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Comparison of Flavonoids from Spina Gleditsiae by Response Surface Method and Orthogonal Design

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Abstract. In this paper, the effects of extraction temperature, extraction pressure and dosage of entrainer on flavonoids yield were studied. The optimal process determined by response surface method was extraction temperature as 48.52°C, extraction pressure as 40MPa, dosage of entrainer as 36.13mL, theoretical predictive yield as 0.791%, actual verified yield as 0.793%, and there was an interaction between the extraction temperature and dosage of entrainer. The optimum process determined by orthogonal design was extraction temperature as 50°C, extraction pressure as 40MPa, dosage of entrainer as 37.5mL, and actual verified yield as 0.779%. Although the two methods obtained similar best processes, the response surface method could provide more accurate information, and the final flavonoids yield was also highest, which was worth promoting.

1 Introduction

Response surface method and orthogonal design are methods commonly used for experimental optimization at present. The two optimization methods have advantages and disadvantages in different applications, so it depends on the specific circumstances instead of being generalized.

In order to avoid blindly choosing optimization methods in the experiment, response surface method and orthogonal design were used to optimize the extraction of flavonoids from spina gleditsiae. In this study, the differences between two methods on the extraction were discussed, so as to provide reasonable guidance for the experimental optimization design.

2 Materials and methods

In this study, supercritical CO_2 fluid extraction was used as the basic process for extraction of flavonoids from spina gleditsiae. A certain amount of 60-80 mesh spina gleditsiae particles was weighed and extracted in supercritical CO_2 fluid extraction tank under different conditions. Flavonoids yield was determined by uv spectrophotometry.

2.1 Single factor test

Extraction of flavonoids from spina gleditsiae by supercritical CO_2 fluid was worked at different extraction temperatures, extraction pressures, and dosage of entrainer (75% ethanol).

2.2 Orthogonal experiment

According to the single factor test results, three factors-three levels of orthogonal test were designed. Three factors were extraction temperature (A), extraction pressure (B) and dosage of entrainer (C). Data were analysed by SPSS software.

2.3 Response surface optimization test

According to the single factor test results, the box-behnken centre combination was used to design a three factor-three level response surface analysis test with extraction temperature (A), extraction pressure (B) and dosage of entrainer (C) as independent variables and flavonoids yield (%) as response value. The data was analysed by the design-expert software.

3 Results and discussion

3.1 Single factor test results and analysis

The effect of each single factor on flavonoids yield was shown in figure 1. Selected the factors contained the highest yield for next tests, the extraction temperature as 30, 40 and 50°C, extraction pressure as 20, 30 and 40 MPa and dosage of entrainer as 25, 37.5 and 50mL.

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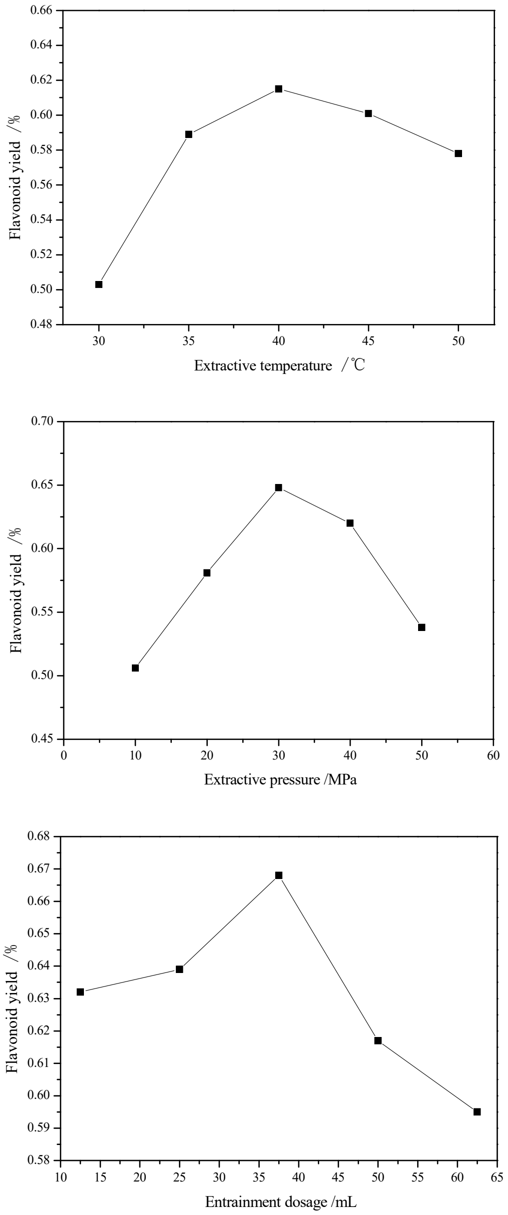

Figure 1. Effects of single factor on flavonoids yield.

3.2 Response surface test design and results

The response surface test design and results were shown in table 1, with each test repeated twice.

Table 1. Box-Behnken design arrangement and corresponding experimental results for response surface analysis

No.	Factor			Yield（%）		
	A	B	C	1	2	Average
1	40	30	37.5	0.774	0.768	0.771
2	50	40	37.5	0.780	0.778	0.779
3	50	20	37.5	0.734	0.736	0.735
4	50	30	50	0.757	0.757	0.757
5	40	20	50	0.726	0.730	0.728
6	30	20	37.5	0.707	0.708	0.707
7	40	20	25	0.708	0.704	0.706
8	40	40	25	0.751	0.747	0.749
9	40	30	37.5	0.772	0.74	0.773
10	30	30	50	0.729	0.729	0.729
11	40	40	50	0.768	0.770	0.769
12	40	30	37.5	0.775	0.773	0.774
13	50	30	25	0.761	0.761	0.761
14	40	30	37.5	0.771	0.767	0.769
15	40	30	37.5	0.752	0.756	0.754
16	30	30	25	0.641	0.641	0.641
17	30	40	37.5	0.707	0.707	0.707

Multiple regression fitting was performed on the experimental data with design expert software to obtain the quadratic regression equation with flavonoids yield as objective function:

$$Y=0.77A++0.031B+0.016B+0.016C+0.011AB-0.023AB-0.001BC-0.026A^2-0.010B^2-0.020C^2$$

The regression analysis results of the model were shown in table 2.

Table 2. Analysis of variance for quadratic polynomial model.

Source	Sum of squares	df	Mean square	F value	P-value
Model	0.020	9	2.202×10^{-3}	15.80	0.0023
A	0.008×10^{-3}	1	7.668×10^{-3}	55.18	0.0001
B	0.002×10^{-3}	1	2.048×10^{-3}	14.70	0.0064
C	0.002×10^{-3}	1	1.984×10^{-3}	14.24	0.0069
AB	4.840×10^{-4}	1	4.840×10^{-4}	3.47	0.1046
AC	2.116×10^{-3}	1	2.116×10^{-3}	15.19	0.0059
BC	1.000×10^{-6}	1	1.000×10^{-6}	7.177×10^{-3}	0.9349
A^2	2.868×10^{-3}	1	2.868×10^{-3}	20.59	0.0027
B^2	4.295×10^{-4}	1	4.295×10^{-4}	3.08	0.1226
C^2	1.701×10^{-3}	1	1.701×10^{-3}	12.21	0.0101
Residual	9.753×10^{-4}	7	1.393×10^{-4}		
Lack of Fit	7.085×10^{-4}	3	2.362×10^{-4}	3.54	0.1268
Pure Error	2.668×10^{-4}	4	6.670×10^{-5}		
Cor Total	0.021	16			

As shown in table 2, F=15.80, P=0.0023<0.01, indicated that the regression of the model was very significant. Lack of fit P=0.1268>0.05, indicated which was not significant. The linear relationship between the dependent variable and the investigated variables was significant, and the model adjusted determination coefficient R^2_{Adj}=0.8928, indicating that the model could explain the change of response value of 89.28 %. The results showed that the model was fit well and the error was small, which could be used to predict the best extraction process of flavonoids from spina gleditsiae.

As could be seen from table 2, P values of A, B, C, AC, A^2 and C^2 were less than 0.05 which indicated significantly different, and the F value of each factor was FA>FB>FC, indicating that the extraction temperature, extraction pressure and entrainer dosage had significant effects on flavonoids yield. There were interaction effects, which could also be verified according to the three-dimensional response surface.

The response surface trend of extraction temperature and entrainer dosage was parabola with maximum value and maximum curved degree, indicating the strongest interaction between AC, as shown in figure 2b. In figure 2a and 2c, the curved surfaces were gentle and the contour lines were close to a normal circle, indicating that the interactions of AB and BC were weak.

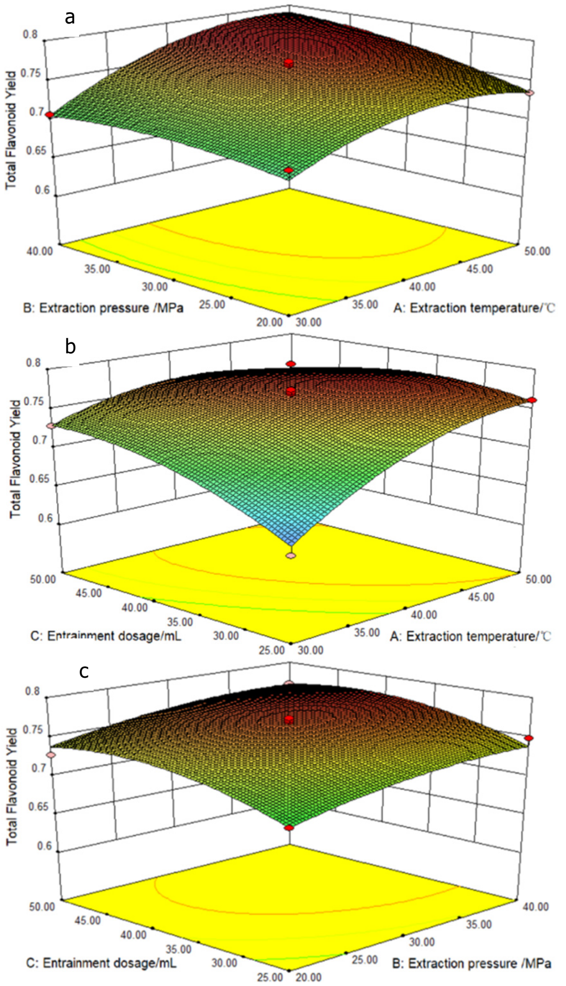

Figure 2. Response surface diagram of flavonoids yield.

According to the model, theoretical value of flavonoids extraction optimum process were as follows: extraction temperature as 48.52°C, extraction pressure as 40MPa, dosage of entrainer as 36.13mL, theoretical predictive yield as 0.791%, actual verified yield as 0.793%. After three parallel tests, the actual flavonoids yield was 0.793%, which was slightly higher than the model prediction. It was shown that the equation was well fitted to the actual situation and the correctness of the model was fully verified.

3.3 Orthogonal test design and results

Orthogonal test was conducted according to the single factor test results, as shown in table 3.

Table 3. Design and results of orthogonal design.

No.	Factor			Yield（%）		
	A	B	C	1	2	Average
1	30	20	25	0.711	0.711	0.711
2	30	30	37.5	0.719	0.715	0.717
3	30	40	50	0.722	0.724	0.723
4	40	20	37.5	0.743	0.743	0.743
5	40	30	50	0.747	0.750	0.749
6	40	40	25	0.752	0.753	0.753
7	50	20	50	0.759	0.759	0.759
8	50	30	25	0.762	0.759	0.761
9	50	40	37.5	0.778	0.779	0.779
K_1	0.717	0.738	0.742			
K_2	0.748	0.742	0.746			
K_3	0.766	0.752	0.744			
Optimum	$A_3>B_3>C_2$					
R	0.049	0.014	0.004			
Prioritize	A>B>C					

Statistics analysis showed that the influence of various factors on the flavonoids yield was: $A_3>B_3>C_2$, which mean the best technology of flavonoids from spina gleditsiae was extraction temperature as 50°C, extraction pressure as 40MPa, dosage of entrainer as 37.5mL. It was found by variance analysis that the extraction temperature had significant influence on the test, while the extraction pressure and entrainer dosage had no significant influence, which was consistent with the response surface analysis results.

3.4 Comparison of response surface method and orthogonal design

Based on single factor analysis, response surface method and orthogonal design could explore the effect of extraction temperature, extraction pressure and entrainer dosage on flavonoids from spina gleditsiae, and obtained the best extraction process. However, both two methods had advantages and disadvantages.

Table 4. Comparison of verification tests.

Method	Factor			Predictive Yield%	Real Yield %
	A	B	C		
Response Surface **M**ethod	48.52	40	36.13	0.791	0.793
Orthogonal **D**esign	50	40	37.5	-	0.779

The response surface method designed the three factor three level tests for 17 groups (Table 1), in which there were 5 groups of repeated centre points, and each test was repeated for at least 2 times, so there were at least 29 tests. The orthogonal design was performed in 9 groups (Table 3), with each group repeating at least 2 times, and the experiment was conducted at least 18 times. The optimal process results obtained by the two methods were basically the same as shown in table 4.

The results of variance analysis showed that the extraction temperature had significant effect on flavonoids yield, while the extraction pressure and dosage of entrainer had little effect. However, the response surface method could analyse could not only know the significant effect of each factor on the extraction rate, but also further analysed the interaction between factors (Table 2 and Figure 2). The existing orthogonal experimental data could not judge whether there was interaction between factors.

The response surface method could simulate the optimal process intuitively and accurately with design-expert software, and the predicted value could be given, while the orthogonal method only selected the factor value that had already occurred, and could not predict the result. The results showed that the actual value of response surface method was 0.002% higher than the predicted value, and 0.022% higher than the orthogonal method.

4 Conclusions

Response surface method and orthogonal design could be used to analyse and optimize the supercritical extraction process of flavonoids from spina gleditsiae. However, the response surface method could provide more accurate data information in the case of a small increase of the number of tests, which was more conducive to finding the best process in production practice, saving cost, and could also simulate and predict the highest yield, which was worthy of promotion. The best extraction process for flavonoids was extraction temperature as 48.52℃, extraction pressure as 40MPa, dosage of entrainer as 36.13mL, and flavonoids yield as 0.793%. The extraction temperature had a significant effect on flavonoids from spina gleditsiae and had an interaction with entrainer dosage.

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