

Optimization of gamma-amino butyric acid biosynthesis in the germ of mung bean (*Vigna radiate*)

Nguyen Xuan Hoan¹, Duong Hong Quan¹, Tien Tien Nam¹, Dinh Huu Dong¹,
Nguyen Thi Phuong¹, Pham Van Thinh², Hoang Thai Ha¹, Dang Xuan Cuong³



Ho Chi Minh University of Food Industry¹, Ho Chi Minh, Vietnam

Nguyen Tat Thanh University², Ho Chi Minh, Vietnam

Nha Trang Institute of Technology Application and Research³, VAST, Nha Trang, Vietnam

Abstract— This study aimed to investigate the optimization of acid gamma-aminobutyric (GABA) content biosynthesis in germinated Mung bean. The effect analysis of soak time, germination temperature, and time on GABA content was using the response surface method (RSM), with a central composite design (CCD) with the support of software JMP version 9.0.2. Results showed that GABA content reaches the highest levels of 3.768 ± 0.041 mg GABA equivalent/g dry matter as soaking the seeds in tap water. GABA content got the highest value of 4.1006 ± 0.0326 mg GABA equivalent/g dry matter at the optimization condition (soaking time of 8 hours, germination at 34°C for 26 hours in tap water) and increase of 9.362 times, compared to non-treatment Mung bean seeds. Soaking time, germination time, and germination temperature affected on acid gamma-aminobutyric biosynthesis. GABA is useful for application in the functional food and pharmaceuticals containing GABA.

Keywords— acid gamma-aminobutyric, germination, Mung bean, optimization, *Vigna radiate*

1. Introduction

Gamma-aminobutyric acid (GABA) is the neurotransmitter amino acid existing in plants or bacteria, having the metabolic role in Krebs cycle and playing the vital role to maintain the normal function in the nervous system, especially in nerves. GABA plays the principal role in reducing neuronal excitability, inhibiting signal transduction of neurotransmitters and cancelling spontaneous muscle action potentials.[1,2] GABA plays as the inhibitor as well as the exciter: antioxidant, relaxing muscle, amnesia, improving consciousness, exciter and anticonvulsant, [3] tranquillizer. [4,5] GABA exists in germinated grain, GABA content in germinated Thai brown rice increased 16,74 times when seeds soaking were in citrate solution for 36 hours. [6] During the germination process, glutamate content decreased where GABA content significantly increased. [7] GABA content in fermented Mung bean flour, germinated Mung bean seeds increased in the range from 7.6 to 27.9 times) and total amino acid content increased from 8.7 to 13 times. [8,9] Nowadays, there are only some notices on GABA from Mung bean, but the GABA content optimization in the germination process is not. Therefore, the study focused on the optimization of the Mung bean germination process for collecting the highest GABA content.

Response surface methodology (RSM) is the method of mathematical and statistical technique for designing experiments, building models, evaluating the relative significance of several independent variables and determining the optimum conditions for desirable responses. The two most common designs extensively used in RSM are the central composite design (CCD) and the Box-Behnken design (BBD). The CCD is ideal for sequential experimentation and allows a reasonable amount of information for testing lack of fit while not involving an unusually large number of design points.

2. Materials and methods

2.1. Materials

Purchasing Mung bean seed was from Xuan Hong Service Trading Co.Ltd (Song Than Industrial Park, Binh Duong province) and Saccharose (99.9%) from Bien Hoa Sugar Joint Stock Company (Bien Hoa 1 Industrial Park, Dong Nai province). γ -aminobutyric acid (Sigma-USA), sodium hypochlorite, disodium tetraborate, boric acid, sodium hydroxide, trisodium citrate, phenol, citric acid, sulfuric acid (Guangdong Guanghua Sci-Tech Co., Ltd, China).The chemical compositions of Mung bean seed were analyzed

(Table 1 and 2).

Table 1. The chemical compositions of Mung bean seed

Chemical compositions	Mung bean flour
GABA content (mg GABA equivalent/g)	0.438 ± 0.0185
Protein content (%)	22.411 ± 0.022
Lipid content (%)	2.136 ± 0.290
Starch content (%)	58.255 ± 0.248
Humidity (%)	11.467 ± 0.042
Ash content (%)	3.487 ± 0.041

Table 2. The indexes of mung beans

Indexes	Value
Absolute dry weight (g/1000 seed)	6.623 ± 0.137
Seed germination (%)	96.196 ± 0.005
Seed germination ratio (%)	96.196 ± 0.005
Average germination time (days)	1.585 ± 0.048

2.2. Sample preparation

Gamma-amino butyric acid biosynthesis in mung beans was according to the method of Banchuen et al. (2009). [6]Mung beans were cleaned and sterilized by using ethanol for 01 minutes.Mung beans were macerated in the solution (pH 7.6) with the mung beans-to-solution ratio of 1:5 (w/v) at 30±2°C for 8 hours. After maceration, mung beans were put in the glass petri having two filter papers for maintaining the moisture of mung beans and done according to the experiments in Table 3.

2.3. Experimental design and optimization

The condition optimum of GABA biosynthesis in mung beans was using the software Stat-Ease Design Expert 9.0.2 with the regression model and the experiment basing on CCD. Three independent variables choosing such as soaking time, germination time, and germination temperature were as the optimization zone for collecting the maximum peak of GABA content in the biosynthesis process. Accordingly, the CCD matrixes using of 16 experiments covering the full design of three factors were for building quadratic models (Table 3 and 4). The experimental data presentation obtaining from the CCD model experiments was in the form of the following equation:

$$Y = B_0 + \sum_{i=1}^n B_i x_i + \sum_{i=1}^n B_{ii} x_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^n B_{ij} x_i x_j + e_i$$

Where Y is the predicted response; n is the number of factors; x_i and x_j are the coded variables; B_0 is the offset term; B_i , B_{ij} , and B_{ij} are the first-order, quadratic, and interaction effects, respectively; i and j are the index numbers for factor, and e_i is the residual error.

Table 3. Experimental range and levels of the independent variables

Variables	Factor	Range and levels		
		-1	0	1
Soaking time (h)	X_1	7	8	9
Germination time (h)	X_2	12	24	36
Germination temperature ($^{\circ}$ C)	X_3	32	34	36

2.4. Quantification of GABA content

The method to determine GABA content was according to the description in previous studies. [10,11] 0.1 g of the sample at different conditions added to 1.5 ml Eppendorf, extracted with 1 ml of 80 % ethanol for 60 min and then centrifuged in 10.000 rpm at 4oC for 10 min for separating the supernatant and the residue. The residue was continuously extracted with 0.5 ml of 80% ethanol for 15 min and centrifuged for collecting the supernatant. All supernatants of centrifugation twice times were evaporated at 80oC until completely dry and added to 1 ml of distilled water, shaken and centrifuged in 10.000 rpm at 4oC for 10 min for collecting the supernatant. 0.6 ml of the supernatant added to 0.4 ml of borate buffer (pH 9.0), 2 ml of 6.0 % phenol, and 1.6 ml of 8% NaOCl, in turn, and vortexing for 1 min. GABA content was analyzed spectrophotometrically on a UV–vis spectrometer at 630 nm by measuring the absorbance of the samples at a maximum wavelength based on the standard range GABA content from 0, 150, 200, 300, 400, and 450 ppm.

2.5. Data analysis

Each experiment was in triplicated ($n=3$), analyzed statistically by the software Stat-Ease Design Expert 9.0.2 that used for the optimization of GABA biosynthesis.

3. Results and discussion

RSM was employed for identifying the simple and interactive effects of operating variables of increased biosynthesis GABA content. On the basis of the CCD, the optimization of GABA synthesis in mung beans occurred four important stages: (1) performing statistically designed experiments for the experimental plan, (2) recommending the mathematical model basis of the experimental data and focus on the data of analysis of variance, (3) controlling the efficiency of the model directly with diagnostic plots and (4) estimating the response and verify the model.

Table 4. Central composite design experiment and results

Experiment number	Experimental design	Experimental plan			GABA content (mg GABA equivalent /g DW)
		X ₁	X ₂	X ₃	
1	---	7	12	32	3.772 ± 0.044
2	+--	9	12	32	3.789 ± 0.107
3	00a	8	24	32	4.025 ± 0.089
4	-+-	7	36	32	3.934 ± 0.105
5	++-	9	36	32	3.768 ± 0.13
6	0a0	8	12	34	3.962 ± 0.114
7	a00	7	24	34	4.028 ± 0.103
8	000	8	24	34	4.143 ± 0.094
9	000	8	24	34	4.162 ± 0.034
10	A00	9	24	34	4.038 ± 0.09
11	0A0	8	36	34	4.058 ± 0.035
12	---+	7	12	36	3.765 ± 0.048
13	+++	9	12	36	3.805 ± 0.121
14	00A	8	24	36	4.010 ± 0.119
15	-++	7	36	36	3.832 ± 0.136
16	+++	9	36	36	3.848 ± 0.081

According to the RSM results, response surface modelling was significant (Table 5) under polynomial regression function, and predicting response (Y) for the GABA content of samples was according to the following Equation:

$$Y = 4.1363 + 0.0347X_2 - 0.0259 X_1X_2 + 0.0258 X_1X_3 - 0.0952X_1^2 - 0.1182X_2^2 - 0.1107X_3$$

Table 5. ANOVA regression model for the optimization of GABA biosynthesis

Source	Sum of Squares	Mean Square	F value	Pr(>F)	
Model	0.2781	0.0309	35.4641	0.0002*	significant
X ₁ (7,9)	0.0007	0.0007	0.7906	0.4082	
X ₂ (12,36)	0.0120	0.0120	13.8179	0.0099*	
X ₃ (32,36)	0.00008	0.00008	0.0900	0.7743	
X ₁ X ₂	0.0054	0.0054	6.1466	0.0479*	
X ₁ X ₃	0.0053	0.0053	6.0284	0.0494*	
X ₂ X ₃	0.0001	0.0001	0.1379	0.7232	
X ₁ ²	0.0239	0.0239	27.4434	0.0019*	
X ₂ ²	0.0369	0.0369	42.2986	0.0006*	
X ₃ ²	0.0323	0.0323	37.1028	0.0009*	
Residual	0.0052	0.00087			

The surface plots showed the interaction of each-pair elements and the optimization value for collecting the

maximum GABA content (Figure 1, 2, and 3).

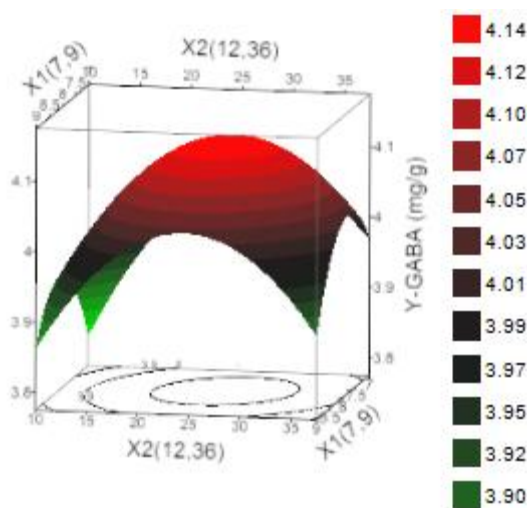


Figure 1. 3D surface plot of increase GABA content versus soaking time (hour) and germination time (hour)

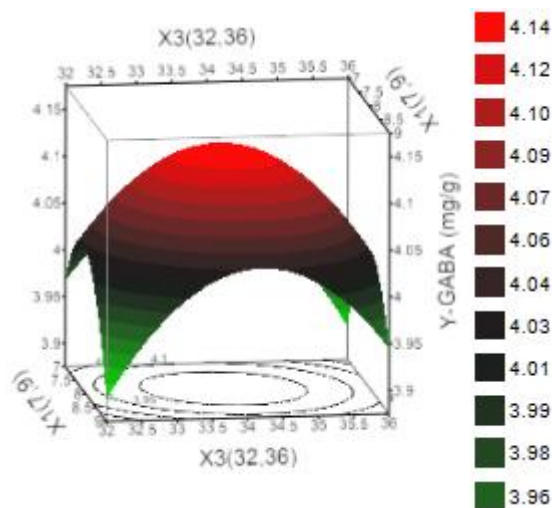


Figure 2. 3D surface plot of increase GABA content versus soaking time (hour) and germination temperature (°C)

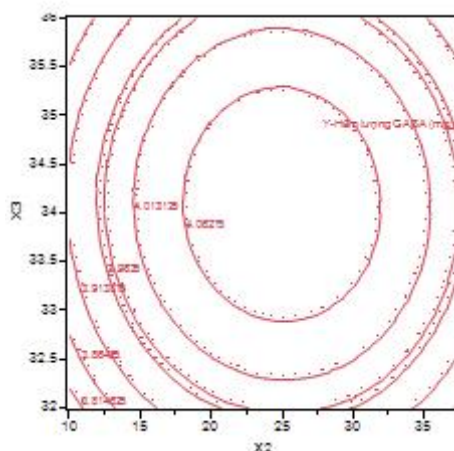


Figure 3. Over surface plot of increase GABA content versus germination time (hour) and germination temperature (°C)

Soaking time, germination time, and germination temperature (34°C) impacted on GABA content was found (Figure 1). GABA content increased slightly when the soaking time was from 7 to 8 hours and got the highest value (4.139 mg GABA equivalent/g) at 7.923 hours. The GABA content decreased when soaking time increased. Similarly, GABA content increased when germination time was from 12 to 24 hours and reached the highest value (4.139 mg GABA equivalent/g) at 25.854 hours of germination time. The germination time continuously increased, the GABA content decreased. The reason for that phenomenon is that soaking time affected water absorption of germination [12] and GAD biosynthesis ability. [13] The GABA content did not depend on the values change of X_1 and X_3 as on the same contours.



Figure 4. Germination mung beans activity was affected by soaking time

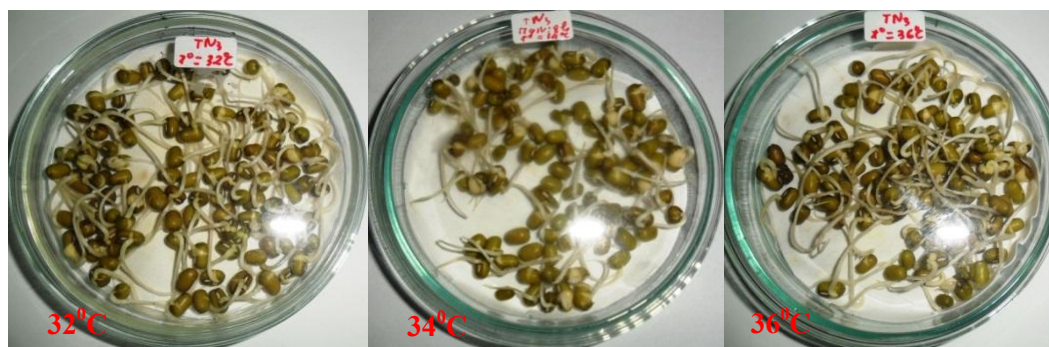


Figure 5. Germination mung beans activity was affected by germination temperature

The soaking time and germination temperature affected GABA content (Figure 2, 4, and 5). GABA content increased when soaking time and germination time increased and reached the highest value (4.138 mg GABA equivalent/g DW) at the time of the soaking (7.8667 hours) and the germination time (33.91 hours). GABA content decreased when the soaking and the germination time continuously increased. The reason is that the germination process increased enzyme activity GAD causing the GABA content increase. GABA content changed according to the non-linear model of level 2 because of the decrease of glutamate content [7] and glutamate conversion into GABA by GAD [14] during the germination process.

The interaction between germination temperature and germination time affected GABA content (mg GABA equivalent/g DW) that reached the highest value (4.1387 mg GABA equivalent/g DW) at the germination temperature (33.954°C) and the germination time (25.584 hours) (Figure 3). GABA content decreased when the temperature increased. The germination temperature affected GABA content in germinating soybean that possessed the highest GABA content at 30°C, optimization temperature for GAD activity at 30°C [15], and optimization germination temperature at 35°C [7]. GABA content changed according to the non-linear model of the maximum peak, similar to the current study.

3. Conclusion

The study presented the optimization condition for collecting the highest GABA content with soaking time (8 hours), germination temperature (34°C), and germination time (26 hours). The prediction (4.139 mg GABA equivalent/g DW) and the actual GABA content (4.1006 ± 0.0326 mg GABA equivalent/g DW) was insignificantly different. The surface model was suitable and significant ($p < 0.05$). The input factors such as soaking time, germination temperature, and germination time impact strongly on GABA content synthesis in mung beans. GABA content synthesis is useful for functional food and pharmaceuticals.

4. References

- [1] Del Castillo J De Mello WC, Morales T. Inhibitory action.n of γ -aminobutyric acid (GABA) on ascaris muscle. *Experientia*.1964;20:4-5.
- [2] Del Castillo J, Sanchez V. Electrophysiology of the somatic muscle cells of *Ascaris lumbricoides*. *FCell Comp Physiol*.1963;62:59-78.
- [3] Bowery NG, Smart TG. GABA and glycine as neurotransmitters: a brief history. *British Journal of Pharmacol*. 2006;147:109-119.
- [4] Jakobs C, Jaeken J, Gibson MK. Inherited disorders of GABA metabolism. *Journal of Inherited Metabolic Disease*. 1993;16:704-715.
- [5] Wong CG, Bottiglieri T, Snead OC. γ -hydroxybutyric acid and neurological disease. *Ann Neurol*. 2003;54:3-12.
- [6] Banchuen J, Thammarutwasik P, Ooraikul B, Wuttijumnong P, Sirivongpaisal P. Effect of germinating processes on bioactive component of sangyod muang phatthalung rice. *Thai Journal of Agricultural Science*. 2009;42(4):191-199
- [7] Komatsuzaki N, Tsukahara K, Toyoshima H, Suzuki T, Shimizu N, Kimura T. Effect of soaking and gaseous treatment on GABA content in germinated brown rice. *J Food Eng*. 2007;78(2):556-560.
- [8] Yeap SK, Ali NM, Yusof HM, Alitheen NB, Beh BK, Ho WY, Koh SP, Long K. Antihyperglycemic effects of fermented and nonfermented mung bean extracts on alloxan-induced-diabetic mice. *J Biomed Biotechnol*. 2012;7 pages.
- [9] Ali NM, Yusof HM, Long K, Yeap SK, Ho WY, Beh BK, Koh SP, Abdullah MP, Alitheen NB. Antioxidant and hepatoprotective effect of aqueous extract of germinated and fermented mung bean on ethanol-mediated liver damage. *BioMed Research Internation*. 2013;9 pages.
- [10] Kitaoka BS, Nakano Y. Colorimetric determination of omega-amino acid. *Journal of Biochemistry*. 1969;66:87-94.
- [11] Ishikawa A, Oka H, Hiemori M, Yamashita H, Kimoto M, Kawasaki H, Tsuji H. Development of a method for the determination of γ -aminobutyric acid in foodstuffs. *J Nutr Sci Vitaminol*. 2009;55:292-295.
- [12] Chung HJ, Jang SH, Cho HY, Lim ST. Effects of steeping and anaerobic treatment on GABA (g-aminobutyric acid) content in germinated waxy hull-less barley. *LWT-Food Sci Technol*. 2009;42:1712-1716.
- [13] Bautista GM, Lugay JC, Cruz LJ, Juliano BO. Glutamic acid decarboxylase activity as viability of artificially dried and stored rice. *Cereal Chem*. 1964;41:188-191.
- [14] Saikusa T, Horino T, Mori Y. Accumulation of γ -Amino-n-butyric acid (GABA) in the rice germ during water soaking. *Biosci Biotech Biochem*. 1994;58(12):2291-2292.
- [15] Ueno Y, Hayakawa K, Takahashi S, Oda K. Purification and characterization of glutamate decarboxylase from *Lactobacillus brevis* IFO 12005. *Biosci Biotechnol Biochem*.1997;61(7):1168-1171.



This work is licensed under a Creative Commons Attribution Non-Commercial 4.0 International License.