Research Article

Effects of a Combined Processes of Low-Pressure Steam Enrichment and Low-Pressure Superheated Steam Drying on the γ-Aminobutyric Acid Content of Japonica Rice

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In order to improve the content of GABA in japonica rice, so as to improve the nutritional composition and enhance the added value of rice, this study explored a combined process of low-pressure steam enrichment of γ-aminobutyric acid (GABA) content in japonica rice and low-pressure superheated steam (LPSS) drying of japonica rice. The low-pressure energy and the energy stored in rice grains were used to promote water absorption and activation of rice germs in a steam environment. Under the action of glutamate decarboxylase, the germ glutamate underwent an enzymatic reaction to generate GABA, which was transferred to and stored in the rice endosperm due to the concentration gradient and water gradient. After germination, LPSS was successively applied to dry the rice grains. The response surface method was used to optimize the parameters of low-pressure wet steam enrichment and LPSS drying of the rice grains. The experimental results showed that when the pressure was 0.026 MPa, the temperature was 60.66°C, the time was 5.01 h, and the flow rate was 0.31 L/h; the GABA content produced by low-pressure wet steam germination of japonica rice peaked at 94.1751 mg/100 g. When the germinated and enriched japonica rice was put through LPSS drying with a pressure of 0.021 MPa, a temperature of 66.55°C, a time of 1.57 h, and a flow rate of 0.28 L/h, the GABA content peaked at 104.161 mg/100 g. The results show that the combined process of low-pressure steam enrichment and drying continuously produced GABA in the soaking enrichment and drying stages, and the content of GABA in the drying stage accounted for 10% of the total content, which effectively increased the content of GABA in japonica rice and shortened the enrichment time. This study provides a technical reference for industrialized production of GABA-rich brown rice, embryonic rice, and polished rice.

1. Introduction

With the continuous improvement of living standards, nutrition has received increasing attention. GABA-rich rice products are the main research direction of functional rice staple food. There are many ways to promote the increase of GABA in japonica rice, such as germination, injury, and salt stress [1, 2]. But the green and pollution-free enrichment method that only uses the energy, water, and pressure of japonica rice grains to promote the increase of GABA content is easier to be accepted by consumers. Domestic and foreign scholars have done a lot of work on GABA enrichment in rice, mainly focusing on the enrichment method of GABA in germinated brown rice [3, 4], but the taste value of germinated brown rice is obviously poor. This paper designed and studied the enrichment mechanism of GABA affected by various factors when japonica rice reached the germination condition and reached the germination state. In order to achieve the purpose of better tasting value of finished products, furthermore, a method was designed to enrich GABA in japonica rice by low-pressure wet steam and dry it by low-pressure superheated steam, expecting GABA generation in the enrichment and drying stages.
γ-Aminobutyric acid (GABA) is a functional nonprotein amino acid that is widely distributed in animals and plants. Purified as a white powder, it is odorless, is slightly acidic, and is easy to dissolve in water. Under normal conditions, GABA does not easily decompose during cooking or processing, and it is one of the main drug components of medicines for hypertension, cerebral hemorrhage, and epilepsy, as well as having the effects of lowering blood pressure, improving nerve function, and activating kidney function [5–7].

GABA functions as a plant signaling molecule during germination, rooting, and resistance to external environmental stresses. That is, the GABA content in a plant can greatly increase when the plant is in the germination stage or when it is subjected to external environmental stress, such as temperature, humidity, and pressure. In the dormant state, rice has a content of natural free GABA per 100 g of only 2–6 mg, and most of it exists in the germ. To enrich GABA from rice requires a suitable environment to activate the germination of rice so the germ absorbs water to form glutamate, and glutamate decarboxylase is activated at the same time. Then, the glutamate accumulated in the germ is made into a large amount of free GABA under the action of glutamate decarboxylase, and GABA gradually diffuses to the endosperm under the action of the water gradient and concentration gradient [8, 9]. The chemical equation for glutamate decarboxylation is as follows:

\[
\text{HOOC} - \text{C} - \text{C} - \text{C} - \text{COOH} \xrightarrow{\text{glutamate decarboxylase}} \text{HOOC} - \text{C} - \text{C} - \text{C} - \text{NH}_2 + \text{CO}_2
\]

The enrichment process of GABA in the industrial production of rice has gone through four successive stages. The first stage was the brown rice soaking enrichment process: After the rice was hulled into brown rice, it was soaked and enriched. The second stage was the slow and continuous humidification process of brown rice: After the rice was hulled into brown rice, it was enriched by adding a small amount of water. The third stage was the warming, humidification, and enrichment of brown rice by humid air: After the rice was hulled into brown rice, it was enriched by the humid air. The fourth stage is the enrichment technology currently used in industrial production, in which the rice is directly heated and humidified with humid air. The above four stages have all induced brown rice or rice soaked and enriched. The second stage was the slow and continuous humidification process of brown rice: After the rice was hulled into brown rice, it was enriched by adding a small amount of water. The third stage was the warming, humidification, and enrichment of brown rice by humid air: After the rice was hulled into brown rice, it was enriched by the humid air. The fourth stage is the enrichment technology currently used in industrial production, in which the rice is directly heated and humidified with humid air. The above four stages have all induced brown rice or rice to reach germination and have used external stress conditions such as temperature to enrich GABA.

In this study, a low-pressure wet steam enrichment process was designed. The low-pressure wet steam served two functions, i.e., to stress the rice grains and to induce rice germination to enrich its GABA content.

After rice germination is activated, the nutrients are transformed, the soluble matter increases, the structure becomes loose, the water content is high, and the physiological activity is high; therefore, the rice must be dried quickly before it can be stored or milled. If rice is not dried in time, the germ of the rice could continue to grow, and the nutrients could be lost, which could easily cause molding and deterioration of the rice [10, 11]. Rice drying is the primary processing stage of rice. It mainly aims to control the cost and reduce the crackle ratio. In the whole rice processing chain, drying is the key link that determines the quality of the finished products.

In the drying process, it is necessary to keep the GABA content in the rice as high as possible or to create conditions for the continuous production of GABA by the rice. Low-pressure superheated steam (LPSS) drying is a drying method that uses superheated steam as the drying medium under low-pressure conduction to directly contact wet materials to take away their moisture. LPSS can dry the wet material at a relatively low temperature, and no oxygen is needed, which can effectively reduce the oxidation and thermal degradation that cause loss of nutrients during the drying process; therefore, LPSS can dry the material to a suitable moisture at a low temperature, ensuring the appearance quality and nutritional content of the products [12–16].

In this study, the effects of low-pressure wet steam enrichment and LPSS drying processes on the GABA content of rice grains were investigated. The best enrichment and drying conditions were found by single-factor analysis and were optimized by the response surface method to provide a reference for extending the processing chain of rice functional products without pollution or nutrient loss.

2. Materials and Methods

2.1. Experimental Materials and Reagents. The test sample was Suijing 18 japonica rice produced in 2020, which was provided by Qing’an Donghe Jingu Grain Reserve Co., Ltd. GABA (≥99.9%) was from Sigma. Sodium hypochlorite, redistilled phenol, ethanol, and sodium tetraborate were purchased from Sinopharm Chemical Reagent Co., Ltd.

2.2. Experimental Equipment. The enrichment and drying experiment platform are shown in Figure 1. It was mainly composed of 20 components, including an acrylic sealed box, a heater made of aluminum foil, a gas superheater, a temperature sensor, a condenser, and a vacuum pump (1550D Taizhou Fujiwara Tools Co., Ltd.).
The JT1003D electronic balance was from Shanghai Hengji Scientific Instrument Co., Ltd. The TD4C centrifuge was from Changzhou Jintan Liangyou Instrument Co., Ltd. The 721G visible spectrophotometer was from Shanghai Precision Scientific Instrument Co., Ltd. The stainless-steel electric distilled water heater was from Beijing Yongguangming Medical Instrument Co., Ltd.

2.3. Methods

2.3.1. Operation. Put 100 g of unshelled intact rice into the material rack. A total of 100 g unshelled rice was put in the material rack. During the enrichment process, the water bath constant temperature heater (2) was turned on, the water bath and water bath thermostat heater were used to heat the vacuum barrel (3), and then, vacuum pump 20 and the cooling drum 18 were turned on. When the system was in operation, low-pressure wet steam was generated, which passed through the rice from top to bottom to achieve the low-pressure steam enrichment of GABA in the rice. The material is humidified and enriched. During the drying process, continue to open the gas superheater 4 and aluminum foil heater 12 and 13; so that the steam reaches the superheated state, the superheated state of steam has drying function. Japonica rice can be dried under low pressure superheated steam. The steam flow is achieved by changing the vacuum barrels 3 of different diameters.

2.3.2. Effect of Low-Pressure Wet Steam Enrichment on the GABA Content of Rice

(1) Single-Factor Experimental Design. Pressure (0.01, 0.015, 0.02, 0.025, and 0.03 MPa), temperature (50, 55, 60, 65, and 70°C), time (1, 1.5, 2, 2.5, and 3 h), and steam flow rate (0.05, 0.1, 0.3, 0.5, and 0.6 L/h) were tested as single factors to investigate the effect of each factor on the GABA enrichment in rice.

(2) Establishment of the Response Model. With the data from the single-factor experiments, Design Expert 8.0.6 software was used to carry out response surface design. The Box-Behnken design was adopted. The independent variables A, B, C, and D were used to represent the four factors of pressure, temperature, time, and flow rate, respectively. The GABA content (Y) of rice was set as the response value. The factor levels and coding values of the Box-Behnken design are shown in Table 1.

2.3.3. Effect of LPSS Drying on the GABA Content of Rice

(1) Single-Factor Experimental Design. Pressure (0.01, 0.015, 0.02, 0.025, and 0.03 MPa), temperature (50, 55, 60, 65, and 70°C), time (1, 1.5, 2, 2.5, and 3 h), and steam flow (0.05, 0.1, 0.3, 0.5, and 0.6 L/h) were tested as single factors to investigate the effect of each factor on GABA enrichment in rice.

(2) Establishment of Response Model. Based on the single-factor experiments, the same method established in Section 1.3.2.2 was used. The factor levels and coding values of the Box-Behnken design are shown in Table 2.

2.3.4. Determination of GABA Content. The raw material was collected, and the surface water was absorbed by filter paper. The raw material was placed in a rice huller to obtain brown rice. The measurements were made as described by Yao [17]. A total of 5.0 g brown rice was ground into a slurry, diluted to 50 mL with distilled water, vibrated and extracted at 30°C for 2 h, and centrifuged at 4000 r/min for 10 min. The supernatant was filtered (0.22 μm) for the determination of GABA. Then, 0.5 mL of GABA solution was added into a 10-mL colorimetric tube, and 0.2 mL of 0.2 mol/L (pH 9.0) sodium tetraborate buffer, 1.0 mL of 6% redistilled phenol, and 0.4 mL of sodium hypochlorite solution with 6.5% available chlorine were successively added. After mixing until uniform, the solution sat in a boiling water bath for 10 min and then immediately in an ice bath for 20 min with shaking. After the solution turned blue-green, 2.0 mL 60% ethanol was added. After mixing until uniform, the solution was allowed to stand for 40 min, and the optical density at 645 nm was measured. By comparing against the standard curve, the GABA concentration was obtained.

2.4. Data Processing. The single-factor experiment was repeated three times for each group, and the results are expressed as the mean ± standard deviation (SD). Origin 8.0 software was used to plot figures. For the response surface experiments, Design Expert 8.0.6 software was used for data analysis, and plotting of three-dimensional figures and contours was plotted.
leading to the accumulation of GABA, and the accumulated GABA in turn stimulates the continuous release of calcium ions from the cells of fine rice grains, thereby increasing the response of rice grains to adversity [20]. Rice grain growth requires many amino acids with which to synthesize proteins. Hypoxia can hinder protein synthesis, which is equivalent to reducing amino acid consumption and increasing amino acid content, thus promoting the synthesis and accumulation of GABA. A low-pressure environment is conducive to the diffusion of water into the interior of rice, a high water diffusion rate is conducive to the diffusion of GABA into the interior of rice, and the reduced concentration of the enzymatic reaction products is conducive to the enrichment of GABA. A low-pressure environment can raise the gene transcription level of glutamate decarboxylase, increase its activity, and facilitate the enrichment of GABA [21]. However, with the continuous decrease of the absolute pressure (vacuum level increasing), the anaerobic respiration of rice is intense, and more H⁺ is produced than consumed by the synthesis of GABA. The high H⁺ level leads to excessive acidity in rice, which reduces glutamate decarboxylase activity, in turn reducing the GABA content [22].

3. Results and Analysis

The main reason for GABA generation is that the germination condition of japonica rice is reached and the germ is activated. Appropriate moisture, temperature, and oxygen content are the necessary conditions for japonica rice germination. However, the steam flow designed in this paper is relatively slow, and oxygen content is related to absolute pressure, so it is expected that temperature and pressure are significant influencing factors.

3.1. Analysis of Low-Pressure Wet Steam Enrichment Results

3.1.1. Effect of each Single Factor in the Low-Pressure Wet Steam Enrichment Process on the GABA Content of Rice

(1) Effect of Pressure on GABA Content. As shown in Figure 2, when the pressure was less than 0.025 MPa, the GABA content of the rice grain gradually increases with increasing absolute pressure (decreasing vacuum level). When the absolute pressure was greater than 0.025 MPa, the GABA content gradually decreased increasing absolute pressure; when the absolute pressure was 0.025 MPa, the GABA content was the highest. Due to the stimulation by temperature and moisture, germination of the rice grains was activated, and their respiration was enhanced. Under low pressure, the possible reasons for the increase in the GABA content of rice are as follows: The rice grain undergoes anaerobic respiration and produces lactic acid, which leads to an increase in H⁺ in the cytoplasm and a decrease in pH. Glutamate decarboxylase has peak activity at the acidic pH of 5.8, which is conducive to the GABA production [18]. Under low pressure, the oxidative phosphorylation of plant mitochondria is weakened, and their reduction potential is increased, which is also conducive to the accumulation of large amounts of GABA [19]. Under low pressure, the concentration of calcium ions rises rapidly,

Table 1: The factor levels and coding values of the Box-Behnken design of wet steam enrichment.

| Factor      | Code  | Level 0 | Level 1  |
|-------------|-------|---------|----------|
| Pressure/MPa| A     | 0.02    | 0.025    |
| Temperature | B     | 55      | 60       |
| Time/h      | C     | 4       | 5        |
| Flow rate/(L/h) | D | 0.1     | 0.3      | 0.5      |

Table 2: The factor levels and coding values of the Box-Behnken design of LPSS drying.

| Factor      | Code  | Level 0 | Level 1  |
|-------------|-------|---------|----------|
| Pressure/MPa| A     | 0.015   | 0.02     |
| Temperature | B     | 60      | 65       |
| Time/h      | C     | 1       | 1.5      |
| Flow rate/(L/h) | D | 0.1     | 0.3      | 0.5      |

(2) Effect of Temperature on GABA Content. As shown in Figure 3, up until 60°C, the GABA content of rice gradually increases with increasing temperature; above 60°C, the GABA content of rice decreases sharply; when the temperature was 60°C, the GABA content of rice is the highest. Temperature is a main factor that determines the transformation of rice from the dormant state to the activated state after grain shedding. When the temperature is suitable, the germination of rice seeds is activated, so they begin a new life cycle. As the temperature rises up to 60°C, the respiration of the rice grains increases, and under low pressure, more H⁺ is produced, which increases enzyme activity [23]. The best pH value for glutamate decarboxylase activity is 5.8, and the best temperature for its reaction is 40°C [24]. However, this study showed that the best reaction temperature of rice glutamate decarboxylase was approximately 60°C under low pressure. At low pressures, acidic substances accumulate, and gene transcription of glutamate decarboxylase occurs, which enhance the activity and increase the suitable
temperature of the enzyme. Increasing the temperature within a certain range can increase the water absorption by rice grains, increase the water diffusion to the interior of rice, accelerate the transfer of GABA produced in the germ to the endosperm, and reduce the GABA concentration in the germ, which is conducive to the occurrence of enzymatic reactions. However, when the temperature is greater than 60°C, the GABA content decreases sharply, which is due to the slowed enzymatic reactions caused by the inactivation of glutamate decarboxylase at higher temperatures.

(3) Effect of Time on GABA Content. As shown in Figure 4, the GABA content peaked at 5 h. Up to 5 h, the GABA content increased with time, and after that, it decreased sharply. An enzymatic reaction occurs at the germ to generate GABA. Under low pressure, anaerobic respiration occurs, a large amount of H⁺ is produced, an acidic environment is generated, and the activity of glutamate decarboxylase is enhanced. Therefore, at 5 h, the most GABA is produced. Low pressure is conducive to the diffusion of water to the interior of rice. When the time is within 5 h, the rice keeps absorbing water, and the GABA is continuously transferred to the endosperm due to the water gradient, which is conducive to the production of more GABA at the germ [23]. After 5 h, the GABA content drops sharply. On the one hand, in a long-term low-pressure environment, the anaerobic respiration of rice grains produces a large amount of H⁺, which exceeds the amount required to synthesize GABA [22]; on the other hand, the rice grains absorb too much water, the cells rupture, the soluble proteins are dissolved, and the enzyme activity is reduced, so the GABA content in the rice is reduced [25].

(4) Effect of Steam Flow Rate on GABA Content. As shown in Figure 5, up to a flow rate of 0.3 L/h, the GABA content in the rice grain increases; at steam flow rates greater than 0.3 L/h, the GABA content decreases with increasing steam flow rate.

Enough moisture is an important condition for the germination of rice grains. When there is too little steam, the rice grains cannot absorb enough water to activate germination, which could cause the rice to absorb water slowly and inhibit the activation of the rice germ. This is not conducive to water absorption and germination of rice germ, thus hindering the production of GABA. We found that when the steam flow rate was less than 0.3 L/h, increasing the steam flow rate was conducive to the absorption of more water by the rice germ, which enhanced the reaction and produced more GABA; when the steam flow rate exceeded 0.3 L/h and kept rising, the rice grain absorbed water too fast under the low-pressure environment, so the rupture of the cells accelerated, soluble proteins dissolved, and the enzyme activity decreased, thus decreasing the GABA content [26, 27].

3.1.2. Determination of the Optimal Low-Pressure Wet Steam Enrichment Process

(1) Response Surface Analysis Scheme and Results. The response surface analysis scheme and results are listed in Table 3, and the variance in quadratic regression based on the response surface is given in Table 4.

According to the analysis of variance, the P value of the model was significant. The model had a coefficient of determination R² (adjusted) of 0.8128, a coefficient of variation
(CV) of 4.85, and $P = 0.6285 (>0.05)$ for the lack-of-fit term, a nonsignificant difference. Unknown factors interfere with the test results somewhat, indicating that the residuals were all caused by random errors. The $R^2 = 0.9064$ indicates that the degree of fit was good, and the experimental error was small, so the model can reflect the change in the response value. The multivariate quadratic regression model based on the response surface is as follows:

$$Y = 93.83 + 3.12A + 1.35B + 0.45C + 0.65D - 1.97AB$$
$$- 0.46AC - 1.64AD - 0.32BC + 5.68BD - 1.07CD$$
$$- 9.20A^2 - 5.54B^2 - 13.88C^2 - 7.82D^2,$$

where $Y$ is the GABA content (mg/100 g), and $A$, $B$, $C$, and $D$ are pressure, temperature, time, and flow rate, respectively.

The Prob > F value shows that the pressure effect was significant. Within the selected ranges of factors, according to the impact on the results, the order of the effects of single factors was pressure ($A$) > temperature ($B$) > flow rate ($D$) > time ($C$). The response surface $Y$ is the 2D contour map of the 3D space composed of the values of factors $A$, $B$, $C$, and $D$, which can intuitively reflect the interaction between various factors. The response surface figure and contour are plotted in Figures 6–11. The figures show that there was an interaction between each pair of factors, and the optimal point was within the test range in this study.

Design Expert 8.0.6 software was used to optimize the combination of process parameters, and the optimal process conditions with GABA content as the target value and pressure, temperature, time, and steam flow rate as the operating conditions were established as follows: The pressure was 0.026 MPa, the temperature was 60.66°C, the time was 5.01 h, and the flow rate was 0.31 L/h. Under these conditions, the GABA content reached 94.1751 mg/100 g. To facilitate real-world operation, the optimal process parameters were corrected to 0.025 MPa, 60°C, 5 h, and 0.3 L/h. Under these conditions, three tests were performed successively:

### Table 3: Response and face analysis scheme and results.

| No. | $A$: pressure/MPa | $B$: temperature/°C | $C$: time/h | $D$: flow rate/L·h$^{-1}$ | Measured value | Predicted value |
|-----|-------------------|---------------------|-------------|------------------------|----------------|----------------|
| 1   | 0.025             | 60                  | 5           | 0.3                    | 89.49          | 93.83          |
| 2   | 0.025             | 60                  | 6           | 0.1                    | 74.91          | 73             |
| 3   | 0.025             | 65                  | 5           | 0.5                    | 89.48          | 88.14          |
| 4   | 0.025             | 55                  | 6           | 0.3                    | 71.05          | 73.84          |
| 5   | 0.03              | 60                  | 4           | 0.3                    | 75.77          | 73.89          |
| 6   | 0.025             | 65                  | 5           | 0.5                    | 71.77          | 74.1           |
| 7   | 0.025             | 60                  | 4           | 0.3                    | 75.77          | 75.63          |
| 8   | 0.02              | 60                  | 5           | 0.1                    | 70.34          | 71.39          |
| 9   | 0.02              | 60                  | 5           | 0.5                    | 78.05          | 75.99          |
| 10  | 0.03              | 60                  | 5           | 0.5                    | 77.34          | 78.94          |
| 11  | 0.025             | 60                  | 5           | 0.3                    | 92.16          | 93.83          |
| 12  | 0.025             | 60                  | 5           | 0.3                    | 91.48          | 93.83          |
| 13  | 0.025             | 65                  | 6           | 0.3                    | 70.91          | 75.89          |
| 14  | 0.025             | 55                  | 5           | 0.1                    | 85.48          | 84.15          |
| 15  | 0.025             | 60                  | 6           | 0.5                    | 74.62          | 72.16          |
| 16  | 0.03              | 65                  | 5           | 0.3                    | 81.76          | 81.59          |
| 17  | 0.025             | 55                  | 4           | 0.3                    | 74.62          | 72.3           |
| 18  | 0.025             | 60                  | 5           | 0.3                    | 96.59          | 93.83          |
| 19  | 0.02              | 65                  | 5           | 0.3                    | 77.62          | 79.28          |
| 20  | 0.03              | 65                  | 5           | 0.3                    | 84.48          | 82.83          |
| 21  | 0.025             | 65                  | 5           | 0.1                    | 80.48          | 75.48          |
| 22  | 0.02              | 60                  | 4           | 0.3                    | 66.77          | 66.72          |
| 23  | 0.02              | 65                  | 5           | 0.3                    | 72.48          | 72.66          |
| 24  | 0.03              | 60                  | 6           | 0.3                    | 76.48          | 73.86          |
| 25  | 0.02              | 60                  | 6           | 0.3                    | 69.34          | 68.55          |
| 26  | 0.025             | 60                  | 5           | 0.3                    | 99.42          | 93.83          |
| 27  | 0.025             | 60                  | 4           | 0.1                    | 67.48          | 69.95          |
| 28  | 0.03              | 60                  | 5           | 0.1                    | 76.2           | 80.92          |
The average value of 92.04 mg/100 g (91.19, 93.33, and 91.62) was seen, which was 2.27% different from the predicted value, indicating that the results of the response optimization model are consistent with the actual values and are feasible.

Through the experimental study on the influence of temperature, pressure, steam flow, and time on GABA content, the conditions of GABA generation in japonica rice germination and low-pressure treatment were comprehensively considered to make japonica rice show stress resistance and produce GABA. Through parameter optimization, the content of GABA in this paper can reach 94.1751 mg/100 g. The final content is significantly better than the GABA content of 31.79-62.3 mg/100 g obtained in other studies [3, 4, 26].

3.2. Results and Analysis of LPSS Drying

3.2.1. Effect of each Single Factor on GABA Content in Rice during the LPSS Drying Process

(1) Effect of Pressure on GABA Content. As shown in Figure 12, when the pressure was greater than 0.02 MPa, increasing absolute pressure (the vacuum level continued to decrease), the GABA content in the rice grain shows a continuous decreasing trend; when the pressure was less than 0.02 MPa, with decreasing the absolute pressure, the GABA content stays basically unchanged; and when the pressure was 0.02 MPa, the GABA content is the highest. By the nature of LPSS drying, the steam first condenses on the material. When the material is wet, due to pressure, the moisture can be moved from the inside to the outside [27–29], which can keep the germ site moist, promote a continued enzymatic reaction, and yield GABA. Therefore, when the absolute pressure is greater than 0.02 MPa, increasing the absolute pressure (weakening the vacuum), the anaerobic respiration of rice grains continues to weaken, the produced H+ continues to decrease, the pH value increases, the activity of glutamate decarboxylase decreases, and thus the production of GABA decreases. When the absolute pressure is less than 0.02 MPa, with decreasing absolute pressure (vacuum level increasing), the anaerobic respiration of rice grains increases, and a large amount of H+ is produced, so the acidity exceeds the suitable range. However, because the LPSS drying removes some acid, the GABA content does not continue to decrease after the initial drop and stays basically unchanged.

(2) Effect of Temperature on GABA Content. As shown in Figure 13, GABA peaks at an LPSS temperature of 65°C. Up to a temperature of 65°C, the GABA content of rice grains increased steadily, and above 65°C, it decreased. In any drying process, temperature is the main factor affecting the outcome. In this study, temperature was the main factor affecting the production of GABA and was also the main influencing factor of the water removal rate [13, 23]. When the temperature was lower than 65°C, the GABA content increased with increasing temperature; that is, the effect of temperature on the GABA production was greater than that of the transfer resistance caused by the increased water removal rate [13, 23]. When the temperature was lower than 65°C, the GABA content increased with increasing temperature; that is, the effect of temperature on the GABA production was greater than that of the transfer resistance caused by the increased water removal rate [13, 23]. When the temperature was lower than 65°C, the GABA content increased with increasing temperature; that is, the effect of temperature on the GABA production was greater than that of the transfer resistance caused by the increased water removal rate [13, 23]. When the temperature was lower than 65°C, the GABA content increased with increasing temperature; that is, the effect of temperature on the GABA production was greater than that of the transfer resistance caused by the increased water removal rate [13, 23]. When the temperature was lower than 65°C, the GABA content increased with increasing temperature; that is, the effect of temperature on the GABA production was greater than that of the transfer resistance caused by the increased water removal rate [13, 23].

### Table 4: Response face quadratic regression ANOVA.

| Source     | Squares | df | Mean square | F value | P value |
|------------|---------|----|-------------|---------|---------|
| Model      | 1977.3  | 14 | 141.24      | 9.68    | <0.0001 |
| A-pressure | 116.75  | 1  | 116.75      | 8       | 0.0134  |
| B-temperature | 21.71  | 1  | 21.71       | 1.49    | 0.2427  |
| C-time     | 2.45    | 1  | 2.45        | 0.17    | 0.6883  |
| D-flow rate | 5.14    | 1  | 5.14        | 0.35    | 0.5624  |
| AB         | 15.44   | 1  | 15.44       | 1.06    | 0.321   |
| AC         | 0.86    | 1  | 0.86        | 0.059   | 0.8112  |
| AD         | 10.79   | 1  | 10.79       | 0.74    | 0.4042  |
| BC         | 0.42    | 1  | 0.42        | 0.029   | 0.8683  |
| BD         | 128.94  | 1  | 128.94      | 8.84    | 0.0101  |
| CD         | 4.6     | 1  | 4.6         | 0.32    | 0.5833  |
| A^2        | 548.65  | 1  | 548.65      | 37.61   | <0.0001 |
| B^2        | 198.95  | 1  | 198.95      | 13.64   | 0.0024  |
| C^2        | 1248.87 | 1  | 1248.87     | 85.61   | <0.0001 |
| D^2        | 396.86  | 1  | 396.86      | 27.2    | 0.0001  |
| Residual   | 204.24  | 14 | 14.59       |         |         |
| Lack of fit| 138.23  | 10 | 13.82       | 0.84    | 0.6285  |
| Pure error | 66.01   | 4  | 16.5        |         |         |
| Cor total  | 2181.54 | 28 |             |         |         |
Figure 6: Response surface and contour diagram of pressure and temperature influence on GABA content.

Figure 7: Response surface and contour diagram of pressure and time influence on GABA content.

Figure 8: Response surface and contour diagram of pressure and flow rate influence on GABA content.
Figure 9: Response surface and contour diagram of time and temperature influence on GABA content.

Figure 10: Response surface and contour diagram of temperature and flow rate influence on GABA content.

Figure 11: Response surface and contour diagram of time and flow rate influence on GABA content.
removal rate, and the rapid outward transfer of water increases the resistance for GABA to transfer inwardly, so GABA may precipitate out of the grain along the direction of the water gradient.

(3) Effect of Time on GABA Content. As shown Figure 14, the GABA content continuously increases up to 1.5 h and decreases after that. Within 1.5 h, the material is in the accelerated and constant rate drying stages; the surface of the material is wet [30], the rice undergoes anaerobic respiration to produce an acidic condition, and the enzymatic reaction continues to occur in the germ to generate GABA. At this time, the produced GABA is transferred to the interior of the rice along the concentration gradient, and GABA overcomes the reverse flow of water to diffuse into the interior of rice, so the GABA content rises. After 1.5 h, on the one hand, the material enters the reduced rate drying stage or reaches equilibrium moisture, the surface of the material dries, and the surface temperature of the material rises above the suitable range of glutamate decarboxylase, resulting in a decrease in the GABA content; on the other hand, prolonged anaerobic respiration leads to greater acidity and lower in enzyme activity, further decreasing GABA content.

(4) Effect of Steam Flow Rate on GABA Content. As shown in Figure 15, the GABA content rises up to a steam flow rate of 0.3 L/h and drops at higher flow rates. When the steam flow rate is less than 0.3 L/h, a faster steam flow can bring more condensed water, which can keep the material in a humid state during the accelerated and constant rate drying stages when moisture is being removed [16, 31, 32]. Since the water content of the germ is maintained, GABA can continue to be produced there and can be transferred inwardly by the concentration gradient; therefore, the GABA content can continue to increase. When the steam flow is too fast, the drying is too fast, so the moisture can precipitate out quickly. GABA is water-soluble, so a high steam flow rate can cause GABA to precipitate out with the moisture, resulting in a decrease in its content.

3.2.2. The Optimal Drying Scheme Was Determined by the Response Surface Method

(1) Response and Face Analysis Scheme and Results. The response surface analysis scheme and results of the low-pressure overheating steam drying test are shown in Table 5, and the secondary ANOVA of the response surface is shown in Table 6.
According to the analysis of variance, the $P$ value of the model was significant. The model had a coefficient of determination $R^2$ of 0.8784, a coefficient of variation (CV) of 2.14, and $P = 0.8733 (>0.05)$ for the lack-of-fit term, a nonsignificant difference. Unknown factors interfere with the test results somewhat, indicating that the residuals were all caused by random errors. The $R^2 = 0.9392$ indicates that the degree of fit was good, and the experimental error was small, so the model can reflect the change in the response value. The multivariate quadratic regression model based on the response surface is as follows:

$$Y = 103.59 + 1.89A + 1.81B + 0.52C - 1.07D + 4.43AB + 2.93AC - 0.20AD + 0.25BC + 0.3BD + 0.22CD - 8.30A^2 - 4.54B^2 - 4.47C^2 - 6.33D^2,$$

where $Y$ is the GABA content (mg/100 g), and $A$, $B$, $C$, and $D$ are pressure, temperature, time, and flow rate, respectively.

The $Prob > F$ value shows that the pressure effect was significant. Within the selected ranges of factors, according to the impact on the results, the order of the effects of single factors was pressure ($A$) > temperature ($B$) > flow rate ($D$) > time ($C$).

The response surface $Y$ is the 2D contour map of the 3D space composed of the values of factors $A$, $B$, $C$, and $D$, which can intuitively reflect the interaction between various factors. The response surface figure and contour are plotted in Figures 16–21. The figures show that there was an interaction between each pair of factors, and the optimal point was within the test range in this study.

Design Expert 8.0.6 software was used to optimize the combination of process parameters, and the optimal process conditions with GABA content as the target value and pressure, temperature, time, and steam flow rate as the operating conditions were established as follows: The pressure was 0.021 MPa, the temperature was 66.55°C, the time was 1.57 h, and the flow rate was 0.28 L/h. Under these conditions, the GABA content reached 104.161 mg/100 g. To facilitate real-world operation, the optimal process parameters were corrected to 0.02 MPa, 65°C, 2 h, and 0.3 L/h.

### Table 5: Analysis scheme and results of the drying response surface of low-pressure overheated steam.

| No. | $A$: pressure/MPa | $B$: temperature/°C | $C$: time/h | $D$: flow rate/L·h$^{-1}$ | Measured value | Predicted value |
|-----|-------------------|---------------------|-------------|--------------------------|----------------|-----------------|
| 1   | 0.02              | 65                  | 1.5         | 0.3                      | 103.96         | 103.59          |
| 2   | 0.025             | 60                  | 1.5         | 0.3                      | 85.06          | 86.4            |
| 3   | 0.02              | 70                  | 1           | 0.3                      | 96.21          | 95.63           |
| 4   | 0.02              | 65                  | 1.5         | 0.3                      | 99.06          | 103.59          |
| 5   | 0.02              | 65                  | 2           | 0.3                      | 105.42         | 103.59          |
| 6   | 0.015             | 65                  | 2           | 0.3                      | 87.57          | 86.53           |
| 7   | 0.02              | 65                  | 1.5         | 0.3                      | 104.32         | 103.59          |
| 8   | 0.02              | 60                  | 1           | 0.3                      | 92.87          | 92.28           |
| 9   | 0.025             | 70                  | 1.5         | 0.3                      | 98.86          | 98.88           |
| 10  | 0.02              | 70                  | 1.5         | 0.5                      | 93.21          | 93.76           |
| 11  | 0.02              | 60                  | 2           | 0.3                      | 94.4           | 93.04           |
| 12  | 0.02              | 65                  | 2           | 0.1                      | 92.25          | 94.17           |
| 13  | 0.025             | 65                  | 1.5         | 0.1                      | 94.21          | 92.12           |
| 14  | 0.025             | 65                  | 1           | 0.3                      | 88.27          | 89.27           |
| 15  | 0.025             | 65                  | 1.5         | 0.5                      | 90.64          | 89.58           |
| 16  | 0.025             | 65                  | 2           | 0.3                      | 95.37          | 96.15           |
| 17  | 0.02              | 60                  | 1           | 0.3                      | 94.14          | 92.5            |
| 18  | 0.02              | 60                  | 1.5         | 0.5                      | 89.24          | 89.53           |
| 19  | 0.015             | 60                  | 1.5         | 0.3                      | 89.52          | 91.48           |
| 20  | 0.02              | 65                  | 1           | 0.1                      | 91.58          | 93.56           |
| 21  | 0.015             | 65                  | 1.5         | 0.1                      | 88.83          | 87.95           |
| 22  | 0.015             | 65                  | 1           | 0.3                      | 92.17          | 91.34           |
| 23  | 0.02              | 65                  | 1           | 0.5                      | 90.92          | 90.98           |
| 24  | 0.015             | 65                  | 1.5         | 0.5                      | 86.04          | 86.19           |
| 25  | 0.02              | 70                  | 2           | 0.3                      | 97.46          | 97.16           |
| 26  | 0.02              | 65                  | 2           | 0.5                      | 92.45          | 92.45           |
| 27  | 0.02              | 70                  | 1.5         | 0.1                      | 95.65          | 95.31           |
| 28  | 0.015             | 70                  | 1.5         | 0.3                      | 85.62          | 86.26           |
| 29  | 0.02              | 65                  | 1.5         | 0.3                      | 105.21         | 103.59          |
Table 6: Analysis of ANOVA with secondary regression of the low-pressure overheated steam response surface.

| Source     | Squares | df | Mean square | F value | P value |
|------------|---------|----|-------------|---------|---------|
| Model      | 869.84  | 14 | 62.13       | 15.45   | <0.0001 |
| A-pressure | 42.79   | 1  | 42.79       | 10.64   | 0.0057  |
| B-temperature | 39.53 | 1  | 39.53       | 9.83    | 0.0073  |
| C-time     | 3.21    | 1  | 3.21        | 0.8     | 0.3865  |
| D-flow rate| 13.85   | 1  | 13.85       | 3.44    | 0.0847  |
| AB         | 78.32   | 1  | 78.32       | 19.48   | 0.0006  |
| AC         | 34.22   | 1  | 34.22       | 8.51    | 0.0113  |
| AD         | 0.15    | 1  | 0.15        | 0.038   | 0.8486  |
| BC         | 78.32E-01| 1| 78.32E-01   | 19.48E-02| 0.8086  |
| BD         | 0.35    | 1  | 0.35        | 0.088   | 0.7711  |
| CD         | 0.18    | 1  | 0.18        | 0.046   | 0.8333  |
| A^2        | 446.8   | 1  | 446.8       | 111.1   | <0.0001 |
| B^2        | 133.67  | 1  | 133.67      | 33.24   | <0.0001 |
| C^2        | 129.65  | 1  | 129.65      | 32.24   | <0.0001 |
| D^2        | 260.17  | 1  | 260.17      | 64.69   | <0.0001 |
| Residual   | 56.3    | 14 | 4.02        |         |         |
| Lack of fit| 29.14   | 10 | 2.91        | 0.43    | 0.8733  |
| Pure error | 27.16   | 4  | 6.79        |         |         |
| Cor total  | 926.14  | 28 |             |         |         |

Figure 16: Response surface and contour diagram of pressure and temperature influence on GABA content.

Figure 17: Response surface and contour diagram of pressure and time influence on GABA content.
these conditions, three tests were performed successively: The average value of 101.09 mg/100 g (101.32, 103.41, and 101.09) was seen, which was 2.13% different from the predicted value, indicating that the results of the response optimization model are consistent with the actual values and are feasible.

The low-pressure superheated steam drying process designed in this paper aims to provide GABA generation conditions for japonica rice in the drying stage. At present, there is no research in this field at home and abroad. It is clearly indicated in the usage manual of Japanese Satbamboo GABA rice production line provided by Qing’an Donghe
Jingu Grain Reserve Co., Ltd. that GABA is no longer produced in the drying stage of its process.

4. Conclusion

(1) Taking advantage of the fact that rice grains that can greatly increase their GABA production in the germination stage and under environmental stress, a new process of low-pressure wet steam enrichment combined with LPSS drying was proposed to prepare GABA-rich rice grains.

(2) The effects of pressure, temperature, time, and steam flow rate on the GABA content of rice under the low-pressure wet steam condition were studied, and the mechanisms of GABA content changes in rice grains under the effects of these factors were discussed. Using the response surface design, a mathematical model of the effects of those four factors on the GABA content of rice was established, and the mathematical model was optimized. The results showed that when the pressure was 0.026 MPa, the temperature was 60.66°C, the time was 5.01 h, and the flow rate was 0.31 L/h; the GABA content produced by low-pressure wet steam germination peaked at 94.1751 mg/100 g.

(3) On the basis of the GABA enrichment in rice by low-pressure wet steam germination, an LPSS drying experiment was carried out to study the mechanism by which those four factors influenced the GABA content of rice grains during drying. A quadratic regression model based on response surface was established, and optimization of the equation was performed. The results showed that the GABA content reached 104.161 mg/100 g when the absolute pressure was 0.021 MPa, the temperature was 66.55°C, the time was 1.57 h, and the flow rate was 0.28 L/h.

(4) The low-pressure wet steam enrichment and LPSS drying processes can use water, pressure, and the energy inside the rice itself to perform the enrichment of GABA with no pollution, and the LPSS drying process can continue to produce GABA in rice during the drying stage. These findings can provide a reference for the industrial production of GABA-rich brown rice, embryonic rice, and polished rice.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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