

Research Article

Optimization of Process Parameters for *Litopenaeus vannamei* Seafood Sauce using Response Surface Methodology

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Abstract: In this study, in order to determine the optimum formulation of *Litopenaeus vannamei* seafood sauce, Response Surface Methodology (RSM) was employed to investigate the effects of contents of thick broad-bean sauce, sweet soybean paste and Shacha sauce on the sensory quality. Statistical analysis of the results showed that the data were adequately fitted into a second-order polynomial model. The independent parameters of contents of thick broad-bean sauce, sweet soybean paste and the quadratics of contents of thick broad-bean sauce, sweet soybean paste and Shacha sauce significantly affected the sensory evaluation scores. Also the interactions between contents of thick broad-bean sauce and sweet soybean paste, sweet soybean paste and Shacha sauce had a significant effect on the sensory evaluation scores. The optimal conditions for higher sensory evaluation scores were thick broad-bean sauce 6.22%, sweet soybean paste 16.32% and Shacha sauce 10.10%. Under such conditions, the maximum predicted sensory evaluation score was 9.49; the prediction accuracy rate of model was up to 97.10%. These optimum conditions were used to evaluate the trial experiment and the maximum sensory evaluation score was recorded as 9.5±0.36.

Keywords: Response surface methodology, seafood sauce, sensory evaluation

INTRODUCTION

Nowadays, shrimp products are no longer limited to traditional processing methods of dried and frozen, the research and development of new products-shrimp paste is moving toward the market gradually. Currently, the commercially available shrimp paste is made by shrimp powder generally and it was a purplish red sticky sauce, delicate organization and tasted smooth after fermentation, it possessed good flavor and certain consumer market. However, this kind of shrimp paste is fermented sauce, not only do the fermentation requires a certain amount of time, but also there is a bottleneck about effective deodorization, this product has high salinity (Liu and Lin, 2003; Qi *et al.*, 2007). Besides, taste flavor of this liquid shrimp paste is relatively single, it only be used as a condiment. Therefore, the requirements about the ready-to-eat products as direct consumption for consumers can not be satisfied. With the more attention being paid to the food quality, the more diverse of flavor food were demanded.

For this purpose, according to the results of the pre experiments that the contents of thick broad-bean sauce, sweet soybean paste and Shacha sauce have significantly influence on the sensory evaluation of *Litopenaeus vannamei* seafood sauce, the optimum

processing conditions were carried out with analyzing the changes in sensory evaluation scores by response surface methodology, component were compounded scientifically, mathematical model was established, the optimum formulation of seafood sauce was obtained and as well as the preparation process was determined. By this production technique, rich seafood flavor and good quality of the sauce were had, a new way to develop and utilize shrimp products was provided, the full use of abundant shrimp resources in China was made, the added value of raw material was raised, economic development was promoted and the demand for convenience foods of the fast pace of modern life was met at the same time.

MATERIALS AND METHODS

Shrimp, broad bean, wheatmeal, peanut and sesame were bought from supermarket; *Aspergillus oryzae* HuNiang 3.042 and *Aspergillus niger* AS 3.350 were from Baoding Huaimao Co., Ltd. of Hebei province in China.

Preparation of thick broad-bean sauce: The chosen complete broad beans were soaked with warm water about 1.5 h, then had an inoculation and made leaven.

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Table 1: Level and code of variables for box-behken design

Variables	Symbols	Coded levels		
		-1	0	+1
Thick broad-bean sauce (%)	X ₁	3	5	7
Sweet soybean paste (%)	X ₂	10	15	20
Shacha sauce (%)	X ₃	8	10	12

The fungus (*Aspergillus oryzae* HuNiang 3.042 and *Aspergillus niger* AS 3.350) of broad bean weight about 0.3% and proper amount of flour were added and mixed when had the inoculation, after that, they were sent into the ventilation system (Liu *et al.*, 2011). Control product temperature 30~4°C, leaven was turned over when materials got agglomerate, so as to provide sufficient oxygen for fungus, regulate the temperature and moisture, make the mycelium propagate uniformly, the enzyme activity highly. After 72~96 h, leaven was mature when it presented color of yellow and green. The low salt solid-state fermentation method was adopted to prepare embryo of thick broad-bean sauce. The broad bean leaven was mixed with 14° saline water, entered insulation pool, the suitable use level of saline water was the liquid level close to the bean surface, temperature of saline water was determined by atmospheric temperature (summer is the normal temperature, winter is about 50°C), fermentation temperature was about 45~50°C, product temperature was 40°C and time was 7~8 days, salt was added after got out of pool to make salt content of sauce embryo reach about 15%, promote after-ripening. The production of thick broad-bean sauce was made up by thick broad-bean sauce embryo and chili paste with the ratio of 60:40.

Preparation of sweet soybean paste: The flour and water were mixed fully with the ratio of 10:3, steamed for 40~60 min and cooled, the fungus about 0.3% of raw material amount were added, after mixed uniformly, they were sent into the ventilation system. Ventilation and static culture of 10 h, the material temperature was controlled in 35°C, the leaven was turned over and cooling re-circulating air was increased when the materials turn white and agglomerate, after twice of turn leaven, it was about 36 h since they entered the pool and leaven was mature when the color of spore was changed from yellow to green. Mature leaven was putted in fermentation tank to ferment,

added the saline water of which concentration and temperature were 17°Be' and 45~50°C, respectively. Saline water use level was about 85% of raw material, product temperature was controlled in 45°C and materials were dipped in 3 days. The first turnover of sauce was carried out after 3 days, product temperature was controlled about 40°C, after this, the sauce was turned over every other day, the fermentation time was about 60 days, the sweet soybean paste was gained (Cao, 2005).

Preparation of *Litopenaeus vannamei* seafood sauce:

A certain amount of thick broad-bean sauce, sweet soybean paste and Shacha sauce were taken to even mix by fry with a little edible oil, then preprocessed shrimp grain were added, adjuvant material like peanut, sesame were also added, then they were bottling and sealing after mixed uniformly, at last, they were sterilized in the autoclave at 121°C for 15 min, the finished product of *Litopenaeus vannamei* seafood sauce was obtained.

Optimum formulation of *Litopenaeus vannamei* seafood sauce:

In this test, single-factor test and RSM were applied to optimize the formulation of *Litopenaeus vannamei* seafood sauce. The *Litopenaeus vannamei* seafood sauce was mainly made up of shrimp, sauce and peanut, the mass ratio among them is 6: 3: 1. Especially, the sauce was made up of different contents of thick broad-bean sauce, sweet soybean paste and Shacha sauce, the sensory evaluation was used as the main index, the effect of contents of thick broad-bean sauce (1, 3, 5, 7 and 9%), sweet soybean paste (5, 10, 15, 20 and 25%) and Shacha sauce (4, 6, 8, 10 and 12%) on product quality were studied, respectively.

According to the result of single-factor experiments about sauce, the response surface method was designed to optimize parameters, also the best ratio of sauce was determined. Box-Behnken design was implemented and three levels were setted for each of these factors. The level and code of variables were shown in Table 1. The sensory evaluation score was taken as dependent variable or response value.

Method of sensory evaluation: Sensory evaluation (Hu *et al.*, 2012), such as color, taste and smell were carried out by a twenty-member panel who have rich

Table 2: Sensory evaluation standards

Index	Score			Weight /%
	1~4	5~7	8~10	
Color	The color is burned black and matt.	The color is dark, glossiness is not enough.	Lubrication and bright, color is reddish brown.	30
Smell	The smell is not coordinated; the aroma is not prominent, fishy smell or other undesirable smell.	Seafood flavor is rich, but the coordination of smell is not very well, no other undesirable smell.	Sauce flavor is rich; seafood flavor is prominent, no undesirable smell.	30
Taste	The taste is inharmonious, softness is poor and some taste is slightly excessively.	The taste is basically harmonious, have no excessively salty phenomenon, soft taste.	The taste is harmonious and moderate, have both salty and fresh fragrance.	40

experience in the sensory testing of food products, using a 10-point hedonic scale according Table 2.

RESULTS AND DISCUSSION

Regression models of response: The Box-Behnken design was used for optimization of ratio of sauce, 17 experimental runs with different combinations of three factors were carried out. The design matrix of the variables in the coded units was shown in Table 3, along with the predicted and experimental values of response. The predicted values of responses (sensory evaluation scores) were obtained from quadratic model fitting techniques using the software Design Expert.

The maximum sensory evaluation score was achieved in run number 11, 13 and 14, while the minimum score was observed in run number 17. Treatment runs 1, 8, 11, 13 and 14 were the center points in the design, which were repeated five times for estimation of error.

The second order polynomial equation was found by applying multiple regression analysis on the experimental data, to explain the empirical relationship between sensory evaluation scores and the test variable in coded units, which can be given as (1):

$$Y = 9.36 + 0.33X_1 + 0.26X_2 - 0.012X_3 - 0.23X_1^2 - 0.31X_2^2 - 0.30X_3^2 - 0.18X_1X_2 + 0.025X_1X_3 + 0.100X_2X_3 \quad (1)$$

where, Y is the predicted response (sensory evaluation scores); X₁, X₂, X₃ are coded values of contents of thick broad-bean sauce, sweet soybean paste and Shacha sauce, respectively.

Analysis of response surfaces: The statistical significance of Eq. (1) was checked by F-test and the Analysis of Variance (ANOVA) for the response surface quadratic model was summarized in Table 4. Table 4 showed that the model was highly significant, as suggested by the model F-value of 60.52 and a low probability value (p model, F<0.0001). Values of “Prob>F” less than 0.0500 indicate that model terms are significant. Among the independent variables,

Table 3: Box-behnken design matrix along with the experimental and predicted values of sensory evaluation scores

Test number	Variables			Sensory evaluation scores	
	X ₁	X ₂	X ₃	Experimental	Predicted
1	0	0	0	9.3	9.36
2	1	0	1	9.2	9.16
3	-1	0	-1	8.5	8.54
4	1	-1	0	9.0	9.06
5	0	1	1	9.1	9.10
6	0	-1	-1	8.6	8.60
7	1	0	-1	9.2	9.14
8	0	0	0	9.3	9.36
9	1	1	0	9.2	9.24
10	0	1	-1	8.9	8.92
11	0	0	0	9.4	9.36
12	0	-1	1	8.4	8.37
13	0	0	0	9.4	9.36
14	0	0	0	9.4	9.36
15	-1	0	1	8.4	8.46
16	-1	1	0	9.0	8.94
17	-1	-1	0	8.1	8.06

X₁ (thick broad-bean sauce) and X₂ (sweet soybean paste) have an extremely significant (p<0.01) effect on sensory evaluation scores, but the impact of X₃ (Shacha sauce) was not significant (p>0.05). In this case, X₁, X₂, X₁X₂, X₂X₃, X₁², X₂² and X₃² were significant model terms. Evidence is the lack of fit (Mannan *et al.*, 2007). The “Lack-of-Fit F-value” of 2.50 implies the lack of fit is not significant relative to the pure error (p>0.05). There is a 19.85% chance that a Lack-of-Fit F-value this large could occur due to noise. Non-significant lack of fit was good and the equation modeling can be good for date analysis in future was illustrated.

The credibility analysis of the regression equation was shown in Table 5. The Coefficient of Variation (CV) indicates the degree of precision with which the treatments were compared. The higher the value of CV, the lower the reliability of experiment is (Li *et al.*, 2007). The better precision and reliability of the experiments were indicated by a lower value of CV% (0.78). And the precision of a model also can be checked by the determination coefficient (R²), the determination coefficient (R²) implies that the sample variation of 98.73% for sensory evaluation score was attributed to the independent variables and only about

Table 4: ANOVA for the response surface quadratic polynomial model

Source	S.S.	df	M.S.	F-value	Probability (p) >F	Significant
Model	2.68000	9	0.30000	60.52	<0.0001	**
A	0.85000	1	0.85000	171.45	<0.0001	**
B	0.55000	1	0.55000	111.85	<0.0001	**
C	0.00125	1	0.00125	0.25	0.6300	-
AB	0.12000	1	0.12000	24.86	0.0016	**
AC	0.00250	1	0.00250	0.51	0.4994	-
BC	0.04000	1	0.04000	8.12	0.0247	*
A ²	0.22000	1	0.22000	45.19	0.0003	**
B ²	0.39000	1	0.39000	79.47	<0.0001	**
C ²	0.39000	1	0.39000	79.47	<0.0001	**
Lack of fit	0.02200	3	0.00750	2.50	0.1985	-
Pure error	0.01200	4	0.00300			
Cor total	2.72000	16				

** : Extremely significant; * : Significant; - : Not significant; S.S.: Sum of square; M.S.: Mean square

Table 5: The credibility analysis of the regression equation

Item	Value	Item	Value
Standard deviation	0.0700	Adj R-squared	0.9710
Coefficient of Variation (CV)%	0.7800	Pred R-squared	0.8607
R-squared	0.9873	Adeq precision	24.0970

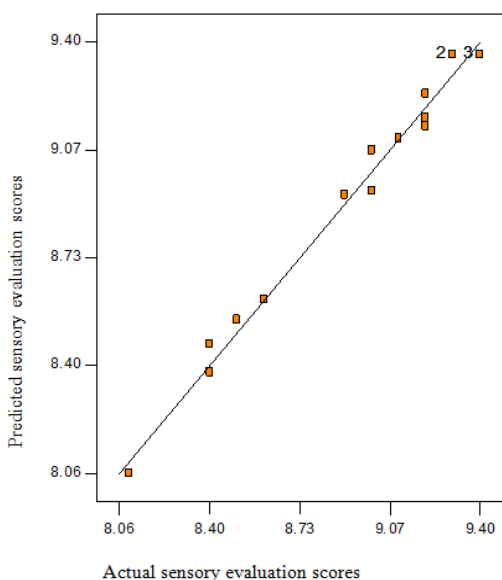


Fig. 1: Actual sensory evaluation scores vs. the predicted sensory evaluation scores under optimum conditions

1.27% of the total variation cannot be explained by the model. The predicted R^2 of 0.8607 is in reasonable agreement with the adjusted R^2 of 0.9710 between the experimental and predicted values of sensory evaluation scores. Therefore, the model adequately represented the real relationship between the parameters chosen.

Figure 1 shows observed the response values (sensory evaluation score) versus those from the empirical model Eq. (1). The figure proves the predicted data of the response from the empirical model is in agreement with the observed ones in the range of the operating variables. The models used in the research were able to identify optimum condition for the sauce.

Localization of optimum condition: The 3D response surface plots described by the regression model were drawn to illustrate the effects of the independent variables and the interactive effects of each independent variable on the response variables. The shape of the corresponding contour plots indicates 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 (Muralidhar *et al.*, 2001).

The 3D plots were depicted in Fig. 2a to c, respectively. The effects of contents of thick broad-bean sauce and sweet soybean paste on the sensory evaluation scores were shown in Fig. 2a, while the content of Shacha sauce was fixed at its middle level

(10%). Figure 2a indicated the sensory evaluation scores increased gradually as the content of sweet soybean paste increased at a low content of thick broad-bean sauce. With the increase in the content of thick broad-bean sauce, the sensory evaluation scores significantly increased at a low content of sweet soybean paste, so the increasing contents of thick broad-bean sauce and sweet soybean paste within the tested range were beneficial to the accumulation of sensory evaluation scores was suggested. These sauces made the *Litopenaeus vannamei* seafood sauce had a unique and rich sauce flavor.

Figure 2b showed the effects of contents of thick broad-bean sauce and Shacha sauce on the sensory evaluation scores, while the content of sweet soybean paste was fixed at its middle level (15%). When the content of Shacha sauce at a low level, increasing the content of thick broad-bean sauce to some extent favored the accumulation of scores. But no significant effect of thick broad-bean sauce content on scores was observed at the content of Shacha sauce exceeded optimal condition. Because the more Shacha sauce has, the worse fish smell. The fish smell could covered sauce flavor and seafood flavor, made production have badly smell and taste. Reasonable content of Shacha sauce can enhance the seafood flavor, to promote the quality of the product.

Figure 2c revealed the effects of contents of sweet soybean paste and Shacha sauce on the sensory evaluation scores, while thick broad-bean sauce content was fixed at its middle level (5%). It is evidence that the sensory evaluation scores steadily increased at a low content of Shacha sauce when increasing the content of sweet soybean paste, this phenomenon was more pronounced when sweet soybean paste content was set at a high level within a certain range. However, the sensory evaluation scores gradually decreased when the content of sweet soybean paste exceeded optimal conditions. This indicated that, under optimal contents of sweet soybean paste and Shacha sauce, an increase in content of sweet soybean paste would not further increase the sensory evaluation scores. Sauce fragrance was so thick that it covered the seafood sauce which itself has the unique seafood flavor, made flavor form single. The favorable flavor and quality of product was acquired with the optimal ratio of sauce. These facts were important in making the whole process economically more feasible and in making it possible to reduce the cost in the potential industry application.

By solving the Eq. (1) using above-mentioned software, the optimum values of the test variables were thick broad-bean sauce 6.22%, sweet soybean paste 16.32% and Shacha sauce 10.10%, respectively. Under this condition, the maximum predicted sensory evaluation score was observed. It was about 9.49.

Validation of the model: The repeat experiments were conducted under optimal conditions for verification of the optimization. Combined with the actual production, there might be slight variation in experimental

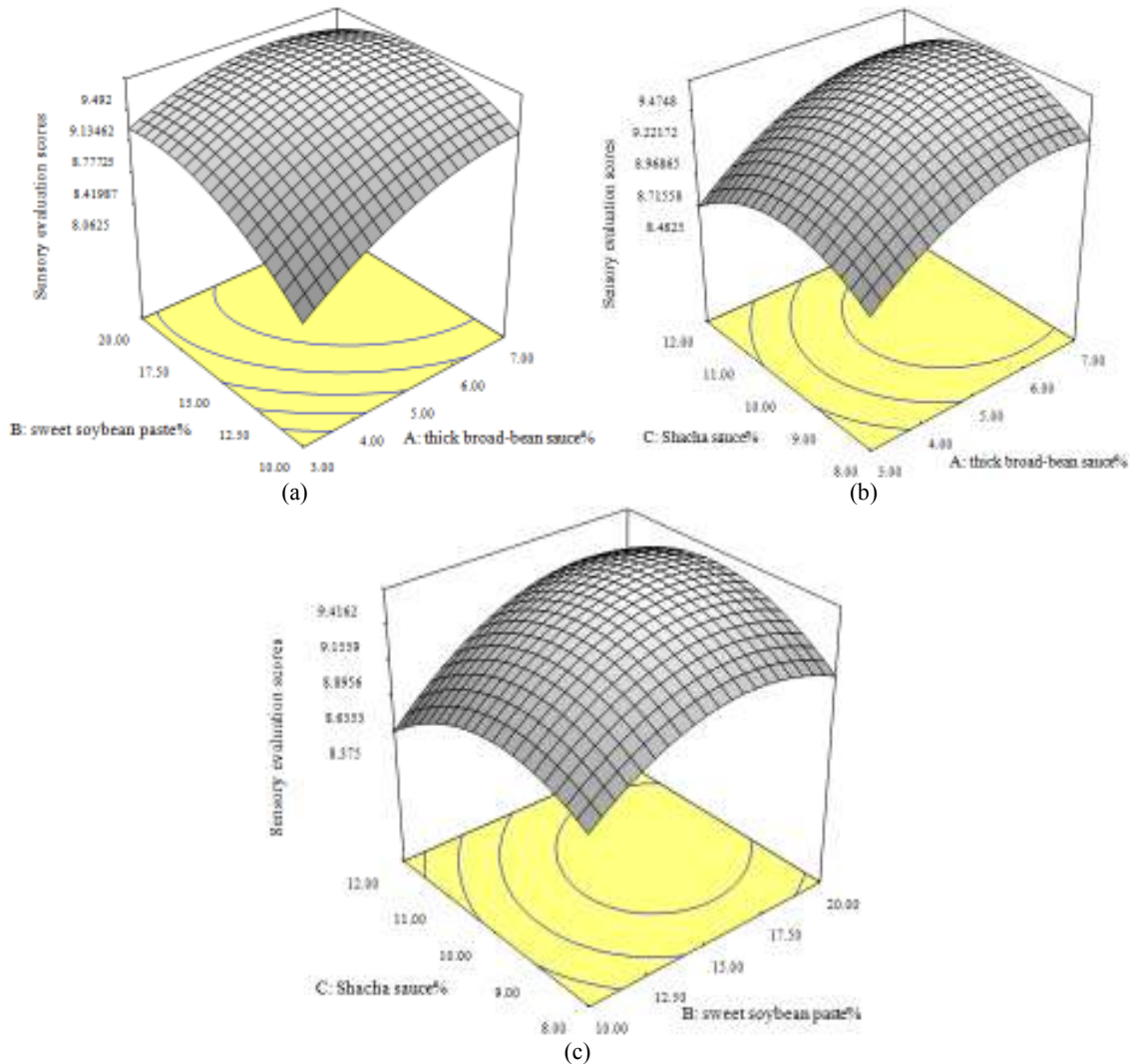


Fig. 2: Response surface plots of the effects of sauces contents on the sensory evaluation scores, (a) the combined effects of contents of thick broad-bean sauce and sweet soybean paste on the sensory evaluation scores with constant content of Shacha sauce (10%), (b) the combined effects of contents of thick broad-bean sauce and Shacha sauce on the sensory evaluation scores with constant content of sweet soybean paste (15%), (c) the combined effects of contents of sweet soybean paste and Shacha sauce on the sensory evaluation scores with constant content of thick broad-bean sauce (5%)

conditions, the maximal sensory evaluation score was obtained at 9.5 ± 0.36 . This value was found to be in agreement with the predicted value. This model can better predict the actual situation of *Litopenaeus vannamei* seafood sauce. Study on the effects of the different parameters of sauce on the sensory evaluation, product quality was improved and production cost was reduced. This model provides good mathematical parameters for the industrial production of *Litopenaeus vannamei* seafood sauce.

CONCLUSION

The optimum condition and model were defined by the central composite experimental design and response surface methodology. The current study showed that the

second-order polynomial model was sufficient to describe and predict the responses of sensory evaluation scores to changes within the experimental ranges, which can be given as:

$$Y = 9.36 + 0.33X_1 + 0.26X_2 - 0.012X_3 - 0.23X_1^2 - 0.31X_2^2 - 0.30X_3^2 - 0.18X_1X_2 + 0.025X_1X_3 + 0.100X_2X_3, R^2 = 0.9873$$

Y is the predicted response (sensory evaluation scores) X_1 , X_2 , X_3 are coded values of contents of thick broad-bean sauce, sweet soybean paste and Shacha sauce, respectively. The independent parameters of the contents of thick broad-bean sauce, sweet soybean paste and the quadratics of contents of thick broad-bean sauce, sweet soybean paste and Shacha sauce

significantly affected the sensory evaluation scores. In addition, the interactions between contents of thick broad-bean sauce and sweet soybean paste, sweet soybean paste and Shacha sauce had a significant effect on the sensory evaluation scores (Xu *et al.*, 2008). The graphical optimization method was adopted to find the best process condition and it predicted that the optimum proportioning parameters within the experimental ranges would be: thick broad-bean sauce 6.22%, sweet soybean paste 16.32% and Shacha sauce 10.10%. Under such conditions, the predicted and verifiable sensory evaluation scores were 9.49 and 9.5 ± 0.36 , respectively. With this formulation prepared *Litopenaeus vannamei* seafood sauce, the product was obtained with red brown color, rich sauce flavor, prominent seafood flavor, had both harmonious salty and fresh taste and smell. This technology not only enrich the product range, but also can meet the demand for convenience foods of the fast pace of modern life and enhance the added value of the raw material at the same time.

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REFERENCES

Cao, B.Z., 2005. Discussion on the production technology of sweet soybean paste. China Brew., 148(7): 1-4.

- Hu, S.S., J. Wang and J.F. Sun, 2012. Optimization of scallop sauce by response surface methodology. J. Chin. Inst. Food Sci. Technol., 12(10): 113-119.
- Li, Y., F.J. Cui, Z.Q. Liu, Y. Xu and H. Zhao, 2007. Improvement of xylanase production by *Penicillium oxalicum* ZH-30 using response surface methodology. Enzyme Microb. Tech., 40(5): 1381-1388.
- Liu, S.Q. and H. Lin, 2003. The preparation of shrimp brei with lower salinity by enzymolysis. Mar. Sci., 27(3): 57-60.
- Liu, Z.W., X.H. Tan and S.G. Yao, 2011. Technology present situation and development directions of pea sauce. China Condiment., 36(3): 13-16.
- Mannan, S., A. Fakhru'l-Razi and M.Z. Alam, 2007. Optimization of process parameters for the bioconversion of activated sludge by *Penicillium corylophilum*, using response surface methodology. J. Environ. Sci., 19(1): 23-28.
- Muralidhar, R.V., R.R. Chirurnamila, R. Marchant and P. Nigam, 2001. A response surface approach for the comparison of lipase production by *Candida cylindracea* using two different carbon sources. Biochem. Eng. J., 9(1): 17-23.
- Qi, C.H., Y.X. Wang and W. Zhang, 2007. The technological conditions of preparation of shrimp sauce by enzymolysis. Food Sci. Technol., 32(4): 108-110.
- Xu, X., Y.X. Gao, G.M. Liu, Q. Wang and J. Zhao, 2008. Optimization of supercritical carbon dioxide extraction of sea buckthorn (*Hippophae rhamnoides* L.) oil using response surface methodology. LWT-Food Sci. Technol., 41(7): 1223-1231.