The Response Surface Method Optimization Process of Water-soluble Flavonoids from Corn Silk

Ying Zhang[a 1]*, Jingjing Xu[b], Ximan Huang[c] and Yang Gao[d]

College of Traditional Chinese Medicine, Jilin Agricultural Science and Technology University, Jilin 132101

[a]*email: 365144737@qq.com, [b]email: 1062319516@qq.com, [c]email: 316137066@qq.com, [d]email: 19607202@qq.com

*corresponding author

Keywords: Corn Silk; Flavonoids; Extraction process

Abstract: In this paper, the water-soluble flavonoids from Corn Silk were studied with Reflux Extraction method. Four effect factors such as ratio of material to liquid, extraction temperature, time and times was analyzed by single factor test with the content of flavonoids as evaluation index. The optimum extraction processes of flavonoids were optimized by the Response Surface Methodology. The results showed that the optimum extraction process of water soluble flavonoids was the ratio of material to liquid at 1:43, extracted in 93.9℃ for 83min and two times. The content of flavonoids was 0.5543% in this condition，The research provided the foundation for the development and utilization of flavonoids in Corn silk.

Introduction

Corn silk is the style and stigma [1] of the gramineous plant Zea mays L. As a traditional Chinese medicine，it has the effects of diuresis, detumescence, regulating middle warmer, stimulating appetite, clearing liver and promoting bile flow, increasing prothrombin and accelerating blood coagulation [2]. Previous studies have shown that corn stigma contains flavonoids, glycosides, polysaccharides, phytosterols, alkaloids, saponins, organic acids and other chemical components [3-4]. Flavonoids are natural compounds with many functions, such as antioxidant, anti-tumor, anti-arteriosclerosis, anti-cardiovascular and cerebrovascular diseases, hypoglycemic, endocrine regulation [5]. As a byproduct of corn, corn silk is rich in resources. However, only a small amount of it is used as medicine, and most of the rest is discarded [6]. The research will provide basis for better development and utilization of corn stigma.

Materials and Methods

Materials and Instruments. Dry the corn stigma in the shade and cut it to pieces for later use. Rutin standard, distilled water, sodium nitrite solution, aluminum nitrate solution, NaOH solution. DZKW-S-4 Electric Heating Constant Temperature Water Bath Pot (Beijing Yongguangming Medical Instrument Factory), UV-5100 UV-Vis Spectrophotometer (Shanghai Yuanlu Instrument Co., Ltd.), AX324ZH Electronic Balance (Changzhou Olhaus Instrument Co., Ltd.), SHB-Ⅲ Circulating Water Multi-purpose Vacuum Pump (Zhengzhou Changcheng Science, Industry and Trade Co., Ltd.).

Drawing Standard Curves. Fractionation 0.0, 1.0, 2.0, 3.0, 4.0, 5.0mL, in the fractionary water-set 10mL capacity bottle, each steaming water soot 5mL, precision subscription degree 5% nitrate solution 0.3mL, mull, standing 6min, additive degree 10 % nitrate solution 0.3mL, .hin, re-standing 6 min , Degree of 1 % hatching solution 4mL, steamed water rare degree for fraction,

[1]Corresponding author. Tel.: 86-432-64703006; e-mail: E-mail: 365144737@qq.com

mull, neglected 15min, The first bottle work blank, for ultraviolet kansa spectroscopic degree of light in 500nm wave sputum constant absorbance degree of absorption, Sitting a certain degree Of the degree of the like, absorbance A sitting, the supposition of the system.

Optimization of Experimental Design by Response Surface Methodology. On the basis of the single factor test results, the three main factors that affect the flavonoid content in corn stigma are continuously optimized. Based on the analysis of Design-Expert8.0.6.1 software. The levels and codes of experimental factors are shown in Table 1.

Table1. Factors and levels

Levels	Factors		
	A:Ratio of material to solution [g/mL]	B:Extraction time [min]	C:Extraction temperature[℃]
-1	1:30	70	85
0	1:40	80	90
1	1:50	90	95

Verification Test. Combined with response surface analysis test results, three validation tests were carried out according to the optimal extraction process, and the average content of flavonoids in the three tests was determined to verify the stability of the extraction process.

Results and analysis

Drawing of Standard Curve. According to item 1.2.2, the standard curve is drawn with concentration C as the abscissa and absorbance A as the ordinate. See Figure 1.

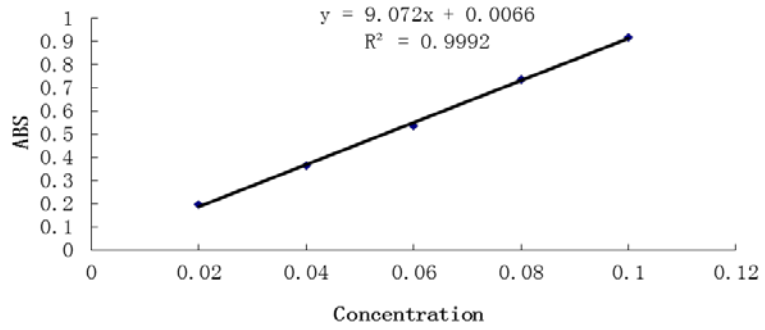

Figure 1. Standard curve

As can be seen from fig. 1, the linear regression equation between absorbance value and concentration is: y = 9.072x+0.0066, and the correlation coefficient R^2 = 0.9992, indicating a good linear relationship.

Design Results of Central Composite Test. Through the central combination test, a three-factor and three-level test design was adopted to conduct 17 tests. The content of flavonoids was used as evaluation index, and the parameters of different extraction time, extraction temperature and solid-liquid ratio were analyzed by Design-Expert 8.0.6.1 software. The experimental design and results are shown in Table 2.

Table 2. Experimental design and results

Numbers	A	B	C	Flavonoids content %	Numbers	A	B	C	Flavonoids content %
1	-1	1	0	0.4284	10	0	-1	-1	0.436
2	0	0	0	0.5308	11	-1	-1	0	0.451

3	0	0	0	0.5576	12	-1	0	1	0.5005
4	1	1	0	0.521	13	0	-1	1	0.5124
5	1	-1	0	0.4661	14	0	0	0	0.5533
6	0	0	0	0.5565	15	0	1	-1	0.4693
7	1	0	-1	0.4327	16	0	1	1	0.5447
8	0	0	0	0.5447	17	1	0	1	0.5307
9	-1	0	-1	0.4144					

Through the analysis of Design-Expert 8.0.6.1 software, taking the flavone content as the dependent variable, the regression equation of water-soluble flavone extracted from corn stigma under reflux was obtained as follows: R1 =+0.55+0.020 * A+0.012 * B+0.042 * C+0.019 * A * B+2.975E-003 * A * C-2.500-004 * B * C-0.051 * A-0.030 * B2-0.028 * C2

The optimized regression model was analyzed by variance (Table 3).

Table 3. Results of variance analysis

Source of variance	Sum of squares	Degrees of freedom	Mean square	F-value	P-value
Model	0.040	9	4.450E-003	25.86	0.0001***
A	3.050E-003	1	3.050E-003	17.72	0.0040**
B	1.198E-003	1	1.198E-003	6.96	0.0335*
C	0.014	1	0.014	81.95	<0.0001***
AB	1.502E-003	1	1.502E-003	8.73	0.0213*
AC	3.540E-005	1	3.540E-005	0.21	0.6639
BC	2.500E-007	1	2.500E-007	1.453E-003	0.9707
A2	0.011	1	0.011	64.87	<0.0001***
B2	3.908E-003	1	3.908E-003	22.71	0.0020**
C2	3.188E-003	1	3.188E-003	18.52	0.0035*
Residual	1.205E-003	7	1.721E-004		
Lack of Fit	7.071E-004	3	2.357E-004	1.89	0.2717
Cor Total	0.041	16			
R2	0.9708				
Adj R2	0.9333				

*Significant at p<0.05；**Significant at p<0.01；***Significant at p<0. 001.

From Table 3, it can be seen that the f value and p value of this model are 25.86 and 0.0001 (P＜0.001), indicating that the selected model is extremely significant. The mismatch term is 0.2717, indicates that the quadratic polynomial equation is not mismatched, but the regression equation fits well. The extraction temperature (C) has a very significant effect, the solid-liquid ratio (A) and the extraction time (B) have significant effec. The main effect relation of each factor is as follows: C> A > B. The correlation coefficient R^2=0.9708, indicating that 97.08% of the changes in response values were from the variables selected in the experiment. This model can be used to analyze the extraction process of soluble flavonoids from corn stigma.

Determination of Optimal Preparation Process. The response surface three-dimensional curved surface diagram can intuitively reflect the influence of interaction of any two factors on flavone content. Through analysis by Design-Expert 8.0.6.1 software, the obtained response surface diagram is shown in Figures 2 to 4.

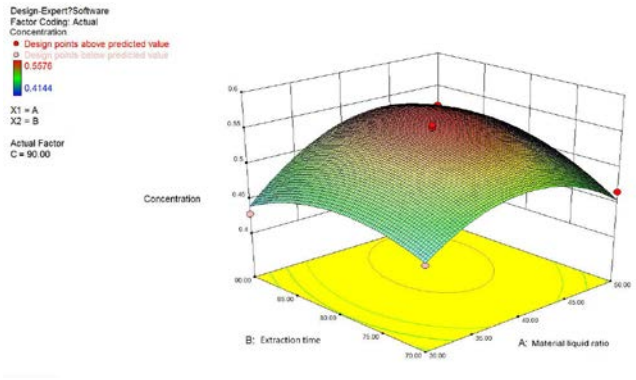

Figure 2. Response surface analysis of the content of flavonoids in Corn Silk by extraction time and material liquid ratio

As can be seen from Fig. 2, the extraction time has a maximum value between 80 and 85 min, and the material-liquid ratio has a maximum value between 1: 40 and 1: 45. The interaction between the two factors is significant.

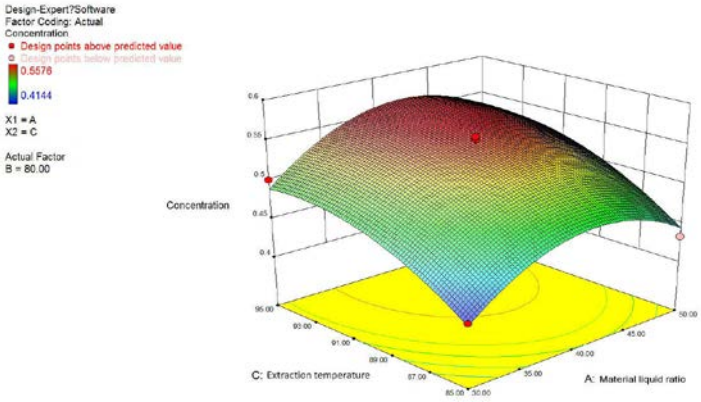

Figure 3. Response surface analysis of the content of flavonoids in Corn Silk by extraction temperature and material liquid ratio

As can be seen from Fig. 3, the extraction temperature has a maximum value between 93 and 95℃, and the material-liquid ratio has a maximum value between 1: 40 and 1: 45. The interaction between the two factors is not obvious.

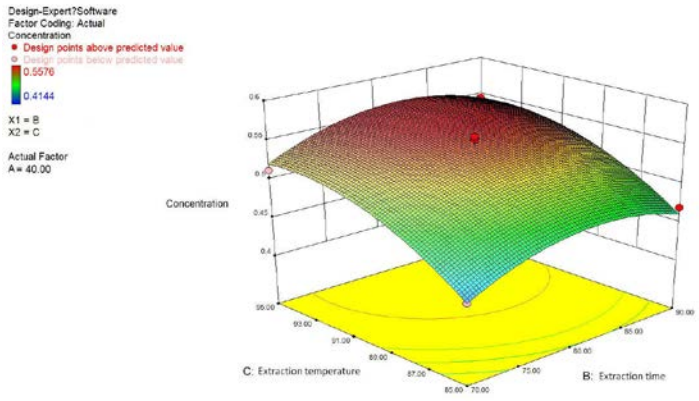

Figure 4. Response surface analysis of the content of flavonoids in Corn Silk by extraction time and temperature

As can be seen from fig. 4, the extraction temperature has a maximum value between 93 and 95℃, and the extraction time has a maximum value between 80 and 85 min. The interaction between the two factors is not obvious.

Through response surface analysis, the best extraction conditions are as follows: solid-liquid ratio 1:42.65, extraction time 82.82min, extraction temperature 93.88℃. Under the optimal conditions, the predicted value of flavone content is 0.569185%. Considering the operability of the actual operation, the reflux extraction conditions of water-soluble flavonoids from corn stigma were determined as follows: the ratio of material to liquid was 1:43, the extraction time was 83min, and the extraction temperature was 93.9℃.

Verification Test. In order to verify the predicted results, according to the optimal extraction process conditions optimized above, the average content of flavone in corn stigma is measured to be 0.5543%, close to the predicted value, and the prediction precision is 97.4%, which indicates that the equation fits well with the actual situation, and also indicates this method is suitable for optimizing the reflux extraction process of flavone in corn stigma.

Discussion

In this experiment, the extraction process of water-soluble flavonoids in corn stigma was optimized by response surface analysis method. Compared with previous experiments, this method has simple operation and accurate results, which lays a foundation for the development of corn stigma.

References

[1] Z. Yan and C.Y. Li. Study on Extraction Technology of Flavonoids from Stigma Maydis. Journal of Jiangxi Agriculture. 09(2007) 108-109+113.

[2] P. Liu. Extraction, Separation and Bioactivity of Flavonoids from Stigma Maydis [D]. Shanxi Normal University, 2006.

[3] N.L. Li, J.Z. Liu and Z.H. Chen. Study on Extraction Technology of Corn Silk Flavonoids. Journal of Foshan University of Science and Technology (Natural Science Edition). 02(2012) 68-70+96.

[4] B.C. Wu, C.X. Yang, D. Zhang and C.L. Hou. Study on Ultrasonic Extraction Technology of Corn Silk Flavonoids. Chemical and Biological Engineering. 07(2011) 67-69.

[5] Y.X. Hu, Y. Yang, S.S. Yang and X.X. Lu. Study on Ultrasonic Extraction of Total Flavonoids from Stigma Maydis. Grain and Oil Processing. 03(2009) 120-122.

[6] N.N. Bo and H. Fu. Extraction and Determination of Total Flavonoids in Stigma Maydis. Journal of Capital Normal University (Natural Science Edition). 04(2009) 44-47.