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Use of response surface methodology to optimize culture medium for production of lovastatin by *Monascus ruber*

Yaw-Nan Chang[a,*], Jen-Chang Huang[a], Chih-Chen Lee[b], Ing-Lung Shih[c], Yew-Min Tzeng[d]

[a]*Department of Food Engineering, Da-Yeh University, Changhua, Taiwan 515, Republic of China*
[b]*Tanque Co., Changhua, Taiwan 515, Republic of China*
[c]*Department of Environmental Engineering, Da-Yeh University, Changhua, Taiwan 515, Republic of China*
[d]*Department of Applied Chemistry, Chaoyang University of Technology, Taichung County, Taiwan 413, Republic of China*

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Abstract

Response surface methodology (RSM) was employed to study the effect of culture medium on the production of lovastatin in mixed solid-liquid state (or submerged) cultures by *Monascus ruber*. The maximal lovastatin yield (131 mg/L, average of three repeats) appeared at the region where the respective concentrations of rice powder, peptone, glycerin, and glucose were around 34.4 g/L, 10.8 g/L, 26.4 ml/L, and 129.2 g/L, respectively. The optimized medium resulted in a significant increase of lovastatin yield, as compared with that obtained by the fermentation of many other *M. ruber* species.

Keywords: Lovastatin production; *Monascus ruber*; Response surface methodology; Fermentation; Medium optimization

1. Introduction

Lovastatin (also known as mevinolin, monacolin K, Mevacor) is a potent competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis. It is active not only *in vitro* to inhibit cholesterol biosynthesis but also *in vivo* to lower plasma cholesterol level in humans and animals [1,2], thereby is effective in the therapy of hypercholesterolemia. Hypercholesterolemia is a primary risk factor for the coronary artery disease, the major cause of death in the western countries [3,4]. In fact, lovastatin has been introduced into commercial use by Merck Sharp and Dohme (known as MEVACOR®) in several countries for the treatment of hypercholesterolemia. Besides, lovastatin has recently been indicated as potential therapeutic agent for the treatment of various types of tumors because of its capability to suppress tumor growth *in vivo* through inhibition the synthesis of nonsterol isoprenoid compounds [5,6].

Lovastatin was first isolated by Endo from *Monascus ruber* [7] and independently, by Alberts et al. from *Aspergillus terreus* [1]. Since then many strains of *Monascus* [8] as well as a variety of other filamentous fungi including some species of *Penicillium* [9], *Hypomyces*, *Doratomyces*, *Phoma*, *Eupenicillium*, *Gymnoascus*, and *Trichoderma* [10] were found to produce lovastatin. However, only a fermentation process with *A. terreus* was developed to manufacture it on large scale [11]. It was found that culture homogeneity, variation of carbon sources, pH of the medium and the speed of agitation all have great effects on the lovastatin productivity by *A. terreu*. Although the productivity of lovastatin is as high as 400 mg/L by *A. terreu* [12], the lovastatin containing broth produced by this microorganism is not generally used directly because *A. terreu* per se is not considered edible. Therefore, tedious procedures for extraction and purification of lovastatin form the broth are needed.

Monascus species are nonpathogenic and frequently used in food processing to add to the aroma, nutrition and color of the fermentation products [13]. Cultivation of *M. ruber* for the production of monacolins has also been studied. The production proceeds for 10–11 days at 25°C in complex media consisting, eg of either glucose, peptone, corn steep and ammonium chloride [7] or glycerin, glucose, soy bean powder, peptone, sodium nitrate, zinc nitrate and olive oil [14]. It is also known that an elevated temperature (35°C) inhibits lovastatin production [8]. Nevertheless, medium

* Corresponding author. Tel.: +86-4-8511-888; fax: +86-4-8511-320.
E-mail address: nelson@mail.dyu.edu.tw (Y.-N. Chang).

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optimization experiments with *M. ruber* for lovastatin production has not been extensively studied.

Previous works on lovastatin fermentation were conducted using "one-factor-at-a-time-technique". Unfortunately, it frequently fails to locate the region of optimum response because the joint effects of factors on the response are not taken into account in such procedure. It was reported that the complexities and uncertainties associated the large-scale fungi fermentation usually come from lack of knowledge of the sophisticated interactions among various factors. The response surface methodology (RSM) has been increasingly used for various phases of an optimization process in fermentation [15–19]. It is a powerful technique for testing multiple process variables because fewer experimental trials are needed compared to the study of one variable at a time. Also, interactions between variables can be identified and quantified by such technique [20]. However, the application of this technique on optimizing the lovastatin production is scant. The present study is to implement such technique on the optimization of lovastatin production by *M. ruber*.

2. Materials and methods

2.1. Chemicals

Reagents for cultivation such as potato dextrose agar (PDA), potato dextrose broth (PDB), were purchased from DIFCO Laboratories Michigan, USA. Glucose, peptone, glycerin, $MgSO_4 \cdot 7H_2O$, KNO_3 were obtained from Sigma Chemical, USA, and rice powder was obtained from local supermarket. Acetonitrile was of HPLC purity (Merck). All other reagents used were of the highest grade available unless otherwise indicated.

2.2. Strain and culture conditions

In our previous study we screened eleven strains of *Monascas* species purchased from the Culture Collection and Research Center (CCRC) Taiwan for lovastatin production [21]. Among these tested strains, only five strains were tested positive. *Monascas ruber* CCRC 31535 (ATCC 18199) was selected for further optimization because of its highest lovastatin production (0.096 mg/L) among the five positive tested strains. *M. ruber* CCRC 31535, obtained as a lyophilized powder in a glass ampoule sealed under vacuum, was first cultured on potato dextrose agar (PDA) containing agar (1.5%), diced potatoes (30%), glucose (2%), to induce spore formation. After cultivation at 30°C for 7 days, colonies of spores that appeared on the plates were transferred ($\sim$1 cm^2) and inoculated into 100 ml of potato dextrose broth (PDB), and incubated at 30°C for 4 days with shaking at 150 rpm. After incubation, 1.25 ml (5% v/v) of the above broth was inoculated into 25 ml of culture medium composed of rice powder (3%), peptone (0.9%), glycerin (3%), glucose (11%), $MgSO_4 \cdot 7H_2O$ (0.1%), KNO_3 (0.2%) in a 250 ml flask. The culture was incubated at 25°C, pH 5.0 with shaking at 150 rpm for 10 days. The production of the lovastatin was monitored by high performance liquid chromatography (HPLC). At the end of the cultivation, the whole broth was filtered. The remained solid was extracted with 50 ml (95%) ethanol for 24 h. The extract was filtered through 0.45 μm membrane and then analyzed by HPLC to quantify the intracellular lovastatin.

2.3. Quantitative analysis of lovastatin

The HPLC system for analysis of lovastatin concentration was composed of a Hitachi L-6200 solvent delivery controller, a Hitachi 4250 UV-VIS detector, a Hitachi-D-2500 Chromato-Integrator, and a hyperbond C18 column (ThermoQuest Hypersil, UK, 300 $\times$ 3.9 mm, 10 μ). The injection volume was 20 μL. The sample was eluted with a mobile phase comprising 65% acetonitrile at a flow rate of 1.0 ml/min. The chromatogram was monitored at 240 nm. Lovastatin standard obtained from United States Pharmacopeia Convention, Inc. was used to construct a calibration curve from which lovastatin concentration in fermentation broth was determined. Although the chromatogram of the culture broth was complicated, the chromatogram of the lovastatin sample after ethanol extraction was quite clean and well resolved (data not shown). The lovastatin (R.T. 8.46 min) peak was so well separated from all the other peaks that make the quantity of lovastatin very easy.

2.4. RSM experimental design

In preliminary experiments, we evaluated various carbon and nitrogen sources for their suitability to sustain good production of lovastatin by *M. ruber* CCRC 31535 [22]. Preliminary data indicated that the major variables affecting the performance of the culture in terms of lovastatin yields are the levels of rice powder, peptone, glycerin, and glucose. Therefore, these four medium ingredients were chosen for further optimization through RSM. Initially, a complete four-factor-two-level factorial design followed the method of steepest ascent was carried out to find the general vicinity of optimum concentration of carbon and nitrogen sources for lovastatin production.

2.4.1. Factorial design

In the first experiment of this series, the ranges of the variables tested were 24.4–44.4 g/L rice powder, 5.8–15.8 g/L peptone, 16.4–36.4 ml/L glycerin, 81.2–177.2 g/L glucose. For the first phase of the optimization process in which the region close to the optimum is to be approached, two-level factorial designs were chosen. In this experimental design, the main effects and interactions of different factors, each at two different levels, can be simultaneously investigated. For a full factorial design, all possible combinations

Table 1
Process variables and their levels for the four-factor two-level (2^4) first-order RSM experiments (above); Matrix corresponding to 2^4 factorial designs together with the observed experimental data (below)

Independent Variables	Symbol	Code Levels		
		−1	0	+1
Rice powder (g/L)	X_1	24.4	34.4	44.4
Peptone (g/L)	X_2	5.8	10.8	15.8
Glycerin (ml/L)	X_3	16.4	26.4	36.4
Glucose (g/L)	X_4	81.2	129.2	177.2

No.	Rice powder (g/L)	Peptone (g/L)	Glycerin (ml/L)	Glucose (g/L)	Observed Lovastatin Yield (mg/L)
1	24.4	5.8	16.4	81.2	24.0
2	44.4	5.8	16.4	81.2	26.0
3	24.4	15.8	16.4	81.2	14.0
4	44.4	15.8	16.4	81.2	5.0
5	24.4	5.8	36.4	81.2	22.0
6	44.4	5.8	36.4	81.2	6.0
7	24.4	15.8	36.4	81.2	20.0
8	44.4	15.8	36.4	81.2	5.0
9	24.4	5.8	16.4	177.2	18.0
10	44.4	5.8	16.4	177.2	16.0
11	24.4	15.8	16.4	177.2	28.0
12	44.4	15.8	16.4	177.2	2.0
13	24.4	5.8	36.4	177.2	9.0
14	44.4	5.8	36.4	177.2	3.0
15	24.4	15.8	36.4	177.2	17.0
16	44.4	15.8	36.4	177.2	7.0
17(C)	34.4	10.8	26.4	129.2	120.0
18(C)	34.4	10.8	26.4	129.2	131.0

No. 17(C), 18(C) were two replication of center points

of the two levels of the independent variables are investigated. For a 2^4 factorial design with four factors at two levels, sixteen experimental runs are required. Two center points were added to estimate the experimental error and check the adequacy of the first-order model. Table 1 shows the four independent variables and their concentrations at the different coded levels of the factorial design experiments. The four factors are rice powder (X_1), peptone (X_2), glycerin (X_3), and glucose (X_4), and their upper and lower levels in this initial design were chosen in reconciliation with the data of our previous work on lovastatin production by *M. ruber* CCRC 31535 [22]. The matrix corresponding to 2^4 factorial design, together with the observed experimental data is also shown in Table 1. To avoid bias, the total of 18 runs was performed in a random order (overall randomization). Statistica, version 5.0 (Statsoft, Inc., Tulsa, OK USA) was used for the regression analysis of the experimental data obtained. The quality of fit of the first-order model equation was expressed by the coefficient of determination R^2, and its statistical significance was determined by an F-test. The significance of the regression coefficients was tested by a t-test.

2.4.2. Path of steepest accent (descent)

The method of steepest ascent (descent) is a procedure for moving sequentially along the path of steepest ascent (descent), that is, in the direction of the maximum increase (decrease) in the response. Based on the results obtained from the factorial design, the fitted first-order model is

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i \quad (1)$$

Y is the predicted response; β_0, β_i, *are constant coefficients, and* x_i *is the coded independent variables or factors*.

The direction of steepest ascent (descent) is the direction in which Y increases (decreases) most rapidly. This direction is parallel to the normal to the fitted response surface. One usually takes as the path of steepest ascent (descent) the line through the center of the region of interest and normal to the fitted surface. Thus, the steps along the path are proportional to the regression coefficients β_i. The path of steepest ascent (descent) started from the center of the first design. To move away from the first design center along the path of steepest ascent (descent), we move −5.25, −0.80, −2.95, −7.20 units in X_1, X_2, X_3, X_4 directions, respectively. These new units were determined from concentration range of unity level from first design and estimated coefficient ratio from equation (1). The design and experimental results along the path of steepest ascent is shown on Table 2.

Table 2
Experimental results along the path of steepest ascent

	Rice powder (g/L)	Peptone (g/L)	Glycerin (m/L)	Glucose (g/L)	Lovastatin (mg/L)
(1) Base Point (Zero level in the 2^4 factorial designs)	34.40	10.80	26.40	129.20	125.5*
(2) Origin Step Unit (Concentration range of unity level)	10.00	5.00	10.00	48.00	
(3) Slope (Estimated coefficient ratio from equation (3))	−10.5	−3.2	−5.5	−3.0	
(4) Correspondent Concentration Range (2) × (3)	−105	−16	−55	−144	
(5) New Step Unit (4) × 0.05#	−5.25	−0.80	−2.95	−7.20	
(6) Experiment No. 1	34.40	10.80	26.40	129.20	123.0
No. 2	29.15	10.00	23.65	122.00	13.0
No. 3	23.90	9.20	20.90	114.80	13.0
No. 4	18.65	8.40	18.15	107.60	11.0
No. 5	13.40	7.60	15.40	100.40	10.0
No. 6	8.15	6.80	12.65	93.20	4.0

* Average value of the observed lovastatin yields of No. 17(C) and 18(C) in Table 1
# 0.05 is a factor determined by experimenter based on process knowledge or other practical consideration, 0.05 is appropriate in this example

2.4.3. Central composite design

The central composite design (CCD) was conducted in the optimum vicinity to locate the true optimum concentrations of rice powder, peptone, glycerol, and glucose for lovastatin production. For the four factors, this trial was essentially a full 2^4 factorial design augmented by eight axial points (or called star points) and three replication of center points, resulting in a total number of 27 experiments. The experimental results of the CCD were fitted with a second-order polynomial equation by a multiple regression technique.

$$Y = \beta_0 + \sum_{i=1}^{K} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i x_j + \sum\sum_{i<j} \beta_{ij} x_i x_j \qquad (2)$$

Y is the predicted response; β_0, β_i, β_{ii}, β_{ij} are constant coefficients, and x_i, x_j are the coded independent variables or factors.

The quality of fit of the second-order model equation was expressed by the coefficient of determination R^2, and its statistical significance was determined by an F-test. The significance of the regression coefficients was tested by a t-test. The computer software used was Statistica, version 5.0 by Statsoft, Inc. (Tulsa, OK USA).

3. Results and discussion

RSM is a sequential procedure with an initial objective to lead the experimenter rapidly and efficiently along a path of improvement toward the general vicinity of the optimum. Although two-level (full or fractional) factorial experiments will only yield data to fit a limited model (equation (1)), they are the most common initial experiments in the application of RSM, because the orthogonality of the design minimizes the variance of the regression coefficients. Besides, any first-order (two-level) orthogonal design is rotational [23,24]. Since the location of the optimum is unknown prior to running RSM experiment, it is conceivable to use a design with rotation that ensures equal precision of estimation in all direction.

3.1. Factorial design

The experimental results of lovastatin productions by a complete four-factor-two level factorial (2^4) experiment design augmented with two center points are shown in Table 1. Repeat observations at the center were used to estimate the experimental error and to allow for checking the adequacy of the first-order model. In order to approach the vicinity of the optimum, a first-order model was fitted to the data obtained from the factorial design experiment. From the analysis of the data in Table 1 by least-squares method, a first-order model was best fit to these data. The main effects of the four factors-rice powder (X_1), peptone (X_2), glycerin (X_3), and glucose (X_4)-were calculated to be −10.5, −3.2, −5.5, −3.0, respectively. We obtained the following model in the coded variables.

First-order Model Equation

$$Y_{(mg/L)} = 26.2 - 10.5X_1 - 3.2X_2 - 5.5X_3 - 3.0X_4 \qquad (3)$$

From the first-order model equation (3), it is predicted that decreasing the concentrations of rice powder (X_1), peptone (X_2), glycerin (X_3), and glucose (X_4) should enhance lovastatin production; however, the effects will not be significant.

Based on the first-order model equation obtained, the path of steepest ascent was determined to find proper direction of changing variables increasing or decreasing the concentration according to the sign of the main effects to improve lovastatin production. The path of steepest ascent started from the center of the factorial design and moved along the path in which the concentrations of rice powder (X_1), peptone (X_2), glycerin (X_3), and glucose (X_4) were

Table 3
Experimental design and results of the central composite design

No.	Rice powder (g/L)	Peptone (g/L)	Glycerin (ml/L)	Glucose (g/L)	Lovastatin (mg/L)
1	19.4	5.8	14.4	79.2	44.0
2	19.4	5.8	14.4	179.2	66.0
3	19.4	5.8	38.4	79.2	36.0
4	19.4	5.8	38.4	179.2	29.0
5	19.4	15.8	14.4	79.2	9.0
6	19.4	15.8	14.4	179.2	24.0
7	19.4	15.8	38.4	79.2	78.0
8	19.4	15.8	38.4	179.2	59.0
9	49.4	5.8	14.4	79.2	16.0
10	49.4	5.8	14.4	179.2	55.0
11	49.4	5.8	38.4	79.2	51.0
12	49.4	5.8	38.4	179.2	19.0
13	49.4	15.8	14.4	79.2	13.0
14	49.4	15.8	14.4	179.2	25.0
15	49.4	15.8	38.4	79.2	17.0
16	49.4	15.8	38.4	179.2	18.0
17	4.4	10.8	26.4	129.2	16.0
18	64.4	10.8	26.4	129.2	12.0
19	34.4	0.8	26.4	129.2	18.0
20	34.4	20.8	26.4	129.2	52.0
21	34.4	10.8	2.4	129.2	108.0
22	34.4	10.8	50.4	129.2	88.0
23	34.4	10.8	26.4	29.2	39.0
24	34.4	10.8	26.4	229.2	51.0
25(C)	34.4	10.8	26.4	129.2	129.0
26(C)	34.4	10.8	26.4	129.2	117.0
27(C)	34.4	10.8	26.4	129.2	146.0

No. 25(C), 26(C), 27(C) were three replication of center points

decreasing. The design and results of the path of steepest ascent experiments are shown in Table 2. It is shown that the highest production response is 123.0 mg/L, at initial point of the path. This suggested that the center of the original factorial design is near the regions of maximum production response.

3.2. Central composite design

To fully explore the sub-region of the response surface in the neighborhood of the optimum, an experimental design with more than two levels of each factor is required, so that a second order approximation to the response surface can be developed. A CCD with five coded levels was used for this purpose. The levels of the variables for the CCD experiments were selected according to the results of the previous experiments. The CCD design and the corresponding experimental data were shown in Table 3. By applying multiple regression analysis on the experimental data, the experimental results of the CCD design were fitted with a second-order polynomial equation (equation (2)). The results of the regression analysis are shown in Table 4. Judging from the regression coefficients and corresponding t values in Table 4 we conclude that all the linear terms and the interaction terms were not significant ($p < 0.05$) and all the quadratic terms exerted a significant effect on lovastatin production. Therefore, the second-order polynomial equation obtained for lovastatin production is

Table 4
Regression results from the data of central composite designed experiments

Parameter	Parameter Estimate	Standard Error	T ratio	Probability
Intercept	130.4998	11.5401	11.3084	9×10^{-8}*
X_1	−11.4804	8.1601	−1.4069	0.1848
X_2	−0.4515	8.1601	−0.0553	0.9568
X_3	1.2368	8.1601	0.1516	0.8820
X_4	4.7267	8.1601	0.5793	0.5731
X_1X_1	−62.7433	8.6550	−7.2493	1×10^{-5}*
X_1X_2	−7.7856	9.9940	−0.7790	0.4511
X_1X_3	−7.9898	9.9940	−0.7995	0.4396
X_1X_4	0.8760	9.9940	0.0877	0.9316
X_2X_2	−52.2337	8.6550	−6.0351	0.0001*
X_2X_3	18.1344	9.9940	1.8145	0.0947
X_2X_4	−1.6956	9.9940	−0.1697	0.8681
X_3X_3	−20.6863	8.6550	−2.3901	0.0341*
X_3X_4	−17.8954	9.9940	−1.7906	0.0986
X_4X_4	−47.0383	8.6550	−5.4348	0.0002*

* Significant at the 5% level ($p < 0.05$); Determination of coefficient $R^2 = 0.876$
X_1: Rice powder
X_2: Peptone
X_3: Glycerin
X_4: Glucose

Second-order Model Equation

$$Y_{(mg/L)} = 130.50 - 62.74X_1^2 - 52.23X_2^2 - 20.69X_3^2 - 47.04X_4^2 \quad (4)$$

This fit of the model was checked by the coefficient of determination R^2, which was calculated to be 0.876, indicating that 87.6% of the variability in the response could be explained by the model. The test statistics F values for the overall regression is significant at the upper 5% level, which further supported that the second-order model is very adequate in approximating the response surface of the experimental design. Since all coefficients of the above equation are all negative, the response surface is suggested to have a maximum point. The optimal concentrations for the four components as obtained from the maximum point of the model were calculate to be 34.4 g/L, 10.8 g/L, 26.4 ml/L, and 129.2 g/L for rice powder, peptone, glycerin, and glucose, respectively. The model predicted a maximum response of 130.5 mg/L lovastatin yield for this point. Verification of the calculated optimum was done with a culture medium representing this maximum point and yielding lovastatin 131.0 mg/L (average of three repeats). The excellent correlation between predicted and measured values of these experiments justifies the validity of the response model and the existence of an optimum point.

There were many reports on the production of lovastatin by *M. ruber* existed in the literature [7,8,12]. However, the production yield is extremely low. In most cases the yields

are generally lower than 20 mg/L. For examples, 87 mg of monacolin K was purified from 5 liters of culture medium when *M. ruber* 1005 was grown aerobically at 28°C in a medium containing 6% glucose, 2.5% peptone, 0.5% corn steep liquor and 0.5% ammonium chloride for 10 days. *M. ruber* M82121 produced monacolin K (10–20 mg/L) when it was grown aerobically at 25°C in a medium containing 1% glucose, 11% glycerol, 5% soy bean powder, 0.8% peptone, 0.1% $NaNO_3$, 0.05% $Zn(NO_3)_2$ and 0.5% olive oil for 11 days. In the present study, the lovastatin yield has increased significantly through application of a mixed solid-liquid state cultivation technique and RSM method. In addition, the use of rice powder in substitution for corn steep or soybean powder previously used as one of the carbon sources for lovastatin production by *M. ruber* is unique.

Recently, Li and coworkers demonstrated that *Monascus purpureus*-fermented rice (red yeast rice), a natural food product, reduced blood cholesterol in three animal models of hypercholesterolemia, and suggested that suitably prepared *M. purpureus* (red yeast rice) could be an effective hypochlesterolemic agent in man [25]. Furthermore, the use of proprietary Chinese red yeast as a dietary supplement in a human clinical trial has shown significant reductions of cholesterol levels, from 254 ± 36 mg/dL to 208 ± 3 mg/dL in eight weeks, in 83 tested individuals [26]. These facts indicate that the production of lovastatin by *Monascus* species might be advantageous with an increased saving in capital costs, because the extraction and purification steps might not be necessary. The whole fermentation broth may be used directly as a health functional food as long as it proves to be nontoxic.

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References

[1] Alberts AW, Chen J, Kuron G, Hunt V, Huff J, Hoffman C, Pothrock J, Lopez M, Joshua H, Harris E, Patchett A, Monaghan R, Currie S, Stapley E, Alber-Schoenberg G, Hensens O, Hirshfield J, Hoogsteen K, Liesch J, Springer J. Mevinolin, a highly potent competitive inhibitor of hydroxymethyl-glutaryl coenzyme A reductase and chlosterol lowering agent. Proc Natl Acad Sci USA 1980;77(7):3957–61.

[2] Kaneko I, Hazuma-Shimada Y, Endo A. Inhibitory effects on lipid metabolism in cultured cells of ML-236B, a potent inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Eur J Biochem 1978; 87:313–21.

[3] Goldstein JL, Brown MS. Progress in understanding the LDL receptor and HMG-CoA reductase, two membrane proteins that regulate the plasma cholesterol. J Lipid Res 1984;25:1450–61.

[4] Lipid Research Clinics Program: The lipid research clinics coronary primary prevention trial results, I. Reduction in incidences of coronary heart disease. J Am Med Assoc 1984;251:351–64. & II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. J Am Med Assoc 1984;251:365–74.

[5] Jones KD, Couldwell WT, Hinton DR, Su YH, He DK, Anker L, Law RE. Lovastatin induces growth inhibition and apoptosis in human malignant glioma cells. Biochem Biophys Res Commun 1994;205:1681–7.

[6] Newman A, Clutterbuck RD, Powles RL, Millar JL. Selective inhibition of primary acute myeloid leukemia cell growth by lovastatin. Leukemia 1994;8:2022–9.

[7] Endo A. Monacolin K, a new hypocholesterolemic agent produced by a *Monascus* species. J Antibiot 1979;32:852–4.

[8] Negishi S, Huang ZC, Hasumu K, Murakawa S, Endo A. Productivity of monacolin K (mevinolin) in the genus *Monascus* (in Japanese). Hakko Kogaku Kaishi 1986;64:584–90.

[9] Alberts AW. Discovery, biochemistry and biology of lovastatin. Am J Cardiol 1988;62:10–5J.

[10] Endo A, Hasumi K, Yamada A, Shimoda R, Hiroshi T. The synthesis of compactin (ML-236B) and monacolin K in fungi. J Antibiot 1986;39:1609–16.

[11] Buckland B, Gbewonyo K, Hallada T, Kaplan L, Masurekar P. Production of lovastatin, an inhibitor of cholesterol accumulation in human. In: Demain AL, Somkuti GA, Hunter-Cevera JC, Rossmoore HW, editors. Novel Microbial Products for Medicine and Agriculture. New York: Elsevier, 1989:161–9.

[12] Szakács G, Morovján G, Tengerdy RP. Production of lovastatin by a wild strain of *Aspergillus terreus*. Biotechnol Lett 1998;20(4):411–5.

[13] Jůzlová P, Martínková L, Křen V. Secondary metabolites of the fungus *Monascus*: a review. J Indus Microbiol 1996;16:163–70.

[14] Endo A, Hasumi K, Nakamura T, Kunishima M, Masuda M. Dihydromonacolin L and monacolin X, new metabolites that inhibit cholesterol biosynthesis. J Antibiot 1985;38(3):321–7.

[15] Buchanan RL, Philips JG. Response surface model for predicting the effects of temperature, pH, sodium chloride content, sodium nitrite concentration and atmosphere on the growth of *Listeria monocutogenes*. J Food Protect 1990;53:370–6.

[16] Haltrich D, Press M, Steiner W. Optimization of a culture medium for increased xylanase production by a wild strain of *Schizophyllum commune*. Enzyme Microb Technol 1993;15:854–60.

[17] Mudahar S, Toledo RT, Floros JD, Jen JJ. Optimization of carrot dehydration process using response surface methodology. J Food Sci 1989;54:714–9.

[18] Prapulla SG, Jacob S, Chand N, Rajalakshmi D, Karanth NG. Maximization of lipid production by *Rhodotroula gracilis* CFR-1 using response surface methodology. Biotech Bioeng 1992;40:965–9.

[19] Zertuche L, Zall RR. Optimizing alcohol production from whey using computer technology. Biotechnol Bioeng 1985;27:547–54.

[20] Box GEP, Wilson KB. On the experimental attainment of optimum conditions. J Roy Statistical Soc (Ser. B) 1951;13:1–45.

[21] Hsieh FL. Production of hypocholesterolemic agent by *Monascus* in solid-liquid culture. Master Thesis. 1998. Changhua, Taiwan, ROC: Department of Food Engineering, Da-Yeh University.

[22] Huang JC, Mu CJ, Zheng YK, Chang YN. Effect of glycerin on monacolin K production by *Monascus ruber*. Poster presented at annual meeting of Chinese Chemical Society. Nov 24–26, 2000. Taichung, Taiwan, P-1B-BI-001.

[23] Mason RL, Gunst RF, Hess JL. Statistical Design and Analysis of Experiments with Application to Engineering and Science. New York: John Wiley & Sons, 1989.

[24] Montgomery DC. Design and Analysis of Experiments. New York: John Wiley & Sons, 1991.

[25] Li C, Zhu Y, Wang Y, Zhu JS, Chang J, Kritchevsky D. *Monascus purpureus*-fermented rice (red yeast rice): a natural food product that lowers blood cholesterol in animal models of hypercholesterolema. Nutri Res 1998;18(1):71–81.

[26] Heber D, Yip L, Ashely JM, Elashoff DA, Elashoff RM, Go VLW. Cholesterol lowering effects of a proprietary Chinese red-yeast-rice dietary supplement. Am J Clin Nutr 1999;69:231–6.