

## Optimization of D-amino acid oxidase production by *Trigonopsis variabilis* using glucose syrup from cassava as carbon source

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**Abstract.** Rusli Z, Suryadi H, Wibisana A. 2018. Optimization of D-amino acid oxidase production by *Trigonopsis variabilis* using glucose syrup from cassava as carbon source. *Bioteknologi* 15: 26-32. Glucose syrup from cassava (GSC) contains glucose as the main sugar suitable for an economical and efficient production of D-amino acid oxidase (DAAO) by *Trigonopsis variabilis*. The aim of this study is to optimize the DAAO production by *T. variabilis* using GSC as the carbon source via response surface methodology (RSM) with Face-centered Central Composite Design (FCCD). In the first step, the effects of six factors (concentration of GSC and DL-alanine, pH, temperature, incubation time, and inoculum concentration) were screened to obtain the significant factors on DAAO production by Plackett-Burman design. As a result, the incubation time, the concentration of GSC and DL-alanine showed to be significant factors for DAAO production ( $P < 0.05$ ). These factors were then optimized using RSM with FCCD. The optimized RSM results demonstrated that a quadratic polynomial model was found suitable to define the relation between the incubation times, concentration of GSC and DL-alanine parameters. Moreover, the observed high  $R^2$  value (0.9782) confirms a strong evaluation of the experimental data. The optimum condition for DAAO production is as follows: GSC and DL-alanine concentration was 12.3% and 0.3%, respectively, and the culture incubation time was 56.1 hours. Using this condition, we got a DAAO activity of 195.38 U/g dry weight of yeast.

**Keywords:** D-amino acid oxidase, face-centered central composite design, glucose syrup from cassava, Plackett-Burman design, *Trigonopsis variabilis*

**Abbreviations:** API: Active pharmaceutical ingredient, DAAO: D-Amino Acid Oxidase, GSC: Glucose syrup from cassava, RSM: Response surface methodology, FCCD: Face-centered central composite design

### INTRODUCTION

The production of pure chiral compounds via a cost-effective method has become an important approach to fine chemical, agrochemical, and pharmaceutical industries. The use of biocatalysts to produce pure chiral compounds has been widely applied in large scale and quickly becoming a cost-effective approach. The recent development of biocatalysts processes was designed to be employed on a large-scale and to produce inexpensive enzyme with good properties, *i.e.*, high activity, stability and selectivity (Caligiuri et al. 2006). Over the last few years, researchers have focused on several enzymes (proteases, oxidases, and hydrolases), investigating and improving the property of the enzyme's protein scaffold to meet the requirement of different applications and utilized in various fields (Li et al. 2012; Gurung et al. 2013).

Chiral compounds containing enantiomeric amine or amino acid groups can be produced using a variety of enzymes, such as amino acid dehydrogenases (EC 1.4.1.X), amine oxidases (EC 1.4.3.22), amino acid oxidases (EC 1.4.3.X), aminotransferases (EC 2.6.4.X), ammonia lyase (EC 4.3.1.X), and lipases (EC 3.1.1.X) (Molla, Melis, and Pollegioni 2017). Deracemization of a DL-mixture to

obtain L-isomer can be done by employing a D-amino acid oxidase (DAAO, EC 1.4.3.3) and non-selective chemical reduction step to obtain enantiopure L-amino acid after numerous cycles (Turner 2004). DAAO catalyzes the oxidative deamination of D-amino acids to produce  $\alpha$ -keto and ammonia.

DAAO is used in the first step of bioconversion of cephalosporin C (CPC) to 7-ACA, whereby the 7-ACA is used as a precursor for the synthesis of API (Pollegioni, Rosini, and Molla 2013). This process involves two-step cleavage with D-amino acid oxidase (DAAO) and glutaryl acylase (GAC) (Barber et al. 2004). The recent innovation has used a one-step mono-enzymatic process using cephalosporin C acylase (Gröger et al. 2017). DAAO has also been used in biosensors, in the resolution of natural (*e.g.* methionine and phenylalanine) and synthetic (*e.g.* naphthylalanine) amino acid racemic solutions (Findrik and Vasić-Rački 2007), and in keto acids production (Song et al. 2016). DAAO can be found in mammalian organs, mainly in the kidneys. DAAO can also be produced by fermentation from microorganisms, such as yeasts *Trigonopsis variabilis*, *Rhodotorula gracillis*, *Candida tropicalis*, and *Neurospora crassa* fungi (Pollegioni et al. 2007). *T. variabilis* DAAO has a high catalytic activity for

CPC oxidation to produce intermediate compound glutaryl-7 amino cephalosporanic acid (Isoai et al. 2002).

DAAO can be produced through fermentation using media that contains carbon, nitrogen, mineral sources, etc. For industrial scale, the utilization of raw materials from local resources for the extractive and fermentative process has to be considered in order to benefit economically (MoH 2016). Indonesia has abundant agro-industrial products that can be used as raw materials for different purposes. Agro-industrial products, such as molasses and cassava, have been used as a substrate in the fermentation industry. Based on preliminary studies, it is known that glucose syrup from cassava is better than molasses or fructose syrup from sorghum; thus, it can be used as an alternative carbon source. Glucose syrup from cassava (GSC) contains glucose as the main sugar (Pontoh and Low 1995), and glucose is the best carbon source for DAAO production (Gupta, Gundampati, and Debnath 2012b). GSC also contain a little amount of nitrogen needed during culture cultivation and enzyme biosynthesis; hence making DAAO production economical and efficient. As an agricultural country, Indonesia is among the top 3 cassava producing countries in the world, producing more than 24 million tons and increasing every year (BPS 2016). Thus, this availability of the main raw materials can be guaranteed for the large-scale production of DAAO in Indonesia.

A statistical technique that combines the Plackett-Burman design (PBD) and central composite design (CCD) is a suitable and the most widely used method for optimization of biological processes (Wibisana et al. 2015; Gupta, Gundampati, and Debnath 2012a). PBD was developed by R.L. Plackett and J.P. Burman in 1946. It was designed to improve the quality control process that could be used to study the effects of design parameters on the system state so that intelligent decisions can be made. Plackett and Burman's devised orthogonal arrays are useful for screening, which yield unbiased estimates of all main effects in the smallest design possible (Vanaja and Shobha Rani 2007). CCD is the most popular among many classes of response surface methodology (RSM). It is widely used for estimating second-order response surfaces. The choice of axial distance  $\alpha$  is based on the region of interest. Choosing the appropriate values of  $\alpha$  specifies the type of CCD. In addition, for biological reasons, one cannot experiment outside the cube, even though experimentation at the extremes in the region is permissible and, in fact, desirable. This scenario, which occurs frequently in many scientific areas, suggests a central composite design in which the eight corners of the cube are centered and  $\alpha = 1$ , often called the Face-centered central composite design (FCCD) (Myers, Montgomery, and Anderson-Cook 2009). FCCD has been widely used in enzyme production (Salihu, Bala, and Alam 2016; Tari, Göğüs, and Tokatli 2007).

The aim of this study is to optimize the production of DAAO by *T. variabilis* using GSC as carbon source. The optimization was done by response surface methodology (RSM) to enhance the production. Important production parameters were screened by Plackett-Burman design and the optimization of significant factors was done using Face-centered Central Composite Design (FCCD).

## MATERIALS AND METHODS

### Materials

*Trigonopsis variabilis* was used as a source of the enzyme obtained from Biotech Center-BPPT (Serpong, Tangerang, Indonesia). Glucose syrup from cassava (GSC) was obtained from PT. Rejo Madusari (Pati, Central Java, Indonesia). DL-alanine were purchased from HiMedia (Manufacturer's city, Country).  $K_2HPO_4$ ,  $KH_2PO_4$ ,  $MgSO_4 \cdot 7H_2O$ , NaCl,  $CaCl_2$ ,  $MnCl_2 \cdot 4H_2O$ ,  $ZnSO_4 \cdot 7H_2O$ ,  $CuCl_2 \cdot 3H_2O$ ,  $H_3BO_3$ ,  $FeCl_3 \cdot 6H_2O$  were purchased from Merck (Darmstadt, Germany). Other chemicals, such as thiamin, biotin, o-phenylenediamine, horseradish peroxidase were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). All experiments were carried out in duplicate.

### Procedures

#### Inoculum preparation

The *T. variabilis* strain was maintained in a yeast malt medium. The cultures were kept at 4°C and subcultured at regular intervals of 30 days. Preculture medium (pH 6) contained: 22 g/L CGS, 4 g/L DL-alanine, 2 g/L  $K_2HPO_4$ , 0.21 g/L  $KH_2PO_4$ , 0.5 g/L  $MgSO_4 \cdot 7H_2O$ , 0.1 g/L NaCl, 0.1 g/L  $CaCl_2$ , 0.105 g/L  $MnCl_2 \cdot 4H_2O$ , 0.0231 g/L  $ZnSO_4 \cdot 7H_2O$ , 0.042 g/L  $CuCl_2 \cdot 3H_2O$ , 0.105 g/L  $H_3BO_3$ , 0.0714 g/L  $FeCl_3 \cdot 6H_2O$ , 0.24 mg/L thiamine and 0.02 mg/L biotin. GSC and DL-alanine were sterilized using a 0.2  $\mu m$  filter. Other components were sterilized by autoclaving at 121°C for 15 min. The inoculum was prepared by taking a loop full of fresh culture (72 h) and suspended in 5 mL of sterile saline water to get an appropriate suspension ( $OD_{600} = 0.1-0.2$ ), then 1 mL of the culture suspension was inoculated to 50 ml pre-culture medium in 250 ml Erlenmeyer flask. The culture was then incubated in an orbital shaker at 30°C and 200 rpm for 24 h for inoculum development.

#### Production of DAAO

Production medium (pH 6) contained: 32 g/L GSC, 6.2 g/L DL-alanine, 2 g/L  $K_2HPO_4$ , 0.21 g/L  $KH_2PO_4$ , 0.5 g/L  $MgSO_4 \cdot 7H_2O$ , 0.1 g/L NaCl, 0.1 g/L  $CaCl_2$ , 0.105 g/L  $MnCl_2 \cdot 4H_2O$ , 0.0231 g/L  $ZnSO_4 \cdot 7H_2O$ , 0.042 g/L  $CuCl_2 \cdot 3H_2O$ , 0.105 g/L  $H_3BO_3$ , 0.0714 g/L  $FeCl_3 \cdot 6H_2O$ , 0.24 mg/L thiamin and 0.02 mg/L biotin. GSC and DL-alanine was sterilized using a 0.2  $\mu m$  filter. The other components were sterilized in an autoclave at 121°C for 15 min.

Production of DAAO was carried out by transferring 10% inoculum ( $OD_{600} = 0.7-0.8$ ) to the production medium and then incubated in an orbital shaker at 30°C and 140 rpm for 72 h.

#### Permeabilization of cell

*Trigonopsis variabilis* cells were harvested and neutralized to pH 6.0 using potassium hydroxide, then centrifuged at 10,000 g at 4°C for 10 min to obtain the cell, and then washed with potassium phosphate buffer (pH 8.0) twice. A certain volume of cells was taken and centrifuged, washed with distilled water and dried at 105°C to reach a

constant mass to determine dry cells weight (Kujan et al. 2001). The rest of the washed cells were suspended using the same buffer. The suspension cells were permeabilized using 5% toluene-ethanol (1:1) and held for 1 h at 37°C. The permeabilized cells were used for enzyme assay.

#### Enzyme assay

DAAO activity was measured by o-phenylenediamine/horseradish peroxidase coupling assay (Wang, Yu, and Kuan 2008). The reaction mixture contained 30 mM D-alanine, 0.03% o-phenylenediamine, 1700 U horseradish peroxidase and DAAO of interest in 100 mM potassium phosphate buffer (pH 8.0). The reaction was monitored by an increase in absorbance at 450 nm for 3 min at 25°C.

One unit of enzyme activity was defined as the amount of enzyme needed to produce 1 micromole of H<sub>2</sub>O<sub>2</sub> per min at 25°C and pH 8.0.

#### Data analysis

Optimization was done in two stages; the first stage was to identify the most significant factors for DAAO production using Plackett-Burman design and the later stage the optimization of significant factors by using RSM with a face-centered central composite design. The experimental design and statistical analysis were done using Design Expert software (version 7.1).

#### Plackett-Burman design

The Plackett-Burman design is very useful for screening the most important factors with respect to their main effects. This model does not describe interaction among factors, it is used to screen and evaluate the important factors that influence the response. Six different independent variables, including carbon (glucose syrup from cassava) concentration, DL-alanine concentration, pH, incubation time (period), temperature and inoculum quantity were selected for screening. Each variable was tested at two levels, namely a high level denoted by (+1) and a low level denoted by (-1) as listed in Table 1. Six variables were screened by twelve experiments conducted by Plackett-Burman design (Table 2). All experiments were carried out in duplicate and the average value of DAAO activity was used for statistical analysis.

**Table 1.** Six factors and their levels used in Plackett-Burman design.

Code	Variables	Low level (-1)	High level (+1)
A	GSC (%)	10	15
B	DL-Alanine (%)	0.3	0.7
C	pH	6	8
D	Temperature (°C)	28	32
E	Incubation time (Hours)	48	72
F	Inoculum (%)	5	10

#### Face-centered central composite design (FCCD)

This step involved the optimization of the levels and the interaction effects between various significant variables. In this study, the experimental plan consisted of 20 trials and the independent variables were studied at three different levels: low (-1), middle (0), and high (+1) (Oyejola and Nwanya 2015). The center points were repeated six times to evaluate the curvature and the experiment replication facilitated the pure error estimation so that the significant lack of fit of the models could be predicted. All the experiments were done in duplicate and the average of DAAO activity (U/g yeast cell dry weight) obtained was taken as the dependent variable or response (Y).

The levels of factors used for experimental design are given in Table 3. For three factors, a fractional factorial design with six replications of center points consists of 20 runs.

**Table 2.** Plackett-Burman screening, representing the response of DAAO production as influenced by GSC (A), DL-alanine (B), pH (C), temperature (D), incubation time (E), and inoculum (F).

Run no.	A	B	C	D	E	F	DAAO activity (U/g YCDW)
1	10	0.3	8	28	72	10	87.6509
2	10	0.3	6	28	48	5	85.8477
3	15	0.3	8	32	72	5	43.8850
4	15	0.7	8	28	48	5	66.3733
5	10	0.7	8	32	48	5	68.5606
6	15	0.7	6	28	48	10	66.6269
7	15	0.7	6	32	72	10	32.6922
8	10	0.7	6	32	72	5	41.7822
9	10	0.7	8	28	72	10	50.2795
10	15	0.3	6	28	72	5	52.4000
11	15	0.3	8	32	48	10	79.5999
12	10	0.3	6	32	48	10	87.0552

**Table 3.** Matrix of Face-centered central composite design.

Run no.	A	B	C
1	1	1	-1
2	-1	0	0
3	-1	1	-1
4	0	0	-1
5	-1	-1	1
6	1	1	1
7	-1	1	1
8	0	0	0
9	1	0	0
10	0	0	0
11	0	0	1
12	0	0	0
13	0	-1	0
14	1	-1	-1
15	0	0	0
16	1	-1	1
17	0	0	0
18	-1	-1	-1
19	0	0	0
20	0	1	0

Note: A: GSC (%), B: DL-alanine (%), and C: incubation time (hours)

## RESULTS AND DISCUSSION

### Screening by Plackett-Burman design

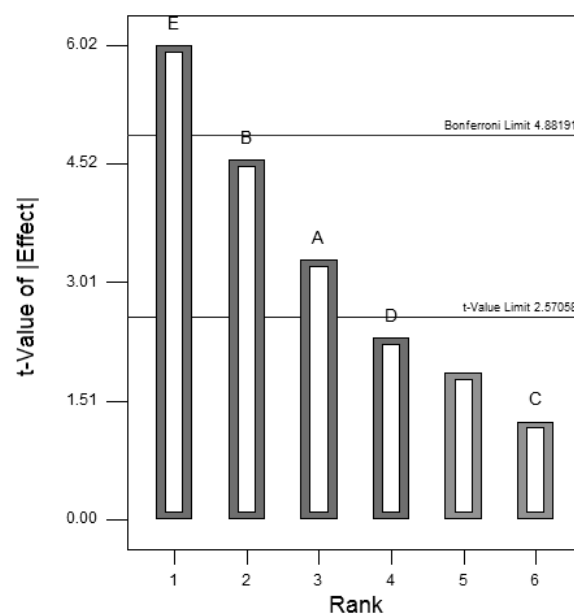
Plackett-Burman design was used to identify the important parameters for DAAO production. Table 3 represents the results of the screening of significant variables for DAAO production and the corresponding response (Y) using Plackett-Burman design. Statistical analysis of DAAO activity was performed and represented in Table 4. pH and inoculum concentration positively affect DAAO production, whereas the other variables, GSC and DL-alanine concentration, temperature, and incubation time negatively affect DAAO production (Table 3). The T and P values were used to identify the effect of each factor on DAAO production, as shown in Table 4. Incubation time, GSC and DL-alanine concentration had a significant effect on DAAO activity ( $P < 0.05$ ). The  $R^2$  values provide a measure of how much variability in the observed response value can be explained by the experimental factors. In this study, the  $R^2$  was 0.9400 which indicates that 94% of the variability in the response were attributed to the given independent variables and only 6% of the total variations are not explained by the independent variables. The Pareto chart illustrates the order of significant variables affecting DAAO production in Plackett-Burman experimental design. We found that incubation period, the concentration of GSC and DL-alanine were the significant parameters in DAAO production (Figure 1).

**Table 4.** Statistical analysis results from Plackett-Burman design showing major effects, coefficient values, t-test (T), P-values for each variable affecting on DAAO production and analysis of variance.

Variables	Main effect	Coef.	T	P value
A-GSC	-13.27	-6.63	-3.30	0.0215
B-DL-alanine	-18.35	-9.18	-4.56	0.0060
C-pH	4.99	2.50	1.24	0.2696
D-Temperature	-55.60	-4.63	-2.30	0.0694
E-Incubation time	-4.04	-12.11	-6.02	0.0018
F-Inoculum	7.51	3.75	1.87	0.1208

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	344.27	6	57.38	12.69	0.0068*
GSC	53.87	1	53.87	11.91	0.0182*
Inducer	87.61	1	87.61	19.37	0.0070*
pH	2.75	1	2.75	0.61	0.4709
Temperature	24.71	1	24.71	5.46	0.0666
Incubation time	161.62	1	161.62	35.73	0.0019*
Inoculum	13.70	1	13.70	3.03	0.1422
Residual	22.62	5	4.52		
Cor Total	366.89	11			

Note: T: Student's test; P: corresponding level of significance; R square 0.9400 and adjusted R square 0.8680



**Figure 1.** Pareto chart illustrates the order of significance of the variables affecting D-amino acid oxidase production by *Trigonopsis variabilis* (A, B, D, E had a positive effect; C and F negative effects; t-value ranging from 1.24 to 6.02); A. Represent GSC concentration; B. Represent DL-alanine concentration; C. Represent pH; D. Represent incubation time; E Represent temperature; and F. Represent inoculum concentration.

### Optimization by face-centered central composite design

The face-centered central composite design was employed to study the optimal levels and the interactions among the selected significant factors. The other factors were maintained at a constant level, which gave maximal yield in the Plackett-Burman experiments. In this study, a total of 20 experiments with different combination of GSC concentration (A), DL-alanine concentration (B), and incubation time (C) were performed and the results of experiments for studying the effect of three independent variables on DAAO activity are presented along with predicted response and residuals (Table 5). The minimum DAAO activity (46,799 U/g YCDW) were observed in run number 6, while the maximum DAAO activity (193.880 U/g YCDW) were achieved in run number 8.

Analysis of variance (ANOVA) was used to analyze the data (Table 6). The goodness of fit of the model was indicated by its coefficient of determination ( $R^2$ ), which found to be 0.9782. The present  $R^2$ -value reflects a very good fit between the observed and predicted responses and implies that the model is reliable for DAAO production. The P-values denote the significance of the coefficients and are also important in understanding the pattern of the mutual interactions between the variables. Interactions between two factors could appear as an antagonistic effect (negative coefficient) or a synergistic effect (positive coefficient).

**Table 5.** The face-centered central composite design represents the response of DAAO production as influenced by GSC (A), DL-alanine (B), and incubation time (C) along with the predicted DAAO production.

Run no.	A	B	C	DAAO activity (U/g yeast cell dry weight)	
				Experimental	Predicted
1	15	0.7	48	115.280	113.984
2	10	0.5	60	155.235	158.368
3	10	0.7	48	132.521	129.619
4	12.5	0.5	48	141.311	147.529
5	10	0.3	72	94.320	93.662
6	15	0.7	72	46.799	44.664
7	10	0.7	72	77.155	77.401
8	12.5	0.5	60	193.880	173.939
9	15	0.5	60	129.079	133.762
10	12.5	0.5	60	176.962	173.939
11	12.5	0.5	72	85.828	87.425
12	12.5	0.5	60	168.451	173.939
13	12.5	0.3	60	189.201	190.930
14	15	0.3	48	130.277	128.077
15	12.5	0.5	60	163.189	173.939
16	15	0.3	72	59.137	60.085
17	12.5	0.5	60	188.640	173.939
18	10	0.3	48	144.371	144.552
19	12.5	0.5	60	168.142	173.939
20	12.5	0.7	60	169.668	175.754

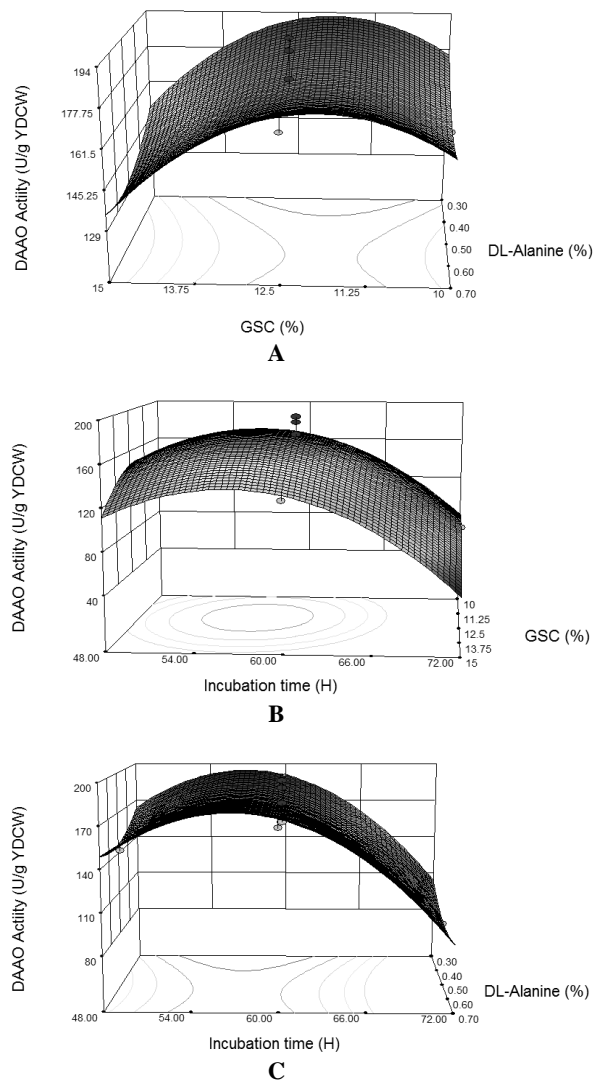
Note: A: GSC (%), B: DL-alanine (%), and C: incubation time (hours)

**Table 6.** Statistical analysis of face-centered central composite design showing coefficient values, standard error (SE) coefficient, t-test (T), P-values and analysis of variance.

Variables	Coefficient	SE coefficient	T	P
A	114.92	24.589	4.673	0.001
B	-269.97	190.093	-1.420	0.186
C	46.40	5.123	9.057	0.000
A*A	-4.46	0.933	-4.778	0.001
B*B	235.08	145.859	1.612	0.138
C*C	-0.39	0.041	-9.678	0.000
A*B	0.42	6.841	0.061	0.952
A*C	-0.14	0.114	-1.250	0.240
B*C	-0.14	1.425	-0.097	0.925

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	36220.10	9	4024.46	42.99	< 0.0001*
A-GSC	1513.64	1	1513.64	16.17	0.0024*
B-DL-alanine	575.85	1	575.85	6.15	0.0325*
C-Incub. time	9031.39	1	9031.39	96.48	< 0.0001*
AB	0.35	1	0.35	0.00	0.9523
AC	146.24	1	146.24	1.56	0.2398
BC	0.88	1	0.88	0.01	0.9246
A <sup>2</sup>	2136.71	1	2136.71	22.83	0.0007*
B <sup>2</sup>	243.16	1	243.16	2.60	0.1381
C <sup>2</sup>	8766.89	1	8766.89	93.65	< 0.0001*
Residual	936.11	10	93.61		
Lack of Fit	174.63	5	34.93	0.23	0.9341
Pure Error	761.48	5	152.30		
Cor Total	37156.21	19			

Note: A: the coded value of GSC, B: the coded value of DL-alanine, and C: the coded value of incubation time. T: Student's test; P: corresponding level of significance; F: Fisher's function. R square 0.9748 and adjusted R square 0.9521



**Figure 2.** Three-dimensional response surface plots showed the effect of several variables on DAAO production. A. The effect of GSC and DL-alanine concentration with 60 H incubation. B. The effect of GSC concentration and incubation time at constant DL-alanine concentration of 0.5%. C. The effect of DL-alanine concentration and incubation time on DAAO activity at constant cassava glucose concentration of 12.5%.

Table 6 shows that the GSC (A), DL-alanine (B), incubation time (C). The quadratic effect of cassava glucose syrup (A) and incubation time (C) are significant. All the interactions (A\*B; A\*C; and B\*C) are not significant, indicating that there is no significant correlation between each variable pair; thus, they do not have a significant effect in increasing the production of DAAO. By applying the multiple regression analysis of the experimental data, the equation that defines the predicted response (Y) can be shown as follows:

$$Y(\text{DAAO activity}) = 173.94 - 12.30 * A - 7.59 * B - 30.05 * C + 0.21 * A * B - 4.28 * A * C - 0.33 * B * C - 27.87 * A^2 + 9.40 * B^2 - 56.46 * C^2$$

The interaction effects and optimal levels of the variables were determined by plotting the three-dimensional response surface curves (Figures 2.A-C) when one of the variables is fixed at an optimum value and the other two are allowed to vary.

Figure 2.A represents the effect of varying GSC and DL-alanine concentration on DAAO production at a constant incubation time of 60 h. DAAO yield increased with the increase of GSC concentration at DL-alanine concentration from 0.3% to 0.4%. Further increase of DL-alanine concentration would decrease the DAAO yield. According to this interaction effects, the maximum yield of DAAO activity was  $\approx 193.88$  U/g at GSC  $\approx 12.5\%$  and DL-alanine concentration  $\approx 0.3\%$ .

Figure 2.B shows the cooperative effect of GSC concentration and incubation time at a constant DL-alanine concentration of 0.5%. As shown in Figure 2.B, at the low and high value of GSC concentration and incubation time, the DAAO activity decreases. The maximum DAAO activity  $\approx 193.88$  U/g was obtained at GSC concentration  $\approx 12.5\%$  and incubation time  $\approx 56$  h.

Figure 2.C shows the effect of both DL-alanine concentration and incubation time on DAAO activity at a constant cassava glucose concentration of 12.5%. It is obvious that with the increase of DL-alanine concentration above 0.3%, the DAAO activity decreases. The maximum DAAO activity  $\approx 193.88$  U/g was obtained at the lowest DL-alanine concentration  $\approx 0.3\%$  and incubation time  $\approx 56$  h.

#### Verification of the model

To determine the accuracy of the model and to verify the result, an experiment under the optimal conditions from RSM was performed and compared with the predicted data. The measured DAAO activity obtained was 195.38 U/g, close to the predicted one 196.38 U/g, indicating a high degree of accuracy. The predicted optimal levels of the process variables for DAAO production by *T. variabilis* were 12.3% GSC, 0.3% DL-alanine, and 56.1 hours incubation time.

In conclusion, Plackett-Burman experimental design is useful to enhance the DAAO production. The design can reduce factors that do not significantly affect the production. Results of Plackett-Burman design showed that the incubation time, concentration of cassava glucose syrup and DL-alanine were significant factors in DAAO production. Optimization of significant factors was done using Face-centered Central Composite Design (FCCD) and the results were evaluated using RSM. The optimum condition for DAAO production as obtained from RSM were as follows: GSC concentration 12.3%, DL-alanine (inducer) concentration 0.3% and 56.1 hours of incubation time. At these optimum levels, DAAO activity reaches 195.38 U/g dry weight of yeast. The results of this study could be used to design a suitable medium using GSC as the carbon source, for an economical and efficient production of DAAO.

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