

Research Article

Influence of germination time on the GABA content and physical properties of germinated brown rice

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Abstract

Germinated brown rice (GBR) is a functional food because it contains gamma amino butyric acid (GABA) which has been reported to promote brain health. The GABA contents and physical properties during germination of two Thai rice varieties, KDML 105 and Chainat 1, were investigated at different times of germination (0-72 h) and temperature combination ($25\pm 1^\circ\text{C}$). GABA content increased dramatically consistent with soaking time. At 72 h of germination in a 50 L tank, the GABA contents were 73.05 and 92.42 mg/100 g. d.b., which were about 13 times higher than in brown rice. Germination time affected darker-coloured grain and a linear relationship between the length of the primary leaf and the GABA content was found. The qualities of cooked rice in terms of water absorption increased, softer textural properties, the expansion ratio in length and volumetric expansion were not significantly different ($p > 0.05$), and the pasting properties of flour decreased as the time of soaking increased were significantly different ($p \leq 0.05$) compared with brown rice. The results suggest that germination time can improve nutritional and physical properties in rice.

Keywords: functional food, GBR, *Oryza sativa*, cooking quality, Thailand.

Introduction

Cereal grain such as rice is an essential component of the daily diet. Rice is one of the most consumed cereals worldwide especially by Asians [1]. The rice kernel consists of a seed coat, embryo and endosperm. The seed coat comprises the husk and also bran tissues. The kernel from which the husk is removed is known as brown rice, which is a functional food. Brown rice contains more nutritional components such as dietary fibre, oryzanol, vitamin E, vitamin B and γ -aminobutyric acid (GABA) than ordinary milled rice. These biofunctional components exist mainly in the germ and bran layers, most of which are removed by polishing or milling. Unfortunately, brown rice takes longer to cook and cooked brown rice is harder to chew and not as tasty as white rice [2].

In recent years, interest in the consumption of germinated brown rice has increased due to increased awareness of its health benefits—mainly the amplified levels of GABA. Germinated brown rice (GBR) is made from brown rice which has been germinated by soaking in water for up to one or two days. The germ produces the physiologically active substances and enzymes to improve the texture of brown rice [3]. During germination, nutrients in the brown rice change dramatically. Nutrients that increase in content include GABA, dietary fibre, inositol, ferulic acid, phytic acid, tocotrienol, magnesium, potassium, zinc, gamma-oryzanol and prolylendopeptidase inhibitor [4].

γ -Aminobutyric acid (GABA) is an amino acid decarboxylation derivative. Glutamic acid is converted to GABA via the enzyme glutamate decarboxylase [5]. GABA is a neurotransmitter in the brain and the spinal cord of mammals [6]. GABA is a four-carbon, non-protein amino acid and is an important component of the free amino acid pool. GABA has an amino group that exists in an unbound form. GABA is highly soluble in water and structurally it is a flexible molecule that can assume several conformations in solution, including a cyclic structure that is similar to proline. GABA is zwitterionic (carries both a positive and negative charge) at physiological pH values (pH values of 4.03 and 10.56) [7].

Many recent studies have reported that GABA provides beneficial effects for human health. It can lower hypertension, promote sleepiness and reduce autonomic disorder observed during the menopausal or presenile period [8]; it has a hypotensive effect, accelerating metabolism in the brain, preventing headaches or depression as after effects of cerebral arteriosclerosis and cerebral apoplexy; it prevents climacteric disorder, preventing presenile derangement such as insomnia and mental irritation; it controls stress [4]; and it inhibits cancer cell proliferation [9]. Intake of GBR instead of white rice is effective for the control of postprandial blood glucose concentration without increasing the insulin secretion in subjects with hyperglycemia [10].

Knowledge of the properties of GBR is used in making secondary products. Procedures for making GBR have been developed to improve the content of some nutrients such as GABA. There have been many studies on products from GBR such as instant nutrition beverages [11], bakery products [12, 13, 14], extruded products [15, 16], rice-balls, rice bread and soups [17], or it can be used in medical treatment such as GABA supplement. Good quality GBR can result in a good quality product. The objective of this research was to study the effect of germination time on the GABA content and some physical properties of germinated brown rice in order to produce GBR with very high GABA content.

Materials and Methods

Materials

Two cultivars of Thai rough rice varieties; *O. sativa* L. cv. Khaodokmali 105 (KDML 105) and Chainat 1 (CNT 1) from the Patumthani Rice Research Centre and the Ayutthaya Rice Research Centre were husked to brown rice (BR) by a laboratory rice mill.

Germination procedure

The experiment was performed by soaking 5 kg of brown rice in a 50 L tank using tap water with the grain-to-water ratio of 1:10 (w/v) at $25\pm 1^\circ\text{C}$ in an air conditioned room for 72 hours, changing the water every four hours. Brown rice was used as a control sample, represented in terms of 0 h germination time. Samples were collected at 12, 24, 36, 48, 60 and 72 hours, respectively. The grain samples after attainment of the required germination period were dried at 60°C with a tray dryer until all final moisture contents were below 13%. All samples were packed in linear low-density polyethylene plastic bags (LLDPE) and stored at -18°C until analyses.

Moisture content analysis and GABA content measurement

The moisture content was determined in triplicate according to the ASAE method [18]. The GABA content was determined by the Institute of Food Research and Product Development consistent with using high performance liquid chromatography (HPLC). The 2.00 g sample was added with 18 mL of deionized water. The mixture was centrifuged at 4,500 rpm for 10 min. The supernatant was mixed with 200 μ L of 0.4 M sodium bicarbonate and 400 μ L 3.98 mM Dabsyl-Cl acetonitrile solution. The reaction was performed at 70°C for 20 min. The sample was filtered into a vial and injected into the HPLC unit (Agilent 1100 series, Agilent Technologies, USA) equipped with a column (Supelcosil-LG-DABS 4.6 mm i.d. and 150 mm length). Acetonitrile was used as the mobile phase with a flow rate of 1 mL/min with the column temperature being 40°C and the ultraviolet detector set at 315 nm [19].

Cooking qualities*Expansion ratio in length*

GBR grain samples were collected and their length measured before heating in boiling water for 10 min. Cooked GBR grain samples were measured for length again. The expansion ratio was calculated by the equation:

$$\text{Expansion length ratio} = \frac{X_L}{Y_L} \quad (1)$$

where X_L and Y_L are the cumulative length of 20 cooked and uncooked grains, respectively [20].

Volumetric expansion

A sample of 1 g of GBR was poured into a 50 mL beaker and 30 mL of water was added and left for 30 min and then boiled at 100°C for 15 min. The leftover water was poured off and the rice was cooled to room temperature. The height of cooked rice in the beaker was measured. The volumetric expansion was determined by the equation:

$$\text{Volumetric expansion ratio} = \frac{V_c}{V_{uc}} \quad (2)$$

where V_c and V_{uc} are the volume of cooked and uncooked GBR grains, respectively [20].

Water absorption

A sample of 20 g of GBR was poured into a 100 mL beaker and 70 g of water was added and left for 30 min and then boiled at 100°C for 15 min. The leftover water was poured into another beaker and the weight of water was measured. The water absorption was determined by the equation:

$$\text{Water absorption (\%)} = \left(\frac{W_c - W_{uc}}{W_{uc}} \right) \times 100 \quad (3)$$

where W_c and W_{uc} are the weight of water before and after cooking GBR grains [20].

Colour

The colour of GBR was measured by a colorimeter (Minolta, CM-3500d, Japan) using the CIELAB system—namely, L^* (lightness), $+a^*$ (redness), $-a^*$ (greenness), $+b^*$ (yellowness), and $-b^*$ (blueness). The colorimetric measurements were performed three times for each sample.

Texture measurements

A texture analyzer (TA-XT plus, England) was utilized to examine the texture of cooked GBR samples. Texture was measured for each sample using seven grains of cooked GBR. The grains were placed on a plate and compressed with a cylinder plunger of 36 mm diameter (P 36R). The

pre-test speed, test speed, and post-test speed of the plunger were set at 1.0, 1.0, and 10 mm/s, respectively. The compression distance was 70% strain. A force-time curve was obtained from the test and the maximum force and area under the curve were determined. (Modified from Perdon *et al.* [21]).

Pasting properties

The pasting properties of the GBR samples were determined with a rapid visco analyser (Newport Scientific, Australia). Rice flour (3 g on a d.b.) was poured into distilled water (25 mL) in a canister and mixed thoroughly. The mixture was stirred at 960 rpm for 10 s and then stirred at 160 rpm. The mixture temperature was first maintained at 50°C for 1.5 min and then raised to 95°C at a rate of 12°C/min. After that, the temperature was maintained at 95 °C for 2.5 min; this was followed by a cooling down period to 50°C with a cooling rate of 12°C/min and was maintained at 50°C for 2.1 min. Each test was done in triplicate. A plot of pasting viscosity in an arbitrary RVA unit (RVU) versus time was used to determine the peak viscosity, temperature at peak viscosity, breakdown viscosity, final viscosity and setback viscosity [22].

Experimental design and statistical analysis

A completely randomized design (CRD) with seven levels of soaking time (0, 12, 24, 36, 48, 60 and 72 h) were investigated. Data of GBR from each rice variety were analyzed by analysis of variance (ANOVA). Duncan's multiple range tests were performed for post hoc multiple comparisons. Statistical significance was determined at $P \leq 0.05$ by the SPSS program, version 16.

Results and Discussion

Changes in appearance and GABA content of germinated brown rice

Germination time affected the appearance of GBR. At 24 h germination, the coleoptile or primary leaf elongated and continued to grow until the final germination time. The length of the primary leaf was 7 mm, as presented in Figure 1. At 60 h germination, the radical or embryonic primary root appeared. Perhaps the fact that the BR was soaked underwater initially may have caused the coleoptile to emerge before the root [23].

The GABA content of the fresh brown rice varieties KDML 105 and CNT 1 was 5.56 and 7.22 mg/100 g d.b., respectively. The GABA content in GBR increased very noticeably (Figure 2). As the soaking time increased, the GABA content increased significantly ($P \leq 0.05$) and continued to increase with time. After 12 h, the GABA contents of GBR cv KDML 105 and CNT 1 were 12.02 and 14.39 mg/100 g d.b. After 24 h, the GABA contents were 32.92 and 27.55 mg/100 g d.b. After 36 h, the GABA contents were 46.55 and 35.53 mg/100 g d.b. After 48 h, the GABA contents were 54.23 and 39.11 mg/100 g d.b. After 60 h, the GABA contents were 59.95 and 87.09 mg/100g d.b. After soaking for 72 h, the highest levels of GABA content of KDML 105 and CNT 1 were 73.05 and 92.42 mg/100 g d.b., respectively. The GABA content of the GBR was about 13 times higher than BR, which was similar to Ohtsubo *et al.* 2005, who produced GBR from BR varieties *Koshihikari*. The GABA content in *Koshihikari* GBR increased during germination and after 72 h was 11.5 times higher than BR. When BR was soaked, the amino acid stored in grains as storage proteins were decomposed by enzymes during germination and then turned into transportable amides and supplied to the growing parts of the rice seedlings [24].

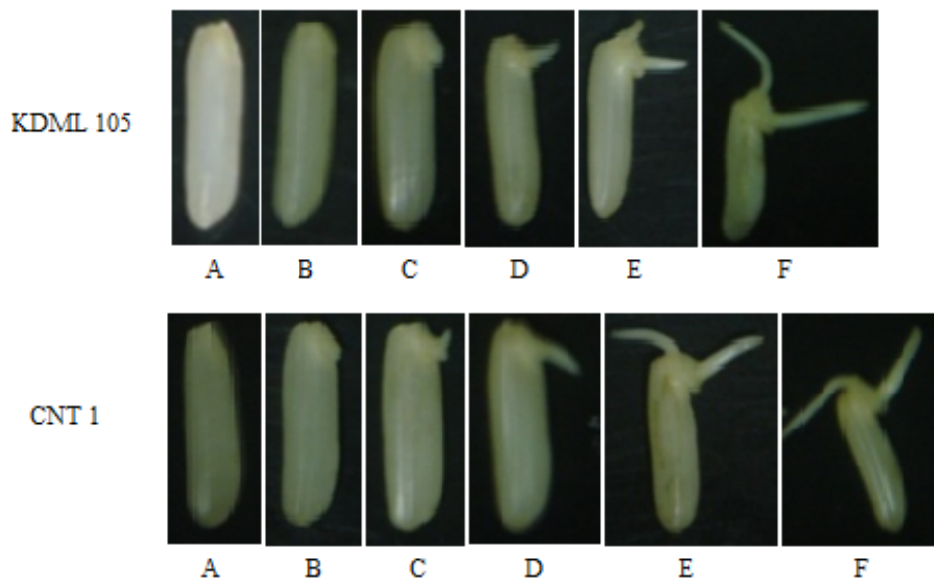


Figure 1. Germinated brown rice varieties KDML 105 and CNT 1 after soaking period of 12h.
(A), 24 h (B), 36 h (C), 48 h (D), 60 h (E), and 72 h (F)

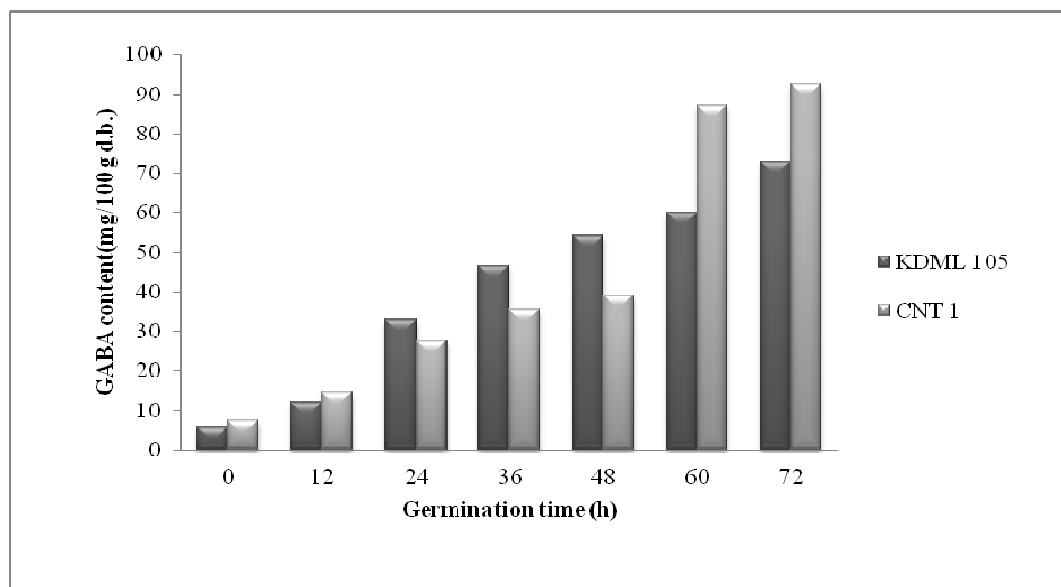


Figure 2. GABA content of GBR varieties KDML 105 and CNT 1 over time of germination at 26°C with a special design of the germination machine and germination process.

Glutamic acid in rice showed an inverse relationship with GABA production; endogenous glutamic acid could be a substrate for GABA production in rice [25]. This is due to the fact that GABA is synthesized from the α -decarboxylation of L-glutamic acid that is catalyzed by the glutamate decarboxylase (GAD) enzyme. The GABA level in plant tissues is low (ranging from 0.03 to 2.00 $\mu\text{mol/g}$ fresh weight) [26], but increases several fold in response to many diverse stimuli including heat shock, mechanical stimulation, hypoxia and phytohormones [7]. In this germination process, brown rice was stressed by cool temperature and changing the water. This can cause an increase in the GABA content. GABA plays a significant role in the growth of plants because GABA is converted to succinic acid by GABA-aminotransferase and a succinic semialdehyde dehydrogenase

enzyme in mitochondria further acted as a metabolizing compound in the TCA cycle [7, 27, 28]. On the other hand, water soaking of rice grains can enrich the GABA content which increased as the soaking time was prolonged [19, 29]. A germination time of 72 h produced GBR with the highest GABA content compared to all other germination times. Figure 3 shows the linear relationship between the length of the primary leaf and the GABA content. The coefficient of determination of the GBR varieties KDML 105 and CNT 1 was 0.7407 and 0.9291, respectively. Therefore, a longer primary leaf indicated a higher GABA content at low temperature.

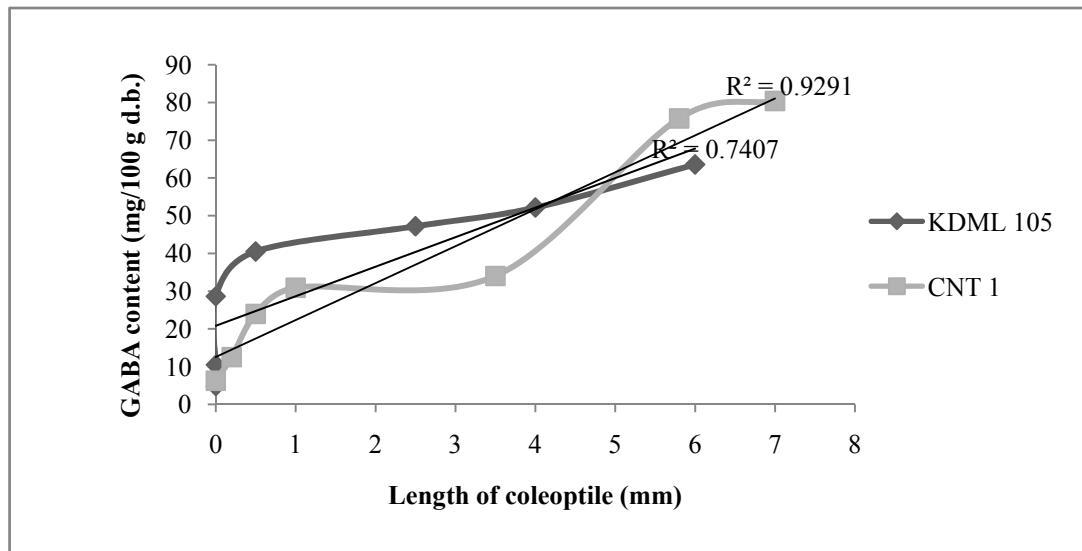


Figure 3. Correlation between GABA content of GBR varieties KDML 105 and CNT 1 and the length of the coleoptile over time of germination at 26°C with a special design of the germination machine and germination process.

Regression analysis for KDML 105 was $y = 7.8253x + 20.802$, $R^2 = 0.7407$; and for CNT was $y = 9.786x + 12.535$, $R^2 = 0.9291$.

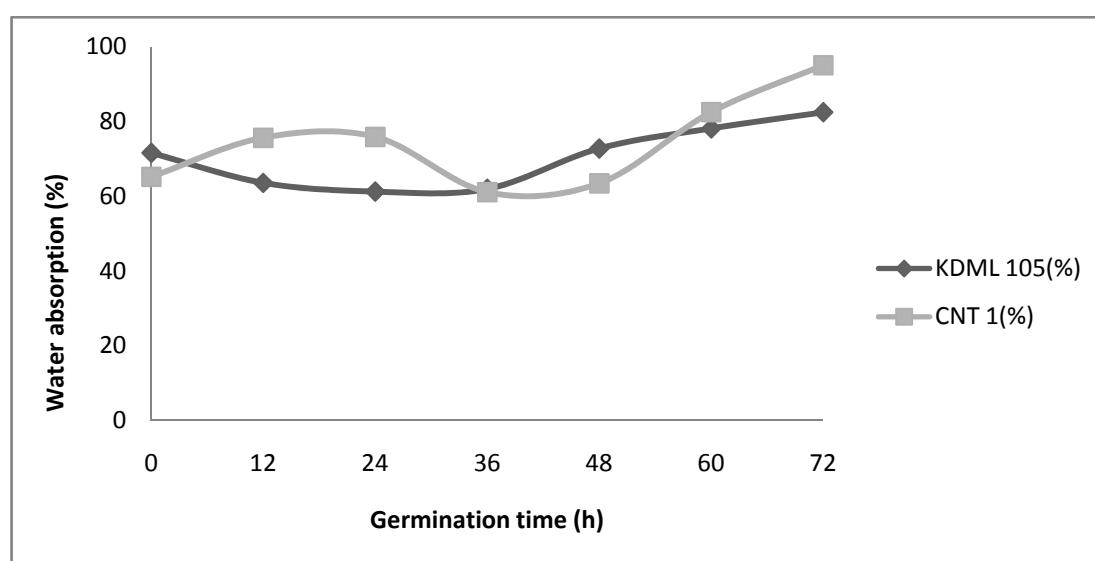
Cooking qualities of GBR

The expansion ratios of GBR in length and volumetric expansion were not significantly different from BR. Table 1 shows the expansion ratios in length and volumetric expansion of rice. The expansion ratio of KDML 105 and CNT 1 was 1.11–1.19 and 1.12–1.47, respectively. The volumetric expansion of KDML 105 and CNT 1 was 1.89–2.14 and 1.56–1.69, respectively. These results may have been due to the outer branny layers around the grains limiting the expansion of BR and GBR [30]. There was a significant decrease and then an increase in water absorption over the time of soaking of GBR for KDML 105 and CNT 1 ($p \leq 0.05$) as presented in Table 1 and Figure 4. Due to the longer germination time, large molecules in grains such as protein and carbohydrate were hydrolyzed to oligosaccharides during the germination process of soaking. Interaction between water and oligosaccharide increased the water absorption of the rice. Therefore, GBR can absorb more water than BR due to the higher sugars [15].

Table 1. Expansion ratio in length and volumetric expansion of GBR over time of germination.

Variety	Value	Germination Time (h)						
		0	12	24	36	48	60	72
KDML 105	Expansion ratio in length (times) ^(ns)	1.19±0.01	1.15±0.08	1.11±0.03	1.14±0.05	1.14±0.03	1.14±0.05	1.15±0.07
	Volumetric expansion (times) ^(ns)	2.11±0.01	2.14±0.43	1.89±0.19	1.89±0.19	1.89±0.19	2.06±0.34	2.06±0.34
CNT1	Expansion ratio in length (times) ^(ns)	1.47±0.08	1.19±0.04	1.13±0.03	1.12±0.01	1.12±0.04	1.13±0.04	1.15±0.04
	Volumetric expansion (times) ^(ns)	1.69±0.04	1.61±0.09	1.56±0.10	1.56±0.10	1.56±0.10	1.65±0.02	1.64±0.07

* ^{ns} with superscript in each row are no significantly different ($P \leq 0.05$), the \pm represents SD value .

**Figure 4. Water absorption of GBR over time of germination.****GBR grain colour**

The L^* , a^* and b^* characteristics of GBR are shown in Figure 5. The colour of the GBR grain darkened significantly ($p \leq 0.05$) over the time of soaking. The result showed that BR grains were lighter (high value of L^*) less red (low value of a^*) and less yellow (low value of b^*). The GBR samples were brownish in colour and with increasing germination time, they became a deeper brown. It has been hypothesized that the variation in colour values among the samples may be attributed to the amount of reducing sugar and amino acid content due to their role in the development of non enzymatic browning [31]. The Maillard reaction (a non enzymatic browning reaction) is a complex set of reactions, initially between a reducing sugar and amino acid [32]. Enzymatic browning reaction from polyphenol oxidase or peroxidase also occurs [33]. GABA increases pigment formation [34]. Thus GBR grains were darker as the soaking time increased.

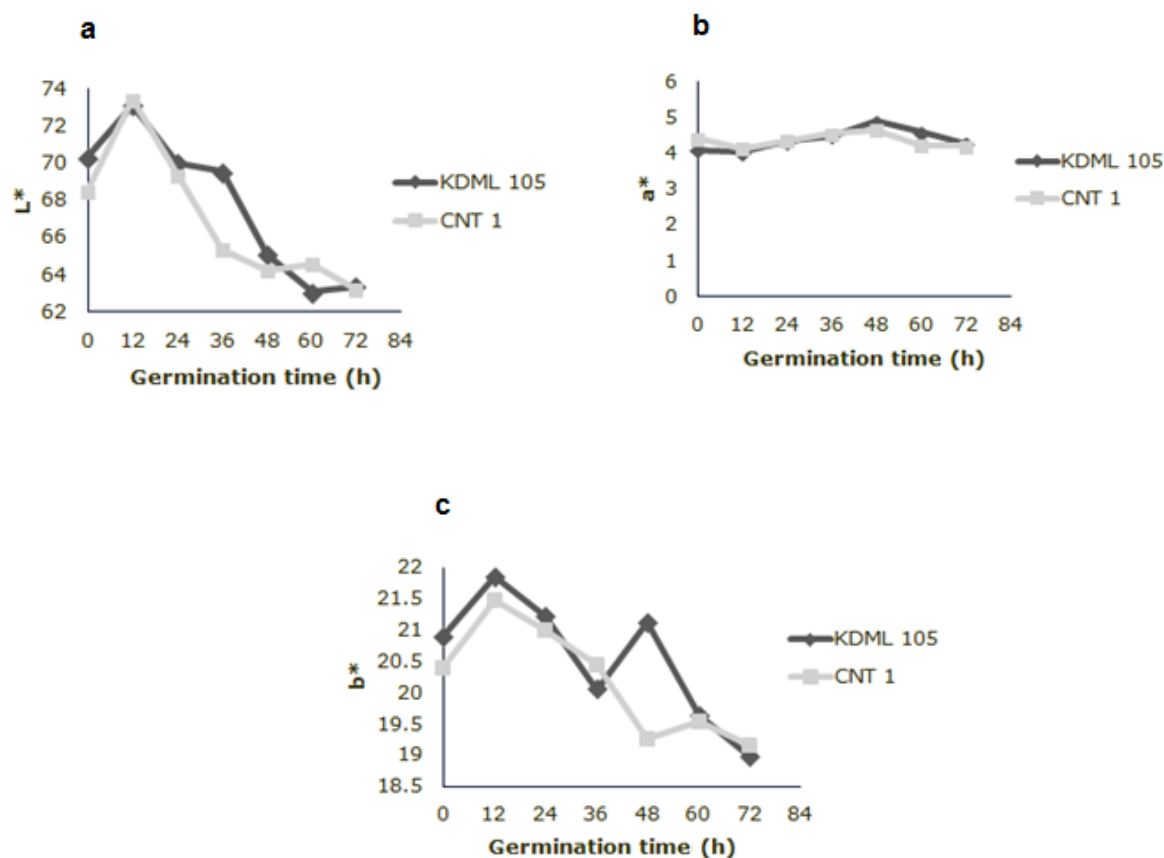


Figure 5. Colour changes as measured by L*, a* and b* of GBR grain over time of germination.

Texture of cooked rice

The texture of cooked GBR was significantly ($p \leq 0.05$) softer as the soaking increased as shown in Table 2. The texture of GBR KDML 105 and CNT 1 for different periods of soaking measured in hardness, stickiness and adhesiveness were the highest and decreased with the germination time. In germinated grains, starch, non-starch polysaccharides and proteins are decomposed and turned into oligosaccharides and amino acids [15]. GBR had partial degradation of the cell wall by xylanases, and protein was degraded by proteases to amino acids [35], and partial digestion of starch granules by amylases to oligosaccharides [15]. The reaction between phytic acid and minerals during the birth of the sprout might be the reason for the softer texture; phytic acid is found predominantly in the bran or outer covering and also in the germ that was hydrolyzed by phytase [4]. Because of the *in vivo* biotransformation, endosperm modification of cereal grains takes place, resulting in softening of the kernel. This indicates that GBR can be easily cooked and will be easier to digest. GBR has a softer texture than BR as the rice granules absorbed water and swelled to a great extent compared to their original size. The granule expansion caused ruptures and hence, amylose leaching. Evidence of amylose leaching in the presence of water above the gelatinization temperature is well documented [36, 37, 38].

Table 2. Textural changes of cooked GBR rice over time of germination.

Variety	Value	Germination Time (h)						
		0	12	24	36	48	60	72
KDM L 105	Hardness (g)	4680.6±242.94 ^d	3986.6±354.35 ^c	3.9414±449.94 ^c	3363.5±210.95 ^b	3378.2±123.38 ^b	3195.8±159.18 ^b	2685.2±173.55 ^a
	Stickiness (N.sec)	-53.29±34.49 ^c	-44.21±111.79 ^b	-62.15±193.21 ^{bc}	-47.21±215.08 ^{bc}	-57.00±205.76 ^{bc}	-49.33±173.83 ^{bc}	-69.32±170.10 ^a
	Adhesiveness (N.sec)	-1.52±1.09 ^c	-8.62±1.67 ^b	-11.29±3.34 ^b	-8.74±3.51 ^b	-12.00±4.33 ^b	-10.27±4.70 ^b	-17.92±4.53 ^a
CNT1	Hardness (g)	8386.3±373.11 ^d	6054.2±512.99 ^c	5888.3±521.27 ^c	5665.7±583.46 ^c	5555.3±525.59 ^{bc}	4940.4±421.16 ^{ab}	4402.5±675.76 ^a
	Stickiness (N.sec)	-41.93±50.55 ^{bc}	-45.32±32.25 ^{bc}	-23.01±7.24 ^c	-50.26±30.90 ^{bc}	-38.05±41.36 ^{bc}	-11.93±49.94 ^{bc}	-17.39±120.92 ^a
	Adhesiveness (N.sec)	-0.90±0.44 ^b	-1.01±0.42 ^b	-0.71±0.25 ^b	-1.12±0.38 ^b	-0.67±0.59 ^b	-2.21±0.77 ^{ab}	-3.58±2.71 ^b

* Means with different superscript letters in each row are significantly different ($P \leq 0.05$), the \pm represents SD value.

Pasting properties of GBR flour

The pasting properties of the two varieties of GBR are shown in Table 3 and Figure 6. The viscosity of GBR flour significantly ($p \leq 0.05$) increased and then decreased over time of soaking. The pasting temperatures of KDML 105 and CNT 1 were around 76.03–78.13 and 79.90–81.00°C. The peak viscosity, breakdown viscosity, final viscosity and set back viscosity of both varieties of rice increased with increasing germination time up to 12 h and then decreased over time. This was probably due to the degradation of starch by enzymes during the soaking process and α -amylase played a significant role in seed germination and was required in starch digestion [39]. The germination process caused a decrease in the protein and starch contents. Protein in rice also had a significant role in the pasting properties of GBR flour by possibly encasing the starch granules and regulating their swelling and resistance to shear at high temperature [40]. The pasting behaviour or rheological properties are also influenced by interaction between the components or by the organization (crystallinity) and also by the size, structure, distribution and water binding capacity of the starch granules. During cooking, the swollen granules are in conflict with each other and some of them (mainly the fragile granules) lose their structural rigidity and form a homogenous mass and as a result, the viscosity decreases [35]. This experiment agreed with an earlier report [41], that studied how germination conditions affect the physicochemical properties of GBR flour and found that the viscosity of GBR flour decreased as the steeping time increased. Thus, the germination time affected the pasting properties of GBR flour.

Table 3. Pasting properties of GBR flour over time of germination.

Variety	Value	Germination Time (h)						
		0	12	24	36	48	60	72
KDML 105	Pasting temp. (°C)	77.85±0.48 ^b	76.03±0.78 ^a	78.13±0.46 ^b	77.62±0.72 ^b	78.10±0.48 ^b	77.47±0.53 ^b	77.88±0.31 ^b
	Peak viscosity (RVU)	209.28±0.92 ^e	265.22±7.43 ^f	114.42±1.88 ^d	40.92±1.09 ^c	13.78±0.43 ^b	4.63±0.61 ^a	2.44±0.59 ^a
	Breakdown viscosity (RVU)	80.56±1.92 ^d	98.47±1.06 ^e	43.89±1.17 ^c	20.11±1.04 ^b	9.09±0.60 ^a	5.01±0.21 ^a	3.22±0.43 ^a
	Final viscosity (RVU)	225.58±4.80 ^e	281.72±3.17 ^f	140.97±1.65 ^d	49.17±1.92 ^c	15.39±0.85 ^b	4.38±0.47 ^a	1.83±0.84 ^a
	Setback viscosity (RVU)	16.30±4.87 ^c	16.50±9.22 ^c	26.56±0.78 ^e	8.25±1.48 ^b	1.61±0.55 ^{ab}	-0.25±0.13 ^a	-0.61±0.64 ^a
	CNT1	Pasting temp. (°C)	81.00±0.43 ^b	79.90±0.05 ^a	80.20±0.48 ^a	79.94±0.07 ^a	80.18±0.45 ^a	80.18±0.41 ^a
Peak viscosity (RVU)		229.64±3.49 ^f	244.11±3.08 ^g	200.09±2.43 ^e	124.93±3.66 ^d	76.89±0.81 ^c	42.19±2.00 ^b	18.90±0.52 ^a
Breakdown viscosity (RVU)		53.08±2.02 ^c	62.75±0.22 ^d	65.81±1.98 ^d	62.09±9.23 ^d	52.94±0.32 ^c	37.75±3.38 ^b	18.65±0.20 ^a
Final viscosity (RVU)		307.28±1.41 ^f	309.00±2.96 ^f	250.67±4.92 ^e	128.61±5.93 ^d	56.83±0.80 ^c	15.72±1.97 ^b	3.47±1.05 ^a
Setback viscosity (RVU)		77.64±2.23 ^f	64.89±1.34 ^e	50.58±2.98 ^d	3.68±8.97 ^c	20.05±0.53 ^a	-26.47±3.37 ^a	15.43±0.56 ^b

* Means with different superscript letters in each row are significantly different ($P \leq 0.05$), the \pm represents SD value.

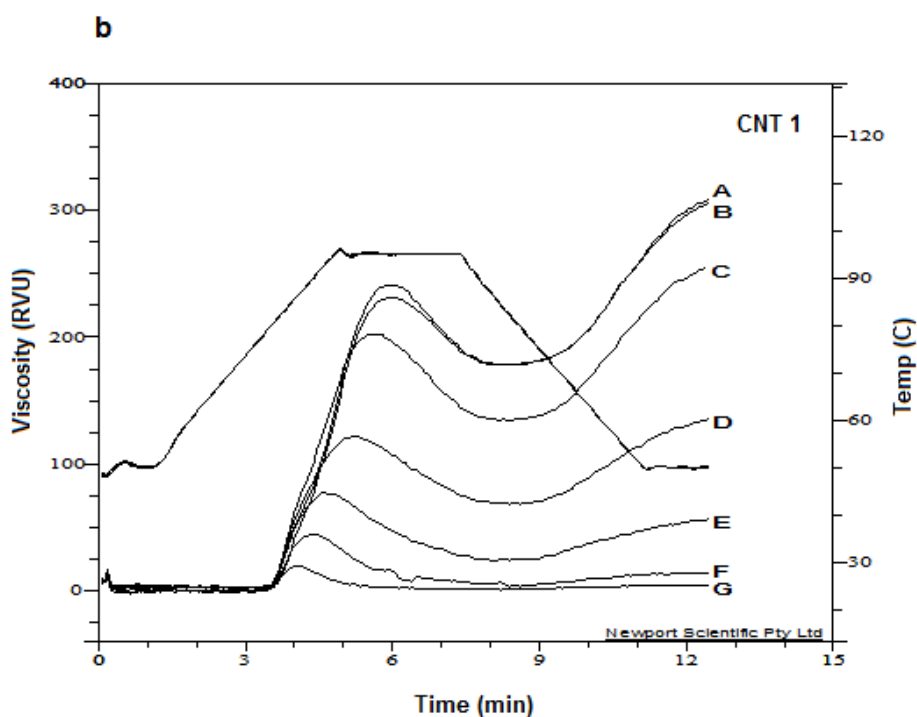


Figure 6. Pasting profile of GBR varieties KDML 105 (a) and CNT 1(b) at various germination times.

0 h (A), 12 h (B), 24 h (C), 36 h (D), 48 h (E), 60 h (F) and 72 h (G)

Conclusion

The results confirmed that the germination time affected the GABA content and some physical properties including appearance and the GABA content. The longer germination time contributed to the higher GABA content and water absorption and the softer texture of cooked GBR which implied that these GBR qualities were effective in lowering the viscosity of GBR flour and changing the appearance of GBR grains to produce a longer leaf and root. The colour of the grain became darker. With high GABA content and other physical properties that differed from BR, GBR may be used as a functional ingredient or secondary functional ingredient in food products such as beverages, confectioneries and bakery products or used in medical treatment to maintain good human health. GBR has the capability to become a foodstuff for humans and also adds value to the rice. Therefore, GBR has high potential to become an innovative rice type by preserving all nutrients in the rice grain for human consumption and to create the highest value.

Further work on GBR is needed to examine greater detail, such as the metabolic activities inside the grain between germination, scale up production of GBR to pilot scale system to produce very high GABA GBR and also to develop products from GBR.

Acknowledgments

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