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Optimization of Total Flavonoids Extraction from *Coreopsis tinctoria* Nutt. by Response Surface Methodology

X. F. Liu, *L. Liu*, *Y. G. Wang*,* *F. F. Leng*, *S. W. Wang*, and *Y. C. Li*

School of Life Science and Engineering, Lanzhou University of Technology, 730 050 Lanzhou, PR China

Response surface methodology (RSM) was applied to predict optimum conditions for extraction of flavonoid from *Coreopsis tinctoria* Nutt. A central composite design (CCD) was used to monitor the effect of extraction temperature, extraction time, and water-to-material ratio on yield of total flavonoids. The optimal extraction conditions were obtained as water-to-material ratio of 55 ml g^{-1}, extraction temperature of 80 °C and extraction time of 70 minutes. Under these conditions, the average total flavonoids yield, according to the mass of raw material, was 9.0 ± 0.6 %, which corresponds to the predicted value of 8.9 %. Thus, the extraction method was applied successfully to extract total flavonoids from *C. tinctoria*.

Key words: Coreopsis tinctoria *Nutt., flavonoid, reflux extraction, response surface methodology*

Introduction

Numerous studies have shown that flavonoids have many biological activities, such as antibacterial, antiviral, anticancer and antioxidant.[1,2,3] Flavonoids, consisting of flavones, flavanone, flavanols, flavonols, and flavanonols, comprise a large group of secondary metabolites in plants,[4,5,6] e.g. vegetables, fruits, flowers, roots, stems and herbs.[7,8,9] Present studies have shown that flavonoids from *Coreopsis tinctoria* Nutt. have a significant amounts of bioactive components.[6,10,11]

C. tinctoria is an annual forb widespread in Canada, Northeast Mexico, much of the United States, especially the Great Plains and Southern states, and is often called "calliopsis" by the native residents.[6,10] *C. tinctoria* plants attain heights of 30 to 100 cm. The leaves are pinnately divided, glabrous, and tending to thin out at the top of the plant where numerous 2.5 to 3.75 cm flowers sit atop slender stems. The flavonoid-rich flowers are brilliant yellow with maroon or brown centres of various size.[6]

Water extraction technology of flavonoid from *C. tinctoria* was studied by response surface methodology (RSM). RSM, first introduced by Box and Wilson, is a very useful tool for this purpose. As a package of statistical and mathematical techniques employed for developing, improving, and optimizing processes, RSM could be used effectively to evaluate the effects of multiple factors and their interaction on one or more response variables.[12,13,14,15,16] In this study, RSM was used to optimize the extraction conditions (water-to-raw-material ratio, extraction temperature, and extraction time). The extraction efficiency was validated by RSM.

* Corresponding author: Prof. Yonggang Wang
e-mail: 18009400938@163.com

Material and methods

Plant material and chemicals

Coreopsis tinctoria Nutt. (Asteraceae) was collected in August 2011 in Hetian County (Xijiang Uygur Autonomous Region, PR China) and identified by Prof. Lin Yang (School of Life Sciences and Engineering, Lanzhou University of Technology). Five hundred grams of *C. tinctoria* was dried, ground to powder, and sieved through a 40-mesh screen.

All analytical grade solvents came from Beijing Solarbio Co., Ltd. (Beijing, PR China), and standard of rutin (> 98 %) was obtained from Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, PR China).

Extraction of flavonoids and determination of extraction yield

The dried flower samples (2.0 g) were extracted for flavonoids by reflux extraction at different temperatures (25 °C to 95 °C) with a water-to-raw-material ratio (ml g^{-1}) (ranging from 1 : 6 to 1 : 21) for a given extraction time (ranging from 0.5 to 3.0 h). The flavanone extracts were added to a defined volume of water (100 ml) and filtered. The concentration of flavonoid extracted from *C. tinctoria* was measured according to the method described by *Wu et al.*[17] The absorbance of solution was measured at 510 nm. The concentration of flavonoids in this sample was calculated according to calibration curve of rutin. The flavonoids yield, *Y*, according to the mass of raw material, m, was calculated as follows:

$$Y = \frac{\gamma N V}{m} \tag{1}$$

where γ is the mass concentration of flavonoid calculated by the calibrated regression equation, N is the dilution factor, and V is the total volume of extraction solution.

Experimental design

In this study, the single factor experimental was employed to guide the preliminary range of variables including X_1 (water-to-raw-material ratio), X_2 (extraction temperature), and X_3 (extraction time). A central composite design (CCD) was used to investigate the effects of three independent variables, X_1, X_2, and X_3 on the yield of flavonoids (Y). The independent variables were coded at three levels (–1, 0, and 1), and the complete design consisted of 20 experimental points, including six replications of the centre points (all variables were coded as zero). In detail, X_1 (45, 55, 65 ml g^{-1}), X_2 (75, 80, 85 °C), and X_3 (45, 60, 75 min) were investigated, respectively.

Statistical analysis

Experimental data showed that the response variables were fitted to a quadratic polynomial model. The general form of the quadratic polynomial model was as follows

$$y = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum_{i<j=1}^{3} \beta_{ij} x_i x_j \tag{2}$$

where y is the measured response associated with each factor-level combination; β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively; x_i and x_j are the coded independent variables. DesignExpert Software (Trial Version 7.1.6., Stat-Ease Inc., Minneapolis, MN, USA) was used to estimate the response of each set of experimental design and optimized conditions. The fit of the quadratic polynomial model was inspected by the regression coefficient R^2. F-value and p-value were used to check the significance of the regression coefficient.

Results and discussion

Effect of extraction temperature on extraction yield of flavonoids

With a fixed water-to-raw-material ratio of 50 ml g^{-1}, extraction time of 60 minutes, the extraction efficiency of flavonoids with the increase in extraction temperature will increase gradually and tend to be gentle (Fig. 1a), which is due to the gradual increase in the molecular diffusion rate with temperature. However, flavonoids are heat-sensitive and excessive temperatures will cause degradation. Low temperature extraction will save energy, therefore 80 °C was selected as the optimal extraction temperature.

Effect of the water-to-raw-material ratio on the yield of flavonoids

At a temperature of 70 °C and extraction time of 60 minutes, the extraction efficiency will increase gradually with the increase in the water-to-raw-material ratio (as shown in Fig. 1b). At water-to-raw-material ratio of more than 55 ml g^{-1}, the extraction yield increases very slowly and steadily. Thus, the value of 55 ml g^{-1} was chosen as optimal.

Effect of extraction time on extraction yield of flavonoids

By setting the water-to-raw-material ratio at 50 ml g^{-1} and extraction temperature 70 °C, the extraction efficiency increased with the extension of extraction time and tended to stabilize (Fig. 1c). The extraction efficiency was highest at 60 minutes.

Optimization of flavonoid extraction conditions

According to the values obtained in the single factor experiment and method of CCD experiment, RSM was applied to optimize the extraction conditions of flavanoids from *C. tinctoria*. Table 1 shows the experiment design matrix with the response values obtained. Multiple regression analysis of

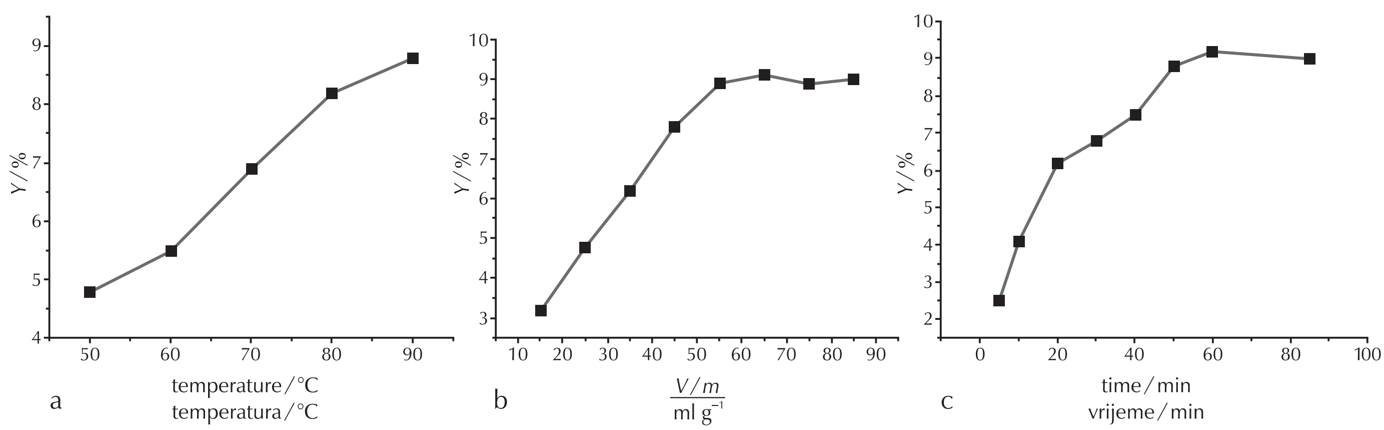

Fig. 1 – *Effect of different parameters on the yield of flavonoid extraction (a – extraction temperature, b – water-to-raw-material ratio, c – extraction time)*

Slika 1 – *Utjecaj različitih parametara na iskorištenje ekstrakcije flavonoida (a – temperatura, b – omjer vode i sirovog materijala, c – vrijeme)* the experimental data yielded the following second-order polynomial equation:

$$Y/\% = -102.91064 + 0.22740\,X_1 + 2.40285\,X_2 + 0.23775\,X_3 + 0.00137500\,X_1X_2 - 0.000591667\,X_1X_3 - 0.000983333\,X_2X_3 - 0.00263636\,X_1^2 - 0.014945\,X_2^2 - 0.000860606\,X_3^2 \quad (3)$$

The ANOVA for the fitted quadratic polynomial model of flavanoid extraction is shown in Table 2. The *F*-value of 33.43 with a low probability *p*-value indicated high significance of the model. The coefficient of determination (R^2) of 0.9678 was the proportion of variability in the data explained or accounted for by the model. The significance of each coefficient was determined using *F*-value and *p*-value. The results are given in Table 3. It could be seen that the independent variables (X_2, X_3), and the quadratic terms (X_1^2, X_2^2) significantly affected the yield of flavanoids ($p < 0.01$). The other independent variables (X_1) and quadratic terms (X_3^2) were significant also ($p < 0.05$). The two-variable interaction X_1X_2, X_2X_3, X_1X_3 had no significant influence ($p > 0.1$) on the extraction yield of flavanoids. Based on linear and quadratic coefficients, it was concluded that the order of factors influencing the response value of the extraction yield was as follows: extraction time > extraction temperature > water-to-raw-material ratio.

Table 1 – *CCD and the response values for yields of flavonoids*
Tablica 1 – *CCD i iskorištenje ekstrakcije flavonoida*

	X_1 temperature / °C temperatura / °C	X_2 time / min vrijeme / min	X_3 (V/m) / ml g^{-1}	Y / %
1	45	75	45	7.25
2	65	75	45	7.42
3	45	85	45	7.49
4	65	85	45	8.15
5	45	75	75	8.18
6	65	75	75	8.21
7	45	85	75	8.34
8	65	85	75	8.43
9	45	80	60	8.27
10	65	80	60	8.51
11	55	75	60	8.25
12	55	85	60	8.31
13	55	80	45	8.14
14	55	80	75	8.78
15	55	80	60	8.71
16	55	80	60	8.81
17	55	80	60	8.80
18	55	80	60	8.79
19	55	80	60	8.84
20	55	80	60	8.86

Table 2 – *Analysis of variance for fitted quadratic polynomial model*
Tablica 2 – *Analiza varijancije odgovarajućeg kvadratnog polinoma*

Source Izvor	Sum of squares Zbroj kvadrata	Degree freedom Stupanj slobode	MSE	*F*	*p*
model	4.27	9	0.47	33.43	<0.0001
X_1	0.14	1	0.14	9.99	0.0102
X_2	0.20	1	0.20	14.02	0.0038
X_3	1.22	1	1.22	85.89	<0.0001
X_1X_2	0.038	1	0.038	2.67	0.1335
X_1X_3	0.063	1	0.063	4.44	0.0613
X_2X_3	0.044	1	0.044	3.07	0.1104
X_1^2	0.19	1	0.19	13.48	0.0043
X_2^2	0.38	1	0.38	27.07	0.0004
X_3^3	0.10	1	0.10	7.27	0.0225
residual rezidualno	0.14	10	0.014		
lack of fit provjera modela	0.13	5	0.026	9.52	0.0136
pure error čista pogreška	0.013	5	0.002697		
cor. total ukupno korig.	4.41	19			

$R^2 = 0.9678$ $R^2_{adj} = 0.9389$

Response surface analysis

The relationship between independent and dependent variables was illustrated by the three-dimensional representation of the response surfaces by the model, as presented in Fig. 2 for the independent variables (the water-to-material ratio, extraction temperature, and extraction time), obtained by keeping one of the variables constant, which indicated the changes in extraction yield under different conditions. Fig. 2a shows the 3D response surfaces, the combined effect of the water-to-material ratio, and extraction temperature on the extraction yield, and it revealed that the extraction yield was minimal at low and high levels of the water-to-material ratio and extraction temperature. When water-to-material ratio was at a certain value, the extraction yield increased with the increase in extraction temperature. However, it was not significant that the increase in extraction temperature affected the extraction yield at a certain water-to-material ratio. From Fig. 2b, the results indicated that the interactions between the water-to-material ratio and extraction time were significant when the other variables were fixed at a constant. As shown in Fig. 2c, extraction temperature, extraction time displayed a quadratic effect on the response. Their interaction effect on the yield was not significant.

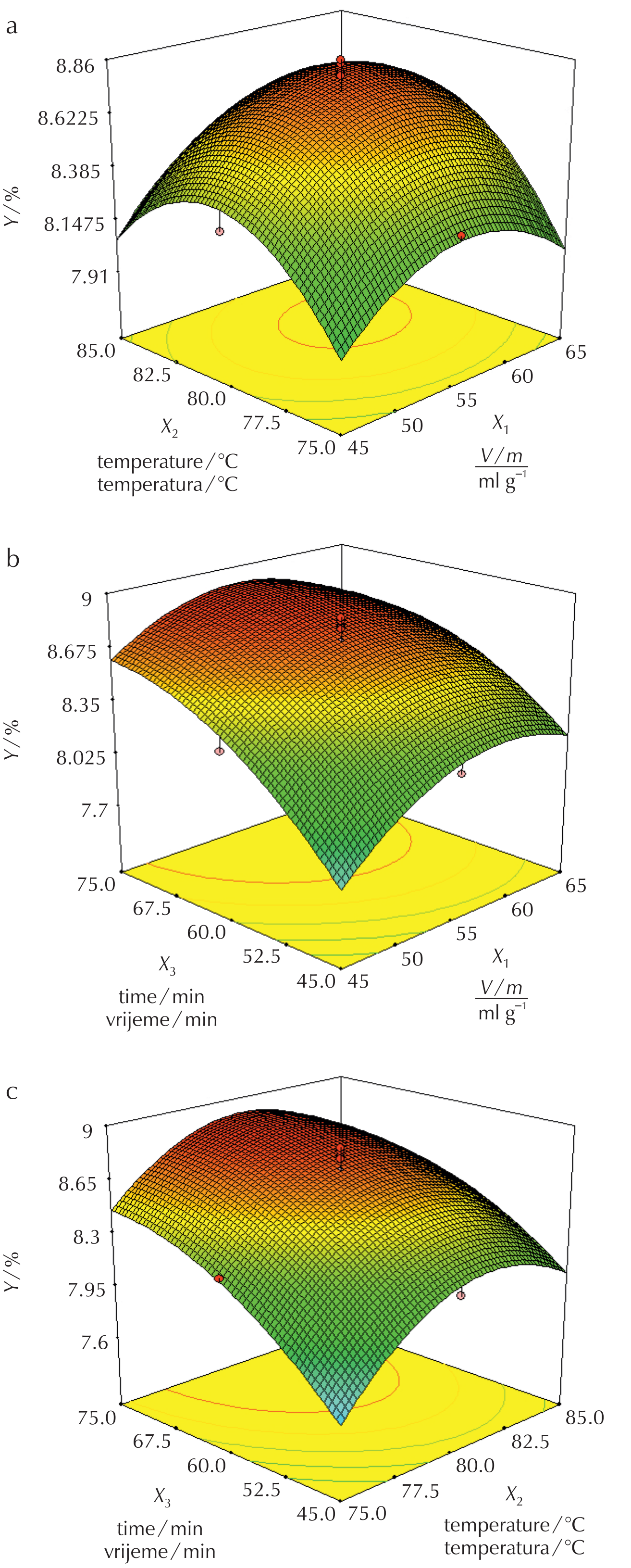

Fig. 2 – *Response surface (3D) showing the effect of different extraction parameters on the yield of flavonoid extraction*

Slika 2 – *Trodimenzionalna odzivna površina koja prikazuje utjecaj različitih parametara na iskorištenje ekstrakcije flavonoida*

Optimization and verification

The optimum condition was obtained by using DesignExpert software, and recommended as follows: water-to-material ratio of 55.96 ml g^{-1}, extraction temperature 80.56 °C, and extraction time 72.87 minutes. The maximum predicted yield of 8.91 % was obtained under these conditions.

To validate the experimental method, the extraction conditions were adjusted according to actual production, as follows: water-to-material ratio of 55 ml g^{-1}, extraction temperature 80 °C, and extraction time 70 min. The result showed that the experimental values of 8.96 % were not only consistent with the predicted values, but were also better than any single factor experiments. The response model was adequate for the optimization of extraction process (Table 2), and the model of Eq. (3) was accurate.

Conclusions

RSM was applied to predict optimum conditions for extraction of flavonoid from *C. tinctoria*. The optimal extraction conditions were obtained as water-to-raw-material ratio of 55 ml g^{-1}, extraction temperature of 80 °C, and extraction time of 70 min. Under these conditions, the average total flavonoids yield was 9.0 ± 0.6 %.

This work clearly showed that the extraction of flavonoids from *C. tinctoria* could be improved by optimizing key extraction parameters (time, temperature, water-to-raw-material ratio). Therefore, this research could be useful for preparation of flavonoids based on *C. tinctoria* for the food and drug industries. In this paper, we chose hot water as the extraction solution rather than methanol or ethanol. The extraction yield was less than methanol or ethanol extraction.[18] The main reason being to provide a foundation for the development of tea beverages. The RSM could be applied to predict optimum conditions for extraction of flavonoid from *C. tinctoria*.

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List of symbols and abbreviations
Popis simbola i kratica

F	– *F*-value – *F*-vrijednost
m	– mass of the raw material, g – masa sirovog materijala, g
N	– dilution factor – faktor razrjeđenja
p	– *p*-value – *p*-vrijednost
R^2	– coefficient of determination – determinacijski koeficijent

R_{adj}	– adjusted coefficient of determination – korigirani determinacijski koeficijent
V	– water volume, ml – obujam vode, ml
X_1	– numerical value of water-to-raw material ratio in ml g^{-1} – brojčana vrijednost omjera vode i sirovog materijala u jedinici ml g^{-1}
X_2	– numerical value of extraction temperature in °C – brojčana vrijednost ekstrakcijske temperature u jedinici °C
X_3	– extraction time in min – ekstrakcijsko vrijeme u jedinici min
x_i, x_j	– independent variables in regression analysis – neovisne varijable u regresijskoj analizi
Y	– yield, % – iskorištenje, %
y	– measured response – mjereni odziv
β_0, β_i, β_j, β_{ij}	– regression coefficients – regresijski koeficijenti
γ	– flavonoid mass concentration, mg ml^{-1} – masena koncentracija flavonoida, mg ml^{-1}
ANOVA	– analysis of variance – analiza varijancije
CCD	– central composite design – središnje kompozitni plan
MSE	– mean square error – srednja kvadratna pogreška
RSM	– response surface methodology – metoda odzivne površine

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SAŽETAK

Optimizacija metodom odzivne površine ekstrakcije ukupnih flavonoida iz *Coreopsis tinctoria* Nutt.

X. F. Liu, L. Liu, Y. G. Wang, F. F. Leng, S. W. Wang* i *Y. C. Li*

Metodom odzivne površine (RSM) utvrđeni su optimalni uvjeti za ekstrakciju flavonoida iz *Coreopsis tinctoria*. Središnje kompozitni plan (CCD) primijenjen je za praćenje utjecaja temperature, vremena te omjera vode i sirovog materijala na prinos ukupnih flavonoida. Optimalni ekstrakcijski uvjeti su omjer vode i materijala 55 ml g^{-1}, temperatura 80 °C i ekstrakcijsko vrijeme 70 min. Pri tim uvjetima prosječno iskorištenje ekstrakcije ukupnih flavonoida u odnosu na sirovi materijal bilo je 9,0 ± 0.6 %, što se slaže s predviđenom vrijednosti 8,9 %. Ekstrakcija je uspješno primijenjena za ekstrakciju ukupnih flavonoida iz *C. tinctoria*.

School of Life Science and Engineering
Lanzhou University of Technology
730 050 Lanzhou
Kina

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