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Original Article

Evaluation of physicochemical properties of Khao Dawk Mali 105 rice during Germination

Jaturong Langkapin[1], Sunan Parnsakhorn[1*], and Orawan Oupathumpanont[2]

[1] *Department of Agricultural Engineering, Faculty of Engineering, Rajamangala University of Technology Thanyaburi, Khlong Luang, Pathum Thani, 12110 Thailand*

[2] *Department of Food and Nutrition, Faculty of Homme Economics Technology, Rajamangala University of Technology Thanyaburi, Khlong Luang, Pathum Thani, 12110 Thailand*

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Abstract

This study investigated the physicochemical properties of the Khao Dawk Mali 105 (KDML 105) rice during germination. The rice was soaked at 40°C for 4 h, followed by incubation at the same temperature and 90% relative humidity for up to 20 h. Key parameters measured included moisture content, γ-aminobutyric acid (GABA) concentration, pasting characteristics, crude fat, and free fatty acids. The results showed that the moisture content increased rapidly and remained at 36-37% during germination. GABA content showed a significant rise, particularly in the part 3, increasing from 5.54 mg/100 g to 20.21 mg/100 g after 20 h. Pasting viscosity analyses revealed a decrease in peak, trough, breakdown, and final viscosities with prolonged germination, attributed to enzymatic activity breaking down starch and crude protein. Crude fat content and free fatty acids also decreased during germination. This study highlights the potential of optimized soaking and germination conditions to enhance the nutritional and functional properties of germinated KDML 105 brown rice, providing valuable insights for future rice production technologies.

Keywords: germination, physicochemical properties, soaking, incubating, KDML 105

1. Introduction

Rice (*Oryza sativa L.*) is a vital source of nutrients and a staple food in many parts of the world. Due to the nutritious richness of the bran, customers today prefer to eat unpolished rice. The reputation for the health benefits and superior nutritional value of consuming this variety of rice has led to an increased demand for brown rice (BR) and germinated brown rice (GBR). Brown rice contains large amounts of proteins, dietary fiber, a number of vitamins and minerals, and vital fatty acids that act as antioxidants and may lower blood cholesterol (Cho & Lim, 2016). The flavor and texture of cooked brown rice have been observed to improve on soaking the grain in water during the sprouting process until a germ grows to a diameter of between 0.5 and 1.0 mm. This has been shown to increase the amount of gamma-amino butyric acid (GABA) in the grain. The result of this process is "germinated brown rice" (Chao, Mitchell, & Fukai, 2021).

The germinated brown rice has a sweet flavor because the sprouting process breaks down proteins and carbohydrates with the help of enzymes. Germinated brown rice contains higher amounts of GABA, ranging from 2 to 5 times higher than in white rice and 2 to 3 times higher than in brown rice (Karladee & Suriyong, 2012). GABA offers numerous health benefits, such as reducing stress and anxiety, promoting relaxation, boosting mood, and enhancing sleep quality. Additionally, it may help regulate blood pressure and exhibit neuroprotective properties (Abdou *et al*., 2006). Brown rice contains glutamate decarboxylase (GAD), which, when activated during water absorption, transforms glutamic acid into GABA (Cáceres, Peñas, Martinez-Villaluenga, Amigo, & Frias, 2017). Soaking brown rice in warm water

*Corresponding author
Email address: sunan.p@en.rmutt.ac.th

between 20 and 40ºC is an efficient way to increase GABA levels, according to Shinmura, Nakagawa, Sasaki, Aoto, & Onishi (2007). Kupkanchanakul, Kadowaki, Kubota, & Naivikul (2018) observed a decrease in lipid content during 50 h of germination at 30°C. Nascimento, Avila, Colussi, & Elias (2020) found that 36 h of germination, combined with stress, increased starch digestibility in brown rice by over 30%.

According to Watcharatparpaiboon, Laohakunjit, & Kerdchoechuen (2010) the Koa Dawk Mali105 (KDML 105) variety is particularly successful in producing brown rice that germinates and may improve the nutritional content of rice, with the texture of cooked GBR being softer than that of cooked brown rice. However, soaking brown rice in the right circumstances can enhance the physicochemical characteristics of products made from germinated brown rice. The scientific literature currently lacks information on the effects of soaking and incubation on the quality of germinated brown rice. This is especially true for changes in GABA concentration, pasting characteristics, crude fat, and free fatty acid that occur throughout the germination process. Consequently, it is imperative to emphasize the significance of researching soaking and germination techniques in the context of enhancing the quality of germinated brown rice. Such investigations are crucial as they can provide essential knowledge and serve as a foundational step towards developing advanced production technologies in the future.

2. Materials and Methods

2.1 Brown rice preparation

This research utilized low-amylose KDML 105 paddy rice, which initially had an average moisture content of 12±1%. Samples were shelled using rubber roll shellers (Model THU 35A, Japan), and broken kernels were separated using graders (Model TRG-05B, Japan).

2.2 Preparation of germinated brown rice

Brown rice was prepared, and 1,000 g rice samples were soaked in 2,000 g of water at 40°C for 4 h before being transferred to plastic boxes with lids. These boxes were then placed in an incubator set at 40°C and 90% relative humidity for 20 h. To prevent fermentation and off flavors, the brown rice samples were additionally cleaned with clean water every 4 h during incubation. Throughout the germination period, brown rice samples were collected every 4 h. Subsequently, these samples were placed in plastic bags and frozen at -10°C for 5 h. Ultimately, the samples were freeze-dried for 15-20 h until reaching a moisture content below 5 %wb. Following one week of storage at room temperature in airtight polyethylene (PE) bags to stabilize hardness and equilibrate moisture, the dried germinated brown rice samples were assessed for physicochemical parameters.

2.3 Physicochemical properties

2.3.1 Moisture content

The conventional oven method was used to determine the moisture content of the rice samples (Association of Official Analytical Chemists [AOAC], 2000).

2.3.2 GABA content

Both the BR and GBR samples were used to determine the GABA concentration. From Figure 1, one grain of rice was identified as part 2. A grain of rice was visually divided into four equal parts, a sharp knife was used to cut off the part of the grain containing the germ, about ¼ of the size. This part of the seed was designated as part 3, and the remaining three-quarters of the seed was designated as part 1. Each of the three parts of rice was prepared to weigh approximately 5 g for testing in the next step. Approximately 5 g was weighed for each part, and then pulverized into rice flour using a manual stone mortar. Subsequently, the flour was passed through a 100-mesh sieve to obtain 3 g of finer rice flour. The rice flour samples were retained in vacuum-sealed polyethylene bags and refrigerated at 4ºC for the subsequent estimation of the GABA content.

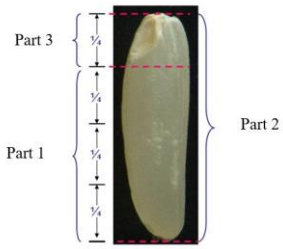


Figure 1. The rice kernels were divided to parts for GABA testing.

1) Extraction method

The extraction procedure, adapted from Tian, Nakamura, Cui, and Kayahara (2005), involved mixing 2.5 g of rice flour with 25 ml of 80 % (v/v) ethanol in screw-cap test tubes. The mixture was then centrifuged at 8,000 rpm for 10 min at a low temperature (4°C). Following the initial step, the supernatant was carefully filtered through No. 2 filter paper and collected into a 50 ml beaker. Subsequently, 25 ml of 80 % (v/v) ethanol were added to the same screw-cap test tube, and the sample underwent two additional extractions using the same procedure, with the supernatant collected each time. The entire collected supernatant was then evaporated using a vacuum dryer set at 40 °C until completely dry, after which it was reconstituted by dissolving it in 3 ml of distilled water.

2) Spectrophotometer method

A modified version of Kitaoka & Nakano (1959) method for measuring γ-aminobutyric acid (GABA) was employed in this work. In this method, 0.1–0.3 ml of the extracted solution was thoroughly mixed with 0.2 ml of 0.2 M borate buffer and 1.0 ml of 6 % phenol. For the standard test, 0.1–0.3 ml of GABA solution, 0.2 ml of borate buffer, and 1.0 ml of phenol reagent were added to test tubes (18x120 mm). The solutions were thoroughly combined and allowed to cool for 5 min in a cooling bath. The solution was then rapidly stirred for 1 min and chilled in a cooling bath for another 5 min before 0.4 ml of 10-15 % NaOCl was added. The solution was then heated for 10 min in a 100 °C water bath before being allowed to cool. Optical density was determined by

spectrophotometry at a wavelength of 630 nm, with 2 ml of ethanol as a blank. GABA content was quantified by comparing the optical density reading with the standard GABA content curve and reported in mg/100 g.

2.3.3 Pasting viscosity

The pasting qualities of the materials were measured using the Rapid Visco Analyzer (Model 4D Newport Scientific, Australia). In an RVA canister, a 3 g sample of rice flour with 12% moisture was dispersed in 25.0 ml of distilled water. A paddle was inserted and used to stir within the canister. The canister, with the paddle, was then placed into the instrument. The RVA canister was initially stirred at 960 rpm for 10 s, followed by continuous stirring at 160 rpm for the remaining duration using the scheduled heating and cooling cycle. The standard temperature profile stages were as follows: 1) 1.00 min at the initial temperature (50ºC); 2) 3.42 min heating up to 95ºC; 3) 2.30 min holding at 95ºC; 4) 3.48 min cooling down to 50ºC; and 5) 2.00 min holding at 50ºC. The entire time for the test was 13.00 min. The pasting profile was used to measure the peak and trough viscosities as well as the final viscosity, pasting temperature, peak duration, breakdown, and setback.

2.3.4 Crude fat and free fatty acid

1) Crude fat

To prepare for extraction, rice samples were ground using an ultra centrifugal mill (Model ZM 100, Retsch, Germany), passed through a 1.00 mm sieve screen, and then dried overnight at 70°C. The method for determining the crude fat was developed and improved from the method of Zhongkai, Chris, Stuart, and Kevin (2003) with the following steps: filter paper was used to compress 3 g of dry rice flour. The compressed flour was then placed in a thimble with an analytical balance and wrapped in fat-free cotton. After adding 150 ml of petroleum ether to the beaker and inserting the thimble, the extraction was completed using a Soxtherm multistat (Model SX PC, Gerhardt, UK), an automatic extraction tool. Once the extraction procedure was finished, the solvent in the flask was evaporated to dryness, and the flask was then dried in an oven at 105°C until a constant weight was attained. The increase in weight after extraction was calculated and reported as a percentage of the dry rice flour, representing the crude fat content of the sample.

2) Free fatty acid

Titration was employed to ascertain the free fatty acid content. After dilution in 50 ml of 0.04% alcohol-phenolphthalein solution, the residue obtained from the extraction flash underwent titration with 0.0178 N potassium hydroxide solution (KOH) for 1 min, using phenolphthalein as an indicator (AOAC, 2000). Equation (1) was utilized to calculate the free fatty acid content as oleic acid.

$$\text{FFA (g/100g oil)} = (a \times t \times M)/(100 \times m) \quad (1)$$

where

a is KOH consumption (ml),
t is KOH concentration (0.0178 N),
M is Molecular weight 288 (oleic acid),
m is weight of oil (g)

2.4 Statistical analysis

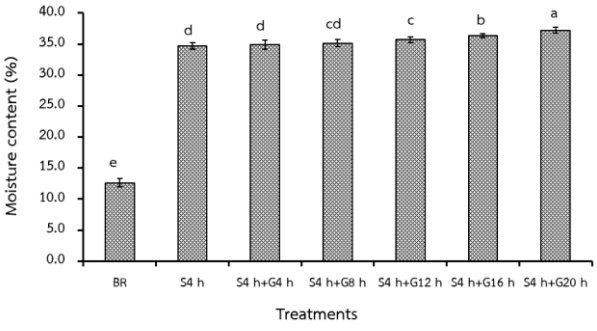
The Statistical Package for Social Sciences (SPSS), analysis of variance (ANOVA), and Duncan's Multiple Range test were used to examine the data at the 95% confidence level.

3. Results and Discussion

The goal of this experiment was to investigate how the physicochemical properties of the KDML 105 variety changed throughout germination. The process was as follows: brown rice (BR) was first soaked in water at 40 °C for 4 h (S4 h), and then it was continuously germinated by soaking brown rice in water at 40 °C, combined with the germination process at 40 °C and 90 % relative humidity for 4 h (S4 h+G8 h). This process continued under the same temperature and humidity conditions for longer durations: 8 h, 12 h, 16 h, and 20 h (S4 h+G8 h, S4 h+G12 h, S4 h+G16 h, and S4 h+G20 h), respectively. Samples for testing were taken every 4 h for a total of 20 h.

3.1 The effect of germination on moisture content

At the outset, brown rice had an average moisture content of 12.62%. The impact of soaking time is illustrated in Figure 2; after being soaked for 4 h at 40°C, the moisture content of the samples quickly increased, reaching 34.68%. Brown rice absorbed moisture quickly at first because air from endosperm micropores was released and replaced by water during soaking (Miah, Haque, Douglass, & Clarke, 2002). Preliminary experiments revealed that bacteria could proliferate in rice seeds if they were not cleaned during the germination process. These problems can be avoided by washing the rice samples with clean water every 4 h until the procedure is complete, resulting in high-quality germinated brown rice. The moisture content remained steady at roughly 36 % during germination from 4 to 16 h, then increased slightly to 37.23 % by the end of the process for 24 h.

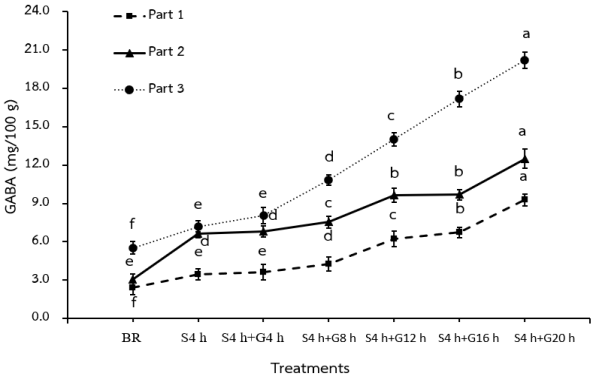


The values are mean ± standard deviation (n=3). a-e Means within each treatment followed by different letters are significantly different ($p \leq 0.05$) using Duncan's new Multiple Range Test (DMRT)

Figure 2. Moisture content of brown rice and germinated brown rice was measured at various times during the germination process.

3.2 The effect of germination on GABA content

Figure 3 presents the analytical data on the GABA content found in both BR and GBR, distinguished across three parts of the rice grain: the part 1, part 2 and part 3. Notably, GBR exhibited significantly higher GABA levels when compared to BR. Initially, the GABA contents in part 1, part 2 and part 3 were recorded at 2.42±0.07, 3.03±0.47, and 5.54±0.49 mg/100 g, respectively. Following 4 h soaking, these levels saw a slight increase to 3.44±0.12, 6.66±0.14, and 7.20±0.42 mg/100 g across all three rice parts. Upon comparing all three parts, it was evident that the greatest increase in GABA content occurred in part 3, commonly referred to as the rice germ. Statistical analysis revealed a significant and consistent rise in GABA content ($p \leq 0.05$), with levels recorded at 8.05±0.63 mg/100 g at 4 h post-germination (S4 h+G4 h) and reaching a peak of 20.21±0.65 mg/100 g at 20 h post-germination (S4 h+G4 h).



The values are mean ± standard deviation (n=5). a-f Means within each treatment followed by different letters are significantly different ($p \leq 0.05$) using Duncan's new Multiple Range Test (DMRT)

Figure 3. GABA content (mean of five determinations) in brown rice and germinated brown rice at different times during the germination process.

An investigation was conducted to analyze the changes in GABA content during the germination process in part 2. After 4 h of germination, the GABA content increased significantly ($p \leq 0.05$), roughly twofold from 3.03±0.47 to 6.66±0.14 mg/100 g. The value of GABA then increased significantly from 6.66±0.14 mg/100 g to 7.54±0.28 mg/100 g when the germination time was increased from 4 to 8 h, and it increased to 9.66±0.35 mg/100 g after 12 h germination. GABA content did not change during germination for 12 to 16 h (9.67±0.38 mg/100 g); however, after 20 h it showed a rapid substantial increase by almost four times compared to the starting content. GABA levels in part 1 were found to be lower after soaking and germination, as illustrated in Figure 3. The initial GABA level in mg/100 g of part 1 was 2.42±0.07, which was much lower than 3.62 to 9.28 after increasing the germination time to 20 h.

The accumulation of GABA and alanine during the soaking and germination of GBR was primarily driven by the synthesis of glutamate through the glutamate synthetase cycle. Glutamate served as a precursor for both GABA and alanine. Enzymatic activities involved in this cycle were enhanced during soaking and germination, resulting in the increased production of these amino acids. These amino acids play crucial roles in stress responses, nutrient mobilization, and metabolic regulation during seed germination (Galili, Avin-Wittenberg, Angelovici, & Fernie, 2014). This cycle, as highlighted by Zhang, Liu, Wang, and Pan (2020), assumed a vital role in anaerobic processes. Furthermore, the role of air during germination was highlighted, with Varanyanond, Tungtrskul, Surojanametakul, Watanasiritham, & Luxiang (2005) reporting that increased air exposure during the GABA formulation procedure led to higher GABA accumulations.

3.3 The effect of germination on pasting profiles of germinated brown rice

Regarding the pasting properties of brown rice flour following germination for various durations (4, 8, 12, 16, and 20 h), alterations in viscosity were observed and are detailed in Table 1 and illustrated in Figure 4.

The peak viscosity was recorded at 294.40±12.12 RVU during the first phase of germination (S4 h+G4 h) and showed a progressive and significant reduction ($p \leq 0.05$) as the germination period increased. Peak viscosity measurements at 8, 12, 16, and 20 h were 260.90±11.69, 245.75±3.28, 198.83±8.15, and 142.28±6.65 RVU, respectively. Saleh & Meullenet (2004) claimed that variations in the protein content of rice grains caused variations in viscosity, particularly the peak viscosity, which represented the capacity for swelling. They found that stronger protein networks had higher peak viscosity. The test findings showed that when rice seeds germinated for 12-20 h, the peak viscosity decreased significantly ($p \leq 0.05$) and was lower than that of non-germinated brown rice. This could have been because the weakened protein matrix could not withstand the same level of starch granule swelling during gelatinization, which occurred when rice was cooked or exposed to heat and moisture (Chung, Liu, Lee, & Wei, 2011). The highest trough value was recorded after 4 h of germination, measuring at 158.85±8.06 RVU, and subsequently showed a slight decline to 140.62±6.73 RVU and 123.10±7.00 RVU after 8 and 12 h of germination, respectively. As the germination time extended to 20 h, marking the end of the process, the trough value continued to steadily decrease, reaching 86.03±3.17 RVU at 16 h and 54.18±4.17 RVU at 20 h. Due to germination, the grain mobilized its stored reserves, such as starch and proteins, to support the growth and development of the embryo. Enzymes like amylases and proteases were activated, leading to the breakdown of starch and proteins, respectively. This breakdown resulted in a decrease in the concentration of these macromolecules, which contributed to a reduction in the trough value (Moongngarm & Saetung, 2010).

When the germination duration was increased from 4 to 20 h, the breakdown viscosity changed dramatically. The lowest value for breakdown viscosity was noted after 20 h of germination. This decline in viscosity is indicative of the mechanical breakdown of starch granules, a phenomenon occurring beyond the point of gelatinization temperature. During this process, the swollen starch granules become highly susceptible to the thermal weakening of bonding forces within the micellar lattice, as suggested by Shuey and Tipples (1980).

The starch molecules had retrograded to the gel or semi-crystalline aggregate, as evidenced by the final viscosity and setback. Brown rice's final viscosity was 182.92±5.02 RVU, but after 4 h of soaking, it increased to 248.05±5.99 RVU. When the germination time was increased from 4 to 20 h, the final viscosity values reduced dramatically from 239.53±9.38 RVU to 104.45±7.49 RVU. Numerous studies have consistently attributed the reduction in final viscosity of GBR flour to the enzymatic hydrolysis of starch. This starch breakdown process was primarily driven by the action of various enzymes, including α-amylase, debranching enzyme, β-amylase, and α-glucosidase, which become active during the germination phase, as outlined by Zeeman *et al.* (2007).

Peak time remained relatively stable during the 4 to 16 h germination process and dramatically lowered ($p \leq 0.05$) to 4.96±0.04 min after 20 h of germination. These findings were aligned with the observations made by Reka, Szilveszter, Timea, and Andras (2005), where it was concluded that the peak time remained relatively consistent during the early phases of the germination process. However, as the process advances to later stages, peak time appears to exhibit greater variability.

The term "pasting temperature" refers to the temperature at which the endosperm's starch granules start to expand and gelatinize as a result of water absorption and starch modification, increasing viscosity. The starch content of rice grains decreases as a result of hydrolysis after being germinated in brown rice samples, resulting in slight differences in pasting temperature ($p \leq 0.05$). At the onset of germination (S4 h+G4 h), the pasting temperature registered at 71.90 °C, with a minor increment to 72.06 °C after 8 h of germination. Nevertheless, towards the conclusion of the germination process, the pasting temperature exhibited relative stability, ranging between 71.26 °C and 71.09 °C during the time span of 12 to 20 h, with no significant variations observed across all conditions.

3.4. The effect of germination on crude fat and free fatty acid of germinated brown rice

The crude fat content of BR and GBR is shown in Figure 5. The BR sample exhibited the lowest crude fat level (2.62 %), however after 4 h of soaking, the level had increased (2.84 %). Crude fat content increased marginally from 3.24 % to 3.26 % after 4-8 h of germination, though not significantly ($p > 0.05$). During extended germination (12-20 h), it remained stable at 2.84-2.88 % without significant variation ($p > 0.05$). Researchers noticed a rise in crude fat content during the early stages of germination after 8 hours. These results corroborated Peterson's (1999) observations that there was increased activity in the synthesis of free fatty acids and in the production and degradation of lipids during germination.

Figure 5 indicates that the initial free fatty acid content of brown rice (BR) was 1.48 % and increased slightly to between 1.49 % and 1.54 % during 4 h of soaking and 8 h of incubation at 40°C and 90 % relative humidity. The differences observed were statistically insignificant ($p > 0.05$). Subsequent germination for 12 h led to an increase, with the free fatty acid content reaching approximately 1.76 %. During extended germination periods (16–20 h), the free fatty acid content remained consistently stable within the range from 1.55 % to 1.58 %. Changes in free fatty acids may be related to enhanced physiological activities of the grain during germination as a result of enzyme action leading to grain expansion (Ayernor & Ocloo, 2007). In germination process involving multiple enzymes, including lipoxidase and lipase, germination, in particular soaking or hydrolysis, acted on the ester bond of fat to release free fatty acids, which can produce unpleasant, rancid flavors (Peterson, 1999). The test results indicated that changes in crude fat and free fatty acid during germination were attributed to lipase enzymes degrading stored triacylglycerols into free fatty acids and glycerol. This process provided energy and carbon to the embryo, increasing free fatty acid levels. As the embryo expanded, its need for lipids to produce new cell membranes increased, resulting in a decrease in free fatty acid and crude fat levels later in germination (Eastmond & Graham, 2001; Baud & Lepiniec, 2010).

4. Conclusions

This research examined the effects of soaking and germination on the physicochemical properties of KDML 105 brown rice. Brown rice was soaked in water at 40 °C for 4 h, followed by germination at 40 °C and 90% relative humidity for varying durations (4, 8, 12, 16, and 20 h). Precise analytical methods assessed changes in moisture content, GABA content, pasting viscosity, crude fat, and free fatty acids at various germination stages. The results revealed that the moisture content of BR increased significantly, stabilizing at approximately 37.23 % after 20 h. GABA content in GBR significantly increased, peaking at 20.21 mg/100 g after 20 h.

Table 1. Pasting characteristics of flours of brown rice and germinated brown rice at different times during the germination process

Treatment	Peak viscosity (RVU)	Trough (RVU)	Breakdown (RVU)	Final viscosity (RVU)	Setback (RVU)	Peak time (min)	Pasting temperature (°C)
BR	233.42±9.65^{d}	112.08±3.81^{e}	121.33±10.49^{ab}	182.92±5.02^{d}	70.83±1.42^{c}	5.44±0.11^{b}	70.27±1.56^{b}
S4 h	309.37±12.47^{a}	169.68±6.71^{a}	139.68±9.10^{a}	248.05±5.99^{a}	78.37±1.76^{ab}	5.61±0.06^{a}	71.87±0.06^{a}
S4 h+G4 h	294.40±12.12^{b}	158.85±8.06^{b}	135.65±5.90^{a}	239.53±9.38^{a}	80.53±1.55^{a}	5.67±0.07^{a}	71.90±0.57^{a}
S4 h+G8 h	260.90±11.69^{c}	140.62±6.73^{c}	120.08±6.36^{ab}	221.58±6.83^{b}	80.92±2.19^{a}	5.58±0.09^{a}	72.06±0.66^{a}
S4 h+G12 h	245.75±3.28^{d}	123.10±7.00^{d}	122.65±4.52^{b}	200.78±8.01^{c}	77.68±1.72^{b}	5.39±0.07^{b}	71.26±0.88^{ab}
S4 h+G16 h	198.83±8.15^{e}	86.03±3.17^{f}	112.90±3.17^{c}	154.35±6.03^{e}	68.42±1.18^{c}	5.09±0.03^{c}	70.90±1.75^{ab}
S4 h+G20 h	142.28±6.65^{f}	54.18±4.17^{g}	88.10±3.75^{d}	104.45±7.49^{f}	50.27±2.09^{d}	4.96±0.04^{d}	71.09±0.57^{ab}

*Values are mean +/- standard deviation determinations.
abc: The means with the same superscripts within each column are insignificantly different at $p \leq 0.05$ by Duncan multiple range test.

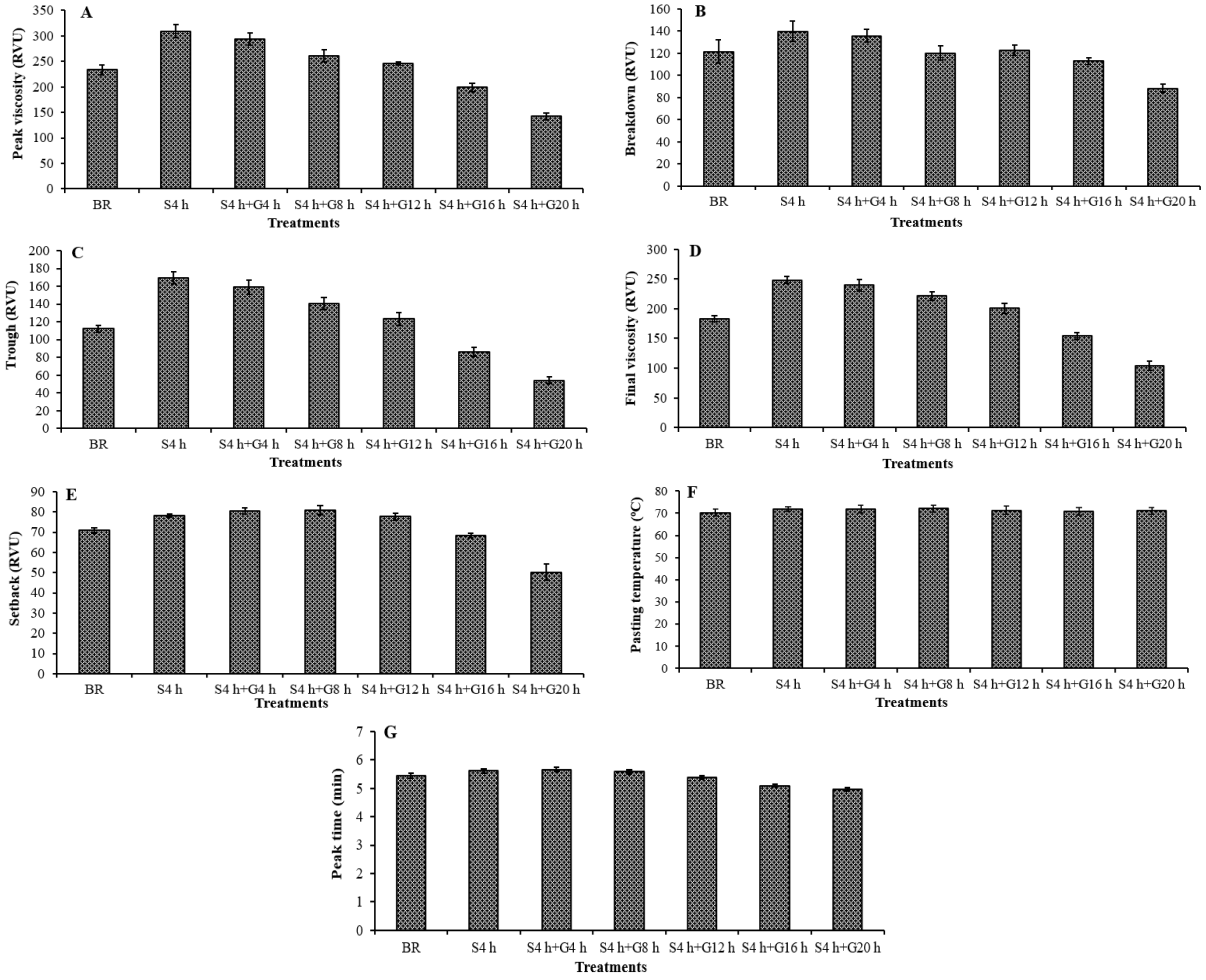
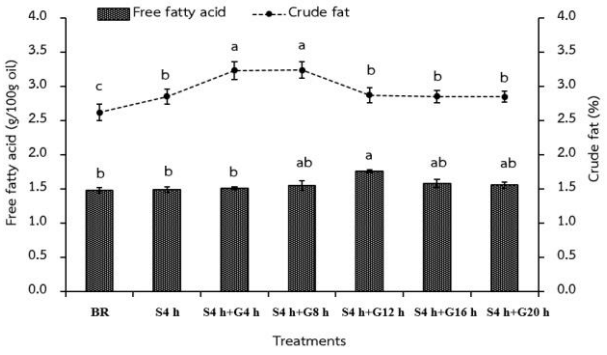


Figure 4. Changes in RVA parameters during germination. A, peak viscosity; B, breakdown; C, trough; D, final viscosity; E, setback; F, pasting temperature; and G, peak time.



The values are mean ± standard deviation (n=3). a-e Means within each treatment followed by different letters are significantly different ($p \leq 0.05$) using Duncan's new Multiple Range Test (DMRT)

Figure 5. Crude fat and free fatty acid of brown rice and germinated brown rice at different times during the germination process

Germination caused a progressive decrease in peak viscosity, trough, breakdown, and final viscosity, with final viscosity dropping from 182.92 RVU in BR to 104.45 RVU after 20 h, and peak time also decreased, suggesting faster cooking properties. Additionally, crude fat content and free fatty acids decreased during germination. These findings offer insights into how soaking, and germination affect the quality of brown rice, especially concerning GABA concentration and pasting characteristics.

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