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Research Article
Lysine and Glutamic Acids as the End Products of Multi-response of Optimized Fermented Medium by *Mucor mucedo* KP736529

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Abstract

Background: Amino acids are important for living organisms, they acting as crucial for metabolic activities and energy generation, wherein the deficiency in these amino acids cause various physiological defects. **Objective:** The aim of this study is to investigate the effect of some nutritional factors on the amino acids production by *Mucor mucedo* KP736529 during fermentation intervals. **Methodology:** *Mucor mucedo* KP736529 was selected according to proteolytic activity. Corn steep liquor and olive cake were used in the fermented medium during Placket-Burman and central composite design to maximize the production of lysine and glutamic acids. **Results:** During the screening by Plackett-Burman design, olive cake and Corn Steep Liquor (CSL) had potential importance for the higher production of amino acids. The individual fractionation of total amino acids showed both lysine and glutamic as the major amino acids associated with the fermentation process. Moreover, the Central Composite Design (CCD) has been adopted to explain the interaction between olive cake and CSL on the production of lysine and glutamic acids. The model recorded significant F-value, with high values of R^2, adjusted R^2 and predicted R^2 for both lysine and glutamic, indicating the validity of the data. Solving equation for maximum production of lysine recorded theoretical levels of olive cake and CSL, being 2.58 and 1.83 g L^{-1}, respectively, with predicting value of lysine at 1.470 μg mL^{-1}, whereas the predicting value of glutamic acid reached 0.805 mg mL^{-1} at levels of 2.49 and 1.93 g L^{-1} from olive cake and CSL, respectively. The desirability function (D) showed the actual responses being 1.473±0.009 and 0.801±0.004 μg mL^{-1} for lysine and glutamic acids, respectively. **Conclusion:** The model showed adequate validity to be applied in a large-scale production of both lysine and glutamic acids.

Key words: Amino acids, lysine, glutamic acid, *Mucor mucedo* KP736529, desirability function

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Amino acids are the most important source of energy for all living organisms, which considered a crucial for the metabolic activities and play an important role in various physiological processes. They are the building blocks of proteins and constitute a major part of the body. Ninety five percent of hormones and neurotransmitters are amino acids in nature[1]. They used as seasonings, flavorings and starting material for pharmaceuticals, cosmetics and other chemicals. However, the deficiency in some essential amino acids such as methionine leads to the development of various diseases and physiological conditions including toxemia, childhood rheumatic fever, muscle paralysis, hair loss, depression, schizophrenia, Parkinson's liver deterioration and impaired growth[2-4]. The deficiency can be overcome by supplementing the diet with these amino acids and therefore, the essential amino acids are of significant interest[2]. The L-lysine is one of the 9 amino acids which are essential for human and animal nutrition. Its demands have been increased in recent years[5]. It is the second produced amino acid in a large industrial scale with 800,000 tons of production per year[6,7]. Generally, lysine is recognized as the most deficient amino acid in the food supply of both man and domestic meat producing animals. However, it can be produced in different ways, including chemical synthesis, extracting from protein hydrolysate, enzymatic method, fermentation method, protoplast fusion technique and recombinant DNA technology[7,5]. Among these methods, fermentation is the most economical and practical means of producing lysine, as in this method low temperature, low pressure and low-cost carbon sources are used[8]. *Corynebacterium glutamicum* is widely used for the industrial production of amino acids especially L-lysine and L-glutamine[9]. Since, the discovery of, glutamic acid producing bacteria by Kinoshita *et al.*[10] eventually led to fermentation processes for producing various amino acids. The industrial production of L-glutamine started with its fermentation in the late 1960s. Currently, it is manufactured for use as pharmaceuticals and health foods at the worldwide, in which the annual production of L-glutamine[11] approximately 2000 t. Fermentation technology has played crucial roles in this progress and currently the fermented amino acids represent chief products of biotechnology in both volume and value[9,12]. Additionally, the microbial production of various amino acids has been developed and almost all protein-constitutive amino acids were produced by microbial biotechnology, fermentation or enzymatic method[13]. On the other hand, optimization of media components by classical methods has some disadvantages, such as time consuming, requirement of more experimental data sets and missing the interactions among variables[14,15]. Therefore, response surface procedures showed efficient experimental strategies to achieve optimal conditions for the multi-variables system. Moreover, these procedures have been successfully applied for the optimization of multiple variables in many fermentation processes and showed satisfactory results[16].

Herein, this study focused on using statistical procedure to optimize economic nutritional fermentation medium to produce lysine and glutamic acids in high amounts by *Mucor mucedo* strain KP736529. This is considered one of the future trends and challenges for alternative food sources.

MATERIALS AND METHODS

***Mucor mucedo* KP736529:** The proteolytic fungus, *M. mucedo* KP736529 was kindly obtained from Department of Microbiology, Soils, Water and Environment Institute, Agricultural Research Center, Giza, Egypt, Which selected according to proteolytic activity. The fungal strain was preserved on PDA slant at 4°C and sub-cultured monthly.

Nutritional components: Olive Cake (OC) and Corn Steep Liquor (CSL) were kindly provided by Dr. Ibrahim A.A. Abou Ayana, Food Technology Research Institute, Agricultural Research Center (Affiliation ID: 60019332), Giza, Egypt. The major components of olive cake (Pendolino) on the dry basis (%) are crude protein (6.61), crude fiber (38.91), oil (4.50) and ash (1.60). The approximate composition of corn steep liquor (%) is lactose (3.5), glucose (0.25), non-reducing carbohydrates (mainly starch) 1.5, total nitrogen (0.175) and total solids (85).

Inoculum preparation and fermentation medium composition: Plates of sabouraud agar were inoculated and incubated at 35°C for 72 h, the inoculum was obtained by scraping the fungal growth on the agar surface in the presence of sterile water, to obtain 10^6 spore per milliliter by Neubauer Chamber.

The composition of submerged Liquid State Fermentation (LSF) medium contained (g L^{-1}) glucose 18, peptone 8, casein 4, KH_2PO_4 2, olive cake 4 and CSL 4 with initial pH of 5.0, after sterilization at 121°C for 15 min, inoculum (10%, v/v) was transferred to 500 mL Erlenmeyer flask containing 90 mL of the medium. Incubation was carried out at 35°C under shaking at 150 rpm (Orbit Environ-shaker, USA).

Screening of nutritional medium components with Plackett-Burman design: The nutritional components of the previous LSF medium were screened for the production of total free amino acids by the examined fungal strain. The statistical design of Plackett-Burman (Table 1) was applied. The relation between the coded and actual values and the main effect of each variable as well as the statistical analysis of the results was performed with the aid of statistical software packages Minitab (version 17, Minitab Inc., USA) to select the significant nutritional factors affecting free amino acids production.

Optimization process by central composite design: The interaction between the significant nutritional components (olive cake and CSL), which was obtained from the previous screening trial, was evaluated based on the full Central Composite Design (CCD) of response surface optimization. Economically, the other nutritional medium components, that showed no significant effect were omitted from the fermentation medium. Each of olive cake and CSL was examined at five different levels; at the center point and the axial point from the design center in each direction on each axis ($\alpha = \pm 1.414$). According to the applied design, 11 combinations were executed (Table 2). Data were statistically analyzed with design expert software package (version 7, State-Ease, USA), then observations of the two nutritional factors were fitted to the second order polynomial quadratic model to calculate the optimum combination of both nutrients. All data were the average of triplicates.

Dual optimization for lysine and glutamic acids production: In order to simultaneously optimizing the production of lysine and glutamic acids production, the desirability function approach was used. In which, instead of optimizing each outcome separately, settings for the predictor variables sought to satisfy all of the outcomes at once. A desirability function was specified for each response (lysine and glutamic acids). The overall desirability of multi-response optimization is

Table 1: Plackett-Burman design showing the effect of nutritional medium components on total free amino acids production by *M. mucedo* KP736529

	Medium component (g L^{-1})				Total free amino acids (μg mL^{-1})			
Run	Glucose	Peptone	Casein	KH_2PO_4	Olive cake	CSL	Response	Residual
1	25 (1)	4 (-1)	6 (1)	1 (-1)	2 (-1)	2 (-1)	723.00	2.29
2	25 (1)	12 (1)	2 (-1)	3 (1)	2 (-1)	2 (-1)	730.00	2.98
3	11 (-1)	12 (1)	6 (1)	1 (-1)	6 (1)	2 (-1)	601.30	0.30
4	25 (1)	4 (-1)	6 (1)	3 (1)	2 (-1)	6 (1)	576.40	1.29
5	25 (1)	12 (1)	2 (-1)	3 (1)	6 (1)	2 (-1)	600.90	-2.98
6	25 (1)	12 (1)	6 (1)	1 (-1)	6 (1)	6 (1)	469.33	-4.94
7	11 (-1)	12 (1)	6 (1)	3 (1)	2 (-1)	6 (1)	571.00	-7.54
8	11 (-1)	4 (-1)	6 (1)	3 (1)	6 (1)	2 (-1)	587.30	8.61
9	11 (-1)	4 (-1)	2 (-1)	3 (1)	6 (1)	6 (1)	451.00	-2.36
10	25 (1)	4 (-1)	2 (-1)	1 (-1)	6 (1)	6 (1)	473.60	1.37
11	11 (-1)	12 (1)	2 (-1)	1 (-1)	2 (-1)	6 (1)	611.00	12.19
12	11 (-1)	4 (-1)	2 (-1)	1 (-1)	2 (-1)	2 (-1)	710.90	-11.20
13	18 (0)	8 (0)	4 (0)	2 (0)	4 (0)	4 (0)	638.20	-8.53
14	18 (0)	8 (0)	4 (0)	2 (0)	4 (0)	4 (0)	657.67	10.93
15	18 (0)	8 (0)	4 (0)	2 (0)	4 (0)	4 (0)	644.33	-2.40

Number between parentheses is the corresponding coded value

Table 2: Values of the independent variables used in central composite design matrix with response values of lysine and glutamic acid by *M. mucedo* KP736529

	Coded value		Actual value (g L^{-1})		Lysine (μg mL^{-1})		Glutamic acid (μg mL^{-1})	
Run	Olive cake (X_1)	CSL (X_2)	Olive cake	CSL	Response	Residual	Response	Residual
1	-1	-1	1.00	1.00	0.272	-0.040	0.218	-0.002
2	1	-1	3.50	1.00	1.050	-0.085	0.593	-0.011
3	-1	1	1.00	3.50	0.315	0.007	0.317	-0.020
4	1	1	3.50	3.50	0.314	-0.037	0.256	-0.028
5	-1.414	0	0.48	2.25	0.071	0.007	0.101	0.009
6	1.414	0	4.02	2.25	0.747	0.070	0.347	0.021
7	0	-1.414	2.25	0.48	1.033	0.072	0.588	0.003
8	0	1.414	2.25	4.02	0.409	0.005	0.470	0.028
9	0	0	2.25	2.25	1.470	0.064	0.789	-0.002
10	0	0	2.25	2.25	1.399	-0.007	0.809	0.018
11	0	0	2.25	2.25	1.350	-0.056	0.775	-0.016

defined as the geometric mean of the desirability for each response. The statistical software package design-expert version 7 (Stat-Ease, Minneapolis, USA) was used for both constructing the design and analysis of experiments at $p<0.05$.

Determination of total amino acids as well as lysine and glutamic acids: The total free amino acids, as well as lysine and glutamic acids, were quantified in the fermented supernatants. The culture samples were filtered and hydrolyzed with 6 M HCL for 24 h at 110°C under a vacuum and amino acids content was measured using Sykam 57130 amino acid reagent organizer[17].

RESULTS AND DISCUSSION

It is recognized that diet is one of the nutritional factors that influences human health and development of certain diseases. Among these food components, protein, which constitutes a major nutrient with daily intake between 95 and 120 g, it hydrolyzed to amino acids that acting the important source of energy for all living organisms. Amino acids play a crucial role in various physiological processes. However, the shortage in these amino acids, especially essential ones is associated with various diseases[2,3,1]. On the other side, the overpopulation and global shortage in the food encourage several investigators to search for alternative procedures to meet these demands of food. Single cell protein is one of these alternatives, however, its high content of DNA that causing gout disease is considered one of the obstacles[18]. For these reasons, the industrial production of amino acids has growing interest, since lysine and glutamic acids had been conducted in the late of 1960s, which their production is depending upon chemically synthesized. Other methods, e.g., enzymatic, fermentation, protoplast fusion technique and recombinant DNA technology had been investigated[5]. Herein, this study has been designed to investigate the amino acids associated with proteolytic strain of *Mucor mucedo* KP736529 during fermentation process using desirability function, which considered the future trend and challenge in searching the alternative procedure of food.

Time course of amino acids production by *M. mucedo* KP736529: Proteolytic activity of *M. mucedo* KP736529 has been conducted in a previous study in our laboratory. The estimation of the associated amino acids during the growth of fungus understudy in suggested medium during intervals time has been investigated. Data (Fig. 1) show that the 3rd day was the best incubation time for a high quantity of total amino acids (641 μg mL^{-1}) during the fermentation process. However, the values of amino acids decreased sharply during 4 and 5 days. It is hypothesized that the biosynthesis of amino acids is clearly affected by the availability of substrates used as carbon and nitrogen sources as well as, their formation may be affected by temperature, pH, agitation and are correlated with the phase of growth. The decreasing of amino acids may be due to the exertion of a biological function in the organism that leads to the presence of high levels of end products of amino acids and NH_4^+, which may repress the biological function of the organism[19].

In the previous study of Salmeron-Lopez *et al.*[20], lysine and glutamic acid were produced in the highest amount after the 3rd day from the growth of *Rhizobium* and *Sinorhizobium* in the fermentation medium. Another study reported that valine, glutamic acid, lysine, proline and other amino acids were produced during the stationary phase of growth[21].

Plackett-Burman design for total free amino acids production: Plackett- Burman design using six independent variables, i.e., glucose, peptone, casein, KH_2PO_4, olive cake

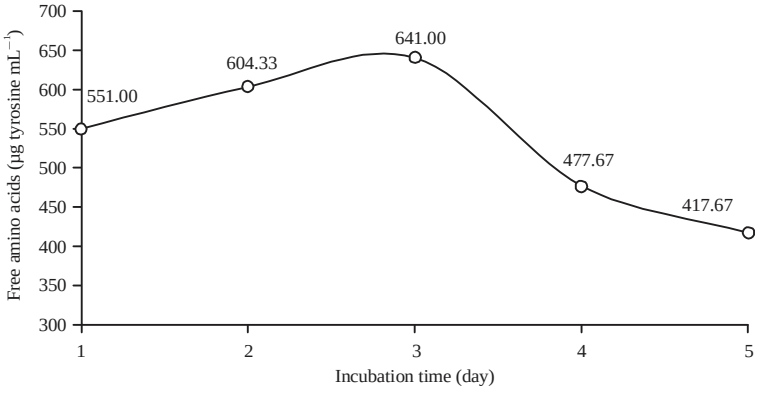

Fig. 1: Profile of the total amino acids synthesized by *M. mucedo* KP736529 as a function of time

and CSL were adopted at 2 levels for screening their potential importance on the total amino acids production by *M. mucedo* KP736529. The design matrix selected for the screening of significant variables for high production of amino acids and the corresponding responses were shown in Table 1. A high degree of similarity was observed between the predicted (fitted) and experimental (response) values of amino acids. The experiment carried out in 15 runs (Table 1), where the run 2 recorded the highest amino acids production. The adequacy of the amino acids production was calculated and the variables evidencing statistically significant effects were screened by ANOVA (Table 3). The main effect of amino acids recorded significant p-value of 0.0. Of the medium components, both olive cake and CSL recorded p-values of zero were the most significant factors. The overall model also showed significant p-value, where confirmed by the high values of coefficient of determination (R^2), adjusted R^2 and predicted R^2 being 99.39, 98.78 and 97.12, respectively, indicating the validity of the data. The values of predicted R^2 and adjusted R^2 are in reasonable agreement. Moreover, the magnitude of the effect of different media components on amino acids production was determined using Pareto chart (Fig. 2). The chart displays the absolute value of the effects and reference line. Effects of olive cake and CSL showed potential importance since both of them extended past the reference line. These data are coinciding with the previous study of Molina-Alcaide and Yanez-Ruiz[22] that showed the content of olive cake, e.g., organic matters, fiber, crude protein and amino acids N may encourage proteolysis and consequently amino acids production. The CSL may stimulate for protease production by *Bacillus* sp.[23] RK43 and may affect amino acids production. In general, some amino acids and nitrogenous compounds are associated with proteolysis process[24]. The other variables (casein, peptone, glucose and KH_2PO_4), which tend to be smaller than the significant ones. These data are in harmony with the previous study of El-Hersh *et al.*[25] who found that both glucose and casein showed a negative effect on proteolysis process and associated amino acids by *Bacillus subtilis* ATCC11774 using the statistical approach. However, both glucose and casein were found to be the best sources of carbon and nitrogen for protease production by *Mucor mucedo* DSM809[26]. Other study pointed out that, lysine and glutamic acids together with other amino acids were produced by *Rhizobium* and *Sinorhizobium* in a chemically defined medium using mannitol as the source of carbon[21]. Commonly, the production of amino acids is influenced by

Table 3: Analysis of variance and coded coefficient of different nutritional medium components on total free amino acids production by *M. mucedo* KP736529 based on Plackett-Burman design

Source	Effect	Coefficient	p-value	Determination coefficient (%)
Overall model				R^2 = 99.39
Model			0.000	Adjusted R^2 = 98.78
Lack of fit			0.590	Predicted R^2 = 97.12
Individual				
Glucose	6.79	3.39	0.265	
Peptone	10.22	5.11	0.111	
Casein	-8.18	-4.09	0.188	
KH_2PO_4	-12.09	-6.04	0.068	
Olive cake	-123.14	-61.57	0.000	
CSL	-133.51	-66.76	0.000	
Constant		592.14	0.000	

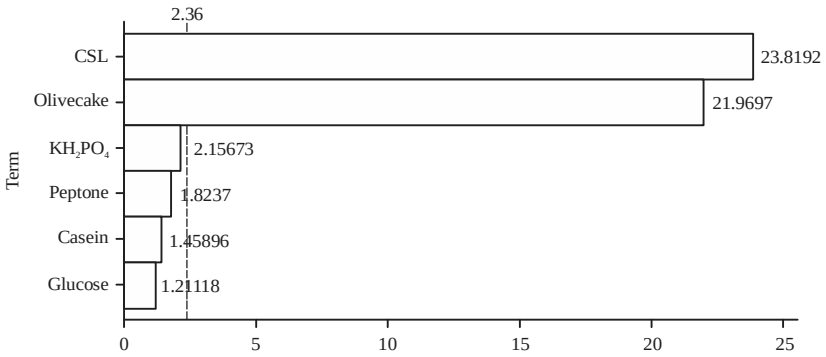

Fig. 2: Pareto chart of the standardized effects of nutritional medium components on total free amino acids (μg mL^{-1}, α = 0.05) by *M. mucedo* KP736529 based on the screening of Plackett-Burman design

the concentration and type of carbon source[27]. Wherein, the insignificant variables were eliminated from the fermentation medium. In the present study, the individual fractionation of total amino acids using Sykam amino acid reagent organizer showed that both lysine and glutamic acids as the major amino acids associated with the fungal growth. Whereas, L-phenylalanine, L-threonine and L-cysteine were produced by *Escherichia coli* strains[9]. As far as, the optimum levels of the two significant variables (olive cake and CSL), that recorded positive effect were further studied on both amino acids (lysine and glutamic) by the Central Composite Design (CCD), of RSM designs.

Quadratic response surface optimization for lysine and glutamic acids production: The CCD has been adopted to study the interaction between the two independent variables, i.e., olive cake and CSL that selected according to Plackett-Burman experiment on the production of lysine and glutamic acids by *M. musedo* KP736529. The response of the two amino acids as the function of CCD design is presented in Table 3. The run number 9 and 10 were the highest ones for lysine and glutamic acids, respectively. The data were fitted using the second order polynomial equation. The analysis of variance (Table 4) indicates that the model terms of X_1, X_2, X_1X_2, X_1^2 and X_2^2 were significant for both lysine and glutamic acids production. The model recorded significant F-value (94.71 and 192.13), high values of R^2 (0.990 and 0.995), adjusted R^2 (0.979 and 0.990) and predicted R^2 (0.939 and 0.968) for lysine and glutamic acids, respectively, indicating the validity of the model. Moreover, the value of predicted R^2 and adjusted R^2 are in reasonable agreement. These values are confirmed by the previous study of Chen *et al.*[28] who reported that a regression model has R^2 value higher than 0.9 is considered a high correlative. Additionally, the models of both lysine and glutamic acids have adequate precision values being 24.415 and 37.307, respectively. The lack of fit F-values of both amino acids are not significant. The model showed standard deviations of 0.074 and 0.025 for lysine and glutamic, respectively, that meaning low variation or dispersion from the average of both amino acids production. The coefficient of variation (CV), which describes the variation of tests percentage of the mean recorded 9.714 and 5.304% for lysine and glutamic, respectively. Finally, the model could be effectively used to measure the particular model fits at each point in the design, since the predicted residual sum of squares (PRESS) values lowered to 0.162 and 0.020 for lysine and glutamic acids, respectively. The efficiency of this design as an indicator of the validity of this model is similar to a previous study that attained optimization of protease production by *B. subtilis* ATCC11774 using CCD design[25]. Generally, the response surface methodology especially CCD design has efficacy in evaluating the factors that building the models and studying interaction as well as select the optimum conditions of variables for desirable response in biotechnological processes[29,30].

Checking the model adequacy: It is of especial importance to check the fitted model to ensure that it provides an adequate approximation to the real system. Unless the model shows an adequate fit, proceeding with the investigation and optimization of the fitted response surface likely give poor or misleading results. Checking of the adequacy of the model needs all of the information on the lack of fit. The model

Table 4: Analysis of variance and coefficient estimated for the response surface quadratic model for lysine and glutamic acids production using olive cake and CSL by *M. mucedo* KP736529, based on CCD

	Lysine (μg mL^{-1})			Glutamic acid (μg mL^{-1})		
Source	Coefficient estimate	F-value	p<0.05	Coefficient estimate	F-value	p<0.05
Model	-2.111	94.71	<0.0001^S	-1.012	192.13	<0.0001^S
X_1	1.946	67.74	0.0004^S	1.062	85.02	0.0003^S
X_2	1.165	55.99	0.0007^S	0.516	31.81	0.0024^S
X_1X_2	-0.125	27.38	0.0034^S	-0.070	73.79	0.0004^S
X_1^2	-0.332	273.40	<0.0001^S	-0.186	743.08	<0.0001^S
X_2^2	-0.232	133.52	<0.0001^S	-0.089	168.49	<0.0001^S
Lack of fit		1.87	0.3670^{NS}		3.01	0.2593^{NS}
R^2	0.990			0.995		
Adjusted R^2	0.979			0.990		
Predicted R^2	0.939			0.968		
Adequate precision	24.415			37.307		
Standard deviation	0.074			0.025		
CV (%)	9.714			5.304		
PRESS	0.162			0.020		

S: Significant, NS: Not significant at p<0.05, CV: Coefficient of variance, PRESS: Predicted residual sum of squares

adequacy was performed using the graph of the actual response values versus the predicted response values, that helps to detect a value or group of values, that are not easily predicted by the model. The data points should be split evenly by the 45 degree line. Figure 3 shows the data points of both amino acids were located close to the line, meaning that all values could be easily predicted by the proposed model.

Single and dual optimization and validation of the models: In order to determine the optimal levels of each variable for maximum production of both investigated amino acids, the three-dimensional response surface graphs were constructed by plotting each amino acid on the Z-axis against the two independent variables (CSL and olive cake). Figure 4 shows that both of the examined amino acids have similar response, the high production of both were at the higher concentration of olive cake than CSL and this is the best combination of both variables, meaning that the production of both amino acids is closely correlated. Accordingly, the fitted value was calculated from the final equation in terms of actual values as follows:

- Lysine (μg mL^{-1}) = -2.111+1.946 (olive cake)+1.165 (CSL) -0.125 (olive cake×CSL)-0.332 (olive cake)^2-0.232 (CSL)^2
- Glutamic acid (μg mL^{-1}) = -1.012+1.062 (olive cake) +0.516 (CSL)-0.070 (olive cake×CSL)-0.186 (olive cake)^2 -0.089 (CSL)^2

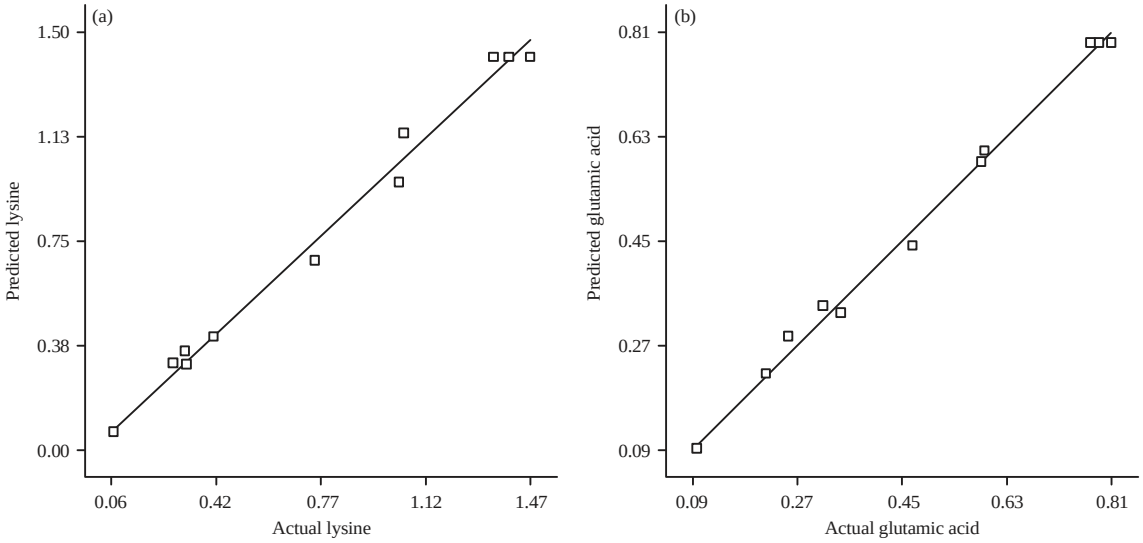

Fig. 3(a-b): Actual versus predicted values of both measured amino acids (a) Lysine and (b) Glutamic acid of the different runs

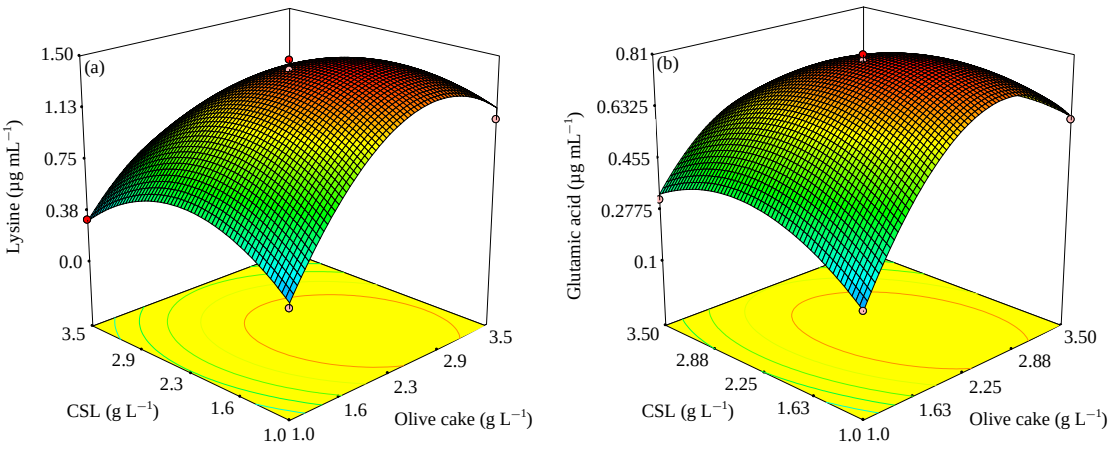

Fig. 4(a-b): Three-dimensional response surface plots showing the interactive effects of olive cake and CSL on (a) Lysine and (b) Glutamic acid production by *M. mucedo* KP736529

Table 5: Experimental validation of the theoretically predicted values for both individual and dual amino acids

	Target optimized amino acid		
Optimization procedure	Lysine	Glutamic acid	Dual optimization of both amino acids
Theoretical value of the tested factor (variable)			
Olive cake (g L^{-1})	2.58	2.49	2.54
CSL (g L^{-1})	1.83	1.93	1.87
Theoretical predicted value of amino acid (response)			
Lysine (μg mL^{-1})	1.470	None	1.469
Glutamic acid (μg mL^{-1})	None	0.805	0.805
Experimental validated value of amino acid (response)			
Lysine (μg mL^{-1})	1.479±0.007	None	1.473±0.009
Glutamic acid (μg mL^{-1})	None	0.813±0.010	0.801±0.004
Desirability function	1.000	0.995	0.997

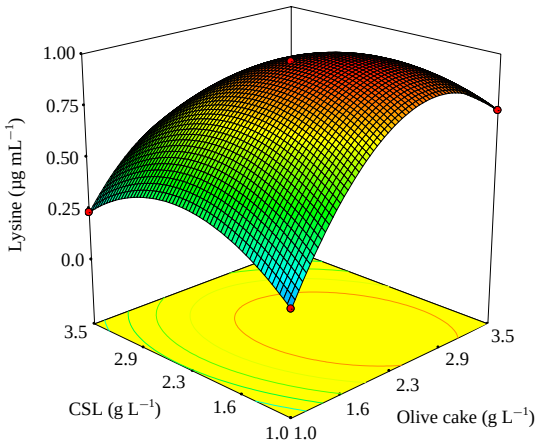

Fig. 5: Three-dimension plot shows the correlative effect of olive cake and CSL on the overall desirability function

Table 5 shows that solving the previous equation for maximum production of lysine led to the theoretical level of olive cake and CSL at 2.58 and 1.83 g L^{-1}, respectively, recording predicting value of lysine at 1.470 μg mL^{-1}. where the predicting value of glutamic acid reached 0.805 μg mL^{-1} at levels of 2.49 and 1.93 g L^{-1} from olive cake and CSL, respectively. These theoretical values were validated experimentally and were found to be 1.479±0.007 and 0.813±0.010 for lysine and glutamic acid, respectively. There are no significant differences between predicted and experimental values for amino acids production, reflecting the validity of the prediction equation and the adequacy of the model.

The dual optimization response of both amino acids was carried out based on total desirability function (D), in which, each of individual response is transformed into a desirability value (d) and the D (lies between 0 and 1) is then computed as the geometric mean of both d. The response approaches the target when D value closer to 1. To calculate D, the olive cake and CSL were set within the experimental range and the two investigated amino acids to be maximized.

The three-dimensional surface response plots of D (Fig. 5) were generated for both amino acids. Performing the multi-response optimization, led to obtaining the theoretical levels of olive cake and CSL at 2.54 and 1.87 g L^{-1}, with predicted values of lysine and glutamic acids at 1.469 and 0.805 μg mL^{-1}. At these levels, the D recorded 0.997. The previously calculated data was experimentally validated; the obtained responses were 1.473±0.009 and 0.801±0.004 μg mL^{-1} for lysine and glutamic acids, respectively. This highly predictive ability of the dual response confirms the adequacy of the model to be applied in large-scale production for both amino acids.

Finally, the tested fungus is supposed to have two pathways for the accumulation of the amino acids; the first is the ordinary proteolysis during the fermentation process, which leads to the liberation of several amino acids in nearly equal concentrations. The other pathway is the ability of this fungus to form the two amino acids (lysine and glutamic acid) from the basic units (nitrogen and carbon) or through the interconversion of amino acids inside the microbial cell that lead to the accumulation of lysine and glutamic acids in high amounts compared with the other amino acids.

CONCLUSION

To the best of our knowledge, it is the first report regarding the optimization of both lysine and glutamic acids. In this study, the Plackett-Burman design and multi-response surface were employed to optimize fermentation medium composition for high production of amino acids understudy by using *M. mucedo* KP736529. Obtained results indicated that the 3rd day is a suitable time for high appreciable amounts of amino acids (641 μg mL^{-1}). The Plackett-Burman design selected olive cake and CSL as potentates for producing amino acids, in which these substrates are economic and readily available. As the results of CCD, there are no significant differences between predicted and experimental values for amino acids production, reflecting

the validity of the prediction equation and adequacy of the model. The desirability function (D) showed the responses of amino acids and adequacy of the model to be valid and could be applied in large scale production of these amino acids. Ultimately, the strategy of microbial production of amino acids is the main choice.

SIGNIFICANCE STATEMENT

Amino acids are the blocks of proteins, which constitute a major part of the body. However, the deficiency in some essential amino acids leads to various diseases and physiological defects. In the current study, both lysine and glutamic acids were produced in maximized amounts by *M. mucedo* KP736529 grown on economic substrates, i.e., corn steep liquor and olive cake with the aid of statistical procedures.

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