

## Research Article

### Evaluation and Optimization of Amino Acids Retention Rate of Japanese Scallop Powder with Different Food Additives in Spray-drying Process by Response Surface Method

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**Abstract:** Spray-drying process for Japanese scallop powder can cause damage because of its heat-sensitive components such as amino acids. Therefore it is important for amino acids to be embedded with the appropriate food additives in spray-drying process. In this study, with Japanese scallop as the raw, Response Surface Method (RSM) with three variables (the addition level of  $\beta$ -cyclodextrin, modified starch and CMC) was used to investigate the effect of food additives on the amino acids retention rate in Japanese scallop powder in spray drying. The equation model to predict amino acids retention rate was reported. The results showed that the optimal addition level of  $\beta$ -cyclodextrin, modified starch and CMC were 25.55, 24.55 and 2.96%, respectively. And the amino acids retention rate was  $56.75 \pm 0.64\%$ . This model was testified to fit the actual situation preferably, therefore it can provide theoretical and practical basis for industrial production of Japanese scallop powder.

**Keywords:** Amino acids retention rate, food additives, Japanese scallop powder, response surface method

## INTRODUCTION

Japanese scallop powder is a product of Japanese scallop through protease hydrolysis by spray drying process, which can be used as the excipient in food process or aquatic food condiment. Protease can hydrolyze protein into amino acids which can enhance protein nutrition value; however, spray-drying process can cause the loss or damage of amino acids and other thermo-sensitive compositions inevitably. For example, Abdul-Hamid *et al.* (2002) found that the temperature of spray drying had obvious effect on the loss of amino acids. Therefore, it is beneficial to embed amino acids with food additive to reduce direct contact with hot air, which help to enhance the amino acids retention rate and provide guarantee of product quality of Japanese scallop powder.

Since Osborne and Clapp (1907a, b, c) found hydrolysis process of phaseolin, excelsin and hordein, the study on amino acids produced by protein have attracted great attention. Murai (1982) improved carp growth rate by feeding embedded amino acids. Murai (1985) found that amino acids embedded by agar were beneficial to the growth of fish. Chen *et al.* (1992) showed that embedded amino acids could enhance body weight gain of Tiger Prawn. Lopez-Alvarado *et al.* (1994) found that the leaching rate of amino acids

could be decreased in solutions with embedded amino acids using protein and lipid as embedded materials.

The embedded function of cyclic dextrin on amino acids was researched by Ramanathan and Porkai (1995). Yufera *et al.* (2002) compared retention effect on amino acid with protein and glutin as wall material. Chiang *et al.* (2009) studied the embedded function of chitosan in hydrogenated fat. Tomás *et al.* (2011) studied the effects of amino acids in a mannitol-based dry powder formulation on particle size, aerosolisation, emitted dose and cohesion. Anderson *et al.* (2013) used using amino acid ionic liquids to remove naphthenic acids from crude oil. Latorre-Moratalla *et al.* (2014) discussed the amino acid availability as an influential factor on the biogenic amine formation. These researches confirmed the great advantages of embedded amino acids on some aspects, such as animals' growth rate, feed availability, releasing effect of microcapsules and other fields. However, the effects of different food additives on the amino acids retention rate in special process were not involved. Moreover, Song and Guo (2001) investigated the essential of embedding technology. Zhou *et al.* (2005) discussed the supramolecular embedding technology of  $\beta$ -cyclodextrin and aromatic amino. These researchers studied embedding mechanism from the angle of molecular chemical; however the suitable dosage of

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wall materials in production process was not discussed. Kurozawa *et al.* (2009) estimated the effect of malt dextrin and arabic gum in spray drying of Japanese scallop powder from the aspect of physical properties including moisture content, bulk density, distribution and mean diameter particle, hygroscopicity and glass transition temperature, however, the effect of different food additives on the amino acids retention rate was not reported.

In this study, Japanese scallop was acted as the raw and the effects of different food additives on the amino acids retention rate in Japanese scallop powder by spray drying with enzymatic hydrolysis (neutral protease and papain) were studied. The target of this research is to provide theoretical and practical basis for industrial production of Japanese scallop powder.

## MATERIALS AND METHODS

**Materials:** Japanese scallop were obtained from Qinhuangdao City, Hebei Province, China. The scallops were kept at the temperature of -18°C, until used.

**Pretreatment of Japanese scallop:** (0.3 kg) Japanese scallops were weighed and homogenized with 5.2 L phosphate buffer solution (pH7). The homogenates were hydrolyzed with 2770 u/g of neutral protease and papain (the proportion of neutral protease and Papain was 3:1, Solorbio, Beijing) and at 46°C for 6 h. And then the hydrolysate was heated at 100°C for 3 min for killing enzyme immediately. And they obtained material was centrifuged by a centrifuge (Anke, GL-20B, Shanghai) for 10 min at 4500 r/min and then filtrated. The filtrate was stored at ambient temperature for the following spray drying process. The content of amino acid was measured using formol-titration method (Dalian Institute of Light Industry, 1994) which was 254.5 mg/100 mL.

**Amino acids retention rate:** Three food additives with different addition levels, which were  $\beta$ -cyclodextrin, modified starch and CMC, were used in the drying process. The filtrate obtained in the pretreatment experiment was poured into several beakers and food additives at different kinds or addition levels were added, respectively. Addition level of  $\beta$ -cyclodextrin varied in the range from 0 to 30%; addition level of modified starch varied in the range from 0 to 35%; addition level of CMC varied in the range from 0 to 4%, respectively (Addition of additive = the soluble solids content of Japanese scallops hydrolysate  $\times$  solution volume  $\times$  add percentage). Samples were blended adequately using a heating magnetic whisk (Guohua, 79-1, Changzhou). Drying processes were carried out with a Spray Drier (Tokyo, SD-1000, Japan) under the condition of that spray dryer inlet temperature, outlet temperature and the air volume were 105°C, 85°C and 0.7 m<sup>3</sup>/min, respectively. Obtained

Japanese scallop powders were collected and amino acid contents were measured using formol-titration method. The amino acids retention rate and the collection rate were calculated using Eq. (1) and (2), respectively.

The amino acids retention rate (%) was calculated using the following equation:

$$\text{The amino acids retention rate (\%)} = \frac{A_1}{A} \times 100\% \quad (1)$$

where,

A<sub>1</sub> : The amino content of Japanese scallops powder after spray drying process

A : The amino content of the filtrate obtained in the pretreatment experiment

The collection rate (%) was calculated using the following equation:

$$\text{The collection rate (\%)} = \frac{B_1}{B} \cdot 100\% \quad (2)$$

where, B<sub>1</sub> is the weight of the powder in collecting bottle after spray drying process and B is the soluble solids content of the filtrate obtained in the pretreatment experiment. Moisture content was determined according to traditional method (Loss on Drying, LOD). The soluble solids content was determined using Electronic Digital Refractometer (ATAGO, PR101, Japan). Data were reported as mean  $\pm$  standard deviation of three replicates. The results were compared through Analysis of Variance (ANOVA) using SPSS (Version 11.5, SPSS, USA). Pairwise multiple comparisons were done by Least Significant Difference (LSD) and Duncan's significant difference test with a family error rate of 0.05.

**Experimental design and statistical analysis:** Before RSM was applied, approximate conditions for addition level of  $\beta$ -cyclodextrin, addition level of modified starch and addition level of CMC, were determined by varying one factor at a time while keeping the other conditions constant (Fig. 1). An appropriate range for each factor was determined for the Response Surface Methodology (RSM).

The effect of the three variables at three variation levels on amino acids retention rate was investigated to determine the optimum combination of variables, using the Box-Behnken design for RSM. The model proposed for the response Y (Amino acids retention rate (%)) is given below:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 \quad (3)$$

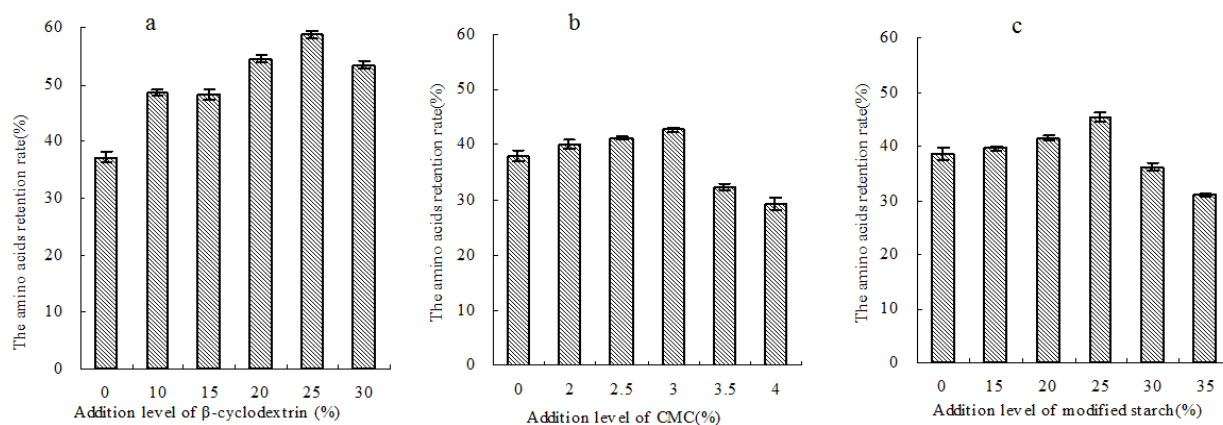


Fig. 1: (a) Effect of β-cyclodextrin, (b) modified starch, (c) CMC on the amino acids retention rate of Japanese scallop powder

Table 1: Design of experiment-levels of various process parameters

Parameter	Level		
	-1.0	0.0	+1.0
$x_1$ : Addition level of β-cyclodextrin (%)	20	25	30
$x_2$ : Addition level of modified starch (%)	20	25	30
$x_3$ : Addition level of CMC (%)	2.5	3	3.5

where,

- $Y$  = The predicted response
- $b_0$  = Model constant
- $x_1, x_2$  and  $x_3$  = Independent variables
- $b_1, b_2$  and  $b_3$  = Linear coefficients
- $b_{12}, b_{13}$  and  $b_{23}$  = Cross product coefficients
- $b_{11}, b_{22}$  and  $b_{33}$  = The quadratic coefficients

As presented in Table 1, the experimental design involved three parameters ( $x_1, x_2$  and  $x_3$ ), each at three levels, coded -1, 0 and +1 for low, middle and high concentrations, respectively. Table 2 represents the design matrix of a 15 trials experiment. For predicting the optimal point, a second order polynomial function was fitted to correlate the relationship between independent variables and response. The adequacy of the polynomial model is expressed by the multiple coefficient of determination,  $R^2$ . The significance of each coefficient was determined by using Student t-test and p value. The software Design Expert (Version 7.0.0, Stat-Ease Inc., Minneapolis, USA) was used for experimental design, data analysis and quadratic model building. The optimal addition levels of three additives for the retention rate of amino acids were obtained by solving the regression equation and also by analyzing the response surface contour plots using the same software. And the optimum condition was verified by conducting experiments under these conditions. The response was monitored and the results were compared with the model predictions.

## RESULTS AND DISCUSSION

### Selection of addition level of β-cyclodextrin, modified starch and CMC: Effect of β-cyclodextrin

on the amino acids retention rate of Japanese scallop powder is presented in Fig. 1a. The amino acids retention rate ranged from 37.22 to 58.81%. The amino acids retention rate of 25% β-cyclodextrin was significantly higher than those of the other treatments ( $p < 0.05$ ). Comparisons between 20% β-cyclodextrin and 30% β-cyclodextrin for the amino acids retention rate were not statistically different ( $p > 0.05$ ). Other comparisons for the amino acids retention rate were statistically different ( $p < 0.05$ ). The addition level of β-cyclodextrin was maximum at the 20-30% range. The design points selected for optimization of the addition level of β-cyclodextrin were 20, 25 and 30%, respectively. Figure 1b shows the effect of modified starch on the amino acids retention rate of Japanese scallop powder. The amino acids retention rate ranged from 30.91 to 45.44%. The amino acids retention rate of 25% modified starch was significantly higher than those of the other treatments ( $p < 0.05$ ). Comparisons between 0% modified starch and 15% modified starch for the amino acids retention rate were not statistically different ( $p > 0.05$ ). Other comparisons for the amino acids retention rate were statistically different ( $p < 0.05$ ). Therefore, variable levels of 20, 25 and 30%, respectively were chosen as the lower, middle and upper points, respectively. Effect of CMC on the amino acids retention rate of Japanese scallop powder is presented in Fig. 1c. The amino acids retention rate ranged from 29.36 to 42.72%. The amino acids retention rate of 3% CMC was significantly higher than those of the other treatments ( $p < 0.05$ ). Comparisons between 2% CMC and 2.5% CMC for the amino acids retention rate were not statistically different ( $p > 0.05$ ). Other comparisons for the amino acids retention rate were statistically different ( $p < 0.05$ ). The design points selected for addition level of CMC were 2.5, 3 and 3.5%, respectively.

**Response surface analysis:** RSM is an optimization procedure which can determine optimum conditions through first or second order polynomial equations in a sequential testing process. RSM can test several

Table 2: Design of response surface method and result of experiments

Experiment no.	Addition level of $\beta$ -cyclodextrin ( $x_1$ )	Addition level of modified starch ( $x_2$ )	Addition level of CMC ( $x_3$ )	Amino acids retention rate (%)	
				Experimental	Predicted
1	25	20	3.5	42.98	42.60
2	25	25	3.0	57.66	56.56
3	20	20	3.0	40.68	40.99
4	30	30	3.0	46.35	46.03
5	25	20	2.5	43.29	43.44
6	20	25	3.5	41.59	42.34
7	25	25	3.0	56.34	56.56
8	20	25	2.5	42.48	42.72
9	30	25	2.5	47.09	47.04
10	30	20	3.0	44.65	45.25
11	25	25	3.0	55.69	56.56
12	25	30	2.5	45.69	46.78
13	20	30	3.0	45.35	44.73
14	25	30	3.5	43.22	43.78
15	30	25	3.5	43.11	43.58

variables with special experimental designs to cut the number of required tests and measure several effects by objective tests at a time. In this study, amino acids retention rate a Box-Behnken design was used (Table 2); Table 1 showed the actual levels of variables used in each experimental run.

Table 2 showed that the highest amino acids retention rate observed in experiment No. 2 was 57.66%, which contained 25%  $\beta$ -cyclodextrin, 25% modified starch and 3% CMC. The lowest amino acids retention rate found in experiment No. 11 was 40.68%, which contained 20%  $\beta$ -cyclodextrin, 20% modified starch and 3% CMC (Table 2). Data obtained from the experiments (Table 2 and 3) were analyzed by software Design Expert version 7.0.0. The following equation was obtained:

$$Y = 56.56 + 1.39x_1 + 1.13x_2 - 0.96x_3 - 0.74x_1x_2 - 0.77x_1x_3 - 0.54x_2x_3 - 6.27x_1^2 - 6.04x_2^2 - 6.73x_3^2 \quad (4)$$

where,  $Y$  is the predicted response variable, namely, the amino acids retention rate (%);  $x_1, x_2, x_3$  are the coded values of the independent variables, namely, addition level of  $\beta$ -cyclodextrin, addition level of modified starch and addition level of CMC, respectively.

Table 3 showed the coefficients and their standard errors. The statistical significance of Eq. (4) was checked by an F-test and the Analysis of Variance (ANOVA) for response surface quadratic model is summarized in Table 4. It is evident from Table 4 that the model is highly significant, as is evident from the model F-value and a very low probability value ( $p = 0.0002$ ). The goodness of the model can be checked by the determination coefficient  $R^2$  and the multiple correlation coefficients  $R$ . The quadratic regression was significant at the level of 0.9948 ( $R = 0.9948$ ). The value of adjusted  $R^2$  (0.9709) for Eq. (4) suggests that the total variation of 97% for demineralization is attributed to the independent variables and only about 3% of the total variation cannot be explained by the model. The value of lack-of-fit was not significant ( $p = 0.5932$ ). Table 4 also showed that the interaction terms of the three factors

Table 3: Coefficient of the model

Variable	Coefficient	Coefficient S.E.
$b_0$	56.56	0.55
$b_1$	1.39	0.33
$b_2$	1.13	0.33
$b_3$	-0.96	0.33
$b_{12}$	-0.74	0.47
$b_{13}$	-0.77	0.47
$b_{23}$	-0.54	0.47
$b_{11}$	-6.27	0.49
$b_{22}$	-6.04	0.49
$b_{33}$	-6.73	0.49

S.E.: Standard error

had no significant effects on amino acids retention rate ( $px_1x_2 = 0.1770 > 0.10$ ;  $px_1x_3 = 0.1631 > 0.10$ ;  $px_2x_3 = 0.3050 > 0.10$ ).

Graphical representations of response surfaces are shown in Fig. 2 to illustrate the main and interactive effects of the independent variables on the dependent one. One of the variables kept at optimum level while the remaining two variables were changed within experimental range. The effect of addition level of  $\beta$ -cyclodextrin and addition level of modified starch on the amino acids retention rate is presented in Fig. 2a. Addition level of  $\beta$ -cyclodextrin and addition level of modified starch have quadratic effect on the amino acids retention rate. The influence of addition level of  $\beta$ -cyclodextrin and addition level of CMC is shown in Fig. 2b; the addition level of CMC has quadratic effects. Influence of addition level of modified starch and addition level of CMC is shown in Fig. 2c; these results have shown that the response surface had a maximum point. Precise coordinates of maximum of the response were gained by an analytical process. If the partial derivatives of regressions Eq. (4) were ordered to zero, three equations would form:

$$\begin{aligned} 1.39 - 0.74x_2 - 0.77x_3 - 12.54x_1 &= 0 \\ 1.13 - 0.74x_1 - 0.54x_3 - 12.08x_2 &= 0 \\ -0.96 - 0.77x_1 - 0.54x_2 - 13.46x_3 &= 0 \end{aligned} \quad (5)$$

Table 4: Analysis of Variance (ANOVA) for response surface quadratic model

Source	Degree of freedom	S.S.	M.S.	p-value
Model	9	426.10	47.34	0.0002
Addition level of $\beta$ -cyclodextrin ( $x_1$ )	1	15.40	15.40	0.0089
Addition level of modified starch ( $x_2$ )	1	10.15	10.15	0.0199
Addition level of CMC ( $x_3$ )	1	7.32	7.32	0.0354
$x_1x_2$	1	2.21	2.21	0.1770
$x_1x_3$	1	2.39	2.39	0.1631
$x_2x_3$	1	1.17	1.17	0.3050
$x_1^2$	1	145.00	145.00	<0.0001
$x_2^2$	1	134.66	134.66	<0.0001
$x_3^2$	1	167.19	167.19	<0.0001
Residual	5	4.47	0.89	
Lack of fit	3	2.45	0.82	0.5932
Pure error	2	2.02	1.01	
Corrected total	14	430.57		

S.S.: Sum of square; M.S: Mean square;  $R^2 = 0.9896$ ; Adjusted  $R^2 = 0.9709$ ;  $R = 0.9948$

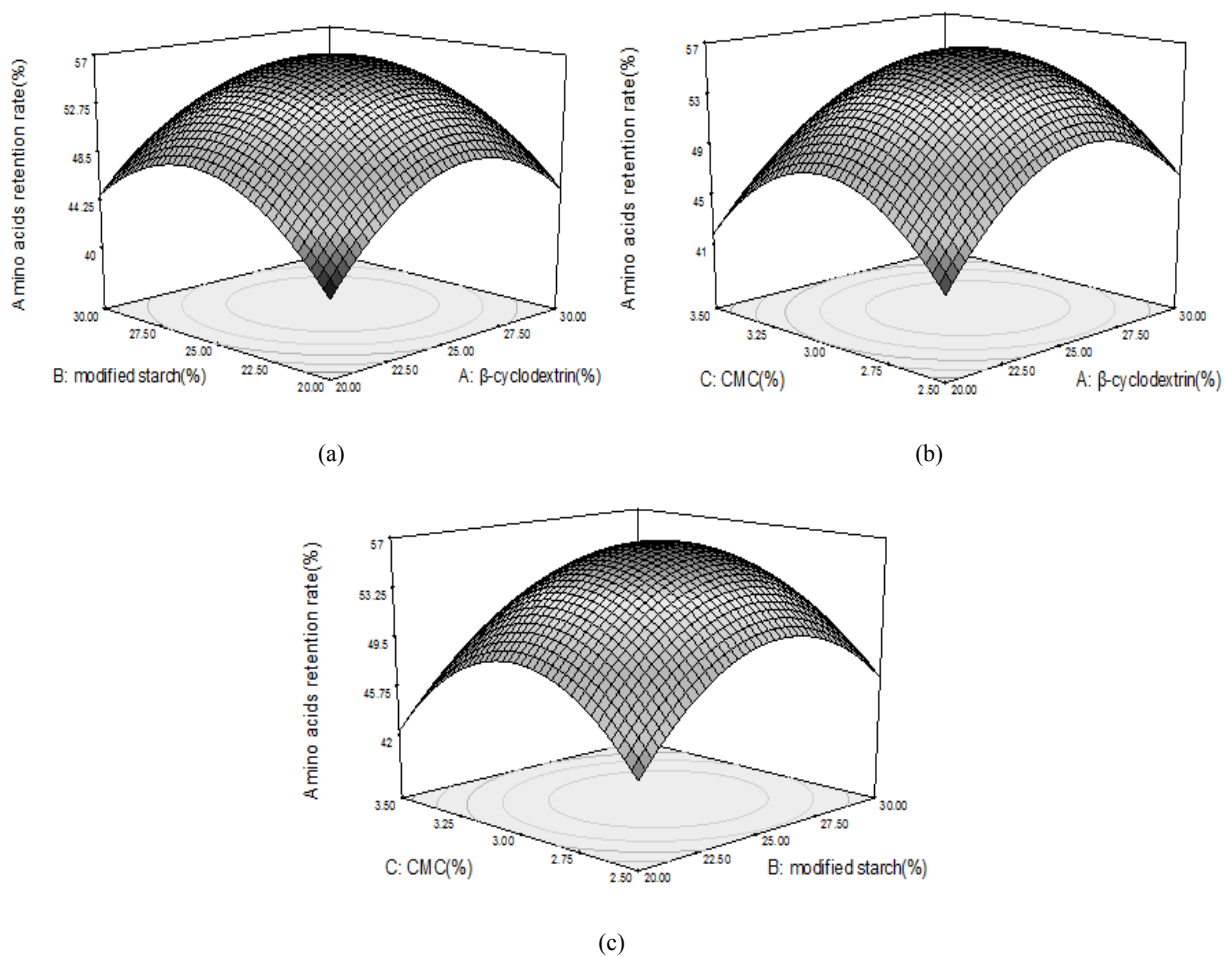


Fig. 2: 3D graphics surface optimization of the amino acids retention rate, (a) effect of  $\beta$ -cyclodextrin and addition level of modified starch, (b) effect of  $\beta$ -cyclodextrin and addition level of CMC, (c) effect of modified starch and addition level of CMC

The system of Eq. (5) was solved and the coded values of the independent variables were obtained, which were  $x_1 = 0.1105$ ,  $x_2 = 0.0904$  and  $x_3 = -0.0813$ . From the coded values of the independent variables, we can know actual values which were that addition level of  $\beta$ -cyclodextrin was 25.55%, addition level of

modified starch was 24.55% and addition level of CMC was 2.96%.

This group of conditions was determined to be optimum by RSM optimization approach. In order to validate this optimal condition, the experiment was carried out under the conditions, which were that

Table 5: Comparison of the predicted and experimental result of amino acids retention rate

Independent variables	Optimum conditions	
Addition level of $\beta$ -cyclodextrin	25.55%	
Addition level of modified starch	24.55%	
Addition level of CMC	2.96%	
Responses	Predicted value	Experimental value
The amino acids retention rate	56.73%	56.75 $\pm$ 0.64%

addition level of  $\beta$ -cyclodextrin was 25.55%, addition level of modified starch was 24.55% and addition level of CMC was 2.96%. The amino acids retention rate was 56.75 $\pm$ 0.64%, which was found to be in high conformity with the predicted one 56.73% which was obtained with Eq. (3) (Table 5). From the results of this study, it can be confirmed that RSM represents an effectual approach for evaluation and optimization of amino acids retention rate of Japanese scallop powder with different food additives in spray-drying process.

In our study, the lowest test had a value of the amino acids retention rate of 29.36% (Fig. 1c) which acted as the control. It can be concluded that the amino acids retention rate under the optimal spray drying parameters improved 27.39% than that of the control. Under these optimum conditions, the final product is milky white powder.

However, Fig. 1a showed that amino acids retention rate under 25% of  $\beta$ -cyclodextrin was a litter higher than that under the optimum conditions. Perhaps this is because the physical and chemical properties of three additives in spray drying process are different. Another conceivable causation rests with that the effects of food additives on the amino acids retention rate in different spray drying parameters are different. No matter what reasons, considered to the moisture content, solubility and stability of the final powder, it is better to use mixture additives in the drying process. Possibly with the improvement of spray drying parameters, the amino acids retention rate with mixture additives can be increased, which needs further investigation.

### CONCLUSION

The results clearly showed the amino acids retention rate reached 56.75% with the addition level of  $\beta$ -cyclodextrin, modified starch and CMC were 25.55, 24.55 and 2.96%, respectively. The regression equation, obtained in this study, can be used as a mathematical model reference to choose the additive contents in production, which can ensure enterprise to get maximize economic profits. It can be considered that the model can provide theoretical and practical basis for industrial production of Japanese scallop powder.

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