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Full Length Research Paper

Optimization of fermentation medium for nisin production from *Lactococcus lactis* subsp. *lactis* using response surface methodology (RSM) combined with artificial neural network-genetic algorithm (ANN-GA)

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Nisin is a bacteriocin approved in more than 50 countries as a safe natural food preservative. Response surface methodology (RSM) combined with artificial neural network-genetic algorithm (ANN-GA) was employed to optimize the fermentation medium for nisin production. Plackett-Burman design (PBD) was used for identifying the significant components in the fermentation medium. After that, the path of steepest ascent method (PSA) was employed to approach their optimal concentrations. Sequentially, Box-Behnken design experiments were implemented for further optimization. RSM combined with ANN-GA were used for analysis of data. Specially, a RSM model was used for determining the individual effect and mutual interaction effect of tested variables on nisin titer (NT), an ANN model was used for NT prediction, and GA was employed to search for the optimum solutions based on the ANN model. As the optimal medium obtained by ANN-GA was located at the verge of the test region, a further Box-Behnken design based on the RSM statistical analysis results was implemented. ANN-GA was implemented using the further Box-Behnken design data to locate the optimum solution which was as follow (g/l): Glucose (GLU) 15.92, peptone (PEP) 30.57, yeast extraction powder (YEP) 39.07, NaCl 5.25, KH_2PO_4 10.00, and $MgSO_4 \cdot 7H_2O$ 0.20, with expected NT of 22216 IU/ml. The validation experiments with the optimum solution were implemented in triplicate and the average NT was 21423 IU/ml, which was 2.13 times higher than that without ANN-GA methods and 8.34 times higher than that without optimization.

Key words: Response surface methodology, artificial neural network, genetic algorithm, nisin titer.

INTRODUCTION

Nisin is a bacteriocin produced by molecular strains of *Lactococcus lactis* subsp. *lactis*. It is composed of 34 amino acids and approved in more than 50 countries as a natural food preservative for various products such as processed cheese, beverages, canned foods, etc. Nisin is reported to act primarily upon the cytoplasmic membrane, and is effective against a broad range of Gram-positive bacteria and their spores. Besides, it can also inhibit the growth of a wide variety of Gram-negative bacteria with ethylenediaminetetraacetic acid (EDTA) (Cheigh and Pyun, 2005; Delves-Broughton et al., 1996; Economou et al., 2009; Soriano et al., 2004). The nisin precursor is synthesized in the early active growth phase, the nisin production rate is maximal towards the end of the exponential growth phase, and cells completely stop nisin biosynthesis when entering the stationary growth phase. Therefore, nisin production is greatly influenced by the growth of bacteria and the fermentation media (Liu et al.,

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2005; De Vuyst and Vandamme, 1993; Mirdamadi et al., 2008; Wardani et al., 2006). Optimal nisin production in batch culture usually requires a complex fermen-ation media. Several literatures presented studies on the optimization of fermentation media for nisin production using statistical methods, such as response surface methodology (RSM), multi-linear regression and logistic model. However, these methods are not suitable for such complex system, and it is difficult to develop satisfying models with high predictive accuracy using these methods (Lv et al., 2004; Lv et al., 2005; Penna and Moraes, 2002; Vazquez and Murado, 2008).

RSM is a collection of statistical techniques for designing experiments, developing models, evaluating the effects of factors, and searching for optimum conditions. Plackett-Burman design (PBD) is a 2-level experimental design that requires fewer runs than a comparable fractional design. It can be used to identify the significant factors among many candidate factors. The path of steepest ascent (PSA) is a procedure for moving sequentially along the path of the steepest ascent, that is, in the direction of the maximum increase in the response. Box-Behnken design and multi-quadric regression could find out the relationship between the variables and response values based on statistical analysis, and the optimum of each variable would be obtained as well. RSM has been extensively used in the optimization of medium composition, conditions of enzymatic hydrolysis, fermentation and food manufacturing processes (Choudhari and Singhal, 2008; Ghosalkar et al., 2008; Mannan et al., 2007; Wang and Liu, 2008; Wu et al., 2007).

Artificial neural network (ANN) is a non-linear computational model based on biological neural networks. It simulates the human brain learning process by mathematically modeling the network structure of interconnected node cells. ANN has been utilized with high success for system design, modeling, optimization and control mainly due to their capacity to learn, filter noisy signals and generalize information through a training procedure. Several literatures have demonstrated that the predictive accuracy of ANN models were superior to RSM model using the same experimental design. The advantages of ANN compared to RSM are: (i) ANN does not require a prior specification of suitable fitting function and (ii) ANN has universal approximation capability, that is, it can approximate almost all kinds of non-linear functions including quadratic functions, whereas RSM is useful only for quadratic approximations. However, ANN is known as a black box modeling approach. Therefore, the effect of factors on response values and the interaction effect among the factors cannot be studied by ANN model. Genetic algorithm (GA) which is an artificial intelligence based stochastic non-linear optimization formalism, is used to optimize the input space of the ANN model. This hybrid methodology will be referred to as ANN-GA hereafter. The GA mimics the principles of biological evolution, namely “survival-of-the-fittest” and “random exchange of data during propagation” followed by biologically evolving species. GA has been considered an ideal technique to solve diverse optimization problems in biochemical engineering. In the present work, RSM and ANN-GA were applied to optimize the fermentation media for nisin production simultaneously. This method can much more efficaciously evaluate both the effect of the individual factor and the interaction effect among the factors, and increase the nisin titer (NT) as well (Chen et al., 2004; Desai et al., 2006; Moreira et al., 2007; Nagy, 2007; Singh et al., 2008; Tramer and Fowler, 1964).

MATERIALS AND METHODS

Microorganism and seed culture preparation

A mutant strain *L. lactis* subsp. *lactis* CGMCC NO. 3050 obtained by treating *L. lactis* subsp. *lactis* ATCC 11454 with diethyl sulfate (DES) was used for the optimization studies. *L. lactis* subsp. *lactis* ATCC 11454 was purchased from the American Type Culture Collection. *L. lactis* subsp. *lactis* CGMCC NO. 3050 was collected in China General Microbiological Culture Collection Center. It was maintained on CM slants medium composed of (g/l): Glucose (GLU) 10, peptone (PEP)10, yeast extraction powder (YEP)10, KH_2PO_4 10, NaCl 2, $MgSO_4 \cdot 7H_2O$ 0.2 and agar 15. The medium was adjusted to pH 6.8 using 5 M NaOH before autoclaving at 121°C for 30 min. GLU was separately autoclaved at 105°C for 10 min. The slants were inoculated to 3 ml sterile CM liquid medium in cuvette (15 ml) and incubated at 37°C in a rotary shaker at 150 rpm for 24 h. Then, the culture was inoculated to Erlenmeyer flask (250 ml) containing 100 ml sterile CM liquid medium, which was incubated at 37°C in a rotary shaker at 150 rpm for 18 h, and the seed culture was prepared.

Production media and cultivation condition

The initial medium for nisin production was CM medium. The concentrations of the components in CM medium were varied depending on the experimental design used for nisin production fermentation. 3% (v/v) inoculum was added aseptically to Erlenmeyer flasks (250 ml) containing 100 ml of designed medium. The fermentation medium was incubated at 37°C in rotary flasks at 150 rpm for 24 h.

Analytical methods

NT was measured by a modified method of Tramer and Fowler (1964). The fermentation broth was adjusted to pH 2 using 10 M HCl, and then centrifuged at 5000 rpmin for 10 min. The supernatant was appropriately diluted with 0.02 M HCl and nisin assay were performed by an agar well diffusion assay. A standard curve (200 - 3000 IU /ml) was plotted using a stock solution of 4000 IU/ml nisin (Sigma, USA). Each assay was performed in triplicate and the average result was presented.

PBD

PBD experiments had 9 factors and 2 levels, employed to select the Significant factors based on statistical analysis. Among the 9 factors, six were the components in the CM medium. The high (+1) and low (-1) level of the six components were GLU (X_1) 12.5 and 10.0 g/l; PEP (X_2) 12.5 and 10.0 g/l; YEP(X_3) 12.5 and 10.0 g/l; KH_2PO_4 (X_4) 12.5 and 10.0 g/l; NaCl (X_5) 2.5 and 2.0 g/l; $MgSO_4 \cdot$

$7H_2O$ (X_6) 0.25 and 0.20 g/l. The other three factors were dummy variables (X_7, X_8, X_9), used for estimating the experimental error and checking the adequacy of the first-order model. Twelve experimental runs were carried out in the present work. Then, a first-order model in coded variables was obtained using SAS (version 8.02) for the regression analysis of the experimental data. At the end of this stage, GLU, PEP, YEP, and NaCl were screened out as significant factors that impact the NT.

PSA method

PSA experiments were applied to approach the optimal regions of the significant factors while the non-significant were set at the lowest level. PSA started from the center of PBD and moved along the path in which the concentration of GLU, PEP, YEP and NaCl increased according to their coefficients in the above mentioned first-order model.

Box-Behnken design

Box-Behnken design was used for further optimization of fermentation media. The low (-1), middle (0) and high level (1) of GLU (U_1), PEP (U_2), YEP (U_3) and NaCl (U_4) in Box-Behnken design were 13.2, 23.2 and 33.2 g/l ; 12.6, 22.6 and 32.6 g/l; 7.2, 17.2 and 27.2 g/l; 4.4, 6.4 and 8.4 g/l, respectively.

The integration of RSM and ANN for modeling

In RSM modeling, the second-order polynomial coefficients were calculated using SAS 8.02 to estimate the responses of the dependent variables. Multilayer perceptron (MLP) is a commonly used feed-forward ANN model that maps sets of input data onto a set of appropriate output data. In this work, the neural network architecture had three layers, namely the input, hidden and output layer. The concentrations of the significant components in CM medium (coded data) were used as the input nodes, and the NTs were used as the output nodes.

The number of hidden nodes greatly affects the capabilities of the ANN model. Generally, when the number of hidden nodes increases, the ANN model fits the training set better. However, too many hidden nodes will lead to ANN model over-fitting. In order to avoid the over-fitting of ANN model, a criterion named the degree of approximation (D_a) (Guo et al., 2006) was employed to select the suitable number of hidden nodes, as defined in Equation 1

$$D_a = \frac{c}{\frac{n_c}{n} \times MSE_c + \frac{n_t}{n} \times MSE_t + \left|MSE_c - MSE_t\right|} \quad (1)$$

Where, MSE_c and MSE_t are the mean-square-errors (MSE) of calibration set and validation set, respectively; n_c and n_v, number of calibration set and validation set; n, sum of the number of calibration set and validation set; c, a constant number (in the present work c was 1000) by which D_a was adjusted to get a good chart.
According to the above equation, it was obvious that the larger D_a obtained, the more the ANN models approached the experimental data. Since D_a achieved the maximum value when there were 15 hidden nodes, the number of hidden nodes was set to 15.
Training of ANN was minimized by adjusting the network weights appropriately using Levenberg-Marquardt algorithm. The ANN model was trained with Levenberg-Marquardt algorithm for 1000 interations. The MSE function, a commonly employed error function, was used in this work and defines as in Equation 2:

$$MSE = \frac{\sum_i^n \left(y_{BPANNi} - y_{ACTUi}\right)^2}{n} \quad (2)$$

Where, n referred to the number of patterns used in the training; i denotes the index of the input pattern (vector); y_{ACTUi} and y_{BPANNi} were the desired and predicted outputs of the nth output node, respectively.

In the present work, the ANN model with a fixed topology structure was developed by neural network fitting tool (nftool) in Matlab 7.6.0. It was trained thirty times with the initial weight given randomly and the best ANN model were selected according to their D_a.

Optimization by ANN-GA

After the optimum ANN model was developed, GA was employed to search for the optimum solution in the input space of the ANN model, which was implemented in Matlab 7.6.0. The values of GA-specific parameters used in the optimization simulations were: Population type as double vector, population size as 20, the initial population as given randomly, selection function as stochastic uniform, elite count as 2, crossover fraction as 0.8, crossover function as scattered, migration fraction as 0.2, penalty factor as 100 and number of generation over which GA evolved as 100. The fitness function was defined as follows:

$$Fitness = \frac{1000}{NT_{ANN \; \mathrm{model}}} \quad (3)$$

Where, $NT_{ANN\ model}$ is the NT calculated by ANN model.

Besides, the GA-based optimization simulations were repeated by using each time a different randomly initialized population of the candidate solutions. Dissimilar initial populations ensure that each time, the GA began its search for the optimal solution from a different search sub-space which helped in locating the lowest local or the global minimum on the fitness function surface.

RESULTS

PBD

Base on the initial medium (CM medium), a PBD experiment was developed. The initial NT was 2568.71 IU/ml obtained by the initial medium. The experimental data of PBD are presented in Table 1 and a linear model was employed to fit these data obtained using SAS 8.02. The first-order model is shown in Equation 4 and the statistical analysis results are shown in Table 1. The coefficient of determination (R^2) was 0.9847, indicating that 98.47% of the variability in the response could be explained by the model. The statistical significance of this model was evaluated by the F-test analysis of variance which reveals that this regression was statistically significant ($P < 0.1$) at 90% confidence level. The F-test was used for identifying the effect of candidate factors on NT. The concentrations of GLU (X_1), PEP (X_2), YEP (X_3) and NaCl (X_5) were selected as significant factors.

Table 1. The matrix of the PBD and the experimental results.

Run	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	NT (IU/ml)
1	1	-1	1	-1	-1	-1	1	1	1	1682
2	1	1	-1	1	-1	-1	-1	1	1	1937
3	-1	1	1	-1	1	-1	-1	-1	1	2079
4	1	-1	1	1	-1	1	-1	-1	-1	1937
5	1	1	-1	1	1	-1	1	-1	-1	2570
6	1	1	1	-1	1	1	-1	1	-1	2231
7	-1	1	1	1	-1	1	1	-1	1	1805
8	-1	-1	1	1	1	-1	1	1	-1	1460
9	-1	-1	-1	1	1	1	-1	1	1	890
10	1	-1	-1	-1	1	1	1	-1	1	1682
11	-1	1	-1	-1	-1	1	1	1	-1	1360
12	-1	-1	-1	-1	-1	-1	-1	-1	-1	829
F values	49.303	46.278	13.984	2.043	6.992	1.602	1.618	6.788	0.371	--
p values	0.020	0.021	0.065	0.289	0.118	0.333	0.331	0.121	0.605	--

Table 2. Experimental design and results of PSA.

Run	GLU (g/l)	PEP (g/l)	YEP (g/l)	NaCl (g/l)	Nisin titer (IU/ml)
1	11.2	11.2	11.2	2.2	3947
2	13.2	13.1	12.2	2.9	4236
3	15.2	15.0	13.2	3.6	4546
4	17.2	16.9	14.2	4.3	6030
5	19.2	18.8	15.2	5.0	6946
6	21.2	20.7	16.2	5.7	8000
7	23.2	22.6	17.2	6.4	9214
8	25.2	24.5	18.2	7.1	8586

$$Y=1705.636+301.202X_1+291.818X_2+160.414X_3+61.307X_4+113.431X_5-54.296X_6+54.572X_7-111.764X_8-26.118X_9 \tag{4}$$

The PSA method

PSA started from the center of PBD and moved along the path in which the concentration of GLU, PEP, YEP and NaCl increased according to their coefficients. The design and results of PSA experiments are shown in Table 2. As can be seen, the maximum NT was 9214 IU/ml, when the media were GLU 23.2 g/l, PEP 22.6 g/l, YEP 17.2 g/l, and NaCl 6.4 g/l, respectively. It was suggested that this point is near the region of maximum response.

The integration of RSM and ANN for modeling

The Box-Behnken design matrix and the corresponding experimental data are shown in Table 3. A multi-quadratic model in coded variables and a two-layer feed forward ANN were applied to fit the Box-Behnken design simultaneously. The multi-quadratic model was as follows:

$$Y=9214.551-511.186U_1+1806.975U_2+1790.820U_3-505. \; 1235.416U_2^2\; 445.821U_2U_3-1169.692U_2U_4-1456.222U_3^2 \; .839U_4-1603.912U_1^2+677.520U_1U_2-1491.210U_1U_3+418.087U_1U_4- \; ^2+354.405U_3U_4-2620.689U_4^2 \tag{5}$$

The statistical analysis results of RSM model are shown in Table 4. According to Table 4, the smaller the magnitude of the P-value, the more significant the corresponding co-efficient. The P-values that were less than 0.05 indicating corresponding model terms were significant. The co-efficient estimates and the corres-ponding P-values suggested that among the test factors, U_2 (PEP), U_3 (YEP), U_1U_3 (interaction effect between GLU and YEP),

Table 3. The matrix of Box-Behnken design and experimental results.

Run	U_1	U_2	U_3	U_4	Experimental NT (IU/ml)	$NT_{RSM\ model}$[1] (IU/ml)	$NT_{ANN\ model}$ (IU/ml)
1	-1	-1	0	0	6946	5757	6937
2	-1	1	0	0	8000	8016	8747
3	1	-1	0	0	4236	3380	4252
4	1	1	0	0	8000	8349	7983
5	0	0	-1	-1	4878	4207	4868
6	0	0	-1	1	2407	2487	2419
7	0	0	1	-1	8000	7080	8013
8	0	0	1	1	6946	6777	6564
9	-1	0	0	-1	5619	6425	5621
10	-1	0	0	1	4878	4577	4890
11	1	0	0	-1	3947	4567	3937
12	1	0	0	1	4878	4391	4884
13	0	-1	-1	0	1947	2479	1064
14	0	-1	1	0	5619	6953	5632
15	0	1	-1	0	8000	6985	7933
16	0	1	1	0	9889	9675	9898
17	-1	0	-1	0	2772	3384	2783
18	-1	0	1	0	9889	9948	9886
19	1	0	-1	0	4878	5344	3263
20	1	0	1	0	6030	5943	6038
21	0	-1	0	-1	2975	2888	3676
22	0	-1	0	1	3947	4215	4201
23	0	1	0	-1	8586	8841	8643
24	0	1	0	1	4878	5490	5831
25	0	0	0	0	9216	9215	9193
26	0	0	0	0	9214	9215	9193
27	0	0	0	0	9212	9215	9193

$NT_{RSM\ model}$[1], Nisin titer predicted by RSM model.

U_2U_4 (interaction effect between PEP and NaCl) and all of the quadratic terms were significant in the RSM model.

The twenty-seven Box-Behnken design experimental data were randomly divided into three sets: nineteen of them as calibration set, four as prediction set, and the last four as test set. The ANN model was trained with Levenberg-Marquardt algorithm for 1000 interations. Since D_a achieved the maximum value when there were 15 hidden nodes, the number of hidden nodes was set to 15.

The R^2 of the RSM and ANN models were 0.9394 and 0.9829, respectively, the MS*E* of them were 342222.1 and 206225.7 indicating that the fits of the RSM model and ANN model were satisfied.

The optimized fermentation medium by RSM and ANN-GA

The optimum concentrations of four components in the fermentation medium obtained by RSM model using partial differentiation method were (g/l): GLU 19.3, PEP 28.6, YEP 24.1 and NaCl 5.9. The best solution was expected to result in NT of 10531 IU/ml. The verified experiments were implemented with this fermentation medium in triplicate, and the average NT was 10070 IU/ml. The relative error between the expected value and the verified was 4.3%.

The three best solutions obtained by GA after conducting numerous (~50) generations were: GLU 16.81, 16.91 and 16.01 g/l; PEP 28.59, 28.82 and 28.32 g/l; YEP 27.20, 27.20 and 27.20 g-/l; NaCl 5.25, 5.23 and 5.22 g/l. It was also observed that despite beginning the search in a different search space, the GA converged to similar optimal solution corresponding to the lowest local or global minimum on the fitness function surface. The expected NTs with the GA-optimized solutions were: 13209, 13206 and 13216 IU/ml. The best set of fermentation medium was expected to result in NT of 13216

Table 4. The statistical results of multi-quadratic regression analysis of the Box-Behnken design.

Source	DF	SS	MS	F	p(Pr > F)
U_1	1	3135738	3135738	4.0811	0.0663
U_2	1	39181924	39181924	50.9941	0.0001
U_3	1	38484447	38484447	50.0863	0.0001
U_4	1	3070479	3070479	3.9961	0.0688
U_1U_1	1	13720188	13720188	17.8564	0.0012
U_1U_2	1	1836134	1836134	2.3897	0.1481
U_1U_3	1	8894825	8894825	11.5763	0.0052
U_1U_4	1	699186.1	699186.1	0.9100	0.3589
U_2U_2	1	8140014	8140014	10.5940	0.0069
U_2U_3	1	795025	795025	1.0347	0.3291
U_2U_4	1	5472713	5472713	7.1226	0.0205
U_3U_3	1	11309770	11309770	14.7193	0.0024
U_3U_4	1	502410.7	502410.7	0.6539	0.4345
U_4U_4	1	36629387	36629387	47.6720	0.0001
Model	14	1.4299E8	10213769	13.2929	0.0001
Linear	4	83872587	20968147	27.2894	0.0001
Quadratic	4	40919888	10229972	13.3140	0.0002
Cross Product	6	18200294	3033382	3.9479	0.0206
Error	12	9220348	768362.3		
Lack of fit	10	9220348	922034.8	5.849E12	0.0001
Pure Error	2	2	3.153E-7		
Total	26	1.5221E8			

IU/ml. The verified experiments of each GA-optimized solution were implemented in triplicate and the average NTs were: 13536, 13428 and 13529 IU/ml. The relative error between the expected values and experiments was lower than 0.25%, which was also lower than that obtained by the RSM model. The maximum NT obtained by ANN-GA increased 34.0% from that obtained by the RSM model.

The further Box-Behnken design experiments and ANN-GA optimization results

The optimized concentration of YEP obtained by ANN-GA was 27.20 g/l which is the same as the highest level in the test region. It indicated the possibility that the optimum solution was beyond the test regions. Therefore, a further Box-Behnken design was developed based on the statistical results of RSM model. According to the statistical results, the effect of NaCl on NT was non-significant and its optimized concentration was located at the center of the test regions. The concentration of NaCl was set at 5.25 g/l, which was obtained by the first ANN-GA model in the second Box-Behnken design. As the linear statistical results of RSM model indicated, the effect of GLU on NT was negative, while the effects of PEP and YEP were positive. Therefore, the optimized concentration of GLU from the first ANN model was set at the highest level and the optimized concentration of PEP and YEP was set at the lowest levels. The step changes of real values of the variables were set based on the coefficients of the linear terms in the RSM model. The low (-1), middle (0) and high level (1) of the GLU (Z_1), PEP (Z_2) and YEP (Z_3) for further Box-Behnken design were 11.17, 13.99 and 16.81 g/l; 18.59, 28.59 and 38.59 g/l; 27.20, 37.11 and 47.02 g/l, respectively, and the results are shown in Table 5. The ANN was applied to model the correlation of the test factor and NT. The process of establishing and optimizing ANN model is similar to that of the first ANN model. The fourteen sets of Box-Behnken design experimental data were randomly divided into three categories: Ten of them as calibration set; two as prediction set and two as test set. The ANN model was trained with Levenberg-Marquardt algorithm and the number of interations was 1000. The most suitable number of hidden nodes selected by D_a was 12. GA was employed to search for the optimum solutions in the new tested regions. The parameters of GA were the same as those used in the first ANN model.

The optimum solution obtained by ANN-GA was: GLU 15.920, PEP 30.572, and YEP 39.074 g/l. The optimum solution was expected to result in NT of 22216 IU/ml. Three validation experiments with the optimum solution were carried out and the average NT was 21423 IU/ml. The relative error between experimental and expected value was 3.6%.

Table 5. The further Box-Behnken design matrix and experimental results.

Run	Z_1	Z_2	Z_3	Experimental NT (IU/ml)	$NT_{ANN\ model}$ (IU/ml)
1	-1	-1	0	15280	14672
2	-1	1	0	12361	12355
3	1	-1	0	14238	14241
4	1	1	0	20270	18654
5	0	-1	-1	5295	5746
6	0	-1	1	16398	16396
7	0	1	-1	18887	17325
8	0	1	1	18887	18879
9	-1	0	-1	4934	4938
10	1	0	-1	15280	15278
11	-1	0	1	10733	10732
12	1	0	1	15280	15292
13	0	0	0	16398	15837
14	0	0	0	15280	15837

DISCUSSION

L. lactis subsp. lactis is a well-known nutritionally fastidious microorganism requiring an abundance of nutrients for cell growth and metabolism (Kim et al., 1997). The results of the PBD data shows that the effect of GLU, PEP and YEP was significant (namely, the carbon sour-ces and the nitrogen sources in the fermentation medium are the key components). The effect of carbon sources and nitrogen sources is positive, indicating that the initial concen-trations were too low. Appropriately increasing the con-centrations of carbon and nitrogen sources will elevate the biomass and enhance nisin production. It was reported that the abundant amino acids such as serine, threonine and cysteine in PEP and YEP highly stimulated nisin production (De Vuyst, 1995), which explains why nisin production can be elevated by increasing the concentrations of PEP and YEP. PSA was employed to search appropriate concentrations of the significant components.

The R^2 of the RSM model and ANN model were 0.9394 and 0.9829 respectively, indicating that the fits of the RSM model and ANN model were satisfied. The fit of ANN model were much better than those of the RSM model. The relative error between the expected value obtained by RSM model and the verified was 4.3%. The relative error between the expected value obtained by ANN-GA and the verified was 0.25%. These results demonstrated that the predictive capability of ANN model was much better than that of the RSM model. These results agreed with the conclusion of several literatures (Desai et al., 2008; Kasiri et al., 2008; Pal and Vaidya, 2009). The maximum NT obtained by ANN-GA increased by 34.0% from that obtained by RSM model. These results demonstrated that the true optimum fermentation medium would not be obtained without ANN-GA. The response surface and contours of RSM model and ANN model, respectively, are shown in Figure 1. As can be seen, the response surface obtained by RSM model was convex and their contours were regular. However, the response surface and contours obtained by ANN model seem to be much more nonlinear and complex. The shapes of contours indicated the mutual interaction effects between the test factors. If the shape of the contour is elliptical, the mutual interaction between the two factors is significant; otherwise, if it is circular, the mutual interac-tion effect is non-significant. From the elliptical contour in Figure 1a, the mutual interaction effect between GLU and YEP was significant. It can also be concluded that the effect of the YEP with low GLU was much more signifi-cant than that with high GLU (namely, GLU inhibit YEP for stimulating nisin product). This phenomenon may be caused by the concentration of GLU being excessive than that needed for cell growth which could lead to the accumulation of the excessive acid metabolites such as lactate which inhibit nisin production (Wardani, et al., 2006). As Figure 1b shows, the optimum regions in the response surface and contours of the ANN model were located at the verge of the test regions. It is possible that the optimum fermentation medium for nisin production was beyond the test regions. A further Box-Behnken design experiments were implemented based on the statistical results of RSM and ANN-GA model, and then a new ANN model was developed. GA was employed to search for the optimum solution. The obtained optimum solution was: GLU 15.92, PEP 30.57, YEP 39.07, NaCl 5.25, K_2HPO_4 10, and $MgSO_4 \cdot 7H_2O$ 0.2 g/l with predicted NT of 22216 IU/ml. When compared to the optimum fermen-tation medium obtained by the first ANN model, the concentrations of PEP and YEP in this fermentation medium increased to 7.95 and 43.62%, respectively. The predicted NT increased to 823%. These results indicated that PEP and YEP can highly stimulate nisin production and dramatically enhance the yield of nisin when the GLU

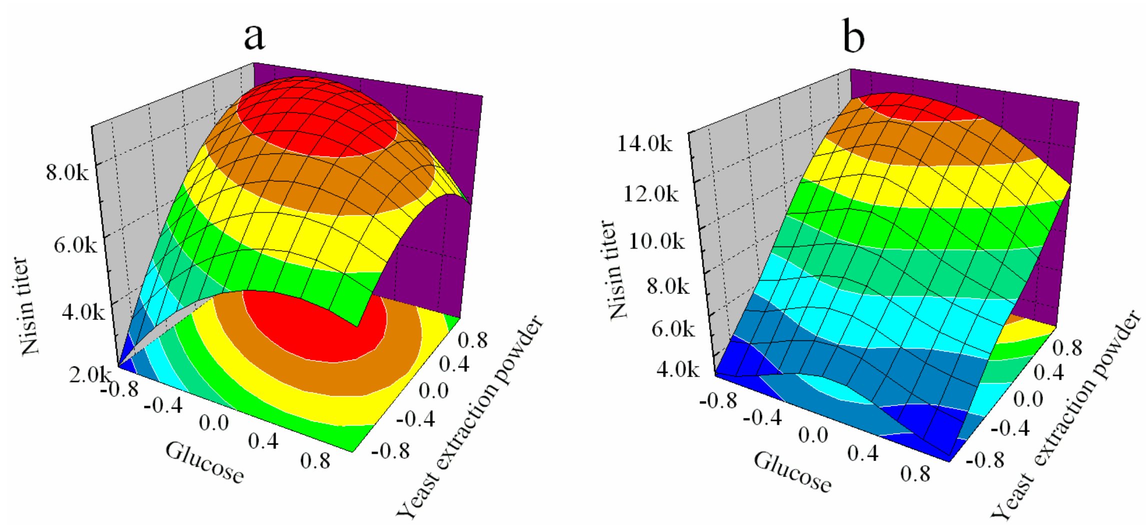

Figure 1. Response surface and contours of the GLU and YEP obtained by RSM model and ANN model (a: RSM model; b: ANN model).

was appropriate. The validation experiments with the optimum solution were implemented in triplicate and the average NT was 21423 IU/ml, which was 8.34 times higher than that without optimization. The relative error between the experimental and expected value was 3.6%, which indicated that the proposed method is feasible.

Abbreviations

RSM, Response surface methodology; **ANN**, artificial neural network; **GA**, genetic algorithm; **PBD,** Plackett-Burman design; **PSA,** path of steepest ascent method; **NT,** nisin titer; **GLU,** glucose; **PEP,** peptone; **YEP,** yeast extraction powder; **EDTA,** ethylenediaminetetraacetic acid; **DES,** diethyl sulfate; **MLP,** multilayer perceptron.

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