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Optimization of Gamma Aminobutyric Acid Production from Various Protein Hydrolysates by *Lactiplantibacillus plantarum* MCM4

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Abstract

Background and Objective: Gamma aminobutyric acid is a non-protein amino acid with various physiological characteristics that is used as a synthetic supplement for healing anxiety, acute stress and sleeping disorders. Due to the demand for natural bioactive components, producing gamma aminobutyric acid enriched foods and supplements via biological methods has been popular in recent years.

Material and Methods: In this study, one factor at a time approach was used to study effects of various protein hydrolysates, including soy protein isolate, whey protein concentrate and casein at different concentrations (1-5% w v^{-1}), fermentation time (24, 48 and 72 h) and inoculum size (1, 3 and 5% v v^{-1}) on gamma aminobutyric acid synthesis. Then, the most effective parameters such as concentration of soy protein hydrolysate, inoculum size and fermentation time at various levels were studied using central composite design based response surface methodology for gamma aminobutyric acid synthesis by *Lactiplantibacillus plantarum* MCM4.

Results and Conclusion: Of various protein hydrolysates, soy protein hydrolysate was more appropriate for gamma aminobutyric acid synthesis. Moreover, higher gamma aminobutyric acid contents were achieved when soy protein with extended enzymatic hydrolysis for 6 h (soy protein hydrolysate 6) was used as the substrate. The polynomial mathematic model could predict gamma aminobutyric acid synthesis successfully. Optimization using central composite design indicated that the maximum gamma aminobutyric acid synthesis yield (19.4 mg 100 ml^{-1}) was achieved under the optimum conditions (fermentation time of 29 h, inoculum size of 3.65% v v^{-1} and Soy Protein Hydrolysate 6 concentration of 3.9% w v^{-1}). Overall, *Lactobacillus plantarum* MCM4 was suggested as a novel lactic acid bacteria species to produce gamma aminobutyric acid from inexpensive sources.

Conflict of interest: The authors declare no conflict of interest.

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1. Introduction

Gamma-aminobutyric acid (GABA) is a non-protein amino acid, which is produced from glutamic acid by the catalytic activity of glutamic acid decarboxylase (GAD) [1]. Furthermore, GABA is a major inhibitory neurotransmitter in the central nervous system of mammals which is currently used as synthetic supplements because of its antidiabetic, antihypertensive, diuretic activities and positive effects in stress management [2]. Due to the recent consumers' demands for natural bioactive components, GABA produced through fermentation processes from naturally growing microorganisms is further preferred [3]. Lactic acid bacteria (LAB) are the major GABA producing microorganisms, which have widely been used in the food industry, especially in production of fermented foods over the centuries [2]. As LAB are generally recognized as safe and due to their high applicability in the fermentation industry, various strains of LAB with GABA-producing activities have been isolated from food sources [4,5]. Fermentation conditions can be optimized to achieve the highest GABA concentration. Optimization of the composition of the media to increase

GABA yield and experiments to prepare cost effective media have been carried out for the mass production of GABA. Composition of the media, particularly nitrogen, carbon and other components, can affect the GABA total yield. Effects of various media such as carbon source (cane sugar, palm, sucrose, lactose, glucose and maltose), nitrogen source (skim milk, soy milk, tofu whey, yeast extract, KNO_3 and NH_4NO_3) and glutamate on the GABA production by *Pediococcus acidilactici* DS15 has been assessed by Anggraini et al. [6]. In another study, soybean meal and sludge powder were used to enhance GABA production by *Lactobacillus* (*L.*) *brevis* PML1 [7]. Moreover, Zareie et al. used inulin and soy protein isolate (SPI) to optimize fermentation conditions to reach the highest GABA concentration by *L. brevis* and showed that SPI concentration included significant effects on the GABA content [8].

In most studies, pure glutamic acid or mono sodium glutamate has been used as precursor to improve the GABA content in the final products or culture media [9,10]. However, inexpensive substrates must be found for GABA synthesis. Overall, protein sources with high glutamate contents can be addressed as good substrates for the GABA enrichment. Soymilk [11,12], SPI [5,8], kimchi [13], mushroom [14], adzuki beans [15], barley proteins or wheat gluten [16] and sourdough [17] have been used for GABA production. Use of cost effective media containing high peptide and amino acid contents rather than pure glutamate can be important greatly. A little information are available on the effects of enzymatic hydrolysis on GABA production. The aim of the current study was to optimize fermentation conditions for GABA production through one factor at a time (OFAT) approaches and response surface methodology (RSM) using cost effective substrates. Various protein hydrolysates from SPI, whey protein concentrate (WPC) and casein were fermented by *Lactiplantibacillus plantarum* MCM4 to produce the highest GABA content. Effects of other variables such as fermentation time, substrate concentration and inoculum size were assessed and optimized as well.

2. Materials and Methods

2.1. Chemicals and Reagents

Phenyl isothiocyanate and GABA (purity≥99%) were purchased from Sigma-Aldrich, Germany. MRS broth, trichloroacetic acid, teriethyl amine, sodium acetate, acetonitrile, sodium hydroxide and other chemicals were supplied from Merck, Germany. Moreover, SPI, casein and WPC were purchased from local suppliers.

2.2. Microbial culture preparation

High GABA-producing *L. plantarum* MCM4 was previously isolated from fermented cabbage [5] and stored at -80 ºC in the Microbial Bank, Department of Food Science and Technology, Gorgan University of Agricultural Sciences and Natural Resources, Iran. Culture was recovered by sub culturing in MRS broth and incubating at 37 °C for 24 h under microaerophilic conditions.

2.3. Optimization of GABA synthesis by OFAT approach

The GABA synthesis by *L. plantarum* MCM4 was optimized through OFAT using various protein hydrolysates, substrate concentrations, hydrolysis times and inoculum sizes. Selected fermentation parameters were used in next steps of optimization process. The most important parameters were further optimized using RSM.

2.3.1. Effects of various protein hydrolysates on GABA synthesis

Various protein hydrolysates with various degrees of hydrolysis were prepared as GABA precursors using enzymatic hydrolysis. Alcalase (2% enzyme/protein) was added to a 6% w v^{-1} suspension of SPI, casein, or WPC in phosphate buffer (pH 7.5, 100 mM) and hydrolysis was carried out at 45 ℃ as the optimum temperature for alcalase activity. Incubation was carried out for 3 and 6 h and then the mixture was heated at 85 ℃ for 10 min to inactivate the enzyme. Mixture was then centrifuged (4220× g, 20 min) and the supernatants were freeze dried as Soy Protein Hydrolysate 3 (SPH3) and SPH6 (SPI was hydrolyzed for 3 and 6 h, respectively), CH3 and CH6 (casein was hydrolyzed for 3 and 6 h, respectively) and WPH3 and WPH6 (WPC was hydrolyzed for 3 and 6 h, respectively). To investigate the appropriate precursor for GABA synthesis, various media containing each protein hydrolysate (1.0%, w v^{-1}) and glucose (0.5% w v^{-1}, as the carbon source) were inoculated with active cultures of *L. plantarum* MCM4 (10^8 CFU ml^{-1}, 2% v v^{-1}). The inoculated media were incubated at 37 ºC for 24 h. After incubation, fermentation was terminated and the media was centrifuged at 2600× g for 10 min. Then, supernatant was collected and used for GABA determination [5,18]. Effects of substrate concentration was assessed using varied levels of the desirable protein hydrolysate (1-5% w v^{-1}).

2.3.2. Effects of fermentation time on GABA synthesis

To investigate the optimal time period in GABA synthesis, *L. plantarum* MCM4 was inoculated (2%, v v^{-1}) to media containing the best protein hydrolysate with appropriate concentrations as determined in OFAT. Samples were collected at 24 h intervals until 72 h and the GABA contents were assessed.

2.3.3. Effects of inoculum size on GABA synthesis

Effects of inoculum size on GABA synthesis was assessed by inoculating various levels of active culture (1-5% v v^{-1}, 10^8 CFU ml^{-1}) into the media. Fermentation media contained the optimal protein hydrolysate, the optimal substrate concentration and the optimal incubation time based on the results from the previous OFAT approach. Then, GABA contents were assessed.

2.4. Optimization of GABA synthesis using central composite design

Central composite design (CCD) was used for the optimization of GABA synthesis by *L. plantarum* MCM4. The three independent variables, including fermentation time (A), inoculum size (B) and SPH6 concentration (C), were used in three various coded levels (-1, 0 and +1) to establish the model (Table 1). Totally, 20 experimental runs were suggested with six center point runs (Table 1). A second order quadratic polynomial mathematical model was suggested to analyze the concentration of GABA (mg 100 ml^{-1}) synthesized in the experiments.

2.5. Assessment of the GABA contents

The GABA contents in fermented samples were assessed based on a method described by Karimian et al [5] and Woraharn et al [14]. Briefly, an aliquot of 10% trichloroacetic acid soluble fraction from the fermented sample was derivatized with Phenyl isothiocyanate. Then, 20 µl of the derivatized solution was injected to an HPLC apparatus equipped with a C18 column (150 × 4.6 mm, 5 Mm) and UV detector at 254 nm. The mobile phase included sodium acetate (20 mM, pH 5.8, Phase A) and acetonitrile (Phase B) at 0.9 ml $(min)^{-1}$ with isocratic elution (A:B = 45:55). For quantitative analysis, standard curves of various concentrations (0.0-0.25 mg ml^{-1}) of the pure GABA were prepared.

2.6. Statistical analysis

The Statistical Analysis Software (SAS, v.9.3) was used to analyze data. All assays were carried out at two replications and data were presented as mean ±SD (standard deviation). Comparisons between the mean values were carried out using Duncan's multiple range test ($p \leq 0.05$). The CCD based RSM was carried out using Design Expert Software v.7.0.0 (State-Ease, USA).

3. Results and Discussion

3.1. Optimization of various parameters using OFAT

In OFAT analysis, effects of various parameters on microbial GABA synthesis were assessed and the major factors with probable ranges were reported (Fig. 1). Based on the results, soy protein hydrolysate was more appropriate substrate than whey and casein hydrolysates (Fig. 1a). Moreover, SPH6 resulted in a higher GABA synthesis than that SPH3 did. Therefore, SPH6 was selected as the best GABA precursor and used in further optimization steps. In substrate concentration, a lower quantity of GABA was produced when SPH6 was used at 1% w v^{-1} (Fig. 1b). The GABA concentrations were 6.0, 13.5 and 14.0 mg 100 ml^{-1} when 1.0, 3.0 and 5.0% w v^{-1} SPH6 was used, respectively. Thus, SPH6 at 3% w v^{-1} was selected as the substrate for the OFAT optimization. When fermentation time increased from 24 to 72 h, GABA concentration decreased from 13.5 to 8.0 mg 100 ml^{-1} (Fig. 1c). Inoculum size was an effective parameter in GABA synthesis as well (Fig. 1d). Significant differences were seen between the GABA content in samples fermented with various inoculum sizes. The highest GABA concentration (13.0 mg 100 ml^{-1}) was achieved in samples inoculated with 3% v v^{-1} starter culture (10^8 CFU ml^{-1}). Over time (after 24 h), protein hydrolysis was extended and solution viscosity decreased. In very low and high viscose solutions, transfer rates of oxygen and nutrients to microorganism decreased and microbial populations and product yields therefore decreased [19].

3.2. Effects of independent factors on GABA production

Effects of SPH concentration on GABA production show that the SPH content included linear significant effects on GABA levels (Table 2) as the higher the SPH level was, the higher the GABA concentration was (Fig. 2b). This could majorly be due to the high levels of glutamic acid in SPH that might include the potential to increase GAD activity of the bacterial species and hence the GABA yield [6]. Soy protein is a source of carbon, which includes significant effects on yields, characteristics, structures and compositions of the bacterial cells; thus, carbon is an important source for the microbial production. In the Krebs cycle, carbohydrate is converted to alpha-ketoglutarate and then L-glutamate is produced in cytoplasm cells. As stated previously, GABA is produced from glutamic acid decarboxylation; hence, high SPH and carbon concentrations could increase the GABA values [20]. Various studies have used soy protein as a potential source for GABA production [21,22]. For example, Karimian et al. reported that the addition of SPH to whey-based media increased GABA production [5]. Furthermore, Zareie et al. reported that fermentation of high quantities of soy protein, as a great source of glutamic acid, could increase the GABA contents [8].

In this study, fermentation time included linear significant effects on GABA production (Table 2). As seen in Fig. 2a, GABA contents increased with increases in fermentation time up to 24 h and then decreased as the fermentation time increased to 72 h. These results could be attributed to the conversion of glutamic acid in soy protein to GABA by *L. plantarum* [8], but simultaneous increases in fermentation time (after 24 h) and inoculum sizes (more than 3%) decreased the GABA yields. This could be mostly because of the limitation of media nutrients and available oxygen, likely decreasing the number of bacteria and sequentially the GAD activity [23].

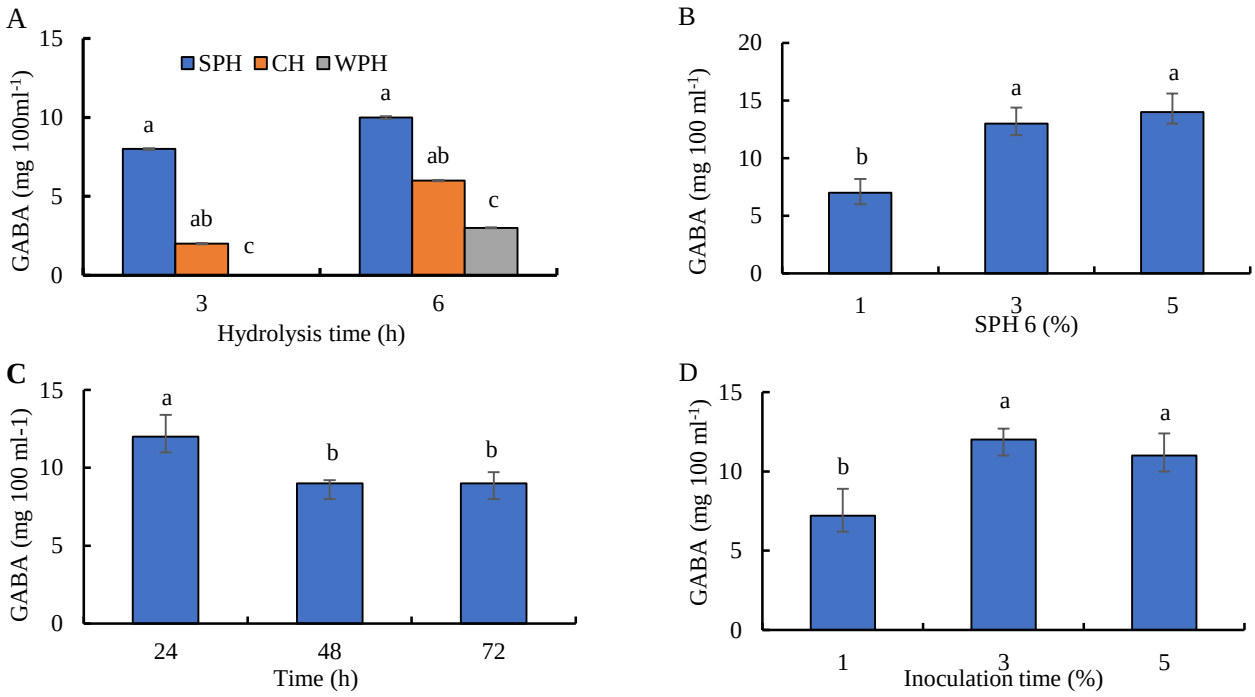

Fig. 1. Effects of various parameters on gamma aminobutyric acid (GABA) synthesis by *Lactiplantibacillus plantarum* MCM4 as determined in OFAT analysis; (A) Various protein hydrolysates, (B) substrate (SPH6) concentration, (C) fermentation time, (D) inoculum size. SPH, soy protein hydrolysate; CH, casein hydrolysate; and WPH, whey protein hydrolysate. Different letters for each parameter show significant differences (p<0.05)

Table 1. Central composite design matrix and response of independent factors, their levels and experimental results for the GABA contents

Factor	unit	levels		
		-1	0	1
Fermentation time (A)	h	12	24	36
Inoculum size (B)	% v v^{-1}	2	3	4
SPH6 concentration (C)	% w v^{-1}	2	3	4
Run	A	B	C	GABA (mg 100 ml^{-1})
1	12	2	4	8
2	24	3	3	15.2
3	12	4	4	8.5
4	12	4	2	5.3
5	24	2	3	16
6	24	3	3	17
7	36	2	4	15.3
8	24	3	3	17.5
9	36	2	2	10.7
10	24	3	4	19
11	12	3	3	7.4
12	36	4	2	12.3
13	36	4	4	18
14	24	4	3	13
15	24	3	3	17.2
16	24	3	2	14
17	24	3	3	16
18	36	3	3	13.1
19	12	2	2	6.7
20	24	3	3	18.1

Table 2. The ANOVA for response surface polynomial quadratic model for gamma aminobutyric acid synthesis by *Lactiplantibacillus plantarum* MCM4

Source	Sum of Mean F p-value Squares	df	Mean square	F value	p-value Prob > F	
Model	331.11	9	36.79	21.83	< 0.01	Significant
A-Time	112.22	1	*112.22*	66.59	*< 0.01*	
B-Inoculum size	0.016	1	*0.016*		0.92	
C-Substrate	39.20	1	39.20	23.26	0.01	
AB	3.38	1	3.38	2.01	0.19	
AC	4.20	1	4.20	2.49	0.15	
BC	1.13	1	1.13	0.67	0.43	
A^2	82.78	1	82.78	49.11	*< 0.01*	
B^2	4.20	1	4.20	2.49	0.15	
C^2	1.60	1	1.60	0.95	0.35	
Residual	16.85	10	1.69			
Lack of Fit	11.28	5	2.26	2.02	0.23	Not Significant
Pure Error	5.57	5	1.11			
Cor Total	347.97	19				
R^2= 0.9516	Adj R^2= 0.9080		Pred R^2 = 0.8656		Adeq Precision= 15.569	

As shown in Table 2, inoculum size included no linear significant effects on GABA production. As stated previously, inoculum sizes of greater than 3% decreased the GABA production (Fig. 2c). Higher inoculum sizes led to high levels of acid production and hence media pH reduction. Since pH 5 was the optimum pH of GAD activity; therefore, it could be concluded that reduction of pH resulted in decreases in GAD activity and hence GABA production [8,24].

3.3. Optimization of GABA synthesis using central composite design based response surface methodology

The GABA synthesis by *L. plantarum* MCM4 was optimized through CCD-based RSM using various ranges of three independent variables, including fermentation time (A), inoculum size (B) and SPH concentration (C). Twenty experimental runs were designed based on the CCD. Variables used for RSM are presented in Table 1 with actual GABA synthesis.

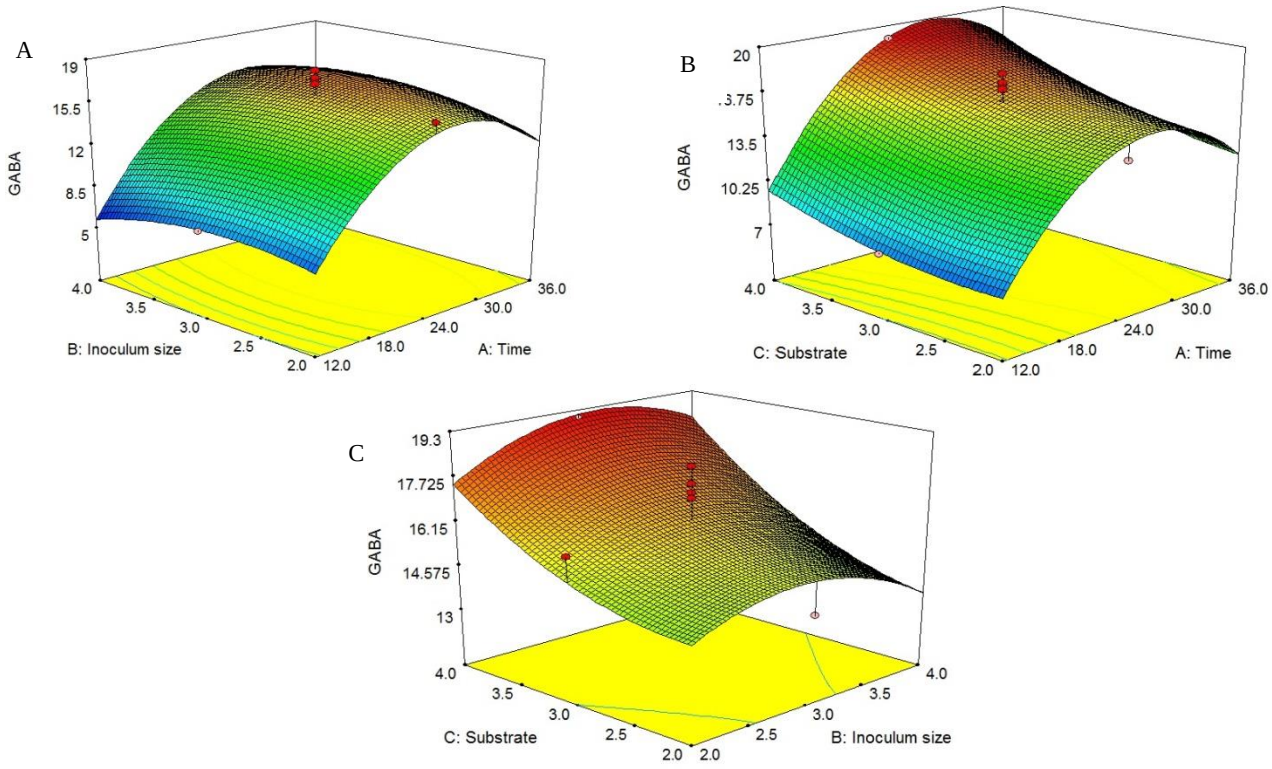

Fig. 2. Response surface 3D contour plots, representing interaction between the variables that affect gamma aminobutyric acid synthesis by *Lactiplantibacillus plantarum* MCM4. (A) Fermentation time and inoculum size, (B) fermentation time and substrate concentration, and (C) inoculum size and substrate concentration

The GABA synthesis yield varied between 5.3 mg 100 ml^{-1} (run no. 4; fermentation time of 12 h, inoculum size of 4% v v^{-1} and SPH6 concentration of 2% w v^{-1}) and 19.0 mg 100 ml^{-1} (run no. 10; fermentation time of 24 h, inoculum size of 3% v v^{-1} and SPH6 concentration of 4% w v^{-1}). Significance of the suggested model was assessed using analysis of variance (ANOVA) (Table 2). Based on ANOVA, the model was highly significant (p<0.0001) to predict GABA concentration as the response variable. Within the model, A (fermentation time), C (SPH concentration) and A^2 (fermentation time^2) were the significant parameters. A polynomial quadratic regression equation was established for the GABA synthesis, as presented in coded (Eq. 1) and actual (Eq. 2) terms as follows:

GABA (mg 100 ml^{-1}) = +16.39 + 3.35 * A + 0.040 * B + 1.98 * C + 0.65 * A * B + 0.73 * A * C + 0.38 * B * C - 5.49 * A^2 - 1.24 * B^2 + 0.76 * C^2 Eq. 1

GABA (mg 100 ml^{-1}) = -10.94045 + 1.76420 * time + 5.03318 * inoculum size - 5.17682 * substrate + 0.054167 * time * inoculum size + 0.060417 * time * substrate + 0.37500 * inoculum size * substrate - 0.038100 * time^2 - 1.23636 * inoculum size^2 + 0.76364 * substrate^2 Eq. 2

Coefficient of determinant (R^2), adjusted R^2, predicted R^2, adequate precision and lack-of-fit were the criteria to assess fitness of the model. The R^2 value of 0.9516 indicated that the model could explain 95.16% of variables in GABA synthesis during fermentation. The predicted R^2 of 0.7651 was in reasonable agreement with the adjusted R^2 of 0.9080. Adequate precision calculated the signal to noise ratio. Based on the analysis, a ratio greater than 4 was desirable. Therefor, the ratio of 15.569 showed an adequate signal. The F-value of 2.02 for the lack-of-fit demonstrated that lack-of-fit was not significant. Therefore, it was concluded that the design space could be navigated by the suggested model successfully [25].

Reasonable correlations between the experimental values and the predicted values were shown by the parity plot (Fig. 3A). The points grouped around the diagonal line indicated that the model fitted well with the observations and deviation between the experimental and the predicted values was low. As shown in Fig. 3B, linear relationships between the normal probability and the externally studentized residuals reflected an acceptable goodness of fit of the model. This verified that the suggested regression model included a normal distribution capable of predicting the experimental results. As reported previously, an appropriate model should include a normal distribution [26]. Formation of a horizontal band in the residuals versus prediction plot (Fig. 3C) revealed the equal variances of the error terms. Moreover, ideal residual plot in this figure suggested no outliers in the model. The plot of residuals versus experimental run order, which was used to search for hidden variables that might affect the response during the experiment [27]. The plot should show the random scatter as in Fig. 3D.

The optimum GABA syntehsis conditions were achieved where fermentaion time was 28.99 h, inoculum size was 3.65% v v^{-1} and SPH6 concnetration was 3.89% w v^{-1} with a maximum GABA yield of 19.4 mg 100 ml^{-1} (Table 3).

Table 3. The optimum fermentation conditions to achieve the maximum level of gamma aminobutyric acid and validation of the regression model

Factor		Level		
A	Time	28.99		
B	Inoculum size	3.65		
C	Substrate	3.89		
Response	Prediction	Experimental	95% PI low	95% PI high
GABA	19.39	17.7	16.02	22.76

The GABA synthesis was further experimentally carried out under the optimized conditions to verify the predicted results. The experimental value for GABA was achieved as 17.7 (mg 100 ml^{-1}) and the perdicted value was highly validated. In fact, GABA production has been reported as a time-dependent process; the greatest concentration of GABA (~255 mM) was yielded after 48 h and furthrer fermentation time did not convert monosodium glutamate to GABA. This might be due to pH reduction as a consequence of cell enterance into the exponential phase, which activated GAD enzyme and triggered GABA synthesis. Upon glutamate conversion to GABA, media pH increased, which suppressed the GAD activity [28]. Falah et al. reported that 6.27% soybean meal (120 h fermentation at 32 °C) was optimal for GABA production (300 mg l^{-1}) and this was linked to the high glutamic acid content in soybean meal (~18% glutamic acid), which was a substrate for the decarboxylation reaction of GABA production [7]. Based on a study by Chi et al., soy-based products could be used in fermentation cultures to synthesize versatile amino acids, owing to their avaible short chains and bioactive peptides [29].

Inoculum size and cultivation time showed significant effects on GABA production. Accordingly, it has been demonstrated that 48 and 72 h cultivation times provided the highest GABA yields, while GABA production decreased significantly at 96 and 120 h [30]. Moreover, 10% (v v^{-1}) inoculum size (*L. brevis* SL9-6) resulted in significantly high GABA yields [30]. Furthemore, it was shown that the maximum GABA production (103.7 g l^{-1}) was yielded by *L. brevis* NCL91 cultivated for 48 h at 10% inoculum [31]. The optimal level of GABA yield from KungSom (10.50 $mgkg^{-1}$ fresh product), optimezed using CCD-based RSM, was achieved at 0.5% monosodium glutamate level and approximately 10^7 CFU g^{-1} inoculum size of *L. futsaii* cells. Moreover, addition of starter culture to media resulted in up to 4× time higer GABA contents in the product, compared to control (without starter culture) or commercial Kung-Som products (10 and 120 mg kg^{-1} product) [32].

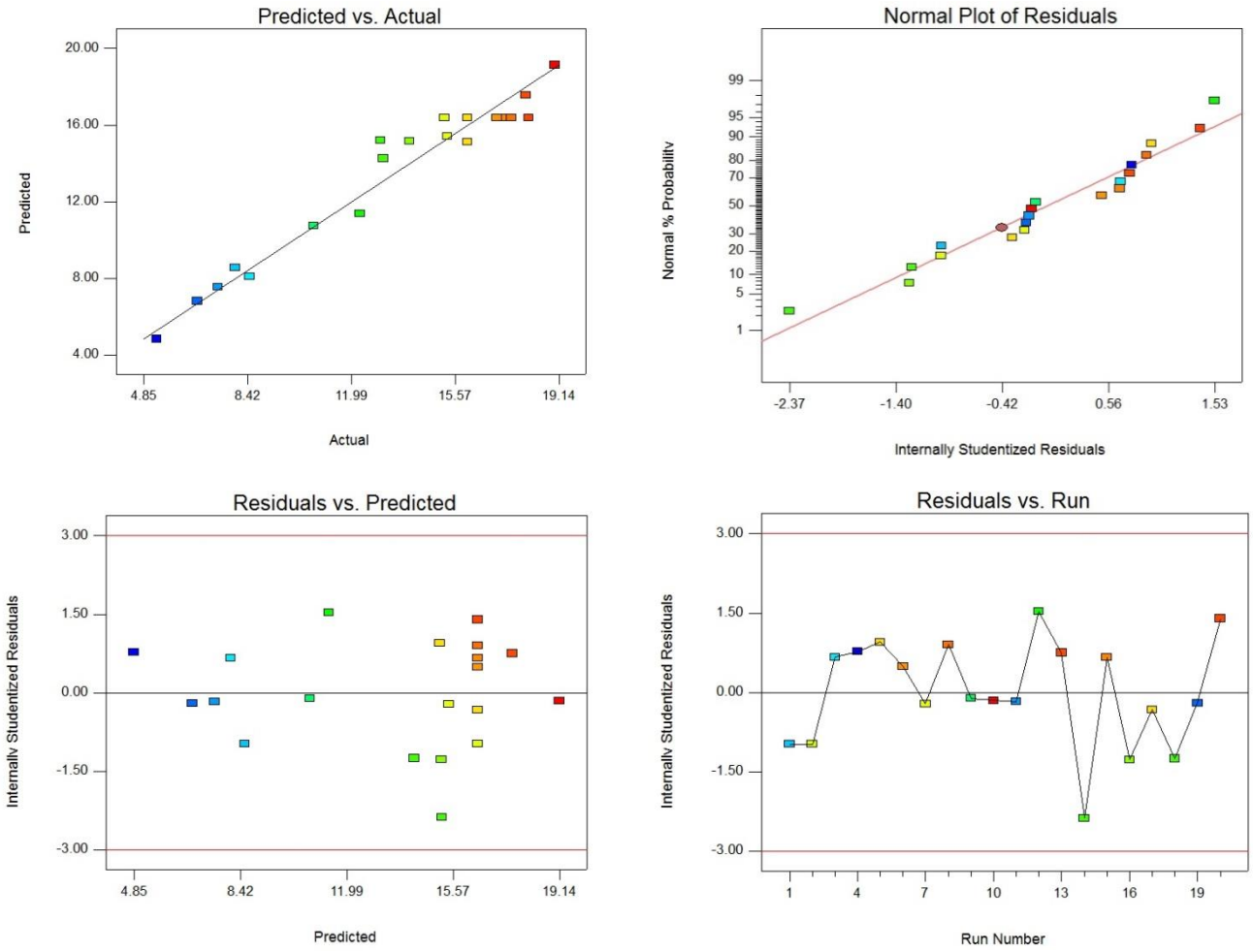

Fig. 3. Assessment of the validity of CCD-based RSM model. (A) Predicted versus actual gamma aminobutyric acid concentrations, (B) normal plot of residuals, (C) internally studentized residuals versus predicted values, and (D) internally studentized residuals versus run numbers

4. Conclusion

Soy protein hydrolysate was used as an appropriate substrate for GABA synthesis by *L. plantarum* MCM4, without using supplements such as cofactors and pure GABA precursors. Extension in the enzymatic hydrolysis (6 h against 3 h) resulted in the production of substrates with higher GABA yields. Results showed that *L. plantarum* MCM4 could be suggested as an efficient bacterial strain to produce bioactive GABA from inexpensive sources. However, further investigation of purification technique effects on the final products is recommended.

5. Acknowledgements

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6. Conflict of Interest

The authors report no conflicts of interest.

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بهینه سازی تولید گاما-آمینوبوتیریک اسید از هیدرولیزشده‌های پروتئینی گوناگون به‌دست آمده از *لاکتی‌پلانتیباسیلوس پلانتاروم* MCM4

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چکیده

سابقه و هدف: گاما-آمینوبوتیریک اسید نوعی آمینواسید غیر پروتئینی با عملکردهای فیزیولوژیک متنوع است که به‌عنوان مکمل‌های سنتزی برای درمان اضطراب، استرس حاد و اختلالات خواب مورد استفاده قرار می‌گیرد. به‌دلیل افزایش تقاضا برای مصرف ترکیبات زیست فعال طبیعی، غنی سازی غذاها و مکمل‌ها با گابا تولید شده به‌روش‌های بیولوژیک در سال‌های اخیر رواج یافته است.

مواد و روش ها: در این مطالعه، ابتدا روش اثر یک عامل- در یک زمان[1] برای بررسی پروتئین‌های آبکافت‌شده[2] گوناگون شامل ایزوله پروتئین سویا،کنسانتره پروتئین آب پنیر وکازئین در غلظت‌های گوناگون ($w\ v^{-1}$ ۵-۱)، زمان‌های تخمیر (۲۴، ۴۸ و ۷۲ ساعت) و حجم مایه تلقیح ($v\ v^{-1}$ ۱%، ۳ و ۵) بر میزان تولید گاما-آمینوبوتیریک اسید مورد بررسی قرار گرفت. سپس، به‌منظور بهینه سازی تولید گاما-آمینوبوتیریک اسید توسط *لاکتوباسیلوس پلانتاروم* MCM4، موثرترین عوامل مانند پروتئین آبکافت‌شده سویا، حجم مایه تلقیح و زمان تخمیر با استفاده طراحی آزمون سطح پاسخ[3] در قالب طرح مرکب مرکزی[4] مطالعه شدند.

یافته‌ها و نتیجه‌گیری: در بین آبکافت‌های پروتئینی گوناگون، پروتئین آبکافت شده سویا رشدمایه مناسب‌تری برای سنتز گابا بود. علاوه بر این، مقادیر بیشتر گاما-آمینوبوتیریک اسید هنگامی‌به‌دست آمد که پروتئین سویا با ۶ ساعت آبکافت آنزیمی (آبکافت پروتئین سویا ۶) استخراج و به‌عنوان رشدمایه مورد استفاده قرار گرفت. مدل ریاضی چند جمله‌ای قادر بود به‌طور موفقیت‌آمیزی ساخت گاما-آمینوبوتیریک اسید را به روش تخمیر پیش‌بینی کند. بهینه سازی در قالب طرح مرکب مرکزی نشان داد که بالاترین میزان گاما-آمینوبوتیریک اسید ($19/4 mg\ 100\ ml^{-1}$) در زمان تخمیر ۲۹ ساعت، حجم مایه تلقیح $3/65\ v\ v^{-1}$و غلظت آبکافت پروتئین سویا ۶، $3/9\ \%\ w\ v^{-1}$ درصد به‌دست آمد. به‌طورکلی، *لاکتی‌پلانتی باسیلوس پلانتاروم* MCM4 به‌عنوان یک گونه جدید باکتری لاکتیک اسید برای تولید اسید گاما-آمینوبوتیریک از منابع ارزان قیمت پیشنهاد شود.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

[1] One factor at a time -OFAT
[2] Protein hydrolysates
[3] Response Surface Methodology (RSM)
[4] Central Composite Design (CCD)