Study on Enrichment of Y -aminobutyric Acid in Fermented Brown Rice

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Keywords: Brown Rice, Lactic Acid Bacteria, γ-Aminobutyric Acid, Orthogonal Test

Abstract: In this experiment, γ-aminobutyric acid was produced by lactic acid bacteria fermentation with germinated brown rice powder as culture medium. Sodium glutamate and vitamin B_6 were added to the fermentation process to increase the yield of GABA. Through single factor experiment and orthogonal experiment, the optimum conditions were obtained as follows: the concentration of vitamin B_6 was 0.3 mmol/L, the concentration of sodium glutamate was 0.6 mg / mL, the culture time was 72 h, and the culture temperature was 30 ℃. The yield of GABA was 7.83 mmol / L. This study has opened up a new way for the preparation of γ-aminobutyric acid.

1. Introduction

γ -aminobutyric acid (GABA),) is also called aminobutyric acid. Its amino acid is located in γ -C position, but not in α -C position of protein amino acid, so it is called non-protein amino acid [1]. L-glutamic acid decarboxylase catalyzed by L-glutamic acid decarboxylase, its molecular formula is NH2CH2CH2CH2COOH [2]. As a natural active factor, gamma-aminobutyric acid exists in animals and plants, and is widely distributed in animals and plants [3] .A large number of GABA in the nerve tissue in animals, especially in the brain The distribution was the most concentrated in the tissue, and the content was about 0.1? 0. 6 mg/g [4, 5]. Immunological studies show that the highest concentration in the substantia nigra is found in the brain. Plants such as the genus Pea, cereals, especially the germ of cereals contain a certain amount of GABA [6]. GABA is an important active substance. It is the primary neurotransmitter inhibin in mammalian central nervous system and participates in many neurophysiological activities in vivo. In the human brain, glutamate can be converted into GABA, under the action of glutamate decarboxylase, but as age and stress increase, the accumulation of GABA becomes very large. Difficulties [9]. In order to improve the accumulation difficulty of GABA, the aim of promoting human health can be achieved by dietary supplement.

Lactic acid bacteria (Lactic acid bacteria) are important probiotics closely related to human daily life. With the development of human society, people begin to pay more and more attention to the quality of life. A large number of studies show that lactic acid bacteria and their metabolites have brought about a change in human life. So that people can ensure the quality of life while protecting their own health. Therefore, the use of lactic acid bacteria in the production of GABA-rich functional foods will bring greater social and economic benefits.

In this study, the colorimetric method based on Berthlot color reaction was used to determine the content of GABA [10] to form colored compounds, and then color reaction was carried out. The content of the components to be determined by comparing or measuring the color depth of the colored substance solution [11]. This method is convenient, fast, low cost, widely used, high sensitivity and strong selectivity is one of the advantages of this method [12-14].

2. Materials and Methods

2.1 Materials.

Raw materials: Brown rice (bought from farmers' market), lactic acid bacteria (kept by the microbiology laboratory of our school).

  DOI: 10.25236/isbsbh.2018.026

2.2 Reagent.

Diammonium citrate, potassium hydrogen phosphate, potassium dihydrogen phosphate, sodium acetate, calcium chloride, magnesium sulfate, GABA standard, phenol, vitamin B6, glucose, sodium glutamate, boric acid, yeast powder, peptone, beef paste, etc.

2.3 Preparation of Media and Reagents.

Fermentation medium (g/L): 5 g brown rice powder was extracted and sterilized at 121 ℃ for 30min and mixed with 10ml aseptic water.

PBS (Phosphate Buffered Saline phosphate buffer.

2.4 Main Apparatus and Equipment.

Visible spectrophotometer (722, Shanghai Spectrometer Co., Ltd.);Constant temperature incubator (HH-S, Jintan Zhengji instrument Co. Ltd.);Clean workbench (Antai Company, Su Jing Group);Electronic balance (JA1203N, Shanghai Precision instrument Co., Ltd.);Electrothermal blast Dryer (DHG-9240A, Shanghai Yiheng Scientific instrument Co., Ltd.);High speed centrifuge (GL-20G- II, Shanghai Anting Scientific instrument Factory);High pressure steam sterilizer (MLS-3750, Sanyo Motor Co., Ltd. Made in Japan).

2.5 Test Methods.

Pretreatment of brown rice:100 g brown rice with full grain and uniform color was selected, then washed with NaClO solution of 0.1mmol/L, then washed with distilled water, soaked in CaCl2 of 0.5mmol/L, soaked at 32 ℃ for 24 hours. Under the condition of constant temperature 28 ℃, relative humidity 90% and 95% RH, the sprout of brown rice will be stopped when it reaches about 0.5cm. After drying, grinding and filtering with 100 mesh screen, the 30min is sterilized at 121 ℃.

2.6 Preparation of Seed Liquid.

Lactic acid bacteria were selected and inoculated in the MRS liquid medium of 5ml for 24 h under the condition of 30 ℃. The activated strain was inoculated into the MRS liquid medium of 100ml with 1% inoculation, and the OD600 value was about 1. The centrifuge was adjusted to 6000 rpm for 5 min. The bacteria were collected and washed with sterile PBS for 2 times. The high concentration of bacterial suspension was obtained by using 1 / 10 times volume of aseptic PBS in the original culture medium, and the high concentration of bacterial suspension was the seed solution.

3. Single Factor Test

3.1 Effect of Culture Temperature on GABA Yield.

Adding 5g sterilized brown rice powder to 10ml aseptic water to mix evenly, then adding 1ml concentration of vitamin B_6 solution of 0.4mmol/L and 0.6mg/mL solution of sodium glutamate 1 ml, inoculating 5% of bacteria. The concentration of GABA in the samples was determined by colorimetric method after 72 h culture at 37 ℃ for 72 h.

3.2 Effect of Culture Time on GABA Yield.

Adding 5g sterilized brown rice powder to 10ml aseptic water to mix evenly, then adding 1ml concentration of vitamin B6 solution of 0.4mmol/L and 0.6mg/mL solution of sodium glutamate 1 ml, inoculating 5% of bacteria. The concentration of GABA in the samples was determined by colorimetric method at 30 ℃ for 3 parallel hours.

3.3 Effect of Sodium Glutamate Concentration on GABA Yield.

The 5g sterilized brown rice powder was added to 10ml aseptic water to mix evenly, and then the 1ml concentration was 0 ~ (0.2) ~ 0.4N ~ (0.6) ~ (0.6) ~ (-1) mg / mL of glutamate sodium solution, and the 1ml concentration was a Vitamin B _ (6) solution of 0.4mmol/L. The concentration of

GABA was determined by colorimetry at 30 ℃ for 72 h after inoculation of 5% strain.

3.4 Effect of Vitamin B6 Concentration on GABA Yield.

Adding 5g sterilized brown rice powder to 10ml aseptic water and mixing it evenly, then adding the vitamin B6 solution with 1ml concentration of 0.2mmol-1 / L and 1ml 0.6mg/mL solution of 0.8mmol-1 / L respectively, and adding the glutamate sodium solution of 1ml 0.6mg/mL. The concentration of GABA was determined by colorimetry at 30 ℃ for 72 h after inoculation of 5% strain.

4. Orthogonal Test

According to the above single-factor experiment, the content of γ -aminobutyric acid was taken as the index, and four major influencing factors were selected to carry out the L9 (34) orthogonal experiment, as in Table 1.

Table 1 Factor level table

horizontal	factor			
	A culture time(h)	B culture temperature (℃)	C vitamin B_6 concentration(mmol/L)	D-glutamate concentration(mg/mL)
1	60	28	0.3	0.5
2	72	30	0.4	0.6
3	84	32	0.5	0.7

4.1 Method for determination of GABA content.

The absorbance of the solution was determined under the wavelength 630nm of the spectrophotometer, and the standard curve of GABA was drawn (see fig. 1). The equation was obtained by using the GABA concentration as the transverse coordinate and the absorptivity (A), with the vertical coordinate as the absorption value. The equation is y=0.1382x-0.0164, and the correlation coefficient is R^2= 0.9983.

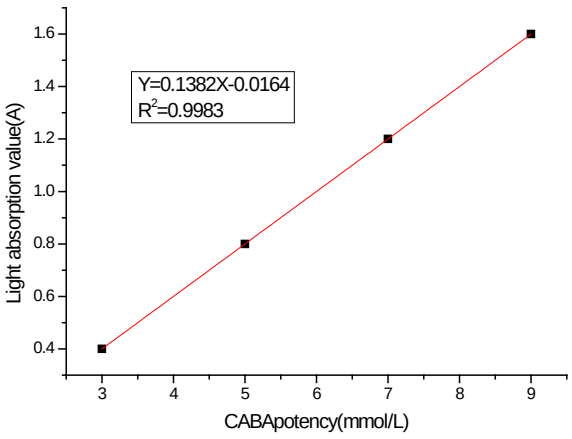

Fig. 1 GABA standard curve

Preparation of GABA crude products: the culture medium was centrifuged under 5000rpm for 10 min, and the supernatant was obtained, that is, the crude product of GABA. 0.6ml, the crude product of GABA, was taken into ice bath and the boric acid buffer (pH 9.0) with 0.4 ml concentration of 0.2mol/L was added to terminate the reaction. Then add 6% phenol 1ml and 0.4ml sodium hypochlorite solution with available chlorine 5.25%, mix evenly and put in boiling water bath for 10 min, then immediately put in ice bath for 20 min, until the solution turns blue and green. Add 2m to solution 60% ethanol was mixed well and the absorbance value of the solution was

determined by 630nm.

4.2 Results and Discussions.

4.2.1 Single factor test results and analysis.

4.2.1.1 Effect of Culture Temperature on GABA Yield.

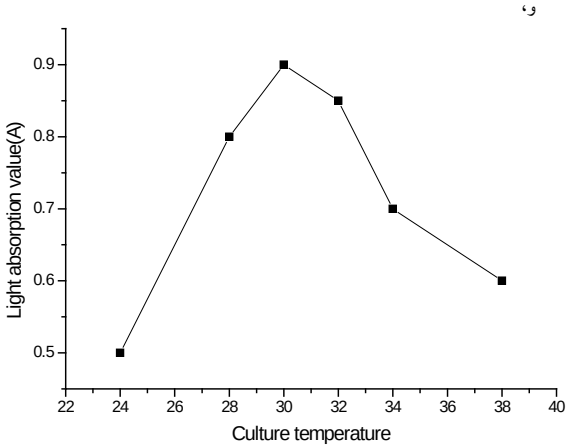

Fig. 2 effect of culture temperature on GABA yield.

Figure 2 shows that the yield of GABA increases with the increase of temperature in a certain range. When the temperature reaches 30 ℃, the absorbance is 0.900, and the yield of GABA is the highest at 6.63 mmol / L, and then the content of GABA decreases gradually with the increase of temperature. Appropriate temperature is a necessary condition to increase the conversion and synthesis of nutrients. Too high or too low temperature will affect the enzyme activity. Therefore, the orthogonal test selected the culture temperature of 2830 Culture temperature 32 ℃.

4.2.1.2 Effect of culture time on GABA yield.

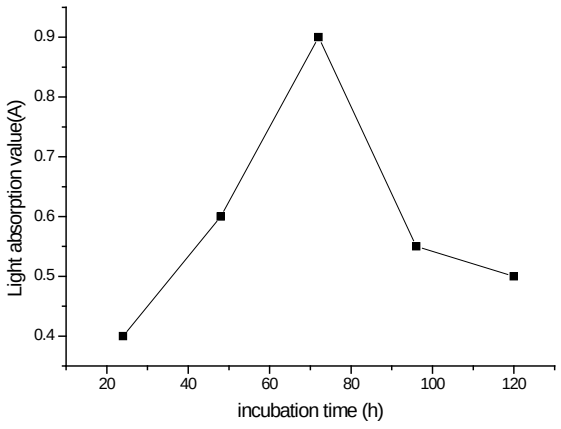

Fig. 3 effect of culture time on GABA yield

Figure 3 shows that when the culture time is 72 hours, the light absorption value is 0.845, corresponding to the concentration of GABA is 6.23 mmol / L, the yield reaches the maximum, and then decreases with the increase of culture time. GABA was catalyzed by transaminase and converted to hemicaldehydes succinate, which resulted in the decrease of GABA content. In addition, the synthesis of the product was mainly in the stable stage of the growth of lactic acid bacteria, the longer the culture time, the more the lactic acid bacteria may enter the stage of decay. Therefore, the three levels of culture time selected in the orthogonal experiment were 607~2~84 h.

4.2.1.3 Effect of Sodium Glutamate Concentration on GABA Yield.

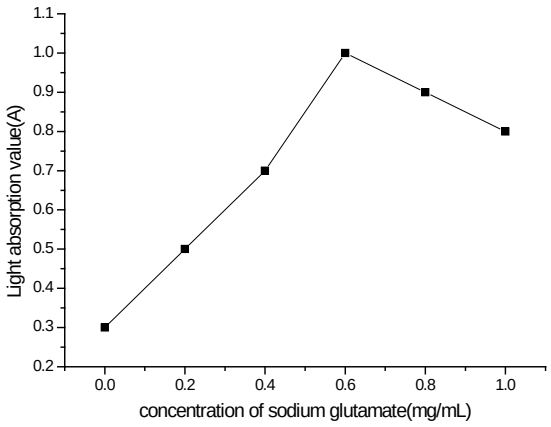

Fig. 4 effect of concentration of sodium glutamate on yield of GABA

Figure 4 shows that the higher the concentration of glutamate, the higher the yield of GABA. When the concentration was 0.6 mg/mL, the corresponding absorbance was 0.962.The maximum yield of GABA was 7.08 mmol / L, and the higher the concentration of sodium glutamate was, the lower the yield of GABA was. The main reason for the decrease may be that the fermentation process belongs to the enzymatic reaction to some extent, when the substrate concentration is very high, the enzyme is saturated. When the concentration of sodium glutamate increases, the ratio of sodium glutamate to sodium glutamate decarboxylase reaches an optimum ratio, and after that, the concentration of sodium glutamate High ion concentration may inhibit the growth of lactic acid bacteria, which is not conducive to the synthesis of the product. Therefore, the three levels of sodium glutamate concentration selected in the orthogonal test are 0.5 0. 6C 0. 7 mg/mL.

4.2.1.4 Effect of Vitamin B6 Concentration on GABA Production.

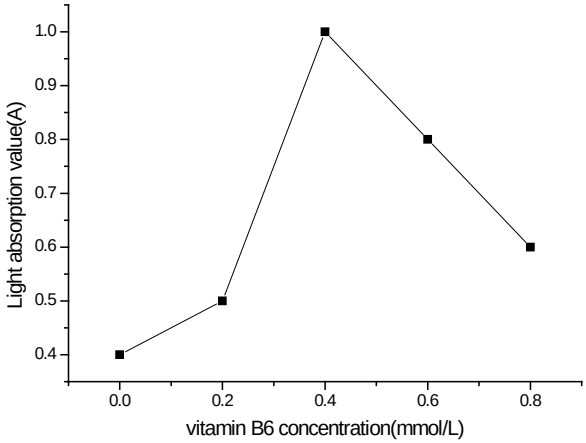

Fig. 5 effect of vitamin B_6 concentration on GABA production

Figure 5 shows that with the increase of vitamin B_6, the yield of GABA increases greatly. When the concentration of vitamin B_6 is between 0~0.4mmol/L, the yield of GABA increases gradually, and when the concentration of vitamin B_6 is 0.4mmol/L, the absorptivity is 0.987. The yield of GABA reached the maximum of 7.26 mmol / L, and the yield of GABA decreased with the increase of vitamin B_6 concentration, which may be due to the presence of a certain amount of transaminase in brown rice. Vitamin B_6 is both a coenzyme of decarboxylase and a coenzyme of aminotransferase. When a certain concentration is reached, the activity of transaminase The activity of decarboxylase is greater than that of decarboxylase, which makes the amount of synthesis less

than the amount decomposed under the action of transaminase, which leads to the decrease of GABA content. Therefore, the three levels of vitamin B_6 concentration selected in the orthogonal experiment were 0.3 ~ 0.4 μ mol ·L ~ (-1) ·L ~ (-1) ~ (-1) ~ (-1).

4.3 Orthogonal test results and analysis.

Based on the single factor test, the orthogonal test of L 9 (34) is carried out. The results are shown in Table 2.

Table 2 L9 (34) orthogonal test results

test number	factor				OD value
	A	B	C	D	
1	1	1	1	1	0.485
2	1	2	2	2	0.979
3	1	3	3	3	0.327
4	2	1	2	3	0.663
5	2	2	3	1	0.365
6	2	3	1	2	1.066
7	3	1	3	2	0.286
8	3	2	1	3	0.565
9	3	3	2	1	
K1	1.971	1.434	2.116	1.319	0.469
K2	2.094	1.909	2.111	2.331	
K3	1.320	1.862	0.978	1.555	
k1	0.597	0.478	0.705	0.440	
k2	0.698	0.636	0.704	0.777	
k3	0.440	0.621	0.326	0.518	
R	0.258	0.158	0.379	0.337	

According to the results of orthogonal experiment, RC > RD > RA > RB, can influence the yield of GABA in the order of vitamin B_6 concentration > glutamate concentration > culture time > culture temperature. The optimum scheme is A 2B 2C 1D 2, and the optimum scheme is A 2B 2C 1 D 2, and the order of effect on GABA yield is as follows: vitamin B_6 concentration > glutamate sodium concentration > culture time > culture temperature. The optimum concentration of vitamin B6 is 0.3 mmol/L, the optimum concentration of sodium glutamate is 0.6 mg / mL, the best culture time is 72 h, the optimum culture temperature is 30 ℃, and the yield of GABA is 7.83 mmol / L.

5. Conclusion

In this experiment, GABA, was enriched by germinating brown rice and then GABA. was produced by lactic acid bacteria fermentation. In the process of fermentation, the yield of GABA was improved by adding sodium glutamate and vitamin B_6, controlling the culture time and temperature at the same time. Finally, the fermentation process was optimized by orthogonal experiment. Compared with the original brown rice, the content of GABA increased significantly. Therefore, the method of using lactic acid bacteria to produce GABA and be used in food production and processing is feasible.

Acknowledgement

Jilin Provincial Education Department "13th Five-Year Plan" Science and Technology Research Project (JJKH20180737KJ).

References

[1] Q.Lin. Overview of physiological function of γ -aminobutyric acid [J]. Journal of Yulin normal University, 2010 / 02: 62: 65.

[2] Y.Ding, H.Hua. The relationship between γ -aminobutyric acid and human health [J]. Chinese Journal of Clinical Health, 2012 / 01: 100 / 103.

[3] C.G.Li, C.C.Wang and H.P.Yang. Enrichment and determination of γ-aminobutyric acid in germinated brown rice [J]. The food and feed industry, 2011,02: 4.

[4] J.J.Xu, B.Jiang and S.Y.Xu. A new functional food factor GABA- [J]. Food Industry Technology, 2003,01: 109 / 110.

[5] B.Jiang. GABA (γ-aminobutyric acid)-a new functional food factor [J]. Chinese Journal of Food, 2008 / 02: 1 / 4.

[6] Horie, Naoto Kan and W.Z.Jin. GABA (γ -aminobutyric acid [J]. Chinese Food Additives, 2010: 169, 173.

[7] X.L.Zhou, L.Zhao. The physiological function of γ -aminobutyric acid and its application in food [J]. Food Industry, 2011 / 10: 58 / 61.

[8] Y.R.Lin, J. Wang. Progress in the study of γ -aminobutyric acid [J]. Modern Food Science & Technology, 2008 / 05: 496 / 500.

[9] H.Jiang, H.Su and J.H.Fu. The function of γ -aminobutyric acid and its enrichment in germinated brown rice [J]. Journal of Jiangxi Agricultural Sciences, 2011 / 11: 1466 / 148.

[10] Y.L.Han, X.L.Tan and Y.L.Tan. Optimization of system for determination of γ-aminobutyric acid in rice by colorimetric method [J]. Agricultural Science, Anhui, 2013, 41 (10): 4629, 4630.

[11] L.S.Huang, L.Q Shen and X.Y.Wang. Determination of γ -aminobutyric acid and its thermal stability in mulberry leaves by modified colorimetric method [J]. Food Technology, 2010: 0828: 331.

[12] X.C.Zheng , Ye.Chen and J.Song, et al. Studies on the enrichment of γ-aminobutyric acid by rice bran fermentation [J]. Chinese Food Additives, 2013, S1: 50, 56.

[13] Z.Z.Wei, M.Zhao and S.G.Li , et al. Feasibility study on determination of GABA in Tea by colorimetric method [J]. Jiangxi Journal of Agriculture, 2010: 08: 56: 58.

[14] H.Zhang. Studies on the properties of glutamic acid decarboxylase from rice embryo and its enrichment of γ-aminobutyric acid [D]. Jiangnan University, 2004.