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Enhancing bioactive compounds and antioxidant properties: role of germination in rice

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Abstract The germination time (24, 48, 72, or 96 hours) on the protein content, protein molecular weight, GABA, phenolic compounds, and antioxidant activity in two rice varieties: Hom Mali 105 and Hom Nil were investigated. Based on the results, a germination time of 72 hours produced the highest levels of protein, GABA, phenolic compounds, and antioxidant activity in both varieties. The following were the values for Hom Mali 105 rice: protein content, 1.77 mg/100 g dry weight; protein molecular weight, 14.4–41.1 kDa; GABA, 254.81 mg/100 g dry weight; phenolic compounds, 258.73 mg/100 g dry weight; and antioxidant activity, 82.92%. The values for Hom Nil rice were protein content, 1.89 mg/100 g dry weight; protein molecular weight, 14.4–97.0 kDa; GABA, 409.79 mg/100 g dry weight; phenolic compounds, 313.59 mg/100 g dry weight; and antioxidant activity, 88.94%. These findings indicated that a germination period of 72 hours was optimal for enhancing the bioactive compound levels in germinated rice of both the Hom Mali 105 and Hom Nil varieties.

Keywords: Protein, Phenolic, GABA, Hom Mali 105, Hom Nil

Introduction

Rice is an essential crop for Thailand, playing a key role in the nation's economy, culture, and diet. Sakon Nakhon province, one of Northeast Thailand's major rice-producing regions, is a prime example of how local knowledge can be incorporated into a variety of rice-based goods, consisting of processed rice products, brown rice, and Hang rice. Rice is not only a rich source of energy due to its high carbohydrate content but also provides a valuable protein component.

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This protein is highly nutritious, non-allergenic (Li *et al.*, 2024), and offers a well-balanced profile of essential amino acids compared to many other plant-based proteins (Jayaprakash *et al.*, 2022). Additionally, because it is gluten-free, it is perfect for gluten-free products and appropriate for people who are intolerant to gluten (Quiñones *et al.*, 2015). The two famous Thai rice varieties Hom Nil rice and Hom Mali 105 are in great demand both domestically and internationally due to their outstanding quality and nutritional advantages. Hom Nil rice is a purple-black variety distinguished by its fragrant aroma and vibrant color that comes from its anthocyanin, an antioxidant that helps shield cells from degeneration. The non-glutinous Hom Mali 105 rice is praised for its unique flavor, soft texture, and fragrant aroma. In addition, Hom Mali 105 rice is a good source of carbohydrate, which are an energy source for the human body (Preecharram *et al.*, 2025).

Germinated rice, commonly referred to as germinated brown rice (GBR), has garnered a lot of attention due to its enhanced nutritional value. During germination, the rice grain goes through biochemical changes that increase beneficial substances such as γ-aminobutyric acid (GABA), phenolic compounds, and antioxidants (Prakhongsil *et al.*, 2025). GABA is found in greater amounts in germinated brown rice and is essential for maintaining good health since it lowers blood pressure, reducing the risk of diabetes, and lessens the symptoms of depression and stress (Wu *et al.*, 2013). Furthermore, GBR's phenolic contents and antioxidant qualities help to reduce the risk of chronic illnesses, including heart disease and cancer (Cho and Lim, 2016). One important element affecting the amounts of bioactive chemicals in germinated rice is the germination time. Understanding the effect of germination duration is essential to determine the optimal time for achieving maximum nutritional value and biological activity. GBR has been demonstrated to prevent colon cancer in rats (Latifah *et al.*, 2010). Additionally, consuming pre-GBR has been found to prevent diabetes in rats by reducing insulin resistance and correcting adipocytokine imbalances that are two major causes of diabetic complications (Torimitsu *et al.*, 2010). It has been demonstrated that extracts from GBR decrease brain amyloid-β levels and lipid peroxidation, which are two important processes in the onset of Alzheimer's disease (Azmi *et al.*, 2023). GBR extract has been shown to improve cerebral blood flow and has anti-allergic, anti-tumor, anti-obesity, and anti-inflammatory properties (Xia *et al.*, 2019). Given these health benefits, germinated rice has become increasingly popular among health-conscious individuals. Additionally, it is a key ingredient in a variety of foods, including cookies, noodles, and breakfast cereals (Cho and Lim, 2016).

Therefore, it is crucial to study the effects of germination time on protein content, protein molecular weight, GABA levels, phenolic compounds and antioxidant activity in germinated rice for the development of high-quality germinated rice products.

This study aimed to compare Hom Mali 105 and Hom Nil rice germinated for 24, 48, 72, and 96 hours with ungerminated rice in terms of protein content and molecular weight, GABA content, phenolic compound content, and antioxidant activity.

Materials and methods

Materials

Hom-Nil and Hom Mali 105 were investigated as two accessible varieties of rice (*Oryza sativa* L.). All samples were obtained from Sakon Nakhon province and were taxonomically identified by the Sakon Nakhon Rice Research Center. All chemicals used were analytical grade. The main reagents were protein marker (LMW-SDS marker kit; Cytiva; USA), Folin-Ciocalteu phenol, γ-aminobutyric acid, and 2, 2-diphenyl-1 picrylhydrazyl (DPPH) that were purchased from Sigma-Aldrich Chemical Co., USA.

Sample preparation

The germinated rice was produced based on Preecharram *et al.* (2025), with modifcations. Briefly, each paddy rice sample was submerged in water at a 1:5 w/w ratio for 12 hours. Then, the soaking water was drained, the rice was rinsed in water, and the paddy rice was evenly spread out on a piece of damp cheesecloth and covered with another piece of wet cheesecloth. The end of the cloth was dipped in 700 mL of clean water in the bottom of an aluminum tray. Different combined samples of the rice and tray were incubated at room temperature (30°C) for 24, 48, 72, and 96 hours. After incubation, each sample was dried for 10 hours at 50°C. Finally, the dried rice was dehusked, yielding GBR. Ungerminated brown rice (UBR) was prepared by dehusking the rice directly, without undergoing the soaking or germination steps.

Research parameters

In this work, an analysis was conducted using rice extraction (Jandaruang and Preecharram, 2020). The protein content was determined using the Bradford procedure, followed Jandaruang and Preecharram, (2020). The protein concentration was measured by comparing the absorbance of the sample to the bovine serum albumin standard curve ($y = 0.1611x + 0.0106$, $R^2 = 0.9958$). The protein levels were expressed as milligrams of protein per 100 grams of dry weight using the formula:

$$C = [(c \times v) / m] \times 100$$

where C is the protein content (mg/100 g dry weight), c is the protein concentration determined from the calibration curve (mg/ml), v is the extract volume (ml), and m is the sample dry weight (g).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to measure the molecular weight of rice protein in accordance with the method of Jandaruang and Preecharram, (2020).

The GABA content, total phenolic content, and antioxidant activity were analyzed, according to (Preecharram *et al.*, 2025), (Preecharram *et al.*, 2023) and (Preecharram *et al.*, 2024), respectively.

Statistical analysis

For all outcomes, the mean ± standard deviation (S.D.) is used. One-way ANOVA was used for statistical analysis, and Duncan's multiple range test was used for post hoc comparisons. A p-value of less than 0.05 was considered statistically significant.

Results

Protein content

An examination of the protein content of the two types of germinated rice (Hom Mali 105 and Hom Nil) revealed that the effects of germination varied depending on the rice variety and the duration of the germination process. The protein content of the ungerminated the Hom Mali 105 rice was 1.58 mg/100 g dry weight (Figure 1). The protein concentration dropped to 1.44 mg/100 g dry weight at 24 hours of germination. However, it increased to 1.60 mg/100 g dry weight at 48 hours and 1.77 mg/100 g dry weight at 72 hours. Subsequently, a slight decrease was observed at 96 hours of germination, with the protein content measuring 1.74 mg/100 g dry weight. The protein content of the Hom Nil rice had similar trends as the Hom Mali 105 rice. The protein content of the ungerminated Hom Nil rice was 1.68 mg/100 g dry weight. The protein concentration dropped to 1.61 mg/100 g dry weight at 24 hours of germination. However, at 48, 72, and 96 hours of germination, it rose to 1.79, 1.89, and 1.94 mg/100 g dry weight, respectively.

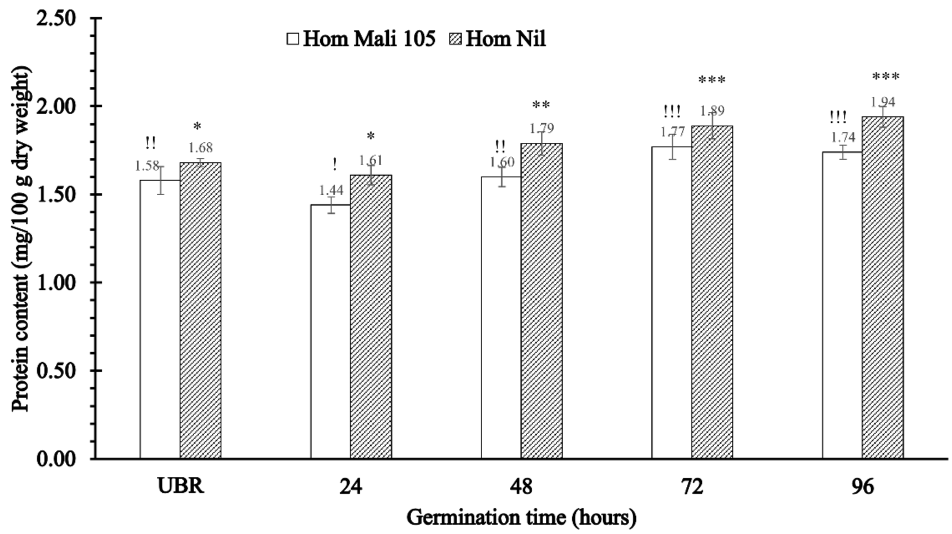

Figure 1. Protein contents for ungerminated brown rice (UBR) and following germination at 24, 48, 72, and 96 hours of Hom Mali 105 rice and Hom Nil rice. The values are shown as mean ± standard deviation (n = 3). Significant differences at $p < 0.05$ are indicated by symbols above the bars.

Molecular weight of rice protein

The molecular weights of the protein samples of both varieties of rice were ascertained using the SDS-PAGE technique. For the ungerminated and germinated Hom Mali 105 rice samples at 24 and 48 hours, protein bands with molecular weights of 14.4, 20.1, and 41.1 kDa were observed (Figure 2A). At 72 hours of germination, these protein bands became more prominent, along with additional protein bands with molecular weights of 23.2 and 76.0 kDa. At 96 hours of germination, the protein bands at 14.4, 20.1, and 41.1 kDa decreased in intensity, while the 23.2 and 76.0 kDa protein bands disappeared. The ungerminated Hom Nil rice and the samples germinated at 24, 48, and 72 hours showed protein bands at 14.4, 20.1, and 97.0 kDa (Figure 2B). The most prominent protein bands were seen at 72 hours. However, within 96 hours of germination, the intensity of these protein bands diminished, with the one at 97.0 kDa band no longer being detected.

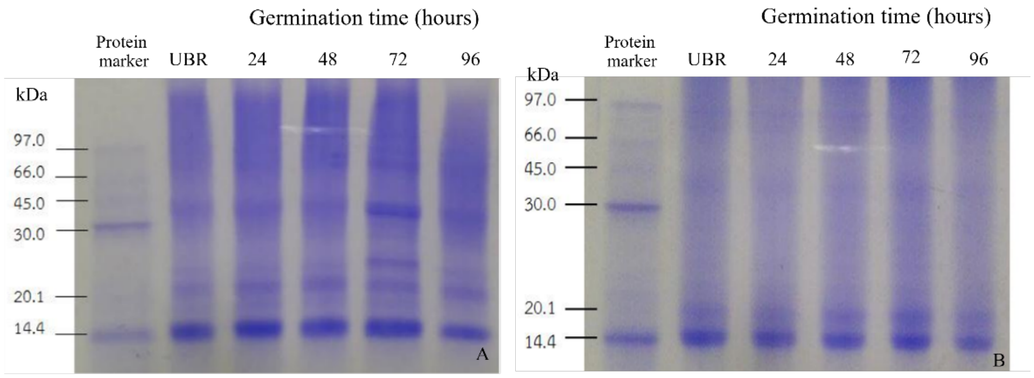

Figure 2. Protein molecular weight of ungerminated brown rice (UBR) and following germination at 24, 48, 72, and 96 hours of Hom Mali 105 rice (A) and Hom Nil rice (B)

GABA content

The GABA content in the ungerminated and germinated Hom Mali 105 rice and Hom Nil rice samples at different time intervals revealed that germination significantly influenced GABA levels (Figure 3). The ungerminated Hom Mali 105 rice contained 160.92 mg/100 g dry weight. During germination, the GABA content increased across all time points, reaching its peak at 72 hours (254.81 mg/100 g dry weight), representing an approximately 58.34% increase compared to the ungerminated rice. However, at 96 hours of germination, the GABA content decreased to 211.14 mg/100 g dry weight. The ungerminated Hom Nil rice GABA content was 210.38 mg/100 g dry weight, which was higher than that of the ungerminated Hom Mali 105 rice. After 72 hours of germination, the Hom Nil rice had the highest GABA content (409.79 mg/100 g dry weight), representing an increase of 94.78% compared to the ungerminated sample. However, by 96 hours of germination, the GABA content had decreased to 221.87 mg/100 g dry weight.

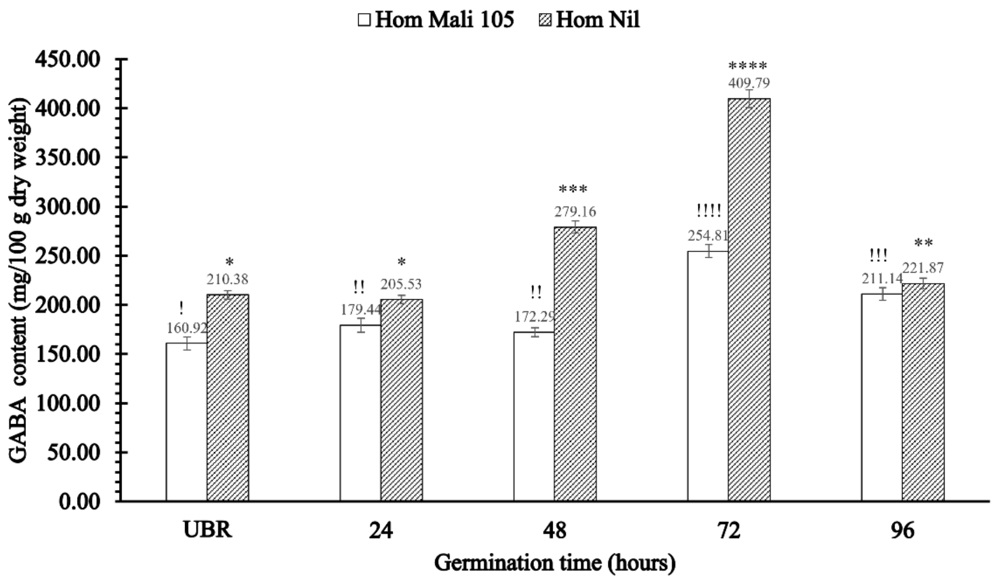

Figure 3. GABA contents of ungerminated brown rice (UBR) and following germination at 24, 48, 72, and 96 hours of Hom Mali 105 rice and Hom Nil rice; The values are shown as mean ± standard deviation (n = 3). Significant differences at $p < 0.05$ are indicated by symbols above the bars.

Phenolic compounds content

The phenolic components in the Hom Mali 105 rice and Hom Nil rice samples at various time points under both ungerminated and germinated circumstances were significantly affected by the duration of germination changes (Figure 4). The ungerminated Hom Mali 105 rice contained 184.28 mg/100 g dry weight. Following germination, the phenolic compounds increased significantly, peaking at 72 hours (258.73 mg/100 g dry weight), a 40.40% increase compared to the ungerminated rice. However, the content had slightly decreased at 96 hours (225.04 mg/100 g dry weight). The ungerminated Hom Nil rice contained 279.19 mg/100 g dry weight, which was higher than for the ungerminated Hom Mali 105 rice. Germination led to an increase in phenolic compounds at all time points, with the highest content observed at 72 hours (313.59 mg/100 g dry weight), representing a 12.32% increase compared to the ungerminated Hom Nil rice. However, at 96 hours of germination, the phenolic compound content had decreased slightly (286.89 mg/100 g dry weight).

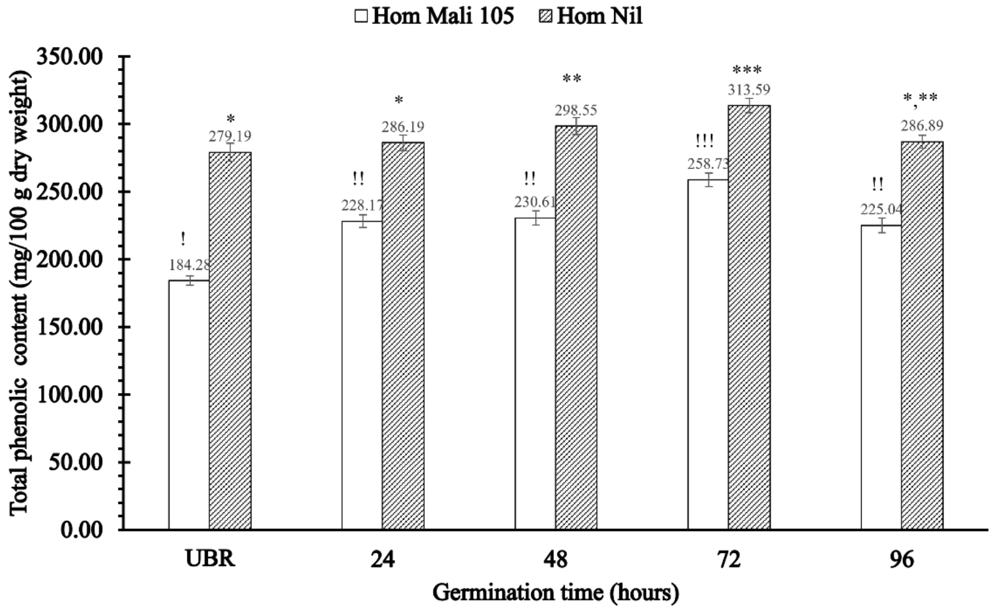

Figure 4. Phenolic compound contents of ungerminated brown rice (UBR) and following germination at 24, 48, 72, and 96 hours of Hom Mali 105 rice and Hom Nil rice; The values are shown as mean ± standard deviation (n = 3). Significant differences at $p < 0.05$ are indicated by symbols above the bars.

Antioxidant activity

The ungerminated Hom Mali 105 rice had a DPPH antioxidant capacity of 71.33% (Figure 5). Upon germination, its antioxidant capacity increased significantly, peaking at 72 hours (82.92%), representing a 16.25% increase compared to the ungerminated rice. However, at 96 hours of germination, the antioxidant capacity had slightly decreased (80.01%). The DPPH antioxidant capacity of the ungerminated Hom Nil rice was 83.44% higher than that of ungerminated Hom Mali 105 rice. At every time point, there was an increase in the antioxidant capacity of the germinated Hom Nil rice, which peaked at 72 hours (88.94%), 6.59% higher than that of the ungerminated Hom Nil rice. A slight decrease in antioxidant capacity (85.01%) was observed during the 96 hours of germination.

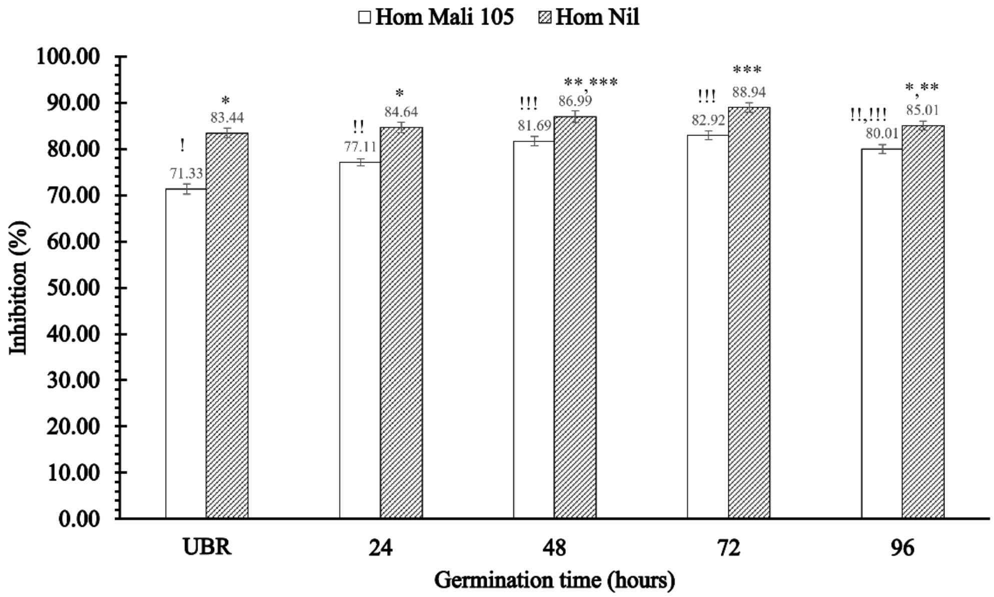

Figure 5. Antioxidant activity of ungerminated brown rice (UBR) and following germination at 24, 48, 72, and 96 hours of Hom Mali 105 rice and Hom Nil rice. The values are shown as mean ± standard deviation (n = 3). Significant differences at $p < 0.05$ are indicated by symbols above the bars.

Discussion

The increase in the protein content during germination may have been due to the breakdown of starch into sugars and the activation of enzymes involved in protein synthesis (Moongngarm and Saetung, 2010; Moongngarm and Khomphiphatkul, 2011). Moongngarm and Khomphiphatkul (2011) reported that the protein content of germination-stage rough rice (*Oryza sativa* L.) was 8.69% and increased to 10.44% after 96 hours of germination. In contrast, Müller *et al*. (2021) observed no significant increase in protein content during germination for 40 hours. However, in the current study, the protein content had decreased by 24 hours of germination. This decline may have been due to the utilization of protein as an energy source for the developing seedlings, prior to the restoration of the protein content through metabolic processes at later stages. This finding was consistent with a reported decrease in the protein content in rough rice (*Oryza sativa* L.) that had germinated following a soaking in water for 48 hours (Moongngarm and Khomphiphatkul, 2011). In the current study, when the two types of rice were tested at the same germination stage, the Hom Nil rice consistently displayed a higher protein content than the Hom Mali 105 rice. This disparity may have resulted from genetic alterations or differences in their chemical composition that impacted their metabolic activities. Based on the current results, germination affected the rice protein content, with the effect varying based on the cultivar and germination duration.

Based on the results of the examination of the molecular weight of the proteins in both rice varieties, there was a distinct protein band at 72 hours of germination. This may have been associated with biochemical processes occurring during germination, such as the activation of enzymes or growth-related proteins. Cho and Lim (2016) reported that during phase II of germination, protein synthesis occurred from both pre-existing mRNAs and newly transcribed mRNAs. In the current study, at 96 hours of Hom Mali 105 rice germination, the protein bands at 14.4, 20.1, and 41.1 kDa had decreased in intensity, while the 23.2 and 76 kDa protein bands had disappeared. This reduction may have been due to the activity of protease enzymes during this stage. Tortayeva *et al.* (2014) reported that during germination between 120 and 168 hours, proteins with molecular weights of 26, 33, and 56 kDa were hydrolyzed by proteases. As a result, germinated rice is considered less likely to cause allergies, as allergenic proteins with molecular masses of 33 and 56 kDa are no longer present.

Based on the current results, germination significantly increased the GABA content in both types of rice, particularly at 72 hours. During this period the enzyme glutamate decarboxylase (GAD) is most active, leading to the efficient conversion of glutamic acid to GABA (Jandaruang and Preecharram, 2020). These findings aligned with another study on the Indian rice varieties Jyothi, Chitteni, and Njavara, which also showed a significant increase in GABA content at 72 hours of germination (Thomas *et al.*, 2023). In the current study, the decreases in the GABA levels observed after 72 hours in both rice species may have been due to the inactivation of the glutamate decarboxylase enzyme or the utilization of GABA in other biochemical processes such as metabolism during seedling growth. The breakdown of GABA occurs via two different routes. In the first, GABA is changed into succinate, which subsequently enters the Krebs cycle. The second pathway converts GABA into γ-hydroxybutyric acid (Yuan *et al.*, 2023). The initial GABA content of the Hom Nil rice was consistently higher than that of the Hom mali 105 rice across all time points, indicating that the type of rice has an impact on the GABA content of the seed. This may have been due to genetic components or chemical factors in the Hom Nil rice that promote GABA synthesis. Similarly, An *et al*. (2010) reported that the GABA content of germinated pigmented rice (*Oryza sativa* L., red) was higher than that of non-pigmented rice.

The levels of phenolic chemicals significantly increased in both types of rice germinated for different durations, especially after 72 hours. Similarly, Baranzelli *et al.* (2023) reported that the greatest concentrations of phytochemicals, such as free flavonoids and free benzoxazinoids, were in wheat that had germinated for 72 hours. The crucial time during germination is when the enzymatic and metabolic processes in the rice grains are greatly accelerated (Chen *et al.*, 2022). This procedure promotes the production of antioxidants, including phenolic acids and flavonoids (Kim *et al.*, 2017), which shield the seedling cells from growth-related stress (Saini *et al.*, 2024). In the current study, the amount of phenolic compounds decreased slightly after 96 hours of germination. This decrease could be explained by the breakdown of phenolic compounds in the germination water environment or their use in the metabolic activities of mature seedlings. The Hom Nil rice had a higher content of phenolic compounds than the Hom Mali 105 rice for both the ungerminated and germinated conditions. This difference was likely due to genetic diversity and the higher pigment content (such as anthocyanins) in the Hom Nil rice. Additionally, there was a slower increase in phenolic compounds in the Hom Nil rice during germination than in the Hom Mali 105 rice, indicating differences in the metabolic rates of the two grain types.

The antioxidant capacity of both types of rice was correlated to their levels of phenolic compounds and GABA. At 72 hours of germination, both the Hom Mali 105 and Hom Nil rice produced their highest levels of GABA and phenolic compounds, resulting in their highest antioxidant capacity. This was consistent with the well-established antioxidant properties of phenolic chemicals and GABA (Li *et al.*, 2023; Kusumawardani *et al.*, 2024). Additionally, Avinash *et al.* (2024) reported that Hom Nil rice contained a high concentration of total phenolic compounds, contributing to its strong DPPH free radical inhibition capacity. Apart from its function in enhancing memory and learning, GABA is also useful in agriculture, where it has improved the antioxidant system and post-harvest quality of strawberries (Zhang *et al.*, 2024). Based on the current results, the Hom Nil rice had a greater DPPH antioxidant capacity than the Hom Mali 105 rice under both the ungerminated and germinated conditions. Germination enhanced the antioxidant capacity in both rice varieties, with the highest levels observed at 72 hours. This peak corresponded to the period when the production of antioxidants, including phenolic compounds, GABA, and other phytochemicals, was most active. However, at 96 hours of germination, the antioxidant capacity slightly declined, potentially due to the utilization of antioxidants in seedling metabolism or the degradation of certain compounds.

Based on the results of the current study, germination significantly influenced the protein content, protein molecular size, GABA levels, phenolic compounds, and antioxidant capacity of rice, with these effects varying by cultivar and germination duration. A 72-hour germination period was optimal for maximizing the protein content, GABA, phenolic compounds, and antioxidant activity. This insight should be very useful for farmers aiming to produce GBR with a high-nutritional-value. Further research is essential on the composition and functional properties of the proteins in different rice varieties to deepen understanding and to unlock the potential of these varieties for use in innovative food products and other applications.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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