PAPER • OPEN ACCESS

Optimization of Supercritical CO_2 Fluid Extraction of Flavonoids from Spina Gleditsiae by Response Surface Method

IOP Conf. Series: Earth and Environmental Science **242** (2019) 042015 doi:10.1088/1755-1315/242/4/042015

Optimization of Supercritical CO_2 Fluid Extraction of Flavonoids from Spina Gleditsiae by Response Surface Method

Xiaojuan Liu[1a*] and Xia Gao[1b]

[1]Jiangsu Provincial Xuzhou Pharmaceutical Vocational College, Xuzhou, China

[*]Corresponding author's e-mail: liuxj2000@126.com, a and b are co-first authors.

Abstract. In order to optimize the supercritical CO_2 fluid extraction of flavonoids from spina gleditsiae, single-factor experiments were utilized to investigate the influences of the extraction temperature, extraction pressure, and entrainment dosage firstly. Then the optimal extraction process for flavonoids extraction was determined by response surface methodology and the quadratic mathematical model was established which reliability was finally verified. The optimal supercritical CO_2 fluid extraction conditions were as follows: entrainment dosage of 36mL, extraction temperature of 48℃and extraction pressure of 40Mpa. Under this condition, the yield of flavonoids from spina gleditsiae was 0.793%.

1. Introduction

Response surface design is an effective statistical and optimization method. This method is able to evaluate and optimize the response value, influencing factors and their interaction relations through finite number of experiments and fitting response model on the basis of previous experiments, and obtain the level and conditions of the best factors.

In this study, the extraction temperature, extraction pressure and entrainment dosage were taken as the influencing factors and the extraction yield as the response value. The box-behnken response surface experiment was designed by using design-expert software to optimize the supercritical CO_2 fluid extraction process of flavonoids from spina gleditsiae, which provided a reliable method for the comprehensive evaluation of the quality of spina gleditsiae.

2. Materials and Methods

2.1Extraction of flavonoids

Spina gleditsiae was dried and crushed into granules, and then 5.0g 60-80 mesh spina gleditsiae granules were added to extraction tank. Supercritical CO_2 fluid extraction was carried out under different conditions finally. The absorbance of flavonoids was determined by ultraviolet spectrophotometry and the yield was calculated.

2.2Single-factor experiments

Supercritical CO_2 fluid extraction was carried out under certain extraction temperature, extraction pressure, and entrainment dosage. Each experiment was repeated three times, the yield of flavonoids from spina gleditsiae was calculated and the average value was taken to draw the chart.

2.3 Response surface optimization test

On the basis of single-factor experiments, the box-behnken model was used to design the response surface experiments, which was shown in table 1.

Table 1. Variables and levels in Box-Benhnken experimental design

Level	Factor		
	A Extraction Temperature /℃	B Extraction Pressure /MPa	C Entrainment Dosage /mL
-1	30	20	25
0	40	30	37.5
1	50	40	50

3. Results and Discussion

3.1 Single-factor experiments.

3.1.1 Effect of extraction temperature on the yield of flavonoids.

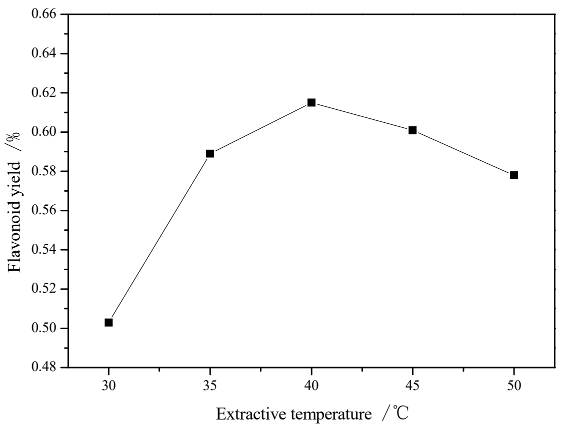


Figure 1. Effect of extraction temperature on flavonoid yield.

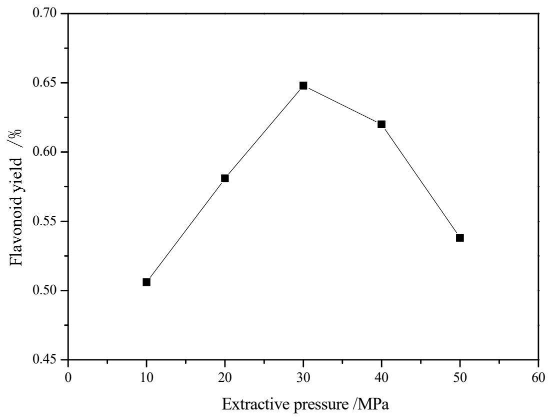


Figure 2. Effect of extraction pressure on flavonoid yield

As shown in figure1, flavonoids yield was significantly increased with the extraction temperature when other conditions were certain. The extraction achieved the best when the temperature was 40℃, and then began to decline when the temperature was more than 40℃.

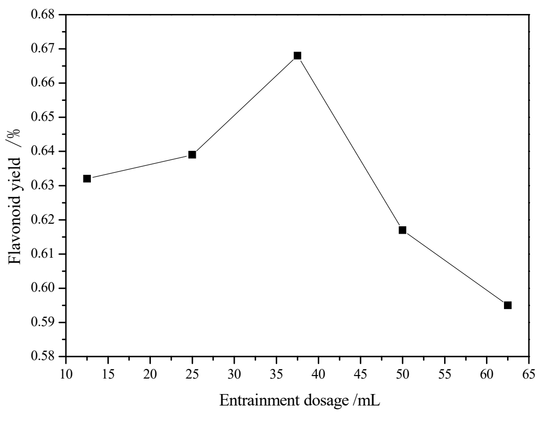


Figure 3. Effect of entrainment dosage on flavonoid yield.

3.1.2 Effect of extraction pressure on the yield of flavonoids.
As shown in figure 2, the yield of flavonoids was increased with the increase of pressure in the initial stage. When the pressure reached 30MPa, the yield was the highest, and began to decline if the pressure continued to increase.

3.1.3 Effect of entrainment dosage on the yield of flavonoids.
As shown in figure 3, flavonoids yield increases gradually with the increase of the entrainment dosage, and reach the maximum when entrainment dosage was 37.5 mL.

3.2 Response surface optimization test results.
Taking the three main factors mentioned above as independent variables and yield of flavonoids from spina gleditsiae as the dependent variable response value, 17 points were designed to investigate the influence of each factor and their interaction on the response value.

Table 2. Box-Behnken design arrangement and corresponding experimental results for response surface analysis

No.	Factor			Yield（%）
	A Extraction Temperature	B Extraction Pressure	C Entrainment dosage	
1	0	0	0	0.771
2	1	1	0	0.779
3	1	-1	0	0.735
4	1	0	1	0.757
5	0	-1	1	0.728
6	-1	-1	0	0.707
7	0	-1	-1	0.706
8	0	1	-1	0.749
9	0	0	0	0.773
10	-1	0	1	0.729
11	0	1	1	0.769
12	0	0	0	0.774
13	1	0	-1	0.761
14	0	0	0	0.769
15	0	0	0	0.754
16	-1	0	-1	0.641
17	-1	1	0	0.707

3.3 Model establishment and significance.
The obtained data were analyzed by design-expert 8.0 software, and the analyses were shown in table 3. The quadratic multiple regression equation of the three influencing factors A, B and C about the response value Y (flavonoids yield from spina gleditsiae) was obtained:

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

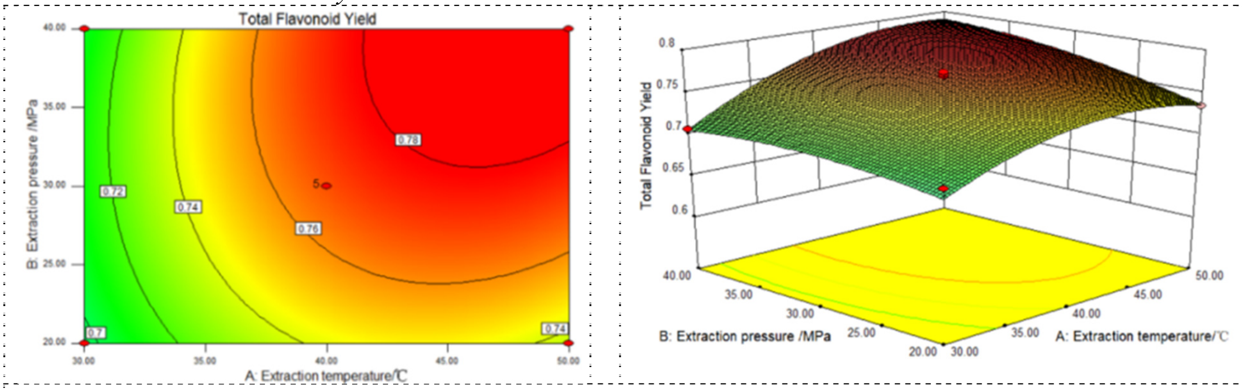
Table 3． Analysis of variance for quadratic polynomial model

Source	Sum of squares	df	Mean square	F value	P-value	
Model	0.020	9	2.202×10^{-3}	15.80	0.0023	significance
A-Extraction temperature	0.008×10^{-3}	1	7.668×10^{-3}	55.18	0.0001	
B-Extraction pressure	0.002×10^{-3}	1	2.048×10^{-3}	14.70	0.0064	
C-Entrainment dosage	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	not significance
Pure Error	2.668×10^{-4}	4	6.670×10^{-5}			
Cor Total	0.021	16				

As shown in table 3, the model F-value of 15.80 and P=0.0023＜0.01 implied the model was significant. There was only a 0.07% chance that a "Model F-Value" this large could occur due to noise. There was a 0.1268 chance that a "Lack of Fit F-value" and non-significant lack of fit was good which we wanted the model to fit.

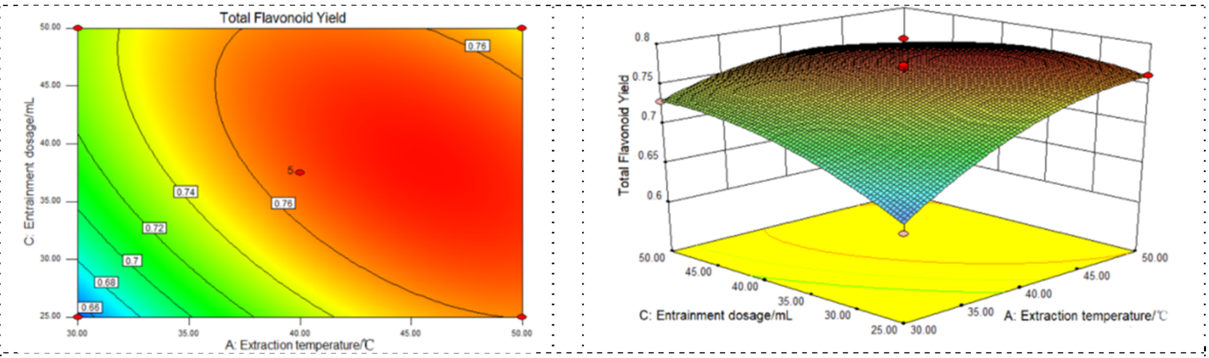
3.4 Factor interaction analysis.



a. Contour map b. 3D surface map

Figure 4. Contour plot and response surface diagram of the interaction of extraction temperature and extraction pressure on flavonoid yield

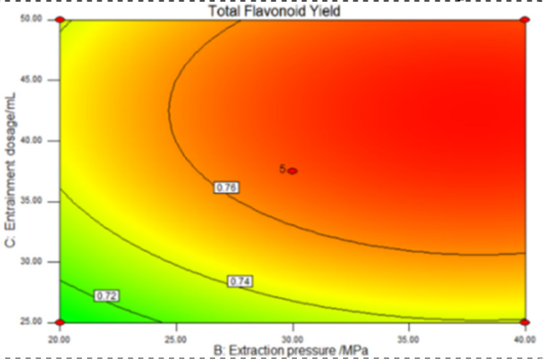
The figure 4 showed response values of extraction temperature A and extraction pressure B along the axial direction when entrainment dosage C was the optimal value, which tended to increase first and then decrease and proved that there were advantages in the experimental range. 3D response surface map was curved, and the contour map was nearly elliptic, indicating that there was a certain degree of interaction between the two factors.



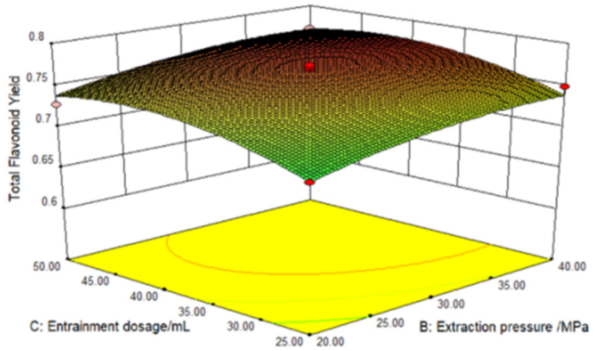
a. Contour map b. 3D surface map

Figure 5. Contour plot and response surface diagram of the interaction of extraction temperature and entrainment dosage on flavonoid yield

It could be seen from figure 5 that when extraction pressure B was fixed as an optimal value, response values of extraction temperature A and entrainment dosage C rise first and then declined slightly, with the maximum bending degree of the overall response surface and the oval contour map at the bottom, which indicated the strongest interaction between the two factors.



a. Contour map



b. 3D surface map

Figure 6. Contour plot and response surface diagram of the interaction of extraction pressure and entrainment dosage on flavonoid yield

Figure 6 showed that when extraction temperature A was fixed, response values of extraction pressure B and entrainment dosage C changed slightly in the axial direction, and 3D response surface was the most gentle, and the contour line at the bottom was close to normal circle, which indicated that the interaction between the two factors was the weakest.

3.5 Best response values and verification

Design - Expert 8.0 software was used to calculate the required parameters of the highest response value Y (flavonoids yield), which were extraction temperature as 48.52℃, extraction pressure as 40MPa, and entrainment dosage as 36.13mL. At the same time, the best response value was predicted to be 0.791%.

In order to verify the reliability and availability of the model, the calculated values of the above optimal process conditions were experimentally verified. Considering the precision of instruments, the optimal conditions of the verification experiment was adjusted for: extraction temperature as 48℃, extraction pressure as 40MPa, and entrainment dosage as 36mL. Three repeated experiments were conducted, and flavonoids yield was 0.793%, which was better than all the other experimental conditions.

4. Conclusions

In this study, the response surface method was used to optimize parameters of supercritical CO_2 fluid extraction of flavonoids from spina gleditsiae. Taking extraction temperature, extraction pressure and entrainment dosage as influencing factors and flavonoids yield as response value, the regression equation model with higher reliability was obtained. At the same time, the effect degree of each factor on yield was determined by response surface model analysis. The model was reliable, good fitting and repeatable, which had practical application value.

Acknowledgments

This work was financially sponsored by Qing Lan Project of Jiangsu Province (2017), Basic Research for Application Projects (KH17022) of Xuzhou, and Natural Science Research Projects of Xuzhou Pharmaceutical Vocational College.

References

[1] Guido R., Mariken J., Bast A. (2006) Structure and activity in assessing antioxidant activity in vitro and in vivo a critical appraisal illustrated with the flavonoids. Environmental Toxicology and Pharmacology, 21:191-198.
[2] Wang H, Gao X., Zhou GC. (2008) In vitro and in vivo antioxidant activity of aqueous extract from Choerospondias axillaris fruit. Food Chemistry, 106:888-895.
[3] Zhang J, Hayat K, Zhang X. (2010) Separation and Purification of Flavonoid from Ginkgo Extract by Polyamide Resin. Separation Science and Technology, 45:2413-2419.
[4] Reverchon E, Antonacci A. (2007) Polymer microparticles production by supercritical assisted atomization. Journal of Supercritical Fluids, 39:444-452.
[5] Wang Q, Guan Y, Yao S. (2011) Controllable preparation and formation mechanism of BSA microparticles using supercritical assisted atomization with an enhanced mixer. The Journal of Supercritical Fluids, 56:97-104.