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Optimization of the Medium Composition for Production of Mycelial Biomass and Exo-Polysaccharide by *Agaricus blazei* Murill DPPh 131 Using Response-Surface Methodology

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Abstract: Mushrooms have become attractive as a functional food and as a source for the development of drugs and nutraceuticals. *Agaricus blazei* is considered one of the best among them. There are several published works on the fruiting body of this mushroom, showing high antitumor activity. The mycelium polysaccharide (EPS) of this mushroom also demonstrated a strong antitumor action. However, there is a little information available in the literature about optimization of culture conditions for production of EPS by liquid submerged culture. A mathematical model was developed to show the effect of each medium composition and their interactions on the production of mycelial biomass and exo-polysaccharide. Study on supplementation of carbon source to the medium revealed that starch was the most effective for EPS production. Yeast extract was the best for EPS among the inorganic and organic nitrogen sources tested. The Box-Behnken model estimated that a maximal yield of mycelial biomass 9.08 g L^{-1} could be obtained when initial pH, temperature, starch and yeast extract concentrations were set at 5.64, 24.60°C, 11.55 and 4.63 g L^{-1}, respectively while a maximal EPS yield of 1.268 g L^{-1} could be achieved when pH, temperature, starch and yeast extract concentrations were set at 6.87, 20°C, 20 and 0.84 g L^{-1}, respectively. These predicted values also verified by validation experiments. Compared with the values obtained by other runs in the experimental design, the optimized medium resulted in a significant increase in the yields of mycelial biomass and EPS.

Key words: *Agaricu blazei*, submerged culture, exo-polysaccharide, optimization, Box-Behnken design

INTRODUCTION

Agaricus blazei mushroom belongs to the family of Basidiomaycetes and is native of Brazil. According to tradition, it helps against a variety of disease, including cancer, diabetes, arteriosclerosis and hepatitis. Its chemical components are including steroids (Osaki *et al*., 1999; Takaku *et al*., 2001) and various polysaccharides (Kim *et al*., 2002a).

Traditional and edible mushrooms are generally produced in solid culture using composts or lignocellulosis waste such straws or wood and it usually takes a long time to culture their fruiting bodies (Solomon, 1975). It is generally recognized that, growing mushroom mycelia in a defined medium by submerged culture is a rapid and alternative method to obtain fungal biomass of consistent quality (Tang and Zhong, 2002). In addition, exo-polymer, which also exhibit similar biological effects as the polysaccharides of the mycelia, can be produced simultaneously (Kim *et al*., 2002b). There were many reports on optimization of culture media for producing microbial metabolites by statistical optimization techniques (Xu *et al*., 2002). However, reports related with submerged fermentation of basidiomycetes and the effects of environmental condition, especially *A. balzei* is limited.

Response Surface Methodology (RSM), which has been extensively applied in optimization of medium composition, conditions of enzymatic hydrolysis, fermentation and food manufacturing process (Mao *et al*., 2005), is a collection of statistical techniques for experimental design, model developing, factors evaluation and optimum conditions searching (Cui *et al*., 2006) RSM could overcome the shortcoming of the classical or empirical methods such as one-factor-at-a-time-method, which is time-consuming process and

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incapable of searching the global optimal condition especially when interaction between independent factor existed.

In this research at the preliminary step we evaluate the suitability of various carbon and nitrogen sources, temperature, initial pH culture medium and inoculum volume for effective production of biomass and exo-polysaccharide and then to optimize the medium composition of different factors to increase the yields of mycelial biomass and exo-polymer using response-surface methodology (Box-Behnken design).

MATERIALS AND METHODS

Microorganism and media: A strain of *A. blazei* DPPh 131 was provided from the School of Pharmacy Sheehed Beheshti University of Medical Science culture collection. The stuck culture was maintained on malt extract agar slants and sub-cultured every two months. The slants were incubated at 25°C for 7 days. The seed cultures medium was containing (in g L^{-1}) glucose 10, yeast extract 3, malt extract 10 and mycological peptone 1.

Inoculum preparation and flask culture: For preparation of inoculum, one pieces (about 4 mm) of mycelia of *A. blazei* was transferred from a slant into each Erlenmeyer flask containing 100 mL seed medium with sterilized self design cutter. The flasks were then placed in a rotary shaker incubator at 120 rpm and 25°C for 7 days.

All basic fermentation cultures were carried out in Erlen flasks (500 mL) containing 100 mL fermentation medium based on the experimental design. Five percent (v/v) of the seed culture was inoculated and the initial pH adjusted to 5.5. The fermentation was carried out in a rotary shaker incubator at 120 rpm and 25°C for 10 days. Various and different medium composition (carbon and nitrogen source), pH and temperature were selected based on requirements. Triplicate experiments were also carried out and the mean values were calculated.

Selection of carbon source: Eight carbon sources, namely galactose, lactose, fructose, starch, sucrose, maltose, glucose and xylose were evaluated. Fermentation medium contained (g L^{-1}) carbon source 10, yeast extract 3, mycological peptone 1, $MgSO_4$. $7H_2O$ 1, K_2HPO_4. $3H_2O$ 1.

Selection of nitrogen source: Eight different sources of organic and inorganic nitrogen were used, i.e., ammonium oxalate, potassium nitrate, ammonium sulfate, ammonium chloride, urea, yeast extract, poly peptone and mycological peptone. Fermentation medium was similar as above but the nitrogen source was replaced at an equivalent concentration of experimental source. Thus, the concentration (%) were, ammonium oxalate 0.3, potassium nitrate 0.3, ammonium sulfate 0.3, ammonium chloride 0.3, urea 0.3, yeast extract 0.3, poly peptone 0.3 and mycological pepton 0.3. Starch (2%) was used as carbon source.

Selection of pH and temperature: For the determining the best temperature and pH for the exo-polysaccharide production, the experiment was carried out at temperature 20, 25 and 30°C and pH 4-8, with the medium containing (g L^{-1}) starch 20, yeast extract 4, $MgSO_4$. $7H_2O$ 1, K_2HPO_4. $3H_2O$ 1.

Selection of inoculum volume: Different inoculum volume ranging from 3 to 9% (v/v) were selected. The fermentation medium was similar as above.

Experimental design and evaluation: Response-Surface Methodology (RSM) was used to investigate the influence of concentration of carbon and nitrogen source, pH and temperature on the yields of biomass and EPS by *A. blazei*. A Box and Behnken (1960) design with 4 factors at three levels including five replicates at the center point was used for fitting data on a second-order polynomial model. Table 1 and 2 show the factors, their corresponding range of variation and their values for each run, respectively. The mathematical method describing the relationships between the process responses (the yield of mycelial biomass and EPS) and the medium contents, was developed. The yield of mycelial biomass and EPS by *A. blazei* was multiply regressed with respect to the medium contents and the fermentation conditions by the least squares method as follows:

$$Y = A_0 + \Sigma A_i X_i + \Sigma A_{ii} X_i^2 + \Sigma A_{ij} X_i X_j \quad (1)$$

Where Y is the predicted response variable; A_0, A_i, A_{ii}, A_{ij} are regression coefficients of the model and X_i, X_j (I = 1, 3; j =1, 3, I≠ j) represent the independent variables (medium composition) in form of coded or real values. The accuracy and general ability of the above polynomial model were evaluated by coefficient of determination (R^2).

Statistical analysis: Data from the Box-Behnken design shown in Table 2 were used for determining the regression coefficient of the second-order multiple regression models. The analysis of regression and variance (ANOVA) was carried out using the Design-Expert software (version 6.0.10, USA). Canonical analysis, which is used to predict the shape of the curve generated

Table 1: Experimental design summary

Study type	Response surface					
initial design	box-behnken					
design model	quadratic		No. of experiments 29			
model	R quadratic					
factor	name	Type	Low actual	High actual	Low coded	High coded
A	pH	Numeric	4.00	7.00	-1.000	1.000
B	Temp	Numeric	20.00	30.00	-1.000	1.000
C	starch	Numeric	0.00	20.00	-1.000	1.000
D	yeast extract	Numeric	0.00	8.00	-1.000	1.000

Table 2: Box-Behnken design matrix and the responses of the dependent variables biomass and exo-polysaccharide

			Starch	Yeast extract	Biomass	Exo-polysaccharide
Run	pH	Temperature (°C)	($g L^{-1}$)			
1	5.5	30	00	4	0.21	0.540
2	5.5	25	10	4	8.85	0.637
3	7.0	25	20	4	5.55	0.436
4	5.5	20	0	4	1.78	0.277
5	5.5	25	20	0	2.89	0.734
6	4.0	25	10	0	2.59	0.954
7	7.0	20	10	4	2.97	0.664
8	4.0	30	10	4	0.10	0.500
9	5.5	25	0	0	1.34	0.167
10	5.5	25	10	4	8.98	0.625
11	7.0	30	10	4	0.93	0.113
12	4.0	25	0	4	2.06	0.227
13	5.5	25	10	4	9.04	0.807
14	5.5	30	10	0	ND	ND
15	5.5	20	10	0	2.04	0.977
16	4.0	25	10	8	4.10	0.085
17	7.0	25	10	8	5.29	0.825
18	5.5	20	10	8	3.12	0.488
19	5.5	25	10	4	9.12	0.521
20	7.0	25	0	4	3.28	0.347
21	5.5	30	20	4	2.83	1.000
22	7.0	25	10	0	3.36	0.547
23	4.0	20	10	4	2.40	0.559
24	5.5	30	10	8	2.63	0.200
25	5.5	25	20	8	5.37	1.035
26	5.5	25	10	4	8.93	0.824
27	5.5	25	0	8	3.16	0.111
28	5.5	20	20	4	2.88	1.437
29	4.0	25	20	4	3.92	0.804

ND = Not detected

by the model, was also carried out. The 3D response surface and contour plot analysis were made by keeping one independent variable at constant level, changing other independent variables and then calculating the response variables using RSRREG analysis.

Preparation of polysaccharides and mycelial biomass: Preparation of the exo-polysaccharides was carried out with slightly modification as explained by Wang and Lu (2005). The fermentation broth was centrifuged at 10000 g for 25 min and the resulting supernatant was filtered through a 0.45 μm membrane filter (Millipore) and mixed with four volumes of absolute ethanol, stirred vigorously and left overnight at 4°C. The precipitated EPSs were collected after centrifugation. The precipitate of EPS was lyophilized and weight. Mycelium dry weight was also measured after repeated washing of the mycelial pellet with distilled water and drying at 70°C overnight to a constant weight.

RESULTS AND DISCUSSION

Effect of carbon sources: For EPS production, starch was the best carbon source with a production of 1.8 g L^{-1} (Fig. 1). Also, it was observed that polysaccharides in general were better than monosaccharides for EPS production, which is probably due to relative ease in polymerization. These results seemed important as only little was known on the effect of carbon source on EPS production by *A. blazei*. In contrast glucose has been referred to as the most suitable carbon source for mycelium growth of majority of mushrooms (Chang, 1998; Wang, 1993). To gain a better insight into the comparison, we also used glucose as a carbon source in subsequent studies, even though starch was more efficient for EPS production. Different concentration of starch was also used to gain a better result for EPS production (Fig. 1b). The result showed that the best EPS production was achieved at the concentration of 20 g L^{-1}.

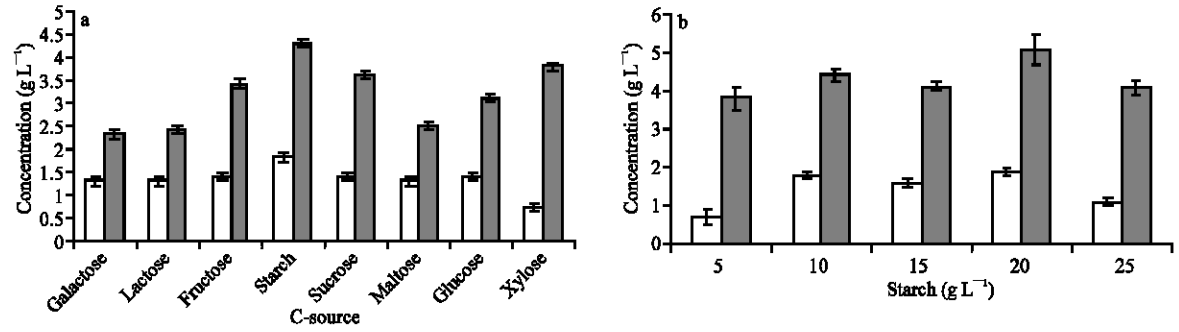

Fig. 1: Effect of different carbon sources (a) and starch concentration (b) on the exo-polysaccharide and mycelial biomass production in *Agaricus blazei*. □ EPS, ■ Biomass. All experimental data are mean±SD of triple determinations

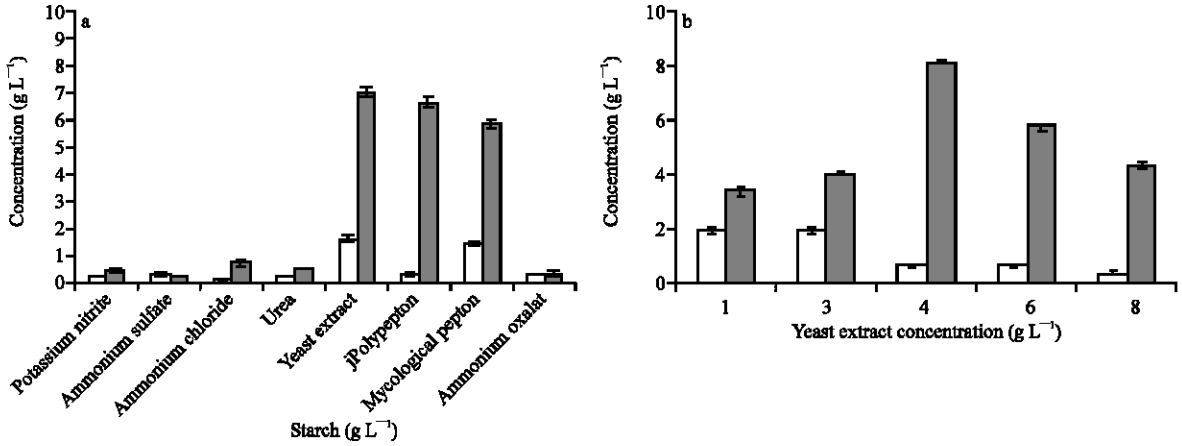

Fig. 2: Effect of different nitrogen sources (a) and yeast extract concentration (b) on the exo-polysaccharide and mycelial biomass production in *Agaricus blazei*. □ EPS, ■ Biomass. All experimental data are mean±SD of triple determinations

Effect of nitrogen sources: To investigate the effects of nitrogen sources on EPS production and mycelial growth, eight different nitrogen sources at the concentration of 3 g L^{-1} were examined (Fig. 2a). Starch was used as carbon source in the basal medium at the concentration of 20 g L^{-1}. In comparison with organic nitrogen sources, inorganic nitrogen sources gave rise to relatively lower mycelial biomass and EPS production. The highest EPS production obtained when yeast extract was used (Fig. 2a). The results obtained indicated that complex nitrogen sources are generally better than inorganic nitrogen source especially for the growth of the fungus and mycelial production. Similarly, other researchers were also reported that organic nitrogen sources are better for EPS and biomass production in different mushrooms (Park *et al*., 2001; Lee *et al*., 2004; Fan *et al*., 2007). Different concentration of yeast extract was also examined. Four gram per liter yeast extract gave the best production of EPS. In contrast, lower concentration of yeast extract resulted in high concentration of biomass. The results were also showed that, increasing the concentration of yeast extract resulted in decreasing the concentration of EPS (Fig. 2b).

Effect of inoculum volume: Inoculum size is also one of the most important factor, affecting mycelial growth and polysaccharide production (Park *et al*., 2001; Lee *et al*., 2004). To find the optimal inoculum size, *A. blazei* was cultivated under different inoculum volume ranging from 3 to 9% (v/v). Consequently, the optimal inoculum size for both mycelial biomass and EPS production by *A. blazei* was found to be 3% (v/v) (Fig. 3).

Effect of initial pH and temperature: The effect of initial pH and temperature on mycelial biomass and EPS production was studied at different initial pH (4.0-8.0) and temperatures (20-30°C). The optimal initial pH was achieved at pH 7 meanwhile the optimal temperature for

growth and mycelial biomass production was observed at 25°C. In contrast the optimal temperature for EPS production was seen at 20°C (Fig. 4 and 5).

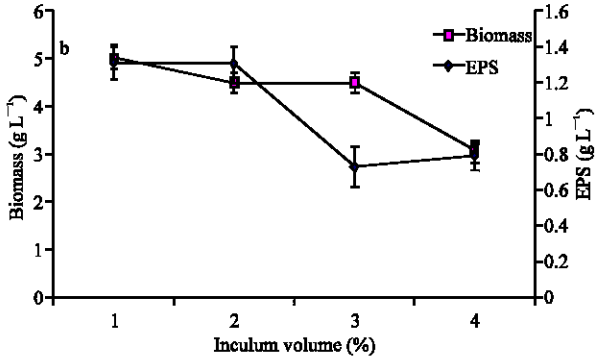

Fig. 3: Effect of inoculum volume on the exo-polysaccharide and mycelial biomass production in *Agaricus blazei*. All experimental data are mean±SD of triple determinations

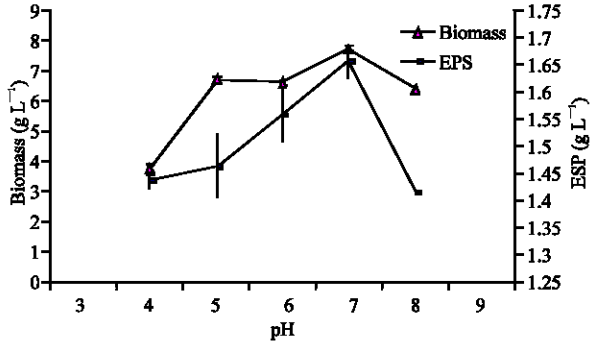

Fig. 4: Effect of initial pH on the exo-polysaccharide and mycelial biomass production in *Agaricus blazei*. All experimental data are mean±SD of triple determinations

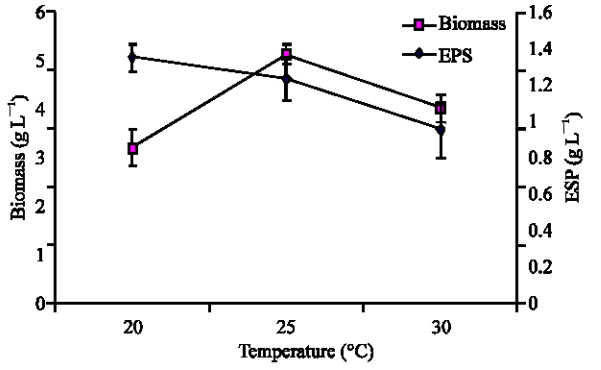

Fig. 5: Effect of temperature on the exo-polysaccharide and mycelial biomass production in *Agaricus blazei*. All experimental data are mean±SD of triple determinations

Optimization results: According to this study starch and yeast extract were chosen as best carbon and nitrogen source to continue the experiment in order to find the optimal submerged culture condition for biomass and EPS production.

Optimization of the yield of mycelial biomass: Table 2 shows the considerable variation in the yields of both mycelial growth and EPS production under different fermentation and medium composition. Minimum biomass concentration was achieved in run No. 8 at the concentration of 0.1 g L^{-1} and maximum biomass was achieved at the concentration 9.04 g L^{-1} (run No. 13), respectively. Ratio of maximum to minimum response is 91.22. A ratio greater than 10 usually indicates a transformation is required. Transformation of the response is an important component of any data analysis. Transformation is needed if the error, (residuals) is a function of the magnitude of the response. The appropriate choice of a response transformation relies on subject matter knowledge and/or statistical considerations. In this study the logit transformation was chosen to transform responses of the mycelial biomass production by *A. blazei*.

$$\text{Logit}(Y) = \ln(Y\text{-lower limit/upper limit} - Y)$$

The logit transformation is useful if the response has a finite range, such as 0 to 100%. Logit spreads out the values near the boundaries. The actual response data collected must be between the lower and upper limit and not equal to either one.

Table 3 shows the analysis of variance (F-test) for the experiment and the coefficient of determination (R^2) was shown as 98.53%. These indicated that, the accuracy and general ability of the polynomial model was good and the analysis of the response trends using the model was considered to be reasonable.

The p-value is used as a tool to check the significance of each coefficient, which also indicates the interaction strength between each independent variable (Table 4). The smaller the p-values, the bigger the significance of the corresponding coefficient (Liu *et al*., 2003). Table 4 shows that the regression coefficients of all nonlinear terms at 1% level were significant. Using the design experimental data (Table 2), the polynomial model for biomass yield $Y_{biomass}$ was regressed by only considering the significant terms and was shown as below:

$$\text{Logit}(Y_{biomass}) = \ln[(Y_{biomass} + 0.90)/(10.12 - Y_{biomass})]$$
$$= -66.89 + 6.32A + 4.02B + 0.17C + 0.39D - 0.56A^2 - 0.08B^2 - 0.01C^2 - 0.08D^2 + 5.16BC + 0.01BD \quad (2)$$

Table 3: Analysis of variance (ANOVA) for the fitted quadratic polynomial model for optimization of biomass production of *A. blazei*

Source	Sum of squares	df	Mean square	F-value	Probability (p) > F
Mean	1.45	1	1.450	-	-
Linear	4.60	4	1.150	0.660	0.6283
2FI	0.37	6	0.062	0.027	0.9999
Quadratic	39.23	4	9.810	193.300	<0.0001*
Total	46.31	28	1.650	-	-

$R^2 = 0.9853$, Adj. $R^2 = 0.9695$

*Significant at 1% level

Table 4: Results of regression analysis of a full second order polynomial model for optimizing of biomass production of *A. blazei*

Source	Sum of squares	df	Mean square	F-value	Probability (p) > F
Model	44.14	10	4.41	104.35	<0.0001**
A	0.64	1	0.64	15.17	0.0012**
B	1.79	1	1.79	42.40	<0.0001**
C	2.19	1	2.19	51.71	<0.0001**
D	1.81	1	1.81	42.90	<0.0001**
A^2	10.06	1	10.06	237.80	<0.0001**
B^2	25.96	1	25.96	613.60	<0.0001**
C^2	10.80	1	10.80	255.26	<0.0001**
D^2	9.63	1	9.63	227.75	<0.0001**
BC	0.27	1	0.27	6.31	0.0224*
BD	0.21	1	0.21	4.91	0.0407*

*Significant at 5% level, **Significant at 1% level

The model (Eq. 2) indicates that pH (A) had a significant effect ($p<0.0001$) on Y as it had the largest coefficient followed by temperature (B), starch (C) and yeast extract concentration (D). Positive coefficient of A, B, C and D indicated a linear effect to increase biomass. However, quadratic terms A^2, B^2, C^2 and D^2 along with interaction terms (BC) and (BD) had negative effects that decrease $Y_{biomass}$.

3D response plots described by the regression model were drown to illustrate the effects of the independent variables and interactive effects of each independent variable on response variables. The shapes of corresponding contour plots indicate whether the mutual interactions between the independent variables are significant or not. An elliptical nature of the contour plots indicates that the interactions between the independent variables are significant. From the 3D response surface plots and the corresponding contour plots, the optimal values of the independent variables could be observed and the interaction between each pairs of independent variables are easily understood. The 3D response surface based on independent variables starch (C) and yeast extract (D) was shown in Fig. 6, while the other independent variables was kept at a constant level. It was obvious that the mycelial growth of *A. blazei* was sensitive even when starch and yeast extract concentration was subject to small alteration.

The interaction between the two independent variables (temperature, starch) and the response variable (mycelial biomass) can be easily understand by examining the contour plots generated by keeping the other independent variables as constants (Fig. 7).

The canonical analysis revealed a maximum yield of biomass of 9.08 g L^{-1} with desirability of 0.99, which was approximately 90 times higher than that of the run No. 8, could be achieved at the concentration point when starch, yeast extract, pH and temperature were set at 11.55 and 4.63 g L^{-1}, 5.64 and 24.60°C (Fig. 8 and 9).

Optimizing of the yield of exo-polysaccharide: Table 2 shows the EPS production ranges from 0.050 to 1.437 g L^{-1}. Ratio of maximum to minimum response as 28.74 so transformation was required and the power transformation was chosen to transform responses of the EPS production. Most data transformations can be described by the power function, $\sigma = fn(\mu\alpha)$, where σ is the standard deviation, μ is the mean and α is the power. In all cases of transformation λ is $1-\alpha$. The power transformation allows transformation to any power in the range -3 to +3, provided the data are positive.

$$Y' = (Y-K)^{\lambda}$$

If the standard deviation associated with an observation is proportional to the mean raised to the power (α), then transforming the observation by the $1-\alpha$ (or λ) power gives a scale satisfying the equal variance requirement of the ANOVA.

Using the Box Cox plot provided in Design Expert software, the appropriate power transformation ($\lambda = 0.52$ and $K = 0$) was chosen.

The polynomial model for EPS yield Y_{EPS} was also regressed by only considering the significant terms and was expressed by Eq. 3:

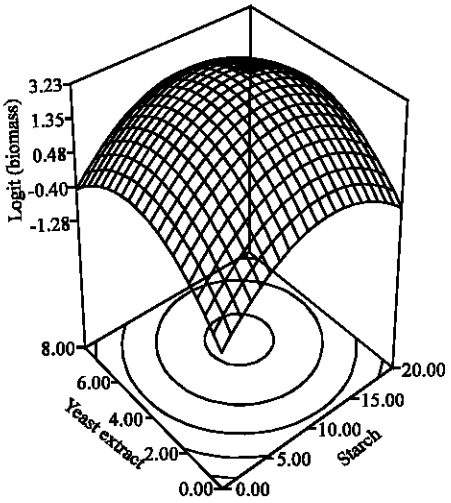

Fig. 6: Surface plot of the combined effects of Starch and yeast extract on the mycelial biomass of *A. blazei*

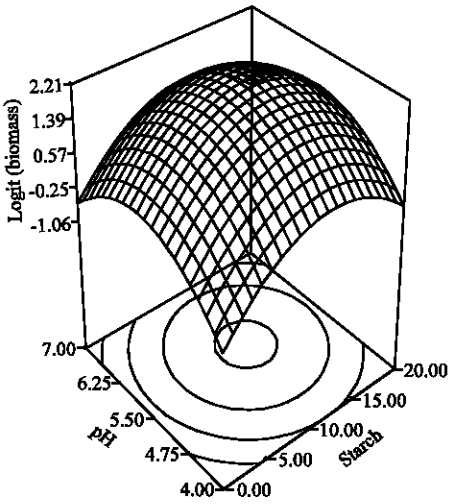

Fig. 8: Surface plot of the combined effects of Starch and pH on the mycelial biomass of *A. blazei*

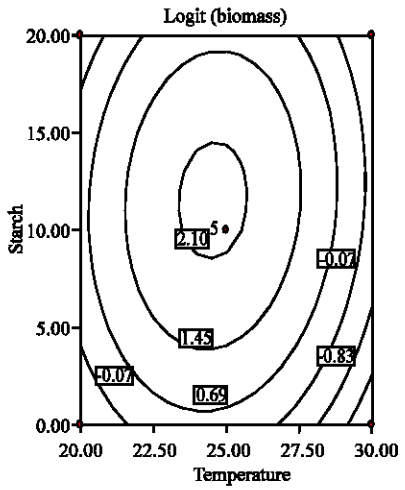

Fig. 7: Contour plot of the combined effects of temperature and starch on the mycelial biomass of *A. blazei*

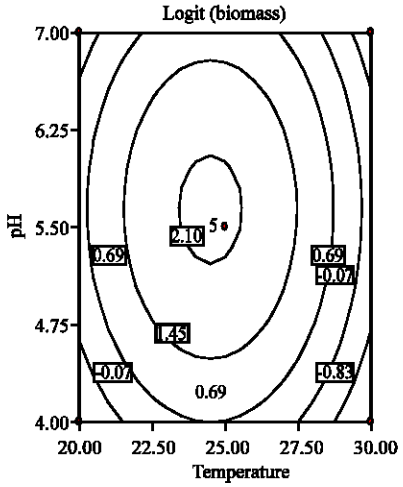

Fig. 9: Contour plot of the combined effects of temperature and pH on the mycelial biomass of *A. blazei*

$$(Y_{EPS})^{0.52} = +49.55-1.37B + 0.95C \quad (3)$$

The model (Eq. 3) indicates that temperature (B) had a significant effect ($p<0.0001$) on Y as it had the largest coefficient followed by starch (C). Positive coefficient C indicated a linear effect to increase EPS. However, linear terms B had negative effects that decrease Y_{EPS}.

The statistical significance of the linear model was checked by F-test (ANOVA) and the results were shown in Table 5. It could be seen that the values of probability < 0.0001, indicating that the model is highly significant.

Table 6 shows that the regression coefficient of linear term of starch (C) and temperature (B) are significant at 1% level.

As an example, the contour plot for EPS production yield by *A. blazei* against temperature and starch concentration in the culture medium was shown in Fig. 10. Despite the results of one factor at a time study in basal medium (with glucose as carbon source), in Box-Behnken study (with starch as the best carbon source) it was found that pH and yeast extract (as nitrogen source) had no significant effect on optimization results (Fig. 11). It was

Table 5: Analysis of variance(ANOVA) for the fitted linear model for optimization of exo-polysaccharide production by *A. blazei*

Source	Sum of squares	df	Mean square	F-value	Probability (p) > F
Mean	17477.43	1	17477.43		
Linear	1692.04	4	423.01	9.94	<0.0001*
2FI	327.16	6	54.53	1.42	0.2632
Quadratic	189.98	4	47.49	1.34	0.3084

*Significant at 1% level

Table 6: Results of regression analysis of a full second order polynomial model for optimization of exo-polysaccharide production of *A. blazei*

Source	Sum of squares	df	Mean square	F-value	Probability (p) > F
Model	1595.34	2	797.67	18.54	<0.0001
B	515.92	1	515.92	11.99	0.0019
C	1079.42	1	1079.42	25.08	<0.0001

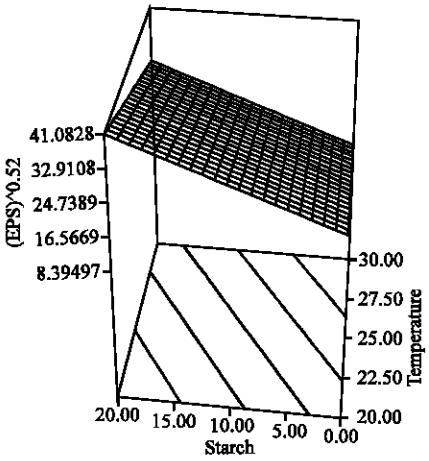

Fig. 10: Surface plot of the combined effects of Starch and temperature on the EPS production of *A. blazei*

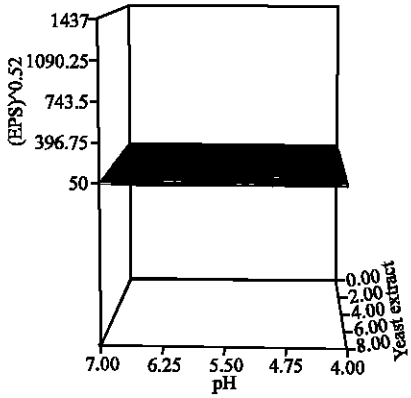

Fig. 11: Effects of pH and yeast extract on the EPS production by *A. blazei*

probably due to the different submerged culture composition which was used in this study.

Canonical analysis revealed maximum production yields of EPS of 1.268 g L^{-1}, could be achieved with desirability of 0.924 when pH, temperature, starch and yeast extract are 9.71, 20°C, 20 and 0.4 g L^{-1}. Shu *et al.* (2004) and recently Fan *et al.* (2007) reported that pH had significant effect on EPS production. Also with their optimized medium composition, the highest level of the EPS of 0. 227 g L^{-1} was obtained, while present predicted results (1.268 g L^{-1}) using the regression model is 5.5 time higher. This was probably due to the differences of the medium composition, analytical and statistical methods used for EPS production.

The mathematical calculation for searching the global maximal points of the RSM model could interpret as follows: Generally a near-optimal point could be deduced by calculating the derivatives of regression models with regards to independent variables or by mapping the response of the model onto a surface contour plot, which could be performed by Design-Expert 6.0.10 soft ware. A set of values of independent variables that leads to maximum mycelial biomass and EPS yield could be obtained by differentiating Eq. 2 or 3 with respect to A, B, C, D and solving the resulting sets of algebraic equations.

Validation of the method: The availability of the regression model (Eq. 2) of the mycelial biomass production by *A. blazei* was tested using the calculated optimal culture composition, starch 11.58 g L^{-1}, yeast extract 4.63 g L^{-1}, initial pH 5.64 and temperature 25°C, with triplicate experiments. The mean value of the mycelial biomass was 9.1±0.15 g L^{-1}, which agreed with the predicted value (9.08 g L^{-1}).

The triplicate experiments were also carried out to verify the validity of the model (Eq. 3) For EPS production in a similar way as that of the mycelial biomass production model. Under the calculated optimal culture conditions, initial pH 9.71, temperature 20°C, starch 20 g L^{-1} and yeast extract 0.4 g L^{-1}, the mean value of EPS production was 1.280±0.063 g L^{-1}, which agreed well with the predicted value, using Eq. 3 (1.268 g L^{-1}). As a result, the model developed was considered to be accurate and reliable for predicting the production of mycelial biomass and EPS by *A. blazei*.

CONCLUSIONS

The results obtained from the above experiment showed that starch is a valuable carbon source for growth and production of exo-polysaccharides which is different

from data presented by other researcher for growth of such basidiomycetes. In contrast a similar result was obtained for growth of the fungus on complex nitrogen source.

Statistical optimization method for fermentation process could overcome the limitations of classic empirical methods and was proved to be a powerful tool for the optimization of the mycelial biomass and EPS production by *A. blazei*. In this study, RSM model was proposed to study the combined effects of culture media compositions. Under optimal condition, the predicted values of the mycelia biomass amount and the EPS production yield were increased to 9.1196 and 1.6174 g L^{-1}, respectively, by canonical analysis. Validation experiments were also carried out to verify the availability and the accuracy of the models and the results showed that the predicted values agreed with the experimental values well. The results of this study provided useful information and reference for the optimization of medium composition for the other submerged mushroom fermentation processes.

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