table 1 Building evaluation terminology

Sensory criteria	Description
Color	Dark orange-yellow, clear
Aroma	Natural aroma of rice and tea, harmonious and distinctive
Flavor	Sweet and slightly bitter taste of tea, pleasant

Table 2 Scores and detailed descriptions for sensory criteria

Score	Color	Aroma	Flavor
5	Clear, no cloudiness, characteristic color	The natural aroma of sprouted rice and tea is harmonious, impressive, distinctive, and free of any strange odors	A harmonious blend of the product's characteristic sweetness and very light bitterness. A pleasant, sweet aftertaste
4	Clear, little sediment, characteristic color	The natural aroma of rice and tea is quite harmonious, distinctive, and free of any strange odors	Relatively harmonious, no strange flavors
3	Fairly clear, little sediment, no characteristic color	Natural aroma of rice and tea, with a stronger scent of rice or tea but not too pronounced, no strange odors	Quite harmonious, with a slight bitterness
2	Still cloudy, sediment present, no characteristic color	Light aroma, with tea and rice scents not overly pronounced	Not harmonious, with bitterness
1	Very cloudy, much sediment present, no characteristic color	Poor aroma (or no aroma) or overly strong scent of rice or tea but not overly pronounced, with strange odors	No characteristic flavor, with a strange flavor characteristic of an ingredient
0	Cloudy, with defective color	Obvious strange odors, defective aromas	A strange flavor causing an unpleasant sensation

scoring sheet alongside the product being tested, assigning scores from 0 to 5 using whole numbers. After each sample test, the panel members used odorless, tasteless water and food that did not affect sensitivity to restore their initial perception. During the test, the panel members were not allowed to engage in casual conversation or other activities. They took a break of 10–15 min after testing a group of the samples or 20–30 min after

Table 3 Importance levels of evaluation criteria

Criterion	Importance factor
Color	1.6
Aroma	1.2
Flavor	1.2

Table 4 Product quality classification

Quality classification	Common scores	Requirements for unweighted average scores for criteria
Good quality	18.6–20.0	Each criterion ≥ 4.8
Fair quality	15.2–18.5	Each criterion ≥ 3.8
Average quality	11.2–15.1	Each criterion ≥ 2.8
Poor quality (does not meet the quality standards specified in the standard but is still marketable)	7.2–11.1	Each criterion ≥ 1.8
Very poor quality (not marketable but can still be used after appropriate recycling)	4.0–7.1	Each criterion ≥ 1.0
Damaged (no longer usable)	0–3.9	–

testing half of the samples (Table 3). The weighted score was calculated using Eq. (4).

$$\text{Weighted score} = \text{Average score} \times \text{Importance coefficient} \quad (4)$$

The importance coefficient was taken from the national standard TCVN 3218:2012.

The total weighted points of all sensory criteria were calculated to obtain the overall score and classify product quality (Table 4).

Q_{10} thermal acceleration for shelf life evaluation.

The shelf life of food products was evaluated according to ISO 22000:2018 (clause 8.5.1.3 – characteristics of the final product).

Principle. Thermal acceleration (or accelerated shelf-life testing, ASLT) is based on the Q_{10} factor. This is one of the techniques for quickly determining the shelf life of food by accelerating the rate of chemical/biological reactions based on temperature, using the Arrhenius law and the Q_{10} factor. The Q_{10} factor represents change in the reaction rate when the temperature increases by 10°C [15].

Procedure. Step 1: defining the objective; Step 2: determining the decay regime; Step 3: identifying the important attributes to monitor (e.g., microbiological, chemical, sensory evaluation); Step 4: selecting the testing method; Step 5: setting storage conditions (e.g., temperature, humidity, light conditions); Step 6: setting the target endpoints and testing frequency; Step 7: identifying appropriate test and control samples; Step 8: conducting the shelf life study; Step 9: Analyze the results;

Step 10: monitoring and confirm the shelf life. The predicted shelf-life time under actual conditions was calculated using formula (5) [15].

$$t_{SL} = t \times Q_{10}^{\frac{T_s - T_r}{10}} \quad (5)$$

where t_{SL} is the predicted shelf-life time under actual conditions; t is the test time under accelerated conditions; T_s is the accelerated temperature, °C; T_r is the actual/storage temperature, °C; and Q_{10} is the thermal acceleration factor.

The Q_{10} factor was calculated using formula (6) [16]:

$$Q_{10} = \left(\frac{k_2}{k_1} \right)^{\frac{10}{T_2 - T_1}} \quad (6)$$

where k_1 , k_2 is the degradation rate constants at temperatures (T_1 , T_2); T_1 , T_2 is the test temperatures, °C.

The decay rate constant (k , day⁻¹) was calculated using the first-order kinetic model [17]:

$$\ln \frac{y_t}{y_0} = -k \times t \text{ or } k = \frac{-\ln \frac{y_t}{y_0}}{t} \quad (7)$$

where y_0 , y_t is the monitoring the value/target function at the initial time and at time (t); t is the test time.

Statistical analysis. The experiments were arranged in a randomized design and conducted in triplicate, with one or two varying factors. Analysis of variance (ANOVA) followed by LSD (Least Significant Difference) testing was used to determine statistical differences among the treatment means. The data were processed using Statgraphics Centurion (version 19.5), and Microsoft Excel was employed for calculating means and standard deviations. The results from preliminary experiments were used as fixed reference parameters for subsequent experimental trials.

RESULTS AND DISCUSSION

The effect of soaking time on the germination process. During the soaking process, enzymes transition from a dormant state to an active state, while the embryo's metabolic activities are restarted to prepare for germination [18]. Therefore, an experiment was conducted to determine the optimal soaking time for rice to achieve the appropriate moisture content for seed germination. For this, brown rice was soaked in water at a ratio of 1:2 at a temperature of 35°C for 0, 6, 12, 18, and 24 h. After soaking, the moisture content of the samples was determined (Fig. 2).

The initial water content in brown rice grains was 11.1%. When soaked in water, rice grains absorb water, significantly increasing their moisture content. In the first 6 hours, the moisture content increased from 11.18 to 26.72%, equivalent to an increase of 15.54%. This indicates a rapid initial water absorption rate, mainly due to the osmotic pressure difference between the grains and the soaking solution. However, after 12 h, the water

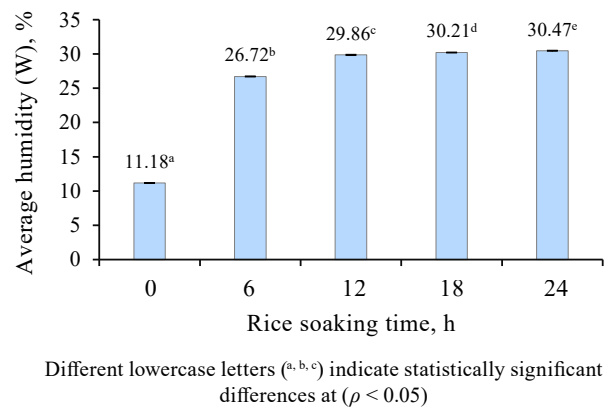


Figure 2 Effect of soaking time on the moisture content of red brown rice

absorption rate decreased significantly. From 6 to 12 h, the germination rate increased by only 3.14%, indicating that the effectiveness of prolonged soaking was no longer apparent. After 24 h of soaking, the moisture content increased insignificantly, indicating that the seeds had reached water saturation. Prolonged soaking not only increases the risk of harmful microbial contamination due to the moist environment that favors their growth but also leads to oxygen deficiency in the seeds, affecting respiration and reducing germination capacity [19].

According to Khang *et al.* [20], the ideal moisture content for brown rice seeds to germinate effectively is between 25–35%. When rice grains reach this moisture level, the physiological processes within the grain, particularly the activity of starch-hydrolyzing enzymes, are optimally activated. This facilitates the conversion of starch into sugar, providing energy for seedling development. Based on the research results, a soaking time of 12 h was recommended as the most suitable.

The effect of germination time on sprout length.

To determine the effect of germination time on sprout length, brown rice grains were cleaned, soaked for 12 h, and germinated at a water:rice ratio of 1:1. The germination process was carried out at approximately 30–35°C (optimal for germination), with the samples incubated for 0, 1, 2, 3, and 4 days (changing the water every 6 h) [2]. The samples were measured for sprout length, germination rate, and polyphenol content (Fig. 3–6).

According to Yan *et al.* [19], the endosperm is the nutritive tissue that nourishes the embryo and provides it with nutrients to develop into a seedling when the seed germinates. According to our results, the 0–3 day germination period showed a significant difference in sprout length. Specifically, at 0, 1, and 2 days of germination, the grain lengths were 0, 6.50, and 9.1 mm, respectively (a significant increase). At 3 to 4 days, the grain lengths reached 12.10 and 14 mm, respectively, showing a negligible difference in sprout length. However, at 3 days of incubation, the sprouts had fully developed. If the rice is further incubated for 4 days, cotyledon will begin to form, and once the rice grains develop cotyledon, the nutrient content will be significantly reduced.

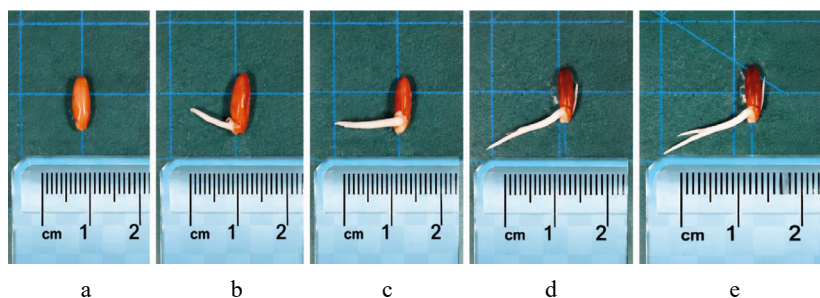
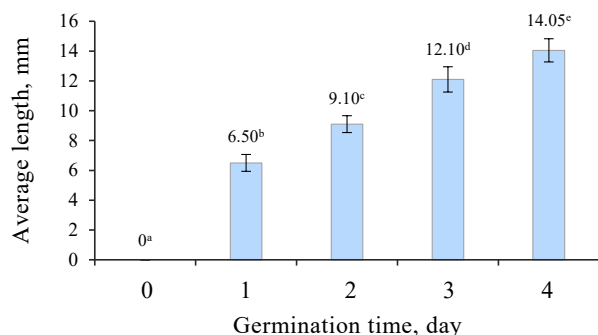
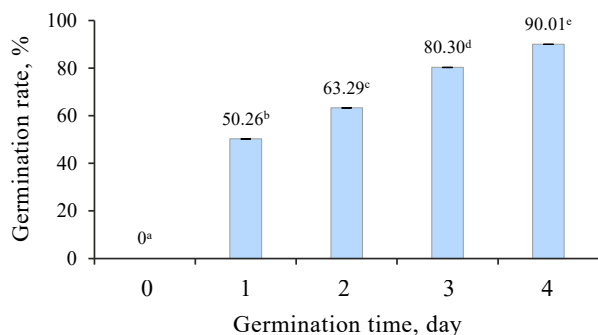


Figure 3 Sprout length during germination periods: 0 days (a); 1 day (b); 2 days (c); 3 days (d); and 4 days (e)



Different lowercase letters (^{a, b, c}) indicate statistically significant differences at ($p < 0.05$)

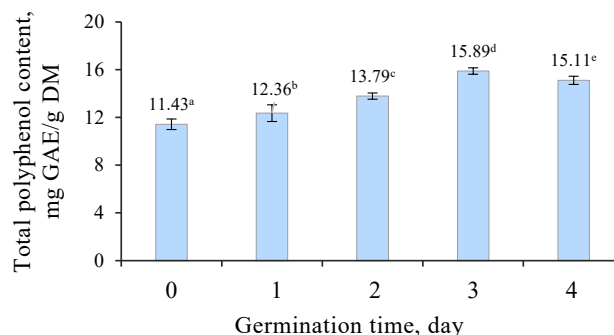
Figure 4 Effect of germination time on sprout length



Different lowercase letters (^{a, b, c}) indicate statistically significant differences at ($p < 0.05$)

Figure 5 Effect of germination time on germination rate

The germination rate of brown rice was strongly influenced by soaking and germination conditions. In particular, the germination rate increased progressively with the germination time, indicating that water absorption and metabolic activation promoted embryo development and radicle emergence. According to our results, the rice grains had the best germination rate when incubated for 4 days. However, by that time, they had already begun to develop cotyledon. As the cotyledon develops, the nutrients in the rice are depleted because they are used to nourish the cotyledon [2]. Therefore, rice should only be incubated for 3 days to achieve the best quality sprouts. Similar findings were reported



Different lowercase letters (^{a, b, c}) indicate statistically significant differences at ($p < 0.05$)

Figure 6 Effect of germination time on total polyphenol content

by Rattanamechaikul *et al.* [21], who demonstrated that optimized moisture conditioning achieved a germination rate of 91.33%, which was significantly higher than that achieved by the conventional soaking method (84.97%).

According to Fig. 4, polyphenols tended to increase significantly in the samples from day 0 to 3 and then decreased slightly in the day 4 sample.

Specifically, the polyphenol content gradually increased from the lowest level of 11.43 mg GAE/g DM in the day 0 sample. This is entirely reasonable since the rice grains had not absorbed sufficient moisture to begin germination. According to Chinma *et al.* [22], water deficiency limits the conversion of bioactive compounds within the grains, including polyphenols, although a significant amount of polyphenols is still concentrated in the bran layer.

Our results showed that the polyphenol content in brown rice reached its highest level (15.89 mg GAE/g DM) in the day 3 sample. This increase indicated the strong activity of cell wall-degrading enzymes [23]. In the early stages of germination, the enzymes were not yet fully stabilized, so the increase in polyphenols was slower. These results are consistent with the previous studies by Wu *et al.* [24] and Hu [25], which reported increased free polyphenol contents during brown rice germination through cell wall degradation. Furthermore, Hu [25] emphasized that germination not only increased the polyphenol content but also improved the overall nutritional value and bioactivity of brown rice.

The polyphenol content in the day 4 sample (15.11 mg GAE/g DM) decreased slightly compared to the day 3 sample. This slight decrease was due to losses during soaking and water replacement during germination. Some phenolic compounds dissolved and escaped into the water environment, leading to a decrease in the remaining phenolic content [26]. The combination of reduced synthesis rate and increased degradation rate led to a decrease in polyphenols in the day 4 sample.

The effect of high-temperature treatment on the quality of sprouted rice. Sprouted rice was subjected to high-temperature treatment at 180°C for 4, 8, 12, and 16 min (counting from the point when the grains reached 180°C). During high-temperature treatment, the rice was continuously stirred to ensure even and stable heat distribution. We aimed to investigate the effect of high-temperature treatment on the polyphenol content, color characteristics, and sensory quality of the final product (Fig. 7–9, Table 5).

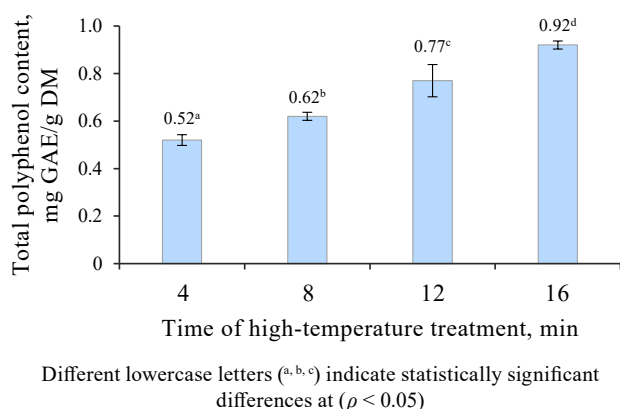


Figure 7 Effect of high-temperature treatment time on total polyphenol content in germinated brown rice

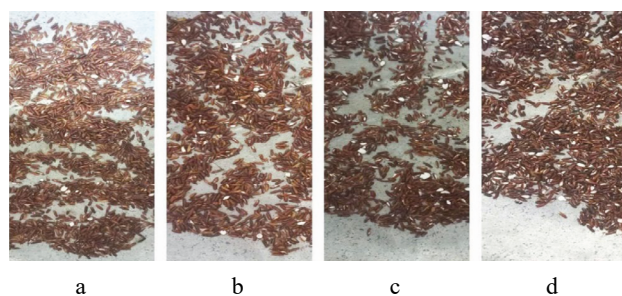


Figure 8 The color of germinated brown rice exposed to high-temperature treatment: 4 min (a); 8 min (b); 12 min (c); and 16 min (d)

Table 5 Color indices of germinated brown rice treated at high temperature for different durations

Sample (high-temperature treatment time)	L^*	a^*	b^*
Control sample	34.25 ± 0.10	4.75 ± 0.03	15.6 ± 0.06
Sample 1 (4 min)	$37.56^a \pm 0.11$	$5.35^a \pm 0.02$	$14.09^a \pm 0.04$
Sample 2 (8 min)	$35.51^b \pm 0.07$	$5.11^b \pm 0.03$	$13.92^b \pm 0.03$
Sample 3 (12 min)	$32.46^c \pm 0.11$	$4.74^c \pm 0.04$	$13.71^c \pm 0.02$
Sample 4 (16 min)	$28.73^d \pm 0.08$	$4.29^d \pm 0.02$	$13.41^d \pm 0.02$

Different lowercase letters (^{a,b,c}) indicate statistically significant differences at ($p < 0.05$)

We found that the total polyphenol content increased significantly throughout high-temperature treatment. Specifically, the total polyphenol content was the lowest in the 4-min sample at 0.52 mg GAE/g DM, increasing to 0.62 and 0.77 mg GAE/g DM in the 8- and 12-min samples, respectively, and reaching the highest level of 0.92 mg GAE/g DM in the 16-min sample.

According to Kang *et al.* [27], high-temperature treatment leads to the breakdown of internal tissue and the rapid release of phenolic compounds. Therefore, the total phenolic content tends to increase with temperature and time during the high-temperature treatment of sprouted rice. Duka *et al.* [28] reported that the increase in the polyphenol content is due to enzyme activation and the hydrolysis of polysaccharides bound to cell walls during high-temperature treatment, which breaks down starch in aerobic conditions and releases phenolic compounds. Alternatively, according to Zhao *et al.* [29], high-temperature treatment reduces enzyme activity (lipase and peroxidase) and increases the synthesis of phenolic compounds and antioxidant activity in the grains. However, recent observations by Yamuangmorn *et al.* [30] indicate that prolonged exposure to high temperatures (specifically, 150–200°C for 20 min) inhibits polyphenoloxidase enzyme activity and significantly reduces the polyphenol content in the rice. Additionally, if the exposure time to high temperatures is too short, it is insufficient to inhibit the activity of polyphenoloxidase. As a result, polyphenols form complexes with copper, which reduces the polyphenol content [31].

Sample 1: Compared to the control sample, Sample 1 showed a slight increase in L^* (lightness), an increase in

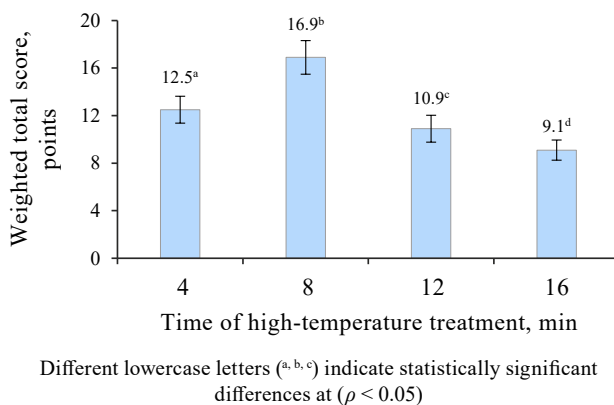


Figure 9 Effect of high-temperature treatment time on sensory evaluation of beverage products based on germinated brown rice

a^* (redness), and a decrease in b^* (yellowness), indicating a tendency toward a brighter, warmer, and slightly bluer color region.

Sample 2: Continued the trend of Sample 1, but the increase in redness slowed down and the decrease in brightness was also insignificant.

Sample 3: Compared to the control sample, brightness decreased significantly, redness decreases slightly, and yellowness also decreased, indicating that the sample shifted toward a darker, cooler, and bluer color region.

Sample 4: Continued the trend of Sample 3, but the L^* , a^* , and b^* values all decreased most significantly, indicating a darker and more opaque color.

The results clearly showed the color gradually shifting from light to dark, from the initial red and yellow hues to green and blue as high-temperature treatment time increased. This indicated that the time of exposure to high-temperature significantly affected the color of the product. Previous studies by Kang *et al.* [27] also showed a significant decrease in L^* , a^* , and b^* values after high-temperature treatment, while noting a significant increase in phenol and flavonoid contents. According to Fukui *et al.* [32], high-temperature treatment of rice at higher temperatures and for longer durations also affected the chemical composition of the product. This is manifested through the Maillard reaction between sugars and proteins during denaturation, resulting in a darker product color. Consequently, L^* , a^* , and b^* values decrease with increasing high-temperature treatment temperature and time. Additionally, the authors reported that the samples with darker colors after high-temperature treatment had higher antioxidant activity.

In addition to determining polyphenol levels and color of brown rice grains, we evaluated their sensory criteria to create beverage products that are both highly nutritious and delicious.

Based on the sensory evaluation results, the sample treated for 8 min had the highest total score of 16.9 and was rated as “fair” quality. At the 8-min high-temperature treatment time, the beverage exhibited the natural color and aroma of sprouted rice, along with its characteristic mild sweetness. This might be because high-temperature treatment for 8 min achieved a balance between developing aromatic compounds and preserving the inherent sensory characteristics of sprouted rice. This result is consistent with previous studies. For example, Shi *et al.* [33] showed that high-temperature treatment not only changed the color but also increased the content of aromatic compounds such as furan and pyrazine, contributing to the characteristic flavor of the product.

A comprehensive analysis of the data, including polyphenol content, color, and sensory characteristics, showed that high-temperature treatment for 8 min at 180°C is optimal for brown rice beverage products. Although the sample treated for 8 min did not achieve the highest polyphenol content, the difference in color compared to the control sample was insignificant and difficult to detect with the naked eye. More importantly, this sample achieved the highest average sensory score, out-

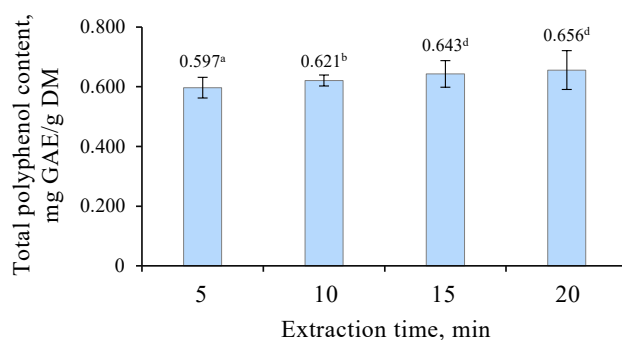
performing the other samples. This indicates that the 8-min high-temperature treatment time created an optimal balance between retaining health-promoting compounds and improving sensory characteristics, meeting both quality requirements and consumer preferences.

The effect of sprouted rice extraction time on beverage flavor. Sprouted rice, after being subjected to high-temperature treatment at 180°C for 8 min, was extracted with water at 85°C using a ratio of 50 g of sprouted rice to 300 mL for different time intervals (5, 10, 15, and 20 min) to produce samples with varying degrees of extraction. These samples were thoroughly analyzed for their polyphenol content, color changes, and sensory properties (Fig. 10–12, Table 6).

According to our results, the total polyphenol content tended to increase as the extraction time increased from 5 to 20 min. However, we found no statistically significant difference between the 15- and 20-min samples. Total polyphenols gradually increased from the 5-min sample to the 15-min one, reaching a maximum of 0.643 mg GAE/g DM in the 15-min sample. This result is consistent with the study by Turkmen *et al.* [34], which reported higher phenol contents after cooking or wet heating. According to Ferracane *et al.* [35], this increase was due to polyphenols being released from intracellular protein complexes, altering plant cell structure and inactivating polyphenol oxidase. The thermal treatment also prevented the oxidation and polymerization of polyphenols, contributing to higher total polyphenol content [36].

However, in the 20-min sample, the polyphenol content only increased slightly by 1.98%. Although polyphenols increased throughout the extraction process, some polyphenols were degraded or lost. This result is consistent with the study by Baeza-Jiménez *et al.* [37], which showed that high temperatures alter the structure of phenolic compounds, leading to a decrease in antioxidant capacity and the formation of complexes with proteins and carbohydrates, thereby limiting their biological activity.

Sample 1 (5 min): The L^* value increased, the a^* value decreased, and the b^* value increased compared to the control sample, indicating a shift toward a brighter, greener, and yellower color region.



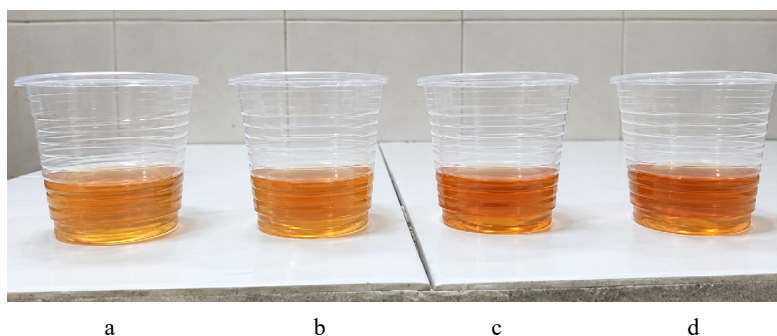
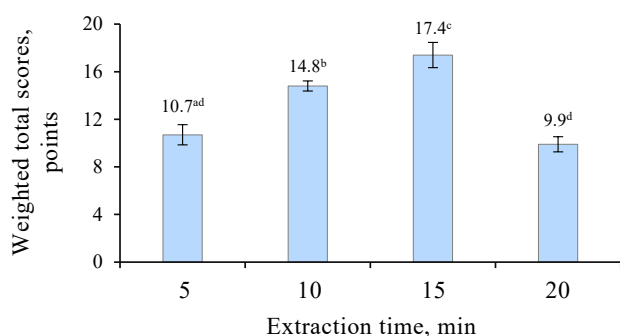
Different lowercase letters (a, b, c) indicate statistically significant differences at ($p < 0.05$)

Figure 10 Effect of extraction time on total polyphenols in germinated brown rice beverages

Table 6 Color changes in brown rice beverage samples at different extraction time

Sample	L^*	a^*	b^*
Control sample	34.25 ± 0.07	4.75 ± 0.05	15.60 ± 0.04
Sample 1 (5 min)	$37.86^d \pm 0.08$	$3.56^a \pm 0.09$	$15.65^a \pm 0.07$
Sample 2 (10 min)	$36.76^c \pm 0.09$	$3.82^b \pm 0.03$	$15.24^b \pm 0.06$
Sample 3 (15 min)	$34.48^b \pm 0.07$	$4.61^c \pm 0.03$	$14.79^c \pm 0.03$
Sample 4 (20 min)	$32.08^a \pm 0.09$	$5.73^d \pm 0.06$	$14.29^d \pm 0.02$

Different lowercase letters (^{a, b, c}) indicate statistically significant differences at ($p < 0.05$)

**Figure 11** Colors of extracts isolated from germinated brown rice: a – Sample 1; b – Sample 2; c – Sample 3; and d – Sample 4

Different lowercase letters (^{a, b, c}) indicate statistically significant differences at ($p < 0.05$)

Figure 12 Effect of extraction time on sensory evaluation of beverages from germinated brown rice

Sample 2 (10 min): Similar to Sample 1, but with a lower magnitude of the change.

Sample 3 (15 min): Insignificant color changes compared to the control.

Sample 4 (20 min): Distinct color changes compared to the previous samples. The L^* value decreased, the a^* value increased, and the b^* value decreased, indicating a shift toward darker, redder, and bluer colors.

According to Hayashi *et al.* [38], proanthocyanidin is the main pigment that determines the characteristic color of red rice. This group of compounds not only plays an important role in coloring but also possesses strong biological activity, especially antioxidant properties. However, thermal processing, especially cooking, causes significant changes in the color of rice.

Previous studies have shown that cooking alters the chemical structure of rice, leading to the release, polymerization, and oxidation of compounds, including proantho-

cyanidins [39]. These changes explain the color changes observed at different extraction times in our study.

At short extraction times (5–10 min), the color of the samples was still faint because proanthocyanidin had not been completely released from the rice grains. At 15-min extraction, the color reached its most stable level, indicating a balance between the release and degradation of proanthocyanidin. At 20-min extraction, the amount of proanthocyanidin was higher, but starch gelatinization reduced the clarity of the sample and made the color darker and cloudier. Starch gelatinization is a physicochemical phenomenon in which starch granules absorb water and swell, breaking down the original crystal structure. As the temperature increases, the gelatinization process occurs faster, affecting the viscosity and clarity of the product [40]. Thus, the extraction time significantly affected the color of the rice due to a complex interaction between proanthocyanidin release, starch gelatinization, and other chemical reactions that occur during thermal processing.

At the next stage, we conducted a sensory evaluation of four sprouted rice samples with different extraction times to select the sample that meets the sensory requirements for sprouted rice tea products.

According to sensory evaluation results, the 15-min sample had the highest sensory score of 17.4. The 15-min extraction time was sufficient to create the product's distinctive color, natural aroma of sprouted rice, along with a light sweetness and slight astringency.

Choosing the appropriate extraction time is important for the product. It greatly affects not only the sensory characteristics of the final product but also its nutritional value. However, Banchuen *et al.* [41] found that the extraction time of sprouted rice did not significantly affect the nutrients in the sprouted rice liquid.

Based on the results of polyphenol content analysis, sensory evaluation, and color assessment, we selected 15 min as the optimal extraction time. Although the polyphenol content was lower than that at 20-min extraction, the difference was insignificant. Furthermore, the 15-min extraction sample showed a color similar to the control sample and received the best sensory evaluation.

The effect of sprouted rice and green tea ratios on beverage quality. After extraction, tea and rice were blended at ratios of 4:6, 5:5, and 6:4. Sensory evaluation was then conducted to determine the preferred tea-to-rice blending ratio among consumers (Fig. 13 and 14).

The analysis results show that the mixing ratio of sprouted rice to green tea significantly affected the sensory characteristics of the product. The test samples showed statistically significant differences in color, aroma, and taste ($p < 0.05$). Among them, the sample with a 6:4 ratio achieved the highest average sensory score of 17.2, ranked as “fair” in quality, and was noted for its harmonious balance of sensory characteristics.

This indicates that the 6:4 mixing ratio is optimal for producing beverages with the best sensory quality. This result is important in determining the optimal product formula to meet the consumer demand.

Evaluation of product shelf life using the Q_{10} method.

After optimizing the production process, we determined the shelf life of the product by applying the accelerated thermal method. The product was placed in a chamber with high temperature and humidity (50 and 60°C, 85% RH) to simulate rapid aging. We monitored changes

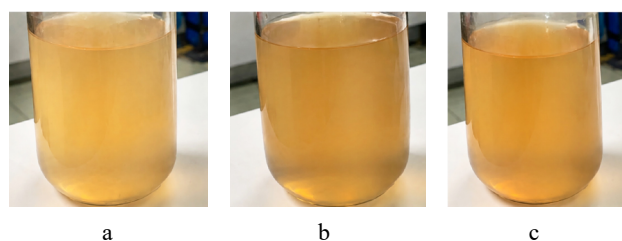
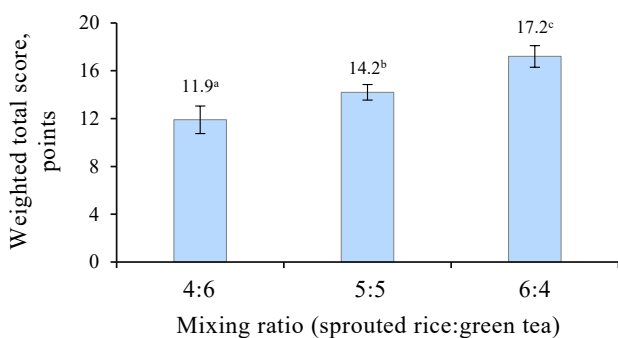


Figure 13 Colors of beverage samples blended from sprouted rice extract and green tea extract: a – Sample 1; b – Sample 2; and c – Sample 3



Different lowercase letters (^{a, b, c}) indicate statistically significant differences at ($p < 0.05$)

Figure 14 Effect of blending ratio on sensory evaluation scores for beverages from germinated brown rice

in quality indicators, total polyphenol content, and color parameters L^* , a^* , b^* over a period of 10 days (Tables 7 and 8).

The results showed significant color changes in the product over time. The brightness L^* gradually decreased, indicating that the product lost its initial brightness. At the same time, the a^* value tended to decrease slightly, shifting the color towards green, while the b^* value increased slightly, shifting the color towards yellow. These changes were visually observable as the product became darker and showed greater color differences.

The color changes occurred in two stages. The ΔE value increased continuously, indicating that the color difference between the initial sample and the samples measured at later times became increasingly large. In the first stage (days 1–4), the color of the product remained relatively stable, with no significant difference from the initial sample. However, from day 5 onwards, the color change accelerated. This was particularly noticeable from day 5 to day 8, although it was only visible to the naked eye for experienced observers.

According to Ananingsih *et al.* [42], oxidation combined with the effects of environmental factors such as temperature, humidity, and light was the main cause of

Table 7 Brown rice beverage color indices over thermal acceleration time

Sample	L^*	a^*	b^*
Initial sample	34.32 ± 0.05	4.84 ± 0.01	15.95 ± 0.02
Day 1	33.83 ± 0.04	4.77 ± 0.02	15.99 ± 0.02
Day 2	33.64 ± 0.03	4.69 ± 0.02	16.06 ± 0.02
Day 3	33.45 ± 0.03	4.63 ± 0.02	16.12 ± 0.02
Day 4	33.28 ± 0.04	4.55 ± 0.02	16.20 ± 0.01
Day 5	33.05 ± 0.07	4.46 ± 0.01	16.28 ± 0.01
Day 6	32.85 ± 0.04	4.36 ± 0.03	16.37 ± 0.01
Day 7	32.63 ± 0.03	4.24 ± 0.02	16.44 ± 0.02
Day 8	32.38 ± 0.03	4.11 ± 0.02	16.54 ± 0.02
Day 9	32.03 ± 0.02	4.06 ± 0.02	16.61 ± 0.02
Day 10	31.88 ± 0.03	4.01 ± 0.02	16.69 ± 0.02

Table 8 Total phenolic content, mg GAE/g DM, over thermal acceleration time

Acceleration time, days	Acceleration temperature	
	50°C	60°C
0	0.683 ± 0.050	0.683 ± 0.040
1	0.629 ± 0.040	0.620 ± 0.040
2	0.608 ± 0.050	0.572 ± 0.020
3	0.579 ± 0.030	0.501 ± 0.030
4	0.563 ± 0.020	0.447 ± 0.020
5	0.547 ± 0.020	0.417 ± 0.020
6	0.528 ± 0.010	0.391 ± 0.020
7	0.504 ± 0.030	0.355 ± 0.010
8	0.483 ± 0.040	0.301 ± 0.030
9	0.457 ± 0.010	0.253 ± 0.020
10	0.421 ± 0.010	0.212 ± 0.020

color changes in tea during storage. This phenomenon was clearly illustrated by the color change from bright green to dull greenish-brown in green tea.

Table 8 shows a significant decrease in the total phenolic content (TPC) in the brown rice beverage samples over time and with accelerated heat exposure. This decrease indicates that phenolic compounds, which play an important role in neutralizing free radicals, have undergone oxidation and degradation. This result is consistent with Abdullah & Mazlan [43], who showed that polyphenols are prone to oxidation during storage, especially when exposed to heat and polymerization reactions.

Changes in TPC are a criterion for evaluating the shelf life of the product. The accelerated testing time at 50 and 60°C was calculated based on the degree of TPC reduction to a safe level. According to studies, this safe level typically ranges from 20 to 30%, meaning that a TPC reduction to 70–80% is acceptable [44].

The Q_{10} quality index, which reflects the rate of product quality degradation over time, is used to estimate the shelf life. If the acceleration temperature increases by 10°C, the rate of degradation reaction increases 2–3 times.

To calculate Q_{10} , the TPC degradation rate constant had to be determined at accelerated temperatures of 50 and 60°C (k_1 and k_2 , respectively). The TPC degradation process follows the first-order Arrhenius reaction kinetics law. The TPC degradation rate constant was calculated as follows:

$$k_1 = \frac{-\ln \frac{y_1}{y_0}}{t_1} = \frac{-\ln \frac{0.478}{0.683}}{8.19} = 0.0435$$

$$\text{and } k_2 = \frac{-\ln \frac{y_2}{y_0}}{t_2} = \frac{-\ln \frac{0.478}{0.683}}{3.42} = 0.104 \quad (8)$$

$$Q_{10} = \left(\frac{k_2}{k_1} \right)^{\frac{10}{T_2 - T_1}} = \left(\frac{0.104}{0.0435} \right)^{\frac{10}{60 - 50}} = 2.395 \quad (9)$$

The shelf life of germinated brown rice beverages at 30°C was calculated as follows:

$$t_{SL} = \left(\frac{k_2}{k_1} \right)^{\frac{10}{T_2 - T_1}} = 8.18 \times 2.395^{\frac{50 - 30}{10}} = 72.68 \text{ days} \quad (10)$$

Thus, if brown rice beverage is stored at room temperature (30°C), its TPC will decrease by 30% after 72.68 days, while storing in the refrigerator (12°C) will extend the expected shelf life to 226 days.

To verify the predicted shelf life values from the Q_{10} model against experimental values, a verification experiment was conducted (Table 9).

According to the prediction model (Fig. 15), the remaining TPC was 0.478 mg GAE/g DM (after 72.68 days of storage at 30°C), while according to the experiment,

Table 9 Experimental verification results of total phenolic content at 30°C

Time, days	Total phenolic content, mg GAE/g DM	% Total phenolic content remaining
0	0.683	100.00
72.68*	0.471	68.96

* 72.68 days = 72 days + 41 min

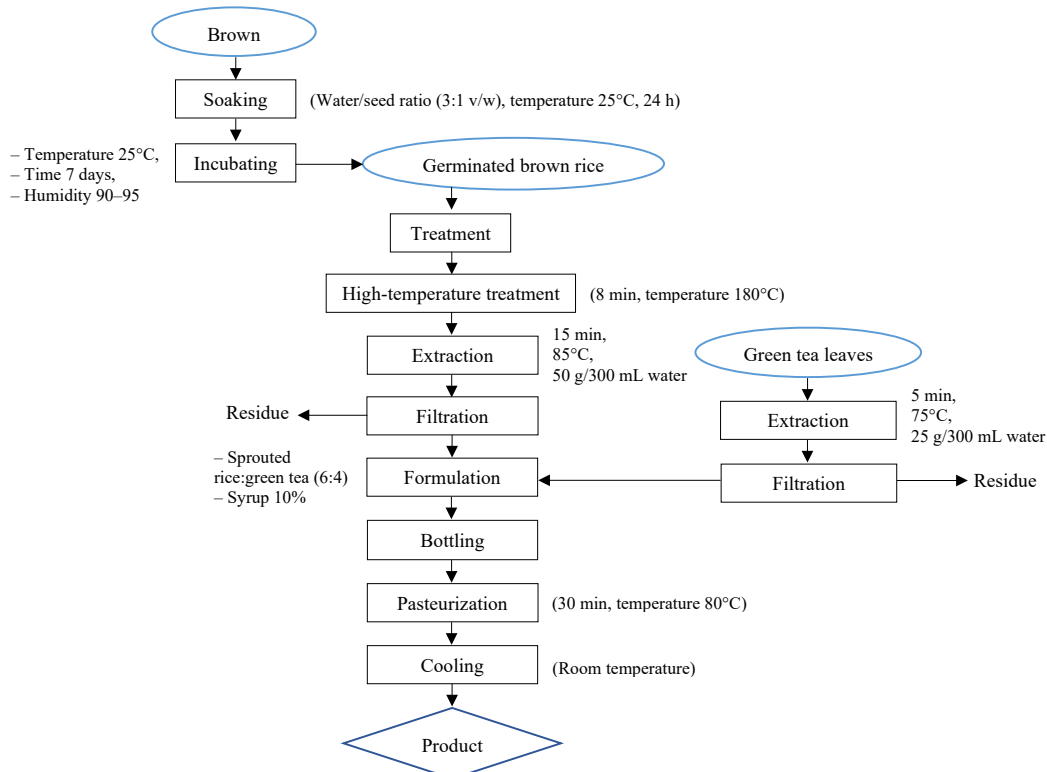


Figure 15 Flowchart of the process for making beverages from germinated brown rice

the remaining TPC was 0.471 mg GAE/g DM, with a result deviation of −1.46%. These verification results show that the longer the storage time, the more the structure and polyphenol composition change. Polyphenols are sensitive antioxidants, so during storage, some polyphenols may participate in oxidase reactions to protect the extract from attack by oxidizing agents present in the sample. According to Gillooly *et al.* [45], the reaction rate is proportional to temperature. Ouaabou *et al.* [46] and Low & Ishida [47] reported that higher storage temperatures lead to higher thermal degradation rates of phenolic compounds. Polyphenolic compounds have been shown to degrade more when exposed to sunlight, temperatures above 40°C, oxygen, and high humidity [48, 49]. However, the deviation of the constructed model from the initial prediction is not significant and can be applied in practice.

CONCLUSION

Our study successfully optimized the germination conditions for brown rice. Specifically, soaking the rice for 12 h achieves maximum moisture content; incubating for 3 days yields the highest germination rate and total polyphenol content; high-temperature treatment for 8 min ensures optimal color and sensory value; and a mixing ratio of sprouted rice liquid to green tea of 6:4 creates a harmonious flavor that meets sensory standards.

The product also demonstrated stability during storage at 30°C, maintaining approximately 70% of total polyphenols after 72.68 days and thus confirming good stability of these bioactive compounds with important physiological activity.

Our results not only provide a scientific basis for producing a new functional beverage from brown rice but also open up the development of healthy beverages with long shelf life without the need for chemical preservatives.

CONTRIBUTION

D.V. Phuong and N.K. Kiem developed the research concept, designed the study, performed the experiments, collected the data and drafted the manuscript. N.K. Kiem wrote the abstract and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Additional information about the authors

Do Viet Phuong ORCID 0000-0002-0081-0930

Nguyen Khac Kiem ORCID 0009-0003-4745-8131