STATISTICAL OPTIMIZATION OF L-ASPARAGINASE PRODUCTION FROM FILAMENTOUS FUNGUS PA3S12MM: INSIGHTS INTO ENZYME ACTIVITY AND INTERACTIONS

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ABSTRACT

L-asparaginase enzyme is used in the treatment of acute lymphoblastic leukemia, as it prevents the replication of cancer cells without affecting healthy cells. Fungi are a promising source for obtaining this enzyme. The objective of this study was to perform the statistical optimization of L-asparaginase production from the filamentous fungus PA3S12MM, using the Central Composite Rotational Design (CCRD) methodology with three variables: glucose (%), asparagus (%), and asparagine (%). Enzyme assays were performed using Nessler's reagent. It was found that all three variables significantly and positively influenced enzyme production in the linear model. Additionally, a positive interaction was observed between the variables glucose and asparagine: the higher the combined concentrations, the greater the enzymatic activity.

Keywords: L-asparaginase. Fungi. Central Composite Rotational Design. Acute lymphoblastic leukemia.

1 INTRODUCTION

The antineoplastic activity of the enzyme L-asparaginase against Acute Lymphoblastic Leukemia (ALL) is based on the inability of tumor cells to synthesize their own asparagine. L-asparagine is an essential amino acid for protein production and cell replication. Cancer cells lack the enzyme asparagine synthetase and thus depend on extracellular asparagine for survival. By administering L-asparaginase to the patient, the enzyme prevents the proliferation of malignant cells while not affecting healthy cells, as the latter possess the enzyme asparagine synthetase and are therefore capable of maintaining their vital activities even in the absence of extracellular asparagine [1,2].

L-asparaginase is produced by various species of bacteria and fungi. Fungi are excellent sources of biomolecules due to the similarities in their biotransformation pathways with those of humans. The filamentous fungi show species that produce the enzyme, and it is found in various types of soil in subtropical climate zones[3,4]. Therefore, the objective of this study was to perform the statistical optimization of L-asparaginase production from the filamentous fungus PA3S12MM.

2 MATERIAL & METHODS

The cultivation of the fungus PA3S12MM was carried out under stationary conditions for 120 hours at 28 °C. The modified Czapek medium was used as the culture medium. Enzymatic activity was determined using Nessler's reagent[5]. One enzymatic unit was defined as the amount of enzyme capable of producing 1 µmol of product per minute under the assay conditions. For the statistical optimization of L-asparaginase production, the Central Composite Rotational Design (DDCR) 2^3 with 17 trials, including 3 central points, was employed. The selected variables were related to the culture medium: concentration of the carbon source (glucose), concentration of the nitrogen source (asparagus), and concentration of the amino acid (L-asparagine). The variables were modified according to the central point (0,0), alternating to positive and negative values. From the DDCR, it was possible to create the Pareto Diagram and response surface plots, which indicate the significance of the variables, whether they interact with each other, and how this affects enzyme production by the fungus. The response for the variables (Y representing L-asparaginase activity) was approximated by the polynomial equation (1) where (β_0) is the intercept, (β_1, β_2, β_3) are the first-order coefficients, (β_{11}, β_{13}, β_{23}) are the interaction coefficients, and (β_{11}, β_{22}, β_{33}) are the second-order coefficients. Data analysis was performed using Statistica 10 software, adopting a significance level of 5%.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^3 \quad (1)$$

3 RESULTS & DISCUSSION

In this experimental design, we studied the effect of three independent variables on the dependent variable, the enzymatic activity of L-asparaginase produced by *C. echinulata*. These variables were the carbon source glucose (X1), asparagus (X2), and the amino acid L-asparagine (X3). Table 1 shows the matrix of the 2^3 Central Composite Rotational Design coded and real (%) values of the evaluated variables and the enzymatic activity obtained in each run. With the optimization performed, the enzymatic activity demonstrated an improvement of 26.6 times when comparing run 12 (0.305 U/mL) and run 6 (8.104 U/mL) from Table 1.

In Figure 1, the Pareto diagram shows that the variables glucose, asparagus, and asparagine were significant and positively influenced the increase in enzymatic activity, as well as the interaction between glucose and asparagine in the linear model ($p<0.05$).

Table 1 Matrix of the Central Composite Rotational Design.

Run	X_1	X_2	X_3	L-asparaginase (U mL^{-1})
1	-1 (1,50)	-1 (0,40)	-1 (0,10)	1,532
2	1 (3,50)	-1 (0,40)	-1 (0,10)	3,714
3	-1 (1,50)	1 (0,60)	-1 (0,10)	2,650
4	1 (3,50)	1 (0,60)	-1 (0,10)	3,768
5	-1 (1,50)	-1 (0,40)	1 (0,50)	2,339
6	1 (3,50)	-1 (0,40)	1 (0,50)	8,104
7	-1 (1,50)	1 (0,60)	1 (0,50)	4,758
8	1 (3,50)	1 (0,60)	1 (0,50)	6,752
9	0 (2,50)	0 (0,50)	0 (0,30)	3,410
10	0 (2,50)	0 (0,50)	0 (0,30)	3,746
11	0 (2,50)	0 (0,50)	0 (0,30)	3,637
12	-1,68 (0,818)	0 (0,50)	0 (0,30)	0,305
13	1,68 (4,182)	0 (0,50)	0 (0,30)	4,998
14	0 (2,50)	-1,68 (0,332)	0 (0,30)	2,630
15	0 (2,50)	1,68 (0,668)	0 (0,30)	3,951
16	0 (2,50)	0 (0,50)	-1,68 (0,0)	1,706
17	0 (2,50)	0 (0,50)	1,68 (0,636)	5,053

Encoded value (actual value %)

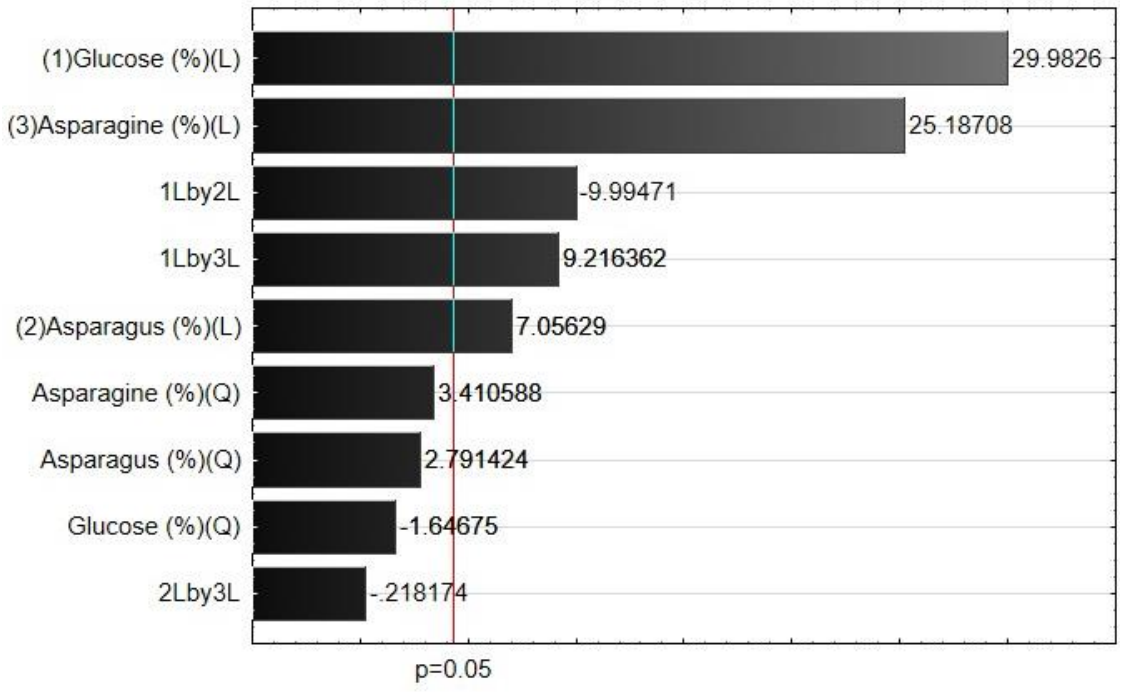

Figure 1 Pareto diagram of the study factors regarding enzymatic activity.

The second-order polynomial equation for the significant variables in L-asparaginase production was:

$$\text{L-asparaginase activity} = 3.52 + 1.39\,X_1 - 0.08\,X_1^2 + 0.33\,X_2 + 0.14\,X_3 - 0.60\,X_3^2 + 0.56\,X_1\,X_2 \quad (2)$$

It can be observed that the model showed significant regression within the 95% confidence interval, with a coefficient of determination (R^2) equal to 0.907, indicating that the model explained 91% of the variation in the experimental data. The calculated F_{calc} for the regression was 2.26 times higher than the tabulated F_{tab}, indicating that the model is predictive. Lack of fit was not significant, as the calculated F_{calc} was lower than the tabulated F_{tab}, indicating that the experimental data fitted the obtained model.

Table 2 Analysis of variance (ANOVA) of the adjusted model for enzymatic activity (U mL^{-1}).

Source of variation	Degree of freedom df	Sum square SS	Mean square MS	F_{calc}	F_{tab} *
Regression	6	46.963	7.827201	7.292005	3.22
Residual	10	10.734	1.073395		
Lack of Fit	8	10.675	1.33443	0.205455	19.37
Pure error	2	12.99	6.495		
Total	16	57.697			

*Tabulated values ([6]).

After analyzing the significance of the model, it was possible to construct three-dimensional response surfaces to visualize the influence of the factors for maximum production of L-asparaginase (Figure 2 a, b, and c). In Figure 2a, it can be observed that at higher concentrations of glucose combined with a higher quantity of asparagus, higher activity is obtained. Conversely, in Figure 2b, it is evident that at both higher concentrations of glucose and asparagine, better activity is achieved. Finally, in Figure 2c, it can be noted that the variation in the concentration of asparagus does not influence enzymatic activity.

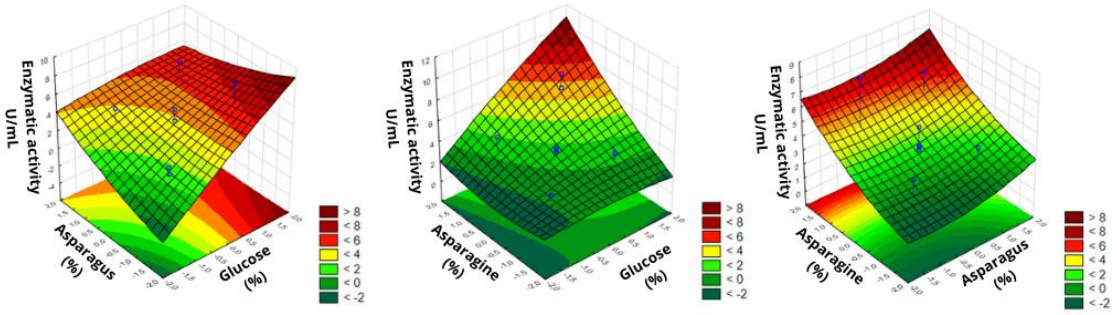

Figure 2 Response surface graph for extracellular production of asparaginase from filamentous fungus PA3S12MM. (a) Asparagus x Glucose; (b) Asparagine x Glucose; (c) Asparagine x Asparagus.

4 CONCLUSION

The use of experimental design by DCCR aided in optimizing the cultivation conditions, resulting in a significant improvement in the production of L-asparaginase by filamentous fungus PA3S12MM.

REFERENCES

[1] ARUMUGAM,N.,THANGAVELU,P. 2022.Purification and anticancer activity of glutaminase and urease free intracellular L-asparaginase from *Chaetomium* sp.Prot. Expres. And Purific.190.

[2] CHOW,Y.,TING,A.S.Y.2015.Endophytic L-asparaginase producing fungi from plants associated with anticancer properties. J. Of Advan.Res. 869-876.

[3] ASHA, S., VIDYAVATHI, M. 2009. *Cunninghamella* – a microbial model for drug metabolism studies – a review. Biotech. Adv. 27 (1). 16-29.

[4] ELKHATEEB, W.A., ELNAHAS, M.O., DABA, G.M. 2021. Biactive metabolites of *Cunninghamella*. J. Of Pharma. And Pharma. Res. 4 (3).

[5] IMADA,A.,IGARASI,S.,NAKAHAMA,K.,ISONO,M.1973. Asparaginase and glutaminase activities of microrganism. Gen. Microbio. 76 (1). 85-99.

[6] BOX, G.E.P., HUNTER, W.G., HUNTER, J.S. 1978. Statistics for Experimenters: An Introduction to Design, Data Analysis and Model Building. John W. and Sons Inc., New York, USA.

ACKNOWLEDGEMENTS

This work was supported by National Council for Scientific and Technological Development (CNPq), Coordination for the Improvement of Higher Education Personnel (CAPES), Araucária Foundation and Universidade Estadual do Oeste do Paraná (UNIOESTE).