Optimization of Extraction Process for Total Flavonoids from Dioscorea Nipponica Makino leaf by Response Surface Methodology

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Abstract. To optimize extraction process of total flavonoids from Dioscorea Nipponica leaf. The effects of influencial factors on total flavonoids concentration were discussed by single factor test and Response Surface designed experiments. The optimal condition for total flavonoids extraction was solid-liquid ratio 1:26, ethanol concentration 70.5%, extraction duration 30min and extraction temperature 51℃. Under this condition, the flavonoids extraction efficiency was 16.54%, the value of 16.31%, the relative error was 0.23%.

Introduction

Dioscorea Nipponica Makino were widely cultivated in various regions. In addition to the medicinal values, its stem also has value in food. Currently, most of the researches has focused on the cultivation and extraction of diosgenin from its tuberous roots, seldom involved extraction of total flavonoids from its leaf. Its leaves were discarded. To reduce the waste of resources and optimize extraction process of total flavonoids from Dioscorea Nipponica leaf, the effects of influence factors on total flavonoids concentration were discussed by single factor test and Response Surface designed experiments.

Methods

Selecting a Template (Heading 2).

Response surface methodology (RSM), as an effective statistical model, has been widely used in pharmaceutical and functional foods research. In this study, twenty-nine experiments were designed and carried out by Box-Behnken design of RSM, which was applied to optimize extraction conditions. On the basis of the single factor experiment results, four independent variables, including the ratio of solution to solid (X_1), ethanol concentration (X_2), extraction temperature(X_3), and extraction time (X_4), were regarded as the response of the design experiments, and each variable was investigated at three levels (Table 1). The results were listed in Table 2. According to a regression analysis of the experimental data, the extraction efficiency could be explained by the following polynomial equations.

Table 1 Level and code of variables for Box-Behnken design.

Independent factor	Symbol	Levels		
		-1	0	1
Ratio of solution to solid	X_1	1:20	1:25	1:30
Ethanol concentration（W）	X_2	60	70	80
Extraction temperature（℃）	X_3	50	60	70
Extraction time（min）	X_4	2	3	4

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Results

On the basis of response surface methodology(RSM), the optimal conditions were determined to be the ratio of solution to solid as 26:1, extraction duration 30min and extraction temperature 51℃. The experimental yield of flavonoids was 16.54%. This study indicated that was an innovative, efficient, and environment friendly method in ultrasonic extraction fields, and had a potential to effectively extract other bioactive constituents.

Table 2 Box-Behnken Design for independent variables and observed responses.

Run No.	X_1	X_2	X_3	X_4	Extraction (%)	
					Experimental	Predicted
1	0	1	0	1	9.99	10.1
2	0	-1	0	-1	11.45	11.28
3	0	1	0	-1	13.96	14.32
4	1	-1	0	0	11.57	11.96
5	1	0	0	1	10.98	10.71
6	0	0	0	0	15.17	15.17
7	0	-1	-1	0	13.01	13.26
8	0	0	0	0	15.17	15.17
9	-1	0	0	1	8.50	9.01
10	1	0	-1	0	11.02	10.91
11	1	0	0	-1	14.05	13.52
12	0	0	1	-1	14.13	14.04
13	-1	0	0	0	10.96	10.65
14	-1	1	-1	0	14.28	13.4
15	1	0	1	0	14.95	15.76
16	0	0	-1	1	11.64	11.82
17	-1	0	0	-1	10.32	10.57
18	0	0	0	1	11.56	11.14
19	0	-1	0	0	15.17	15.17
20	0	0	0	0	15.17	15.17
21	-1	0	0	0	8.49	8.86
22	0	0	0	1	12.04	11.95
23	0	-1	1	0	12.02	11.61
24	0	1	1	0	14.57	14.30
25	-1	0	1	0	8.58	8.63
26	0	0	-1	-1	13.91	14.09
27	1	1	0	0	12.47	12.18
28	0	1	-1	0	12.17	12.57
29	0	0	0	0	15.17	15.17

As shown in Table 3, the *F*-value of the model was 26.14, respectively, which suggested the model was significant. The coefficients X1, X4, X1X2, X1X3, X2X4,$X1^2$, $X2^2$, $X3^2$and $X4^2$ were significant ($P < 0.05$), and the other coefficients were insignificant($P > 0.05$). The value of determination coefficient ($R^2 = 0.9632$) for this model was close to 1, indicating the effective correlation between predicted values and actual ones. The value of adjusted determination coefficients (Adj R^2) was also close to 1, which indicated the experimental values could be significantly predicted by the model.

Table 3 ANOVA of response surface quadratic model analysis for the extraction yield.

Source	Sum of squares	DF	Mean squares	*F*-value	*P*-value Prob > F
Model	126.03	14	9.00	26.14	< 0.0001
X_1	23.46	1	23.46	68.12	< 0.0001
X_2	0.71	1	0.71	2.05	0.1738
X_3	1.240E-003	1	1.240E-003	3.602E-003	0.9530
X_4	14.59	1	14.59	42.36	< 0.0001
X_1X_2	5.21	1	5.21	15.13	0.0016
X_1X_3	23.19	1	23.19	67.35	< 0.0001
X_1X_4	0.30	1	0.30	0.87	0.3658
X_2X_3	2.85	1	2.85	8.29	0.0121
X_2X_4	3.93	1	3.93	11.42	0.0045
X_3X_4	0.025	1	0.025	0.072	0.7923
X_1^2	33.67	1	33.67	97.78	< 0.0001
X_2^2	14.38	1	14.38	41.77	< 0.0001
X_3^2	2.32	1	2.32	6.74	0.0211
X_4^2	21.58	1	21.58	62.65	< 0.0001
Residual	4.82	14	0.34		
Lack of fit	4.82	10	0.48		
Pure error	0.000	4	0.000		
Cor total	130.85	28			

Three-dimensional (3D) response surface, as an essential part of regression equation, could vividly expound the interactions between two variables and determine their optimal levels(Fig. 1).The detailed descriptions were as follows: (1) The interactions between X1 (ratio of solution to solid) and X2 (ethanol concentration) was investigated while other variables were fixed. When X1 was fixed, the yield of flavonoids continuously increased until X2 reached the maximum, and then decreased. In the same way, a variation of yield caused by X1 was also observed. Hence, the interactions between X1 and X2 was obvious. (2) Effect of X1(ratio of solution to solid) and X3 (extraction temperature) was studied when other variables were constant. The yield of flavonoids was nearly unchanged. Thus, the interactive effect of X1 and X3 was insignificant. (3) The function of X1 (ratio of solutionto solid) and X4 (extraction time) was studied when other variables were constant. The yield of flavonoids constantly improved with the increase of both X1 and X4, and reached the maximum when X1 and X4 became 25:1 and 30min, respectively. Beyond this level, the yield reduced with the increase of X1 and X4. Hence, the interactive effect of X1 and X4 was remarkable; (4) Effect of X2 (ethanol concentration) and X3 (extraction temperature) was insignificant, because the yields was invariant with increased X3. (5) The interactions between X2 (ethanol concentration) and X4 (extraction time) was obvious. When X4 was fixed, the yield of flavonoids continuously increased until X2 reached the maximum, and then decreased. In the same way, a variation of yield caused by X4 was also observed. (6) When X4 was set, the yield of flavonoids was nearly unchanged, while X3 was fixed, the yield of flavonoids continuously increased until X4 reached the maximum, and then decreased.

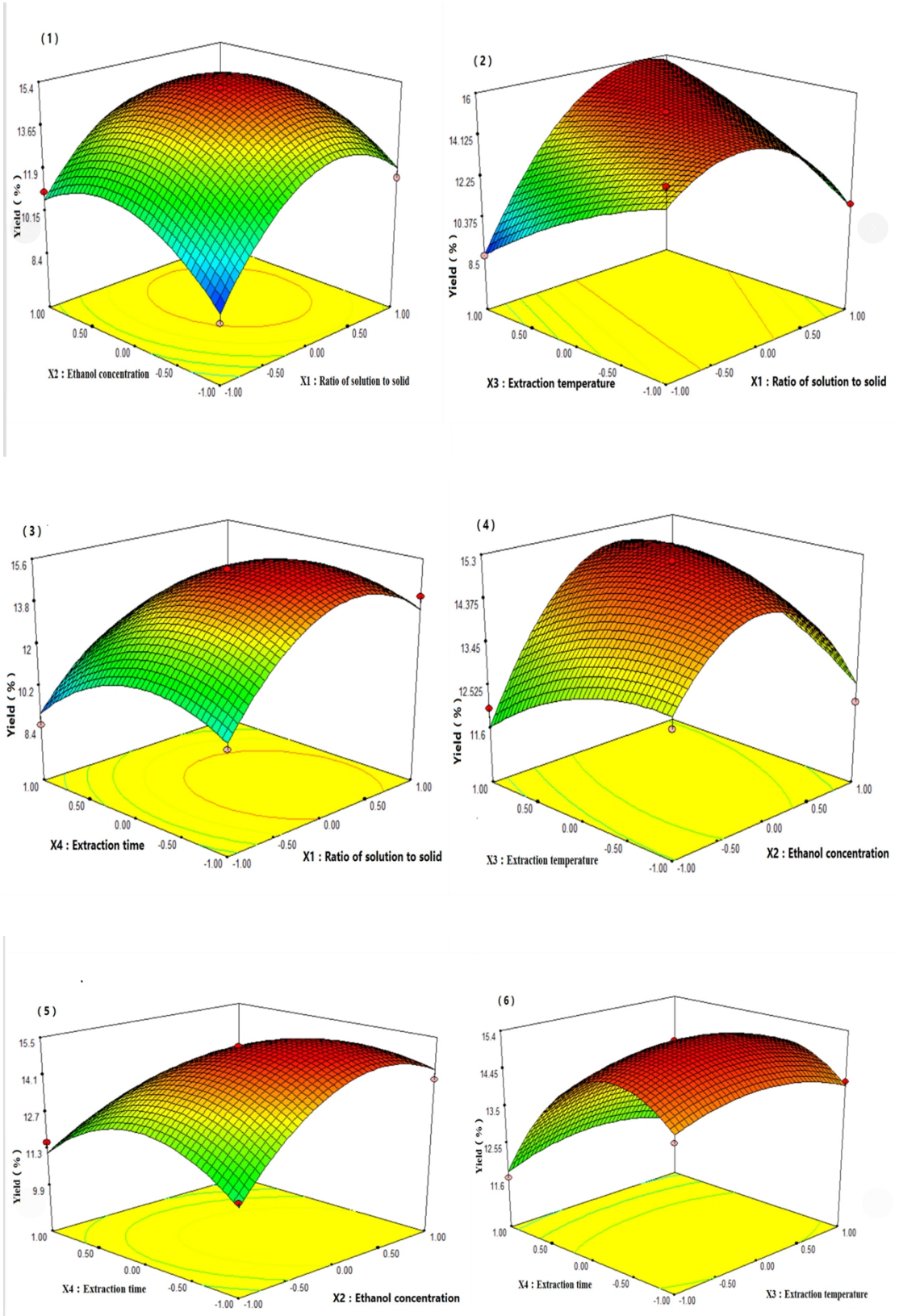

Fig. 1 The 3D response surface of total flavonoids affected by the ratio of solution to solid, ethanol concentration, extraction temperature, and extraction time.

(1) The interactions between X1 (ratio of solution to solid) and X2 (ethanol concentration) was investigated while other variables were fixed. When X1 was set, the yield of flavonoids was nearly unchanged. Thus, the interactive effect of X1 and X2 was insignificant. (2) Effect of X1(ratio of solution to solid) and X3 (extraction temperature) was also slight by means of the same analysis. (3) The function of X1 (ratio of solutionto solid) and X4 (extraction time) was studied when other

variables were constant. The yield of flavonoids constantly improved with the increase of both X1 and X4, and reached the maximum when X1 and X4 became 25:1 and 3.0 s, respectively. Beyond this level, the yield reduced with the increase of X1 and X4. Hence, the interactive effect of X1 and X4 was remarkable; (4) the interactions between X2 (ethanol concentration) and X3 (extraction temperature) was obvious. When X3 was fixed, the yield of flavonoids continuously increased until X2 reached the maximum, and then decreased. In the same way, a variation of yield caused by X3 was also observed.(5) Effect of X2 (ethanol concentration) and X4 (extraction time) was insignificant, because the yields was invariant with increased X4.(6) The yield of flavonoids increased linearly with the increase of X4 at a fixed X3, while a marked quadratic effect of X3 was obtained.

Summary

On the basis of response surface methodology(RSM), the final optimal extraction conditions were determined as follows: the ratio of solution to solid of 26:1, ethanol concentration 70.5%, extraction duration 30min and extraction temperature 51℃. To verify the accuracy of the response model, verification experiments were performed under optimum conditions, the flavonoids extraction efficiency was 16.54%, the value of 16.31%, the relative error was 0.23%.

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