Effects of germination and aeration treatment following segmented moisture conditioning on the γ-aminobutyric acid accumulation in germinated brown rice

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Abstract: Brown rice was treated by segmented moisture conditioning to reach the suitable water content and aerated with air for germination. The effects of germination and aeration treatment on the γ-aminobutyric acid (GABA) content in germinated brown rice were studied. The results showed that the germination rate, glutamic acid, glutamate decarboxylase activity and GABA content in germinated brown rice increased significantly and then decreased with the increase of germination and aeration treatment parameters. Correlation analysis also revealed that there was a significant positive correlation between GABA accumulation and glutamic acid content, glutamate decarboxylase activity. These results suggested that the aeration treatment during germination following segmented moisture conditioning could contribute to a high GABA content of germinated brown rice.

Keywords: germinated brown rice, segmented moisture conditioning and aeration treatment, GABA, glutamic acid, glutamate decarboxylase

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1 Introduction

Brown rice has physiological activity because of its complete rice germ[1,2]. It can germinate under appropriate conditions[3]. During germination, a large number of endogenous enzymes are activated and released[4,5]. The starch, protein and other macromolecular substances are degraded, which generates the reducing sugar and amino acid easily digested and absorbed by the human body[6]. Germinated brown rice (GBR) is rich in many physiological active components, such as γ-aminobutyric acid (GABA), glutathione and oryzanol[7,8]. Among the components, GABA in particular is more representative due to its unique bioactivity[9]. It has the functions of physiological activity, which can lower blood pressure, enhance brain cell metabolism and promote long-term memory[10,11]. The content of GABA in GBR is ten times that of white rice, and over twice as much as the brown rice[12].

The endogenous glutamate decarboxylase (GAD) is activated during the brown rice germination. Glutamic acid (Glu) in brown rice is transformed into GABA[13]. GABA content significantly increases. The content of GABA in GBR is affected by many factors. The main influencing ones are the content of free amino acids and endogenous GAD activity in brown rice[14]. GAD activity is also affected by germination conditions, oxygen content and other factors[15].

The study and development of GBR or other cereals rich in GABA have become a research focus in recent years[16,17]. Water absorption and germination treatment are beneficial to the accumulation of GABA content in brown rice or other grains[18,19]. It has been reported that the soaking treatment of brown rice in water can increase GABA content[20]. Furthermore, several studies confirm that acid immersion environment is helpful to the accumulation of GABA[21]. For example, Das et al.[22] have indicated that the GABA content of brown rice treated with cellulases solution will increase. Further study indicates that the germination treatment of brown rice after water absorption can significantly promote the accumulation of GABA[23]. Water absorption and germination processes can greatly increase the GAD activity and the content of Glu, and impel GABA accumulation. Some researches have suggested that the aeration treatment of brown rice after soaking can increase the GAD activity and promote the transformation of Glu into GABA[24]. However, some other researches have found that anaerobic treatment is helpful to the accumulation of GABA[25,26]. Although extensive studies have been carried out on the optimization of soaking and germination conditions to increase the GABA content of GBR, few studies have tried to find out the enrichment of GABA in GBR by germination and aeration treatment following segmented moisture conditioning[27,28].

GABA in brown rice is quite soluble in water. In the soaking process, part of GABA may be dissolved in the soaking solution and lost[29]. Therefore, the aims of this study are to a) investigate the effects of the germination and aeration conditions on the GABA

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content of GBR after segmented moisture conditioning treatment and b) study the relationships between GABA accumulation and germination rate, Glu content, GAD activity.

2 Materials and methods

2.1 Brown rice samples

The variety of the paddy rice in the experiment was Early 944 provided by the paddy rice test station of Huazhong Agricultural University. The paddy rice was dried to 14.5% (wb) moisture content after the harvest and then stored at the room temperature of 20 ℃. Brown rice was obtained by using a sheller (THU-35B type, Satake, Tokyo, Japan) to remove the paddy rice hull before the experiment. The brown rice grains with uniform size were selected after the screening and impurity removal. They were disinfected for 30 min with 0.5% sodium hypochlorite solution. Then, they were rinsed three times with distilled water and reserved in a refrigerator at 4 ℃.

2.2 Germination test and sample treatment

GBR was produced by reference to the method of Liang et al.[30] with a slight modification. Two hundred grams of brown rice samples were evenly placed into culture dishes. Then, referring to the method developed by Cao et al.[23], cyclic spray humidification was conducted according to the optimal segmented moisture adding rate. Meanwhile, they were stirred uniformly. After each spray humidification, the brown rice samples were placed in a constant temperature incubator (CTHI-150(A) B type, temperature fluctuation ±0.2 ℃, Shi Dukai Equipment Co., Ltd., Shanghai, China) for 1 h of conditioning treatment at 30 ℃. Moisture conditioning for the brown rice samples was carried out until the target moisture content set in the experiment was reached. They were then evenly distributed in culture dishes covered with gauze. Following that, the dishes were placed in a constant temperature incubator (CTHI-150(A) B type, temperature fluctuation ±0.2 ℃, Shi Dukai Equipment Co., Ltd., Shanghai, China) for germination at the temperature and time set in the experiment. Thereafter, GBR was dried for 6 h at 50 ℃. After cooling, the samples were packaged using self-sealing bags and preserved in a refrigerator at 4 ℃ in reserve.

2.3 Experimental design

2.3.1 Target moisture content

Following the method of Cao et al.[23], the target water content of the brown rice samples reached 27%, 28.5%, 30%, 31.5% and 33% (wb), respectively. According to the above germination method, the brown rice samples were put in a constant temperature incubator (CTHI-150(A) B type, temperature fluctuation ±0.2 ℃, Shi Dukai Equipment Co., Ltd., Shanghai, China) for 44 h germination at 25 ℃. The interval aeration treatment time was 6 h. The volume of aeration was 1.0 L/min. The aeration lasted for 20 min each time. After germination, the germination rate, GABA content, Glu content and GAD activity were measured. Three parallel experiments were performed at each target moisture content level.

2.3.2 Germination temperature

Based on the optimal segmented moisture adding rate, the target water content of the brown rice samples reached 30% (wb). The brown rice samples were put in a constant temperature incubator (CTHI-150(A) B type, temperature fluctuation ±0.2 ℃, Shi Dukai Equipment Co., Ltd., Shanghai, China) for 44 h germination at 15 ℃, 20 ℃, 25 ℃, 30 ℃, and 35 ℃, respectively. The interval aeration treatment time was 6 h. The volume of aeration was 1.0 L/min. The aeration time was 20 min. The germination rate, GABA content, Glu content and GAD activity were determined by sampling. Three parallel experiments were conducted at the level of each germination temperature.

2.3.3 Germination time

The target water content of the brown rice samples reached 30% by the method of Cao et al.[23]. According to the above germination method, the brown rice samples were placed in a constant temperature incubator (CTHI-150(A) B type, temperature fluctuation ±0.2 ℃, Shi Dukai Equipment Co., Ltd., Shanghai, China) and germinated for 36 h, 40 h, 44 h, 48 h and 52 h at 25 ℃. The interval aeration treatment time was 6 h. The volume of aeration was 1.0 L/min. The aeration time was 20 min. After germination, the germination rate, GABA content, Glu content and GAD activity were determined. Three parallel experiments were performed at each germination time.

2.3.4 Aeration treatment

Based on the optimal segmented moisture adding rate, the target water content of the brown rice samples reached 30% (wb). The brown rice samples were then put in a constant temperature incubator for 44 h germination at 30 ℃. During germination, aeration treatment was carried out at the interval of 0 h, 2 h, 4 h, 6 h and 8 h. The volume of aeration was 1.0 L/min. The aeration time was 20 min. The germination rate, GABA content, Glu content and GAD activity were measured by sampling. Three parallel experiments were carried out at the level of interval aeration treatment time.

2.4 Determination of germination rate

The germination rate was determined using the method of Chung et al.[26]. One hundred grains of GBR were randomly selected from the prepared GBR samples. The grain number of the brown rice with the germ breaking through the seed coat up to 0.5-1.0 mm in the germination test was counted. Then its percentage to the total grain number of the brown rice was calculated.

2.5 Analysis of the content of GABA and Glu in GBR

Analysis of the content of GABA and Glu referred to Zhang et al.[21]. Ten grams of the GBR samples were taken to be ground and then were screened through a hole sizer of 0.25 mm. Two grams of the GBR powder was weighed and stored in an Erlenmeyer flask. Following that, it was dissolved in a hydrochloric acid solution of 0.02 mol/L to which a 6% sulfosalicylic acid solution was added. The heating reflux operation lasted for 5 min in the boiling water bath. After oscillation for 30 min, the solution was moved into a 50 mL volumetric flask and diluted to the calibration with the citrate buffer solution of pH 2.2. Then, after standing for 1 h at room temperature, it was centrifuged at 1000 r/min for 15 min. A filtration membrane of 0.45 μm was used for filtering. Thereafter, the GABA content and Glu content were measured with an amino acid analyzer (L-8800, Hitachi, Hitachinaka, Japan).

2.6 Measurement of GAD activity in GBR

Briefly, to obtain the crude enzyme, 50 g of the GBR samples was added to 100 mL phosphate buffer saline. The mixture was then homogenized by grinding. The supernatant was obtained by centrifugation for 5 min at 4000 r/min.

The determination of GAD activity in GBR was carried out by the colorimetric method[31]. The phosphate buffer (200 μL, 50 mmol/L, pH 5.7) containing 1% sodium glutamate and 0.2 mmol/L Pyridoxal 5-phosphate monohydrate was mixed with 100 μL crude enzyme and 100 μL distilled water. The mixture was incubated in thermostatic water of 37 ℃ for 30 min and

quickly put in ice-bath. Thereafter, 200 μL borate buffer (0.2 mmol/L, pH 9.0), 1000 μL re-steaming phenol solution (6%) and 400 μL sodium hypochlorite (effective chlorine 9%) were added. The mixture was shaken vigorously with a shaker and put in a boiling water bath for 10 min. It was then immediately put in ice-bath for 20 min and shaken constantly until the blue-green compound appeared. Following that, 2 mL of ethanol (60%) was added to the mixture. It was then determined using colorimetric analysis at the wavelength of 645 nm with a spectrophotometer. GABA at known concentrations was used as a control. Under the above determination conditions, the amounts of enzyme required to produce 1 μmol GABA/min were taken as a unit of the enzyme activity.

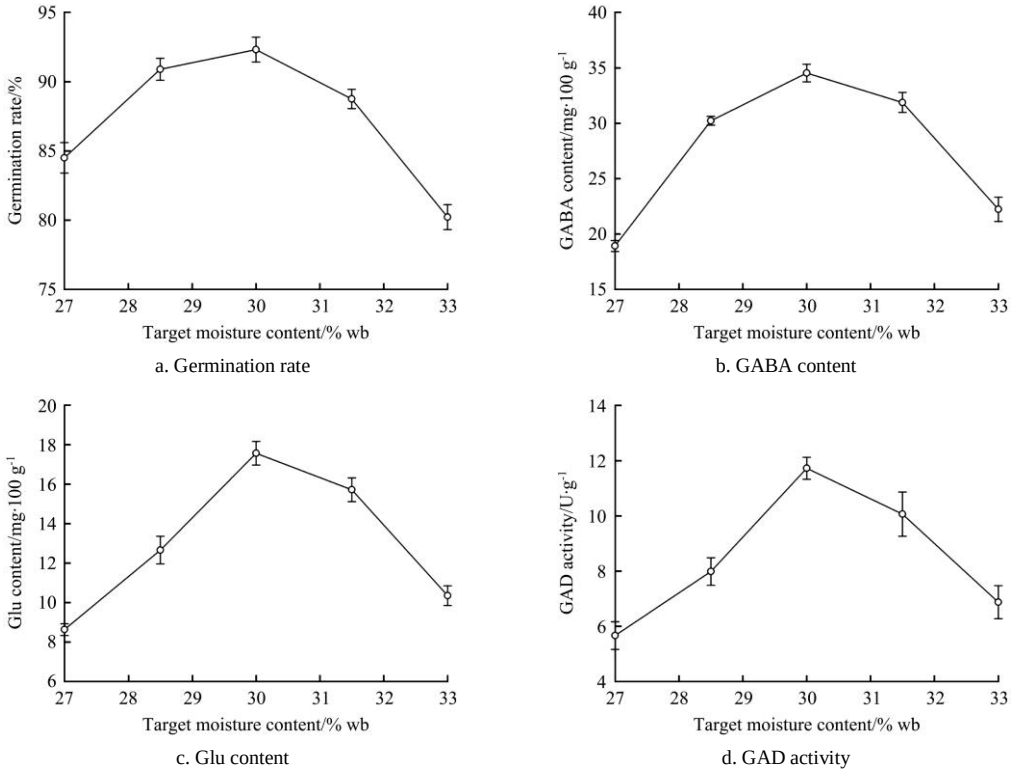
2.7 Statistical analysis

The experiment results were expressed as mean±standard deviation of three repeated experimental determinations. Statistical analysis was carried out using the SPSS program version 16.0 (SPSS Inc., Chicago, IL. USA). Duncan's multiple range tests were employed to determine the significant differences at the level of $p<0.05$.

3 Results and discussion

3.1 Effect of target moisture content conditions on the physiochemical indexes of GBR

As shown in Figure 1a, the germination rates at different target moisture contents were significantly different ($p<0.05$). The germination rate first increased with the increase of the target moisture content. The highest germination rate was achieved at the target moisture content of 30% (wb). As the target moisture content was over 30% (wb), the germination rate decreased. The result also showed that when the target moisture content was 33% (wb), the germination rate was the lowest (80.21%). This indicated that excessive target moisture content affected the germination of brown rice.



a. Germination rate b. GABA content

c. Glu content d. GAD activity

Note: Germination time was 44 h at 25℃. Aeration treatment was at speed of 1.0 L/min for 20 min; interval aeration treatment time was 6 h.

Figure 1 Effect of target moisture contents on physiochemical indexes of GBR

Figure 1b showed the effect of target moisture content on the content of GABA in GBR. The content of GABA first increased with the increase of the target moisture content. At the target moisture content of 30% (wb), the GABA content was 34.53 mg/100 g. When the target moisture content was over 30% (wb), the GABA content decreased. The results also showed that the target moisture content had a significant effect on the content of GABA ($p<0.05$). Figure 1b also indicated that at the lowest target moisture content (27%) (wb), the lowest GABA content was produced. This may be because brown rice needed to absorb water sufficiently during germination. Target moisture content affected the various physiological activation reactions during the brown rice germination[25]. The low target moisture content caused the insufficient water absorption of brown rice, which was not conducive to the production of GABA. Therefore, the optimal target moisture content was 30%.

As illustrated in Figure 1c, within the target moisture content of 27%-30% (wb), the higher target moisture content was more conducive to the accumulation of Glu in brown rice. The effect of target moisture content on Glu content was significant ($p<0.05$). It was clear that when the target moisture content of brown rice was 30% (wb), the content of Glu was the highest (17.56 mg/100 g). When the target moisture content was over 30% (wb), the Glu content decreased. Figure 1c also showed that the Glu content was the lowest at the lowest target moisture content. This indicated that the optimum target moisture content for the Glu accumulation in GBR was 30% (wb).

The target moisture content had a significant effect on GAD activity, shown in Figure 1d. There was no significant difference in GAD activity between the target moisture content of 30% and 31.5% (wb) ($p>0.05$). The GAD activity was the lowest at the target moisture content of 27% (wb) and the highest at the target moisture content of 30% (wb). When the target moisture content was over 30% (wb), a rapid decline in GAD activity was found.

The reason was that the excessive target moisture content caused the brown rice to absorb too much water and swell, resulting in structural damage. The germination of the brown rice germ was affected, thereby influencing the GAD activity.

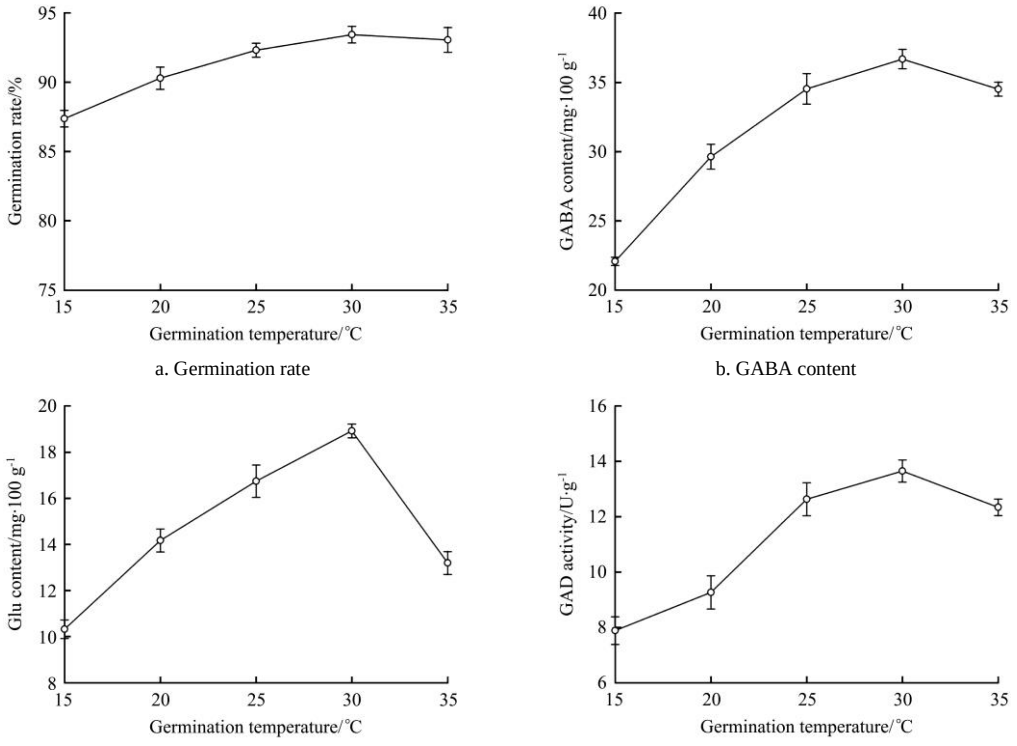
3.2 Effect of germination temperature conditions on physiochemical indexes of GBR

Figure 2a showed that germination temperature had a significant effect on the germination rate (p<0.05). When germination temperature was between 15 ℃-30 ℃, the germination rate of brown rice increased with the increase of germination temperature. When the germination temperature was 30 ℃, the germination rate was the highest. Figure 2a also indicated that the germination rate was the lowest at the lowest germination temperature (15 ℃). The results demonstrated that the enzymatic activity of the endogenous enzyme in brown rice was too low at the temperature below 20 ℃, which was unfavorable to the brown rice germination[27]. Some endogenous enzymes were inactivated at the germination temperature above 30 ℃, which affected the germination of brown rice.

As illustrated in Figure 2b, the highest GABA content obtained at the germination temperature of 30 ℃ was 36.68 mg/100g. The results also showed that GABA content was significantly affected by germination temperature. Figure 2b also indicated that the GABA content at the germination temperature of 35 ℃ was significantly higher than that at 15 ℃ and 20 ℃ (p<0.05). This may be because the GAD catalysis was closely related to germination temperature[28]. When the germination temperature was lower (15 ℃-25 ℃), the catalytic effect of GAD was relatively weak. Thus, the GABA content was low. When the germination temperature was higher (30 ℃-35 ℃), the GABA content was also higher. This temperature range may be suitable for the enzyme catalyzed reaction during the brown rice germination.

At the germination temperature of 15 ℃-30 ℃, the Glu content in GBR increased with the increase of germination temperature, shown in Figure 2c. At the germination temperature of 30 ℃, the Glu content was the highest. When the germination temperature was over 30 ℃, the increase in temperature made the Glu content lower instead. Figure 2c also showed that when the germination temperature was lower (15 ℃), the Glu content was the lowest. This indicated that a relatively high germination temperature was beneficial to the generation and accumulation of Glu.

As shown in Figure 2d, the GAD activity at the lower germination temperature (15 ℃-25 ℃) was significantly lower than that at the higher germination temperature (30 ℃-35 ℃). The research results were consistent with the changing trend of the effect of germination temperature on the GAB content. The results indicated that germination temperature had a significant effect on GAD activity (p<0.05). The catalytic effect of GAD was significantly affected by the germination temperature. Figure 2d also demonstrated that GAD activity was the highest at the germination temperature of 30 ℃.



a. Germination rate b. GABA content

c. Glu content d. GAD activity

Note: Target moisture content was 30% (wb) and germination time was 44 h. Aeration treatment was at speed of 1.0 L/min for 20 min; interval aeration treatment time was 6 h.

Figure 2 Effect of germination temperatures on physiochemical indexes of GBR

3.3 Effect of germination time conditions on physiochemical indexes of GBR

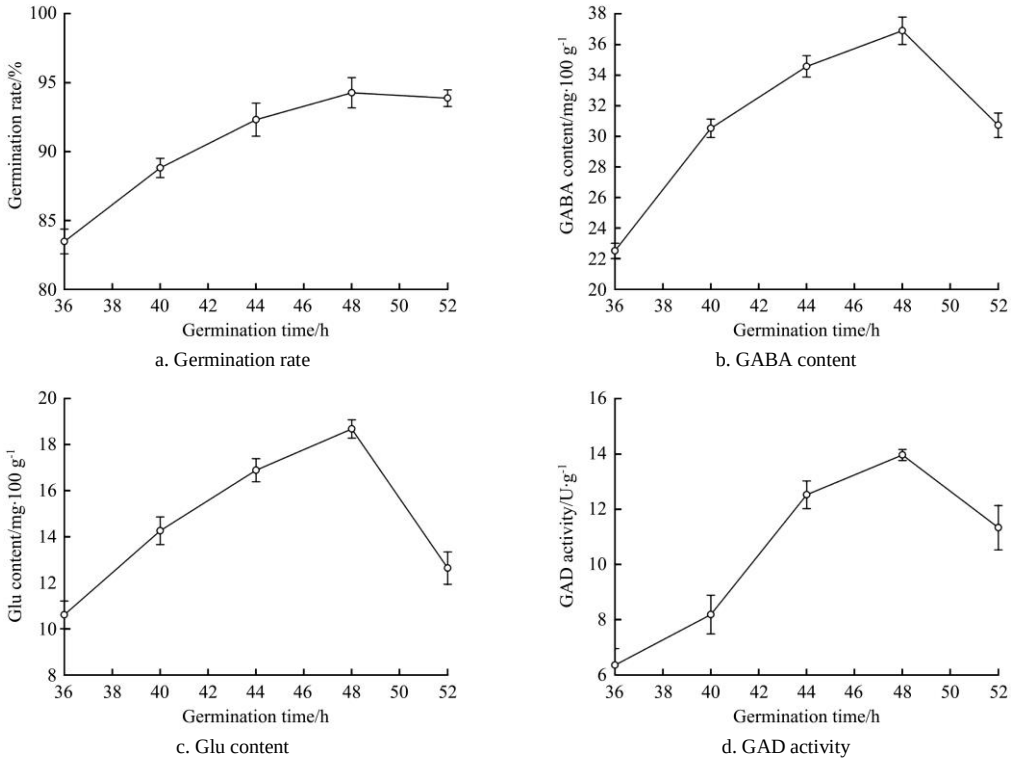
The germination rate increased and reached the maximum value with the germination time, shown in Figure 3a. It was probably because a longer germination time led to more vigorous life activities and material metabolism. The more fully the brown rice germinated, the higher the germination rate was. The results also showed that germination time had a significant effect on the germination rate of brown rice (p<0.05). When the time exceeded 48 h, the change of germination rate was not significant.

With the germination time, GABA content changed significantly during germination, shown in Figure 3b. GABA content gradually increased with the germination time. At the germination duration of 48 h, GABA content was the highest.

This may be because the activated GAD in brown rice continuously acted on glutamic acid during germination, which made GABA gradually accumulate[32]. When the germination time was over 48 h, the GABA content decreased. The possible reason was that with the germination time, GABA was degraded under the action of transaminase.

The effect of germination time on the Glu content was shown in Figure 3c. The Glu content in GBR was significantly affected by germination time (p<0.05). It increased with the germination time. When the germination time was 48 h, the Glu content in GBR was the highest. It indicated that the prolongation of germination time was beneficial to the accumulation of Glu. When brown rice germinated for 52 h, the Glu content was significantly lower than that at the germination duration of 48 h (p<0.05). It showed that the overlong germination time was not conducive to the accumulation of Glu.

Figure 3d showed the effect of germination time on the GAD activity. With the germination time, the GAD activity in brown rice presented a gradually increasing trend. It reached the maximum at the germination duration of 48 h. When the germination time was over 48 h, GAD activity decreased. From Figure 3d, it could also be seen that the GAD activity at the germination duration of 52 h was significantly higher than that at 36 h (p<0.05). The results indicated that germination time had a significant effect on GAD activity (p<0.05). A large number of enzymes were activated and released during germination, including GAD. Therefore, the relatively long germination time helped to improve the GAD activity.



a. Germination rate b. GABA content

c. Glu content d. GAD activity

Note: Target moisture content was 30% (wb) and germination temperature was 25 ℃. Aeration treatment was at speed of 1.0 L/min for 20 min; interval aeration treatment time was 6 h.

Figure 3 Effect of germination times on physiochemical indexes of GBR

3.4 Physiochemical indexes during brown rice germination under different interval aeration treatment times

After the brown rice was humidified based on the optimal segmented moisture adding rate, the GBR subjected to aeration treatment during germination was obtained. Some changes in the physicochemical indexes during germination were found. The physicochemical indexes were significantly influenced by interval aeration treatment time (p<0.05), displayed in Table 1. It was also found that interval aeration treatment time had no significant effect on the germination rate of brown rice (p>0.05). The germination rate of brown rice by aeration treatment was higher than that of the control group. It was the highest at the interval aeration treatment duration of 4 h. It was thus clear that the aeration treatment after segmented moisture conditioning treatment was beneficial to the germination and growth of brown rice. As shown in Table 1, the GABA content could be significantly increased by aeration treatment during brown rice germination after segmented moisture conditioning treatment. This may be related to the increase of the GAD activity in GBR by aeration treatment[33], or be related to the increase of Glu content by aeration treatment, which provided the substrate Glu for GAD decarboxylation reaction[34]. Table 1 also indicated that the Glu content in GBR varied significantly due to the different interval aeration treatment times (p<0.05). With the interval aeration treatment time, the Glu content first increased and then decreased. This indicated that appropriate aeration treatment was beneficial to the generation and accumulation of Glu. When interval aeration treatment time increased, the GAD activity first increased significantly (p<0.05). It reached the highest at the interval aeration treatment duration of 4 h. However, when the interval aeration treatment time was over 4 h, the GAD activity decreased with the interval aeration treatment time.

The results of the correlation analysis were shown in Table 2. There was a significant positive correlation between GABA accumulation and Glu content (r=0.990), GAD activity (r=0.987) (p<0.01). There was no significant correlation between GABA accumulation and germination rate (p>0.05). These results showed that the aeration treatment during germination was

beneficial to the Glu generation and improvement of GAD activity, which contributed to the accumulation of GABA.

Table 1 Effect of interval aeration treatment time on physiochemical indexes during brown rice germination

	0 h	2 h	4 h	6 h	8 h
Germination rate	88.73±3.89^{a}	92.51±2.55^{a}	92.57±2.04^{a}	91.31±4.89^{a}	91.05±2.48^{a}
GABA	26.72±1.02^{e}	35.64±1.39^{b}	39.21±0.69^{a}	32.78±0.96^{c}	29.83±0.83^{d}
Glu	12.22±0.56^{d}	18.45±0.53^{b}	20.59±0.55^{a}	15.62±0.32^{c}	14.93±0.57^{c}
GAD activity	7.83±0.89^{d}	12.83±0.96^{b}	15.62±0.70^{a}	10.55±0.41^{c}	9.86±0.87^{c}

Note: Target moisture content was 30% (wb). Germination time was 44 h at 30 ℃. Aeration treatment was at speed of 1.0 L/min for 20 min. Data were expressed as mean±SD of triplicate determinations. Means within rows followed by the same letter were not significantly different at $p<0.05$. The units of each index are as follows: Germination rate (%), GABA (mg/100 g), Glu (mg/100 g), GAD activity (U/g).

Table 2 Correlation coefficients (*r*) between GABA and physiochemical indexes

r	Germination rate	Glu	Activity of GAD
GABA	0.876	0.990**	0.987**

Note: Correlation coefficient between GABA and physiochemical index was calculated by five time points (interval aeration treatment time of 0, 2, 4, 6, and 8 h in Table 1). ** Extremely significant at $p<0.01$.

The germination process of cereal grains was closely related to the degradation of substances, the formation of plant tissue and the activation of enzymes[35]. During seed germination, oxygen supply was required to ensure normal respiration[36]. Energy was released to meet the needs of various physiological activities. Soaking for too long resulted in anoxia and excessive accumulation of alcohol, which caused cell intoxication and affected embryo germination. This symptom could be significantly improved by increasing the moisture content of brown rice through segmented moisture conditioning treatment combined with aeration treatment. The results of this study showed that the GABA content in GBR increased after aeration treatment, which was consistent with the findings of Guo et al.[24]. The aeration treatment was beneficial to the generation of Glu. The substrate of GAD was more sufficient, which promoted the synthesis and accumulation of GABA.

4 Conclusions

The GABA content in GBR could be significantly increased by increasing the moisture content of brown rice through segmented moisture conditioning treatment, combined with aeration treatment during germination. The germination conditions, including target moisture content, germination temperature and germination time, had significant effects on the physicochemical indexes of GBR, including GABA content, germination rate, Glu content and GAD activity. The target moisture content of 30% and germination at 30 ℃ for 48 h achieved the highest GABA. The results of this study show that GABA accumulation is positively correlated with Glu content and GAD activity ($p<0.01$). Aeration treatment is beneficial to the accumulation of Glu and the improvement of GAD activity, which promotes the synthesis and accumulation of GABA. The present research provides a novel method for the production of GBR rich in GABA.

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