Hindawi Publishing Corporation
Journal of Chemistry
Volume 2016, Article ID 4576758, 9 pages
http://dx.doi.org/10.1155/2016/4576758

Research Article

Effects of Aeration Treatment on γ-Aminobutyric Acid Accumulation in Germinated Tartary Buckwheat (*Fagopyrum tataricum*)

Yuanxin Guo, Yunhui Zhu, Chunxu Chen, and Xiaoman Chen

College of Food and Drug, Anhui University of Science and Technology, Bengbu 233100, China

Correspondence should be addressed to Yuanxin Guo; guoyuanxiner@163.com

Received 23 February 2016; Revised 22 May 2016; Accepted 29 May 2016

Academic Editor: Hongshun Yang

To explore the optimum condition of γ-aminobutyric acid (GABA) accumulation in germinated tartary buckwheat, effects of some factors including aeration treatment, physiological indexes, air flow rate, culture temperature, and pH value of cultivating solution under hypoxia on GABA in germinated tartary buckwheat were investigated. The results showed that the dark cultures with distilled water at 30°C, 2 days, and aeration stress with 1.0 L/min air flow rate at 30°C were optimal for GABA accumulation. Under these conditions, the predicted content of GABA was up to 371.98 μg/g DW. The analysis of correlation indicated that there was a significant correlation ($P < 0.01$) between GABA accumulation and physiological indexes. Box-Behnken experimental analysis revealed that optimal conditions with aeration treatment for GABA accumulation in germinated tartary buckwheat were air flow rate of 1.04 L/min, culture temperature of 31.25°C, and a pH value of 4.21. Under these conditions, the GABA content was predicted as high as 386.20 μg/g DW, which was close to the measured value (379.00 ± 9.30 μg/g DW). The variance analysis and validation test suggested that this established regression model could predict GABA accumulation in tartary buckwheat during germination.

1. Introduction

It has been more than 2000 years since bud was turned into human's food in the ancient oriental culture. As a kind of exotic, fashionable, and healthy product, food such as bean sprouts and alfalfa sprouts also received great popularity in the European market [1–3]. In China, there is a special vegetable with the value of medicine: tartary buckwheat (*Fagopyrum tataricum*) sprout which has a long history of being a food but has not obtained the due attention it deserved [4, 5]. Tartary buckwheat belongs to Polygonaceae, whose contents of protein, fat, vitamins, minerals, and polyphenol are generally higher than those of the bulk of the rice, wheat, corn, and other crops [6]. What is more important is that tartary buckwheat is rich in flavonoids such as rutin, which many other cereals do not contain, while the content of tartary buckwheat is about 40~50 times more than that of common buckwheat (*Fagopyrum esculentum*) [7, 8]. For thousands of years, tartary buckwheat has been widely planted and has been made into various foods in the arid area of the northwest and southwest of China, and it is one of the main foods for some poor people in the regions [9–11]. With the development of science and technology, tartary buckwheat has been widely accepted by consumers for its special functions such as lowering blood pressure, hypolipidemic effect, and reducing blood glucose. Thus, the research of tartary buckwheat sprouts is of great academic and practical value [12–14].

Previous research showed that complex biochemical and physiological changes occurred during seed germination or fermentation; as a consequence, some macromolecular substances such as starch and protein are decomposed; the protease inhibitor, phytic acid, and other antinutritional factors are digested or reduced. All these made the protein and amino acid composition become more reasonable [15–17]. Recent studies show that, through the control of the germination and fermentation conditions, we can also enrich γ-aminobutyric acid (GABA) and other functions of those plants which are low or do not have the functional components [18–20].

GABA, the four-carbon nonprotein amino acid, widely exists in eukaryotic and prokaryotic organisms. It contributes to lowering blood pressure, improving the brain function, regulating cardiac arrhythmia, and relieving pain, anxiety, and so on. And, therefore, the development of food rich in GABA has attracted the favor of the majority of scholars [16, 21]. GABA production is mainly synthesized by the impact of GABA shunt, in which glutamate decarboxylase (GAD, EC 4.1.1.15) is the limited enzyme [22].

Previous research has proved that there can be strong activation of GAD activity under the stress of hypoxia, cold, salt stress, and mechanical stimulation in plants, which leads to accumulation of GABA [18, 23, 24]. Among those stresses, the hypoxia stress is the most effective way [25]. Under hypoxia stress, the GABA accumulation of germination soybean [26], millet [27], rice [28], and fava bean [29] had increased greatly compared to that of control. In addition, studies have shown that it can activate GABA synthetase activity through reducing intracellular pH and thus achieve the purpose of enrichment of GABA [23]. At present, the study of tartary buckwheat is mainly concentrated on flavonoids [13, 30, 31], but the accumulation of GABA in germinated tartary buckwheat under hypoxia stress by aeration treatment has not been reported.

In this study, the germinated tartary buckwheat was placed in the medium for the formation of hypoxic environment to establish a method for accumulating GABA in tartary buckwheat sprouts. The effects of optimal opportunities of aeration treatment, the correlation with GABA accumulation, and the other physiological-biochemical indexes on GABA accumulation were investigated. And the aerial culture condition was optimized. In addition, we founded the effective mathematical model so as to provide the theoretical basis for the industrialized production of the functional tartary buckwheat sprouts.

2. Materials and Methods

2.1. Materials. Tartary buckwheat seeds (cultivar yu 6–21) obtained from Ulanqab city, Inner Mongolia province, north of China, in the autumn of 2014, were kept in reserve at −20°C till being used. GABA reference substance (>99%) and dimethylaminoazobenzene sulfonyl chloride (dabsyl chloride, 99%) were bought from Sigma Chemical Co. Ltd. (St. Louis, MO, USA). Reagents used in experiment were of chromatographic grade for HPLC, while other reagents and chemicals used were of analytical grade.

2.2. Germinating and Gaseous Treatment. The tartary buckwheat seeds were disinfected by dipping them into 1% sodium hypochlorite solution for 15 min and then washed and soaked in distilled water at 30 ± 1°C for 4 h. After soaking, the seeds were put in the culture dish covered with 2 layers of filter paper and germinated in the dark for 2 days at 30°C, spraying with distilled water to keep the culture moist every 8 h. They were then put into cultivated pots (diameter 6 cm by 18.5 cm), 10 mmol/L citric acid buffer was added, and then the pots were covered with lids. The culture medium was aerated by a pump and renewed at 24 h intervals until the culture process finished. The air flow rate was controlled by a flow meter (Yuyao Jintai Meter Co., Ltd., Zhejiang, China). Two days later, the seeds germinated under hypoxia were rinsed with distilled water and rubbed down with filter paper. Clean instruments and materials were used to gauge the length of the sprout, GAD activity, and respiration rate. The sample of GABA, free amino acid analysis, and soluble protein were treated by means of vacuum drying method, and then the samples were ground through an 80~100-mesh sieve.

2.3. Decisive Factor of Physiochemical Indexes. A centimeter ruler was used to gauge the sprout length of germinated tartary buckwheat, and thirty sprouts were gauged as a group of samples. An infrared gas analyser (Model CD-3A, Ametek Applied Electrochemistry, Pittsburgh, PA) was employed to measure the respiration rate; the content of soluble protein in the products was decided by Coomassie Brilliant Blue (G250) method; the content of free amino acid was researched by the ninhydrin reaction.

2.4. Determination of GAD Activity. GAD activity was decided according to Guo et al. [19]. One gram of germinated tartary buckwheat was homogenised on an iced-water bath with 6 mL of potassium phosphate buffer (70 mmol/L, pH 5.8), which consists of 2 mmol/L β-mercaptoethanol and 2 mmol/L ethylenediaminetetraacetic acid (EDTA), together with 0.2 mmol/L PLP. The homogenate was transferred to a centrifuge to separate at 10,000 ×g for 20 min at 4°C; after that, the supernatant was gleaned for the determination of enzyme activity. The reaction mixture solution was composed of 200 μL of crudely extracted enzyme liquid and 100 μL of culture medium (1% of Glut, pH 5.8), and the supernatant was cultured at 40°C for 2 h and then heated up to 90°C for 5 min to terminate it. The suspension resulting from the centrifugal sedimentation separation was filtered with a 0.45 μm membrane filter. Then, GABA content in filtrate was analysed. One unit of the enzyme activity was defined as 1 mol per 1 h at 40°C GABA release.

2.5. Determination of GABA Component. Dry powder of germinated tartary buckwheat (0.25 g) was mulled with 5 mL of 7% acetic acid. The purification method of GABA was adopted from Bai et al. [27]. To volatilize the acetum acid and ethanol, the purified supernatant was evaporated at 45°C at 0.1 MPa. The residue was dissolved in 1 M $NaHCO_3$ solution (pH 9) and centrifugal sedimentation separation was carried out at 2,500 ×g for 10 minutes. GABA was determined by HPLC (Agilent 1200, USA) with a ZORBAX Eclipse AAA reversed-phase column (3.5 μm), 4.6 × 150 mm i.d. as described by Syu et al. [32]. 1 mL of dabsyl chloride (2 mg/mL, in acetone) was added to the amino acid solution (1 mL, pH 9.0) and the mixture was heated at 67°C for 10 minutes. Then, the reaction of the mixture was terminated by an ice bath and soon afterwards was detected by UV-vis DAD (diode-array absorbance detection) at 425 nm. Mobile phase B was 0.045 M sodium acetate buffer solution (pH 4), while mobile phase A was acetonitrile, and GABA was demerged from the mixture in 30 min at invariable temperature of 30°C.

2.6. Box-Behnken Design. RSM is deployed to the identification of three independent variables on the GABA content of germination tartary buckwheat and to the optimization of culture conditions for the production of GABA. The experimental design is carried out by using the Statistical Ease software (Design-Expert version 8.0.6 Trial, Delaware, USA, Echip, 2014). On the basis of single factor experiment, the grade of temperature of culture medium (A), pH of culture medium (B), and air flow rate (C) were measured (Table 2). The content of GABA (Y) was considered to be the response variable. The entire design was composed of 17 combinations, five copies of the center points included (Table 1). GABA content data were fitted by multiple regression analysis of this second-order equation below:

$$Y = \beta_0 + \sum_{i=1}^{k} B_i X_i + \sum_{i=1}^{k} B_{ii} X_i^2 + \sum_{i>j}^{k} B_{ij} X_i X_j, \quad (1)$$

where Y refers to GABA content and β_0 stands for the model intercept, while X_i and X_j represent grade of the independent variables, and k denotes the number of the factors being tested ($k = 3$); B_i, B_{ii}, and B_{ij} refer, respectively, to the regression coefficient of linear variable, quadratic regression term, and interaction regression term. The ANOVA (analysis of variance) table is produced to judge quadratic regression coefficient, interaction regression coefficient, and single linear variable.

2.7. Statistical Analysis. Of the three independent experiments, except for special instructions, each value was represented as ±SE, and SPSS (version 16, Chicago, IL, USA) was used to carry out analysis of variance and to assess the data on the basis of it (ANOVA). In order to determine the statistical significance, Duncan's multiple comparison and correlation analysis is deployed in this analysis; meanwhile, a second-order polynomial regression equation based on the analysis of the experimental data using Box-Behnken design (Design-Expert, version 6.0.10, Stat-Ease, Inc.) was established as well; $P < 0.05$ was thought to be significant and $P < 0.01$ was considered to be very significant.

3. Results and Discussion

3.1. GABA Accumulation in Germinated Tartary Buckwheat under Different Opportunities of Aeration Treatment. The right opportunities of aeration treatment have contributed to the accumulation of GABA (Figure 1). The GABA content of germinating tartary buckwheat increased slowly when seeds were treated directly with hypoxia stress after soaking for 4 h at 30°C, and the content of GABA reached 209.08 μg/g in germinating tartary buckwheat with stress when treated for 6 days. However, when the tartary buckwheat was treated with stress after 1-day normal germination, the GABA content increased rapidly to the extent of 240.89 μg/g, which was 1.36 times that of 4-day normal culture. And while the tartary buckwheat was treated with stress after 2-day normal germination, the GABA content increased rapidly to the extent of 371.98 μg/g, which was 2.10 times that of 4-day normal culture. This phenomenon showed that, with the appropriate timing of hypoxia stress, the antireversion force of germinating tartary buckwheat was enhanced, and enzymes like GAD that are related to the GABA generation were activated considerably. But the GABA content of tartary buckwheat did not increase significantly as culture time went on, which indicated that an appropriate stress time was helpful to accumulate GABA. The dark culture with distilled water at 30°C, 2 d, and then aeration stress with 1.0 L/min air flow rate at 30°C was optimal for GABA accumulation.

Table 1: Factors and levels of response surface methodology design.

Variable factors	Symbol	Code levels		
		−1	0	+1
Air flow rate (L/min)	X_1	0.5	1.0	1.5
Temperature (°C)	X_2	25	30	35
pH	X_3	3.0	4.5	6.0

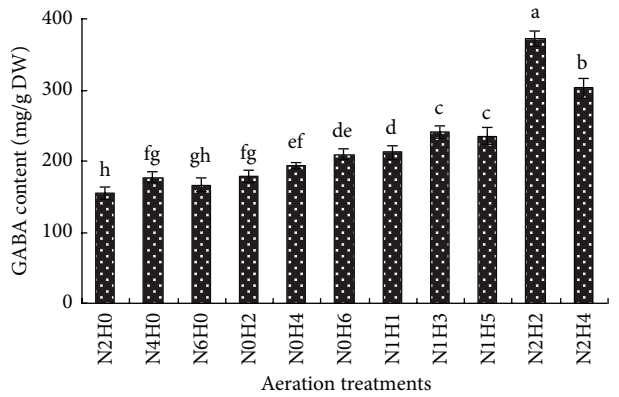

Figure 1: GABA accumulation in germinated tartary buckwheat under different aeration treatments. *N indicates normal dark culture with distilled water at 30°C; H denotes aeration stress with 1.0 L/min air flow rate at 30°C. Therefore, N0H2 denotes normal culture at 0 d and then stress at 2 d, where N2H2 represents normal culture at 2 d and then stress at 2 d.

3.2. Analysis of Physicochemical Properties during Tartary Buckwheat Germination under Hypoxia Stress. The germinated tartary buckwheat seeds cultured for 2 days at 30°C were treated with aeration stress with 1.0 L/min air flow rate at 30°C and pH of 1.0. And the physicochemical indicator of germinating tartary buckwheat had a significant change ($P < 0.05$) (Figure 2). The sprouts length and respiratory rate increased significantly in germination period constantly, reaching 3.58 cm and 1.48 mg CO_2/(g·FW·h) after 48 h under hypoxia stress, 2.00- and 2.20-fold increase over the 0 h sample, respectively. When compared to the normal culture (control), the sprouts length reduced by 18.54%, which inferred that the sprout length of tartary buckwheat was inhibited by hypoxia stress; the respiratory rate significantly increased by 29.83%. The soluble protein content reduced by 29.15% from 0 h to 48 h during stress; however, the free amino acid demonstrated a very significantly increasing trend ($P < 0.01$), which increased significantly by 64.91% compared with control ($P < 0.01$). The decomposition of

Table 2: Correlation analysis among physiological activity, GABA, and other essential substances in germinated tartary buckwheat.

Physicochemical index	Sprout length	Respiratory rate	Free amino acid	Soluble protein	GAD
Respiratory rate	0.99**				
Free amino acid	0.98**	0.97**			
Soluble protein	−0.95**	−0.97**	−0.89**		
GAD	0.98**	0.96**	0.99**	−0.88**	
GABA	0.97**	0.99**	0.95**	−0.97**	0.94**

** Very significant correlations among different indexes ($P < 0.01$).

proteins was promoted by hypoxia stress, which was helpful to accumulate GABA. Meanwhile, the GAD activity had a significant increase during germination ($P < 0.05$), and the GAD activity under stress was 1.36-fold higher than that of the control at 48 h, with a higher significant difference ($P < 0.01$). Particularly, the content of GABA from germinating tartary buckwheat kept a significant increasing trend ($P < 0.05$); the maximum value of 381.92 μg/g appeared under the 48 h stress, which was 2.35 times that of 0 h germination and 1.93 times that of the control group.

The analysis of correlation indicated that the GABA accumulation had a significant positive correlation ($P < 0.01$) with sprout length ($r = 0.97$), respiratory rate ($r = 0.99$), free amino acid ($r = 0.95$), and GAD activity ($r = 0.94$) but had a significant negative correlation ($P < 0.01$) with content of soluble protein ($r = -0.97$) (Table 2). The research of Yao illustrated that GABA yield had a significant positive correlation with sprout length and GAD activity [33]. With increasing the sprout length of germinated tartary buckwheat under hypoxia, the respiratory rate was enhanced, which might be attributed to the stimulation of GAD activity strongly. Meanwhile, the free amino acid was degraded by macromolecular proteins under the action of the protease, and so forth, and it was propitious to the GABA accumulation. The results are in agreement with previous research [26, 29].

3.3. Analysis of Box-Behnken Experiment. The RSM design and the corresponding experimental data were presented in Table 3. Multivariate analysis shows that the model of RSM design was consistent with the second-order polynomial equation referred to in (1). The second-order polynomial model describing the correlational effects of air flow rate (X_1), culture temperature (X_2), and pH of culture solution (X_3) on GABA content from germinated tartary buckwheat was listed

$$\begin{aligned} Y = {} & -2132.95 + 725.0076X_1 + 115.47X_2 \\ & + 160.2159X_3 - 1.20477X_1X_2 - 41.9034X_1X_3 \\ & + 0.558931X_2X_3 - 244.966{X_1}^2 - 1.86525{X_2}^2 \\ & - 15.92319{X_3}^2. \end{aligned} \quad (2)$$

The statistical analysis demonstrated that the model is significant with a desirable value of R^2 (0.9723) in Table 4, and there was fitness of actual and predicted value (Figure 3). ANOVA indicated that the model was most significant ($P < 0.001$) with an F-value of 22.92, the lack of fit was not significant ($P > 0.05$), and the adequate precision of 12.03 displayed an adequate signal. All these results testified to the validity of the experimental model.

3.4. Effect of Air Flow Rate, Culture Temperature, and pH of Buffer Solution on GABA Accumulation. In order to get the optimal level and interrelation of three independent variables during accumulating GABA from germinated tartary buckwheat, the response surface plots were structured, according to (2), which can make it easy to understand the interaction between two variables.

The curves in Figure 4(a) reveal the effects of air flow rate and culture temperature on GABA accumulation in germinated tartary buckwheat at pH of 4.5. Air flow rate had quadratic effects ($P < 0.0001$) on GABA accumulation, and temperature had significant linear ($P < 0.01$) and quadratic ($P < 0.001$) effects on GABA content. However, a significant interaction was not found between air flow rate and temperature ($P = 0.6921$) (Table 4). At a certain temperature, GABA content increased slowly with the rate of air flow increasing and reached a maximum at an air flow of 1.04 L/min. When the air flow rate was fixed, the accumulation of GABA had noticeable enhancement with the increase of temperature, and a maximum of GABA was found at 31.25°C. Previous studies reported that the content of GABA was promoted rapidly under hypoxia, such as soybean, millet, and fava bean using aeration treatment for culture, which led to a sharp increase in GABA accumulation [26, 27, 29].

The effects on GABA accumulation in germinated buckwheat by air flow rate and pH at 30°C were shown in Figure 4(b). Both the linear ($P < 0.05$) and quadratic ($P < 0.01$) effects of temperature on GABA accumulation in germinated tartary buckwheat were significant. The effect of interaction between air flow rate and pH on GABA accumulation was significant ($P < 0.01$) (Table 4). The accumulation of GABA increased with increasing value if the rate of air flow was fixed, and the optimal value of pH was 4.21. The most optimal pH of GAD was 5.5 to 6.0, which suggests that it was helpful to accumulate GABA under sour environment. In this study, the ideal pH value for accumulating GABA from tartary buckwheat in the buffer solution was 4.21, which was higher than fava bean [29] and soybean [26], but lower than millet [27].

Figure 4(c) shows the effects of temperature and pH on accumulation of GABA in germinated tartary buckwheat. The effect of interaction between temperature and pH on GABA accumulation was not significant ($P = 0.5835$) (Table 4). With the increase of temperature and pH, GABA

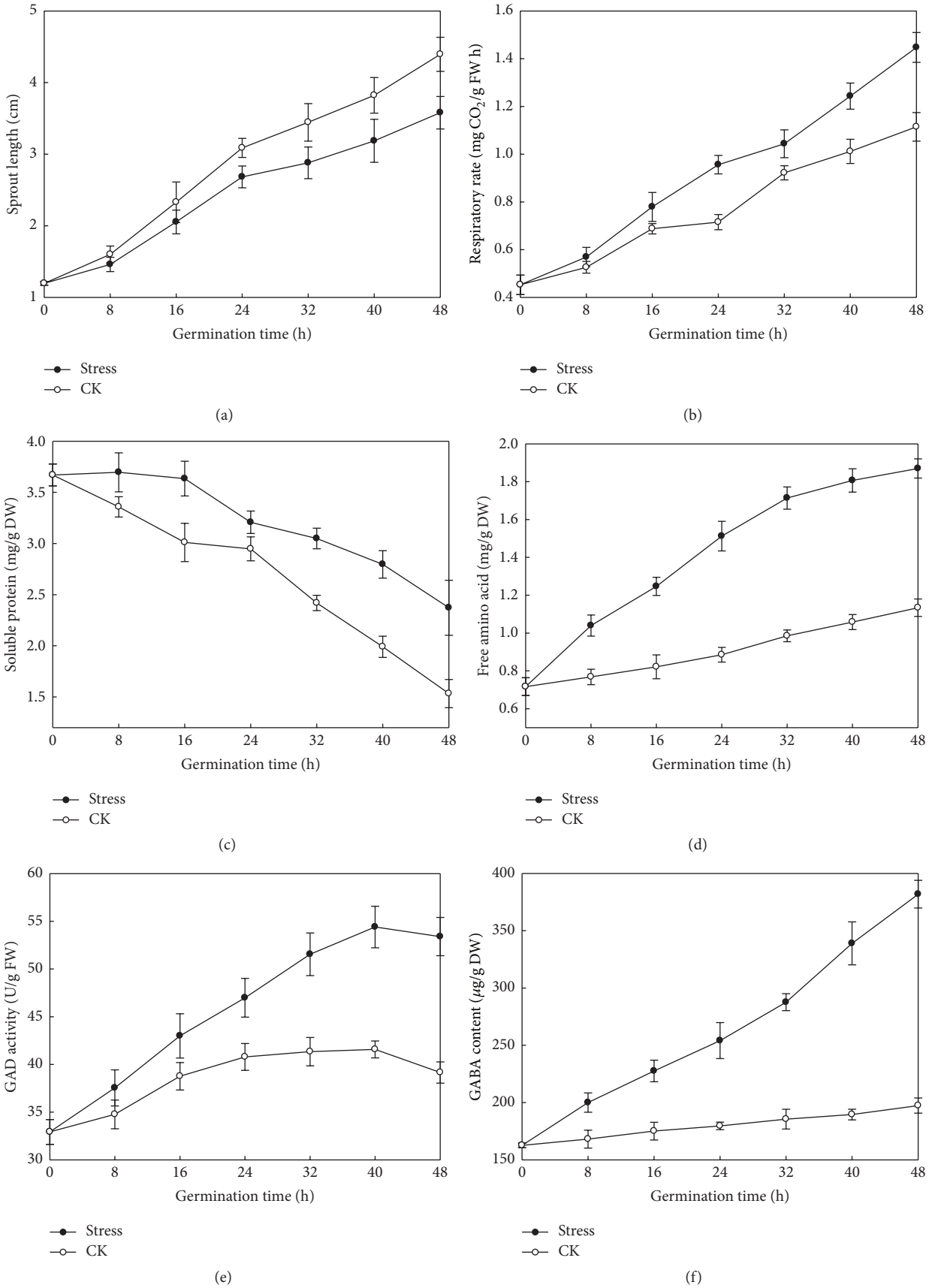

Figure 2: Effects of germination time on the physiological indexes of tartary buckwheat.

Table 3: Box-Behnken design and experiment data for GABA content from germinated tartary buckwheat.

Trial	X_1 Air flow rate (L/min)	X_2 Temperature (°C)	X_3 pH	GABA content (μg/g)	
				Observed value	Predicted value
1	0.5	25	1.0	243.79 ± 7.63	241.34
2	1.5	25	1.0	265.05 ± 6.45	257.73
3	0.5	35	1.0	288.70 ± 7.72	296.02
4	1.5	35	1.0	297.92 ± 5.97	300.36
5	0.5	30	0.5	256.20 ± 7.82	260.40
6	1.5	30	0.5	324.56 ± 6.79	333.62
7	0.5	30	1.5	307.63 ± 11.48	298.57
8	1.5	30	1.5	250.28 ± 7.91	246.08
9	1.0	25	0.5	293.24 ± 10.75	291.49
10	1.0	35	0.5	343.27 ± 15.23	331.76
11	1.0	25	1.5	246.90 ± 12.59	258.42
12	1.0	35	1.5	313.70 ± 4.20	315.45
13	1.0	30	1.0	385.99 ± 13.26	381.74
14	1.0	30	1.0	403.01 ± 17.04	381.74
15	1.0	30	1.0	382.69 ± 7.97	381.74
16	1.0	30	1.0	374.74 ± 10.07	381.74
17	1.0	30	1.0	362.25 ± 5.80	381.74

Table 4: Analysis of variance (ANOVA) for the regression equation.

Source	Sum of squares	Degree of freedom	Mean squares	F-value	P value
Model	43918.46	9	4879.83	22.92	0.0002
X_1	215.00	1	215.00	1.01	0.3484
X_2	4734.41	1	4734.41	22.24	0.0022
X_3	1218.69	1	1218.69	5.72	0.0480
X_1X_2	36.29	1	36.29	0.17	0.6921
X_1X_3	3950.76	1	3950.76	18.56	0.0035
X_2X_3	70.29	1	70.29	0.33	0.5835
X_1^2	15791.61	1	15791.61	74.18	<0.0001
X_2^2	9155.68	1	9155.68	43.01	0.0003
X_3^2	5404.58	1	5404.58	25.39	0.0015
Residual	1490.24	7	212.89		
Lack of fit	589.90	3	196.63	0.87	0.5253
Cor. total	45408.69	16			

R^2 = 0.9723; adj. R^2 = 0.9723; adequate precision = 12.030.

content was increasing as well. But excessively high temperature or pH was disadvantageous to the accumulation of GABA.

3.5. Verification of the Model. According to the experimental data above, the optimal conditions obtained from the model were identified as follows: air flow rate 1.04 L/min, culture temperature 31.25°C, and cultivating solution pH 4.21; the highest yield of GABA predicted by model was 386.20 μg/g DW under these conditions. Previous researchers optimized the conditions of accumulating GABA in germinated millet, soybean, and fava bean under hypoxia stress [26, 27, 29]. The air flow rate, culture temperature, and pH value reported were similar, but the optimum culture conditions for GABA concentration were different for the different plants mentioned above, for the reason that the tolerance level of different plants towards stress differed.

To verify the feasibility of the regression model, the optimal combination experiments and a randomly selected combination for GABA accumulation were investigated, and the experiment design and results were displayed in Table 5. The GABA content observed in germinated tartary buckwheat reached 379.00 ± 9.30 μg/g DW under optimal condition, which was quite close to the predicted value. The experimental results of random assortment proved that the model was valid.

Table 5: Arrangement and result of confirmatory trials.

Trials	Air flow rate (L/min)	Temperature (°C)	pH	GABA content (μg/g DW)	
				Actual value	Predicted value
Optimum conditions	1.04	31.25	4.21	379.00 ± 9.30	386.20
Random condition	1.2	28	4.5	360.47 ± 5.24	357.30

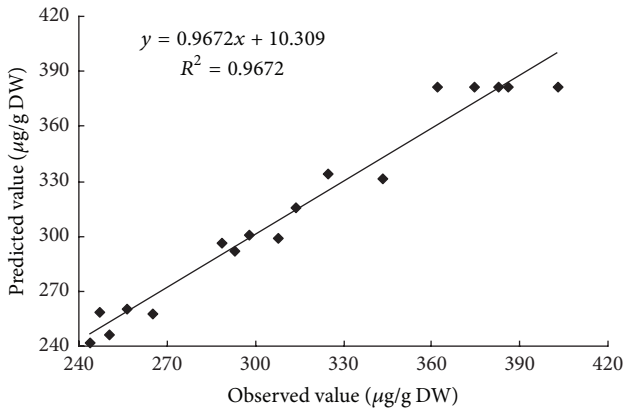

Figure 3: Correlation between predicted and observed values of GABA content.

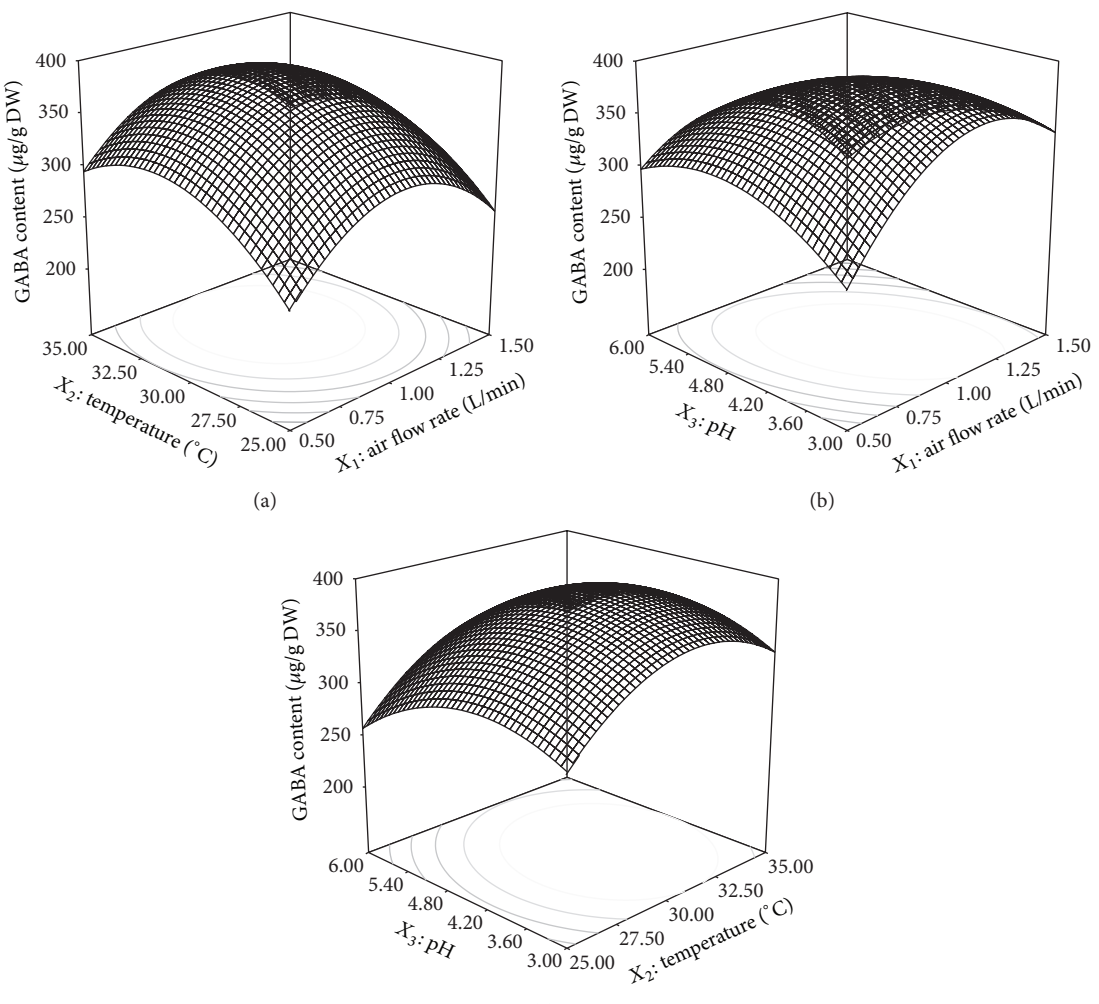

Figure 4: Effect of interaction between pairs of variables on GABA content: (a) air flow rate 1.0 L/min; (b) pH 4.5; (c) temperature 30°C.

4. Conclusions

The effects of different opportunities of aeration treatment and the culture conditions on GABA accumulation in germinated tartary buckwheat were studied. It turned out that the best stress treatment way was aeration stress for 2 days in darkness after dark culture for 2 days at 30°C with distilled water, and the observed value reached 371.98 μg/g. Physiological indexes had a significant ($P < 0.05$) change during germination under hypoxia. Correlation analysis showed that there was a significant correlation ($P < 0.01$) between the GABA accumulation and the other physicochemical indexes. The maximum GABA accumulation in germinated tartary buckwheat was predicted as 386.20 μg/g with the following conditions: air flow rate 1.04 L/min, culture temperature 31.25°C, and pH of cultivating solution 4.21. Under these conditions, the physical production of GABA was 379.00 ± 9.30 μg/g. The results suggest that the model can effectively predict the GABA production.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

Authors' Contributions

Yuanxin Guo and Yunhui Zhu share an equal contribution.

Acknowledgments

This study was financially supported by the Anhui Provincial Natural Science Foundation (1308085MC32).

References

[1] Y.-H. Hong, W.-W. Chao, M.-L. Chen, and B.-F. Lin, "Ethyl acetate extracts of alfalfa (*Medicago sativa* L.) sprouts inhibit lipopolysaccharide-induced inflammation *in vitro* and *in vivo*," *Journal of Biomedical Science*, vol. 16, article 64, 2009.

[2] C. Martínez-Villaluenga, Y.-H. Kuo, F. Lambein, J. Frías, and C. Vidal-Valverde, "Kinetics of free protein amino acids, free non-protein amino acids and trigonelline in soybean (*Glycine max* L.) and lupin (*Lupinus angustifolius* L.) sprouts," *European Food Research and Technology*, vol. 224, no. 2, pp. 177–186, 2006.

[3] S. Chen, C. Wang, H. Chen, and Y. Qiu, "The effect of Zn-added nutrient fluid on the quality of tratary buckwheat bud," *Journal of Chinese Institute of Food Science and Technology*, vol. 6, no. 6, pp. 47–51, 2006 (Chinese).

[4] S.-L. Kim, S.-K. Kim, and C.-H. Park, "Introduction and nutritional evaluation of buckwheat sprouts as a new vegetable," *Food Research International*, vol. 37, no. 4, pp. 319–327, 2004.

[5] K. Kitazaki, S.-I. Watanabe, A. Okamoto, M. Matsuo, S. Furuya, and K. Sameshima, "Far-red light enhances removal of pericarps in tartary buckwheat (*Fagopyrum tataricum* Gaertn.) sprout production under artificial lighting," *Scientia Horticulturae*, vol. 185, pp. 167–174, 2015.

[6] X.-Y. Huang, F. J. Zeller, K.-F. Huang, T.-X. Shi, and Q.-F. Chen, "Variation of major minerals and trace elements in seeds of tartary buckwheat (*Fagopyrum tataricum Gaertn.*)," *Genetic Resources and Crop Evolution*, vol. 61, no. 3, pp. 567–577, 2014.

[7] N. Gupta, S. K. Sharma, J. C. Rana, and R. S. Chauhan, "Expression of flavonoid biosynthesis genes vis-à-vis rutin content variation in different growth stages of *Fagopyrum* species," *Journal of Plant Physiology*, vol. 168, no. 17, pp. 2117–2123, 2011.

[8] D. Li, X. Li, and X. Ding, "Composition and antioxidative properties of the flavonoid-rich fractions from tartary buckwheat grains," *Food Science and Biotechnology*, vol. 19, no. 3, pp. 711–716, 2010.

[9] C. Li, K. Kobayashi, Y. Yoshida, and R. Ohsawa, "Genetic analyses of agronomic traits in Tartary buckwheat (*Fagopyrum tataricum (L.) Gaertn.*)," *Breeding Science*, vol. 62, no. 4, pp. 303–309, 2013.

[10] S.-Q. Li and Q. H. Zhang, "Advances in the development of functional foods from buckwheat," *Critical Reviews in Food Science and Nutrition*, vol. 41, no. 6, pp. 451–464, 2001.

[11] X. Guo, K. Zhu, H. Zhang, and H. Yao, "Anti-tumor activity of a novel protein obtained from tartary buckwheat," *International Journal of Molecular Sciences*, vol. 11, no. 12, pp. 5201–5211, 2010.

[12] L. M. Paucar-Menacho, M. A. Berhow, J. M. G. Mandarino, E. G. de Mejia, and Y. K. Chang, "Optimisation of germination time and temperature on the concentration of bioactive compounds in Brazilian soybean cultivar BRS 133 using response surface methodology," *Food Chemistry*, vol. 119, no. 2, pp. 636–642, 2010.

[13] L.-X. Peng, L. Zou, J.-L. Zhao, D.-B. Xiang, P. Zhu, and G. Zhao, "Response surface modeling and optimization of ultrasound-assisted extraction of three flavonoids from tartary buckwheat (*Fagopyrum tataricum*)," *Pharmacognosy Magazine*, vol. 9, no. 35, pp. 210–215, 2013.

[14] T. Ma, F. Shan, and C. Jia, "Effects of tartary buckwheat particle medicine on the blood glucose of diabetic mice," *Journal of Chinese Institute of Food Science and Technology*, vol. 11, no. 5, pp. 15–18, 2011 (Chinese).

[15] Y.-H. Kuo, P. Rozan, F. Lambein, J. Frias, and C. Vidal-Valverde, "Effects of different germination conditions on the contents of free protein and non-protein amino acids of commercial legumes," *Food Chemistry*, vol. 86, no. 4, pp. 537–545, 2004.

[16] C. Vidal-Valverde, J. Frias, I. Sierra, I. Blazquez, F. Lambein, and Y.-H. Kuo, "New functional legume foods by germination: effect on the nutritive value of beans, lentils and peas," *European Food Research and Technology*, vol. 215, no. 6, pp. 472–477, 2002.

[17] M. Zhang, J. Zhang, and X. Hu, "Studies on the change of trypsin inhibitor activity and relativity with digestibility rate of protein in germinated buckwheat bud," *Journal of Chinese Institute of Food Science and Technology*, vol. 6, no. 5, pp. 34–39, 2006 (Chinese).

[18] N. Bouché, B. Lacombe, and H. Fromm, "GABA signaling: a conserved and ubiquitous mechanism," *Trends in Cell Biology*, vol. 13, no. 12, pp. 607–610, 2003.

[19] Y. Guo, R. Yang, H. Chen, Y. Song, and Z. Gu, "Accumulation of γ-aminobutyric acid in germinated soybean (*Glycine max* L.) in relation to glutamate decarboxylase and diamine oxidase activity induced by additives under hypoxia," *European Food Research and Technology*, vol. 234, no. 4, pp. 679–687, 2012.

[20] X. Yu, R. Yang, Z. Gu, S. Lai, and H. Yang, "Anti-tumor and immunostimulatory functions of two feruloyl oligosaccharides produced from wheat bran and fermented by *Aureobasidium pullulans*," *BioResources*, vol. 9, no. 4, pp. 6778–6790, 2014.

[21] A. M. Abdou, S. Higashiguchi, K. Horie, M. Kim, H. Hatta, and H. Yokogoshi, "Relaxation and immunity enhancement effects

of γ-Aminobutyric acid (GABA) administration in humans," *BioFactors*, vol. 26, no. 3, pp. 201–208, 2006.

[22] B. J. Shelp, A. W. Bown, and M. D. McLean, "Metabolism and functions of gamma-aminobutyric acid," *Trends in Plant Science*, vol. 4, no. 11, pp. 446–452, 1999.

[23] A. M. Kinnersley and F. J. Turano, "Gamma aminobutyric acid (GABA) and plant responses to stress," *Critical Reviews in Plant Sciences*, vol. 19, no. 6, pp. 479–509, 2000.

[24] Y. Yin, R. Yang, Q. Guo, and Z. Gu, "NaCl stress and supplemental CaCl2 regulating GABA metabolism pathways in germinating soybean," *European Food Research and Technology*, vol. 238, no. 5, pp. 781–788, 2014.

[25] R. Reggiani, C. A. Cantu, I. Brambilla, and A. Bertani, "Accumulation and interconversion of amino acids in rice roots under anoxia," *Plant and Cell Physiology*, vol. 29, no. 6, pp. 981–987, 1988.

[26] Y. Guo, H. Chen, Y. Song, and Z. Gu, "Effects of soaking and aeration treatment on γ-aminobutyric acid accumulation in germinated soybean (Glycine max L.)," *European Food Research and Technology*, vol. 232, no. 5, pp. 787–795, 2011.

[27] Q. Bai, G. Fan, Z. Gu, X. Cao, and F. Gu, "Effects of culture conditions on γ-aminobutyric acid accumulation during germination of foxtail millet (*Setaria italica L.*)," *European Food Research and Technology*, vol. 228, no. 2, pp. 169–175, 2008.

[28] N. Aurisano, A. Bertani, and R. Reggiani, "Anaerobic accumulation of 4-aminobutyrate in rice seedlings—causes and significance," *Phytochemistry*, vol. 38, no. 5, pp. 1147–1150, 1995.

[29] Y. Li, Q. Bai, X. Jin, H. Wen, and Z. Gu, "Effects of cultivar and culture conditions on γ-aminobutyric acid accumulation in germinated fava beans (*Vicia faba* L.)," *Journal of the Science of Food and Agriculture*, vol. 90, no. 1, pp. 52–57, 2010.

[30] P. Qin, Q. Wang, F. Shan, Z. Hou, and G. Ren, "Nutritional composition and flavonoids content of flour from different buckwheat cultivars," *International Journal of Food Science & Technology*, vol. 45, no. 5, pp. 951–958, 2010.

[31] D. Zielińska, M. Turemko, J. Kwiatkowski, and H. Zieliński, "Evaluation of flavonoid contents and antioxidant capacity of the aerial parts of common and tartary buckwheat plants," *Molecules*, vol. 17, no. 8, pp. 9668–9682, 2012.

[32] K.-Y. Syu, C.-L. Lin, H.-C. Huang, and J.-K. Lin, "Determination of theanine, GABA, and other amino acids in green, oolong, black, and Pu-erh teas with dabsylation and high-performance liquid chromatography," *Journal of Agricultural and Food Chemistry*, vol. 56, no. 17, pp. 7637–7643, 2008.

[33] S. Yao, T. Yang, L. Zhao, and S. Xiong, "The variation of γ-aminobutyric acid content in germinated brown rice among different cultivars," *International Journal of Molecular Sciences*, vol. 4, no. 12, pp. 3974–3982, 2008 (Chinese).