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Research Article

Ultrasound-Assisted Extraction of Total Flavonoids from Corn Silk and Their Antioxidant Activity

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Object. Ultrasound-assisted extraction of total flavonoids from corn silk and their antioxidant activities were studied. *Methods*. Response surface methodology was adopted to optimize the extraction conditions and antioxidant activities of the extracted total flavonoids were detected through ferric reducing antioxidant power (FRAP) assay. *Results*. Through a three-level, three-variable Box-Behnken design of response surface methodology (RSM) adopting yield as response, the optimal conditions were determined as follows: ultrasonic power 500 W, extraction time 20 min, material solvent ratio 1 : 20, and ethanol concentration 30%. Under the optimum conditions, the extraction yield of total flavonoids was 1.13%. FRAP value of total flavonoids extracted from corn silk was 467.59 μmol/L. *Conclusion*. The total flavonoids of corn silk could be developed as food natural antioxidant reagents.

1. Introduction

Corn silk (*Zea mays* L.) is one of the Chinese traditional herbals and contains flavonoids, sterol, alkaloids, carbohydrates, inorganic elements, vitamins, and other chemical constituents [1, 2] which show remarkable bioactivities, such as diuresis, hypoglycemic, bacteriostatic, antihypertensive, enhancing immune, and anticancer activity [3]. The extracts of corn silk are approved by FDA as OTC drug [4].

Flavonoids are effective components of many Chinese herbal medicine with the function of antihypertensive, reducing blood lipid, being antibacterial and antitumor, enhancing immune, antioxidant, and eliminating free radicals [5]. Therefore, it is helpful to look for an effective method to extract flavonoids from corn silk.

However, the literature on efficient extraction of corn silk is limited. Traditional methods (distillation or liquid solvent extraction) for the extraction of flavonoids from plant often need long extraction times, use of large amounts of solvent, and low efficiencies. Moreover, flavonoids are thermally unstable and easily degrade during the extraction. In order to increase the extraction yield, ultrasound-assisted extraction [6–9], which is inexpensive, simple, and efficient alternative to conventional extraction technique, was adopted in our study. In addition, ultrasound also shows a mechanical effect, allowing greater penetration of solvent into the sample matrix, increasing the contact surface area between solid and liquid phases. Meanwhile, Box-Behnken design of response surface methodology (RSM) was applied in the determination of the optimal extraction conditions with the independent variable of material-solvent ratio, extraction time, and ethanol concentration. The antioxidant activities of extracted total flavonoids were also determined using reliable method [10, 11].

2. Materials and Methods

2.1. Instruments. UV-1100 spectrophotometer, ultrasonic extractor, rotary evaporator, multiuse recycle water vacuum pump, grinder, and electronic balance.

2.2. Reagent. Corn silk was harvested in Sichuan Agricultural University green nursery garden, Rutin standard was obtained from Sichuan Institute for Food and Drug (99% purity, batch number 130910), ethanol, sodium hydroxide,

aluminum nitrate, sodium nitrite, ferrous sulfate, concentrated hydrochloric acid, and sulfuric acid were of analytical grade and purchased from Chengdu Kelong Chemical Reagent Company. TPTZ was purchased from Beijing Solarbio Science and Technology Company for the preparation of the FRAP solution.

2.3. Determination of Standard Curve. A total of Rutin standard solution was prepared by dissolving Rutin reference material in 70% ethanol; Rutin solution (0.0 mL, 1.0 mL, 2.0 mL, 4.0 mL, 6.0 mL, 8.0 mL, and 10.0 mL) was accurately pipetted into 25 mL volumetric flasks, respectively. Then, 12 mL of the 70% ethanol and 2 mL of the sodium nitrate solution were added. The mixture was shaken up and placed for 10 min, followed by the addition of 2 mL 10% nitric acid aluminum solution, and was shaken up. After 10 min, 20 mL sodium hydroxide was added to scale. The mixture was deposited for 5 min, and then the absorbance of the solution was measured at 500 nm with the reagent blank as reference. Make the standard curve with the concentration of Rutin standard solution as abscissa and absorbency as vertical; the regression equation was $Y = 8.3968x - 0.0043$, $R^2 = 0.9998$.

2.4. Single Factor Experiment. Fixed extraction conditions are as follows: ultrasonic time 20 min, material solvent ratio 1 : 20, ethanol concentration 30%, ultrasonic temperature 60°C, ultrasonic power 500 W, and one-time extraction. The supernatant was taken after being centrifuged for 10 min under 3000 r/min, and then the absorbance was measured according to 2.1. Extraction time (10 min, 15 min, 20 min, 25 min, and 30 min), material liquid ratio (1 : 5, 1 : 10, 1 : 15, 1 : 20, and 1 : 25), and ethanol concentration (20%, 30%, 40%, 50%, and 60%) were selected as the key variables. Their impact on the yield of the total flavonoids was tested separately:

Among them, the flavonoids content C (mg/mL) = X ∗ diluted multiples; flavonoids yield (%) = $[C * V/1000\,W] * 100\%$;

X: the concentration of flavonoids, calculated by standard curve (mg/mL);

W: sample quality (g);

V: the original volume of extracting solution (mL).

2.5. The Response Surface Analysis Factor Levels Design [12]. According to single factor experiments, coded level of the three factors for Box-Behnken design of RSM were settled as follows: ethanol concentration (20%, 30%, and 40%), extraction time (15 min, 20 min, and 25 min), and material liquid ratio (1 : 10, 1 : 15, and 1 : 20).

2.6. FRAP Method to Determinate the Antioxidant Activity of Flavonoids [13, 14]

2.6.1. Antioxidant Activity Determination. The total flavonoids were vacuum-concentrated and dried after being extracted under the optimal extraction conditions, and then the crude flavonoids were obtained and certain concentration of flavonoids solution was prepared with distilled water. A volume of 0.3 mL sample solution was pipetted, and 2.7 mL FRAP solution (preheated to 38°C, prepared by 10 mmol/L TPTZ working solution, 20 mmol/L $FeCl_3 \cdot 6H_2O$, 0.3 mol/L Hac buffer at 1 : 1 : 10, pH = 3.6) was added. The mixture was shaken up and placed for 15 min. We keeping record of the absorbance values at 593 nm. Absolute ethanol was used as blank control for zeroing. $FeSO_4$ concentration (μmol/L) could be obtained from the standard curve according to the absorbance and defined as FRAP value. Sample with higher FRAP has higher antioxidant ability.

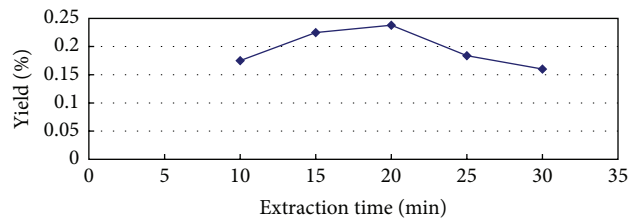

Figure 1: Effects of extraction time on yield of total flavonoids.

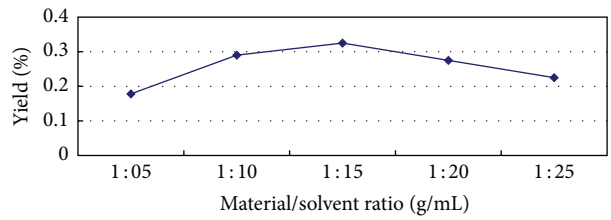

Figure 2: Effects of material solvent ratio on yield of total flavonoids.

2.6.2. Determination of $FeSO_4$ Standard Curve. A volume of 6.08 mg $FeSO_4$ was dissolved with distilled water, 0.25 mL 18 mol/L H_2SO_4 was added, and the mixture was diluted with distilled water to the scale of 50 mL, and then an iron nail was put into it. 5 mL of the solution was pipetted into 50 mL volumetric flask and was diluted with distilled water to the scale to make 800 μmol/L $FeSO_4$ standard solution. The 200, 400, and 600 μmol/L standard solutions were prepared sequentially. Regression equation of the standard curve was obtained as $y = 0.0021x + 0.3210$, $R^2 = 0.9996$.

3. The Results and Analysis

3.1. Single Factor Results

3.1.1. Effect of Extraction Time. From Figure 1, the extraction yield of total flavonoids increased with the extension of time and reached maximum value at 20 min, but after 20 min, the yield decreased, which might be due to denaturation of the total flavonoids through long period of ultrasonication.

3.1.2. Effect of Solid Liquid Ratio. From Figure 2, as ethanol scaled up, the extraction yield increased with the increase of the dissolved extracts, which reached top at ratio of

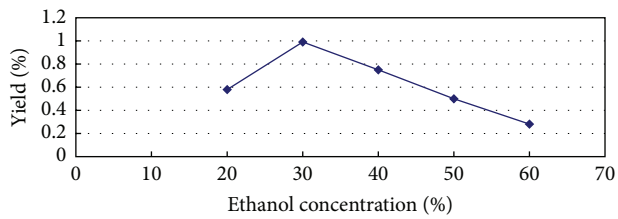

Figure 3: Effects of ethanol concentration on yield of flavonoids.

Table 1: Experimental design and data for response surface analysis.

Run	Factors			Flavonoids extraction yield (%)	
	A	B	C	Experimental data	Predictive value
1	−1	0	−1	1.01	1.08
2	1	1	0	0.97	0.93
3	0	1	−1	0.98	1.00
4	0	0	0	1.06	1.08
5	1	−1	0	0.91	0.89
6	0	0	0	1.08	1.08
7	0	0	0	1.01	1.08
8	0	−1	1	0.99	0.97
9	0	0	0	1.03	1.08
10	−1	0	1	0.94	0.91
11	−1	−1	0	0.75	0.79
12	−1	1	0	1.00	1.00
13	1	0	1	1.07	1.09
14	1	0	−1	0.82	0.84
15	0	1	1	1.07	1.09
16	0	−1	−1	0.91	0.91
17	0	0	0	1.22	1.08

1 : 20 (g/mL). Then the extraction yield decreased due to a dilution effect on flavonoids.

3.1.3. Effect of Ethanol Concentration. From Figure 3, the extraction yield of total flavonoids increased gradually and reached top at 30%. Then the yield dropped sharply, caused by enhanced volatilization with high ethanol concentration.

3.2. The Response Surface Method to Optimize the Extraction

3.2.1. The Response Surface Analysis Factor Levels of Design Results. Based on single factor experiments, extracting time, material solvent ratio, and ethanol concentration were selected, to study the effects of different combinations of the three factors by Box-Behnken design of RSM adopting Design-Expert V8.0.6 (Stat-Ease, Inc.) (Table 1).

3.2.2. Regression Model of Flavonoids Extraction Yield and the Significance Test. The regression equation was obtained as

$$\begin{aligned} R_1 = {} & 1.08 + 8.750e - 003 * A + 0.058 * B + 0.044 * C \\ & - 0.048 * A * B + 0.080 * A * C + 2.500E \\ & - 003 * B * C - 0.01 * A_2 - 0.073 * B_2 - 0.020 \\ & * C_2. \end{aligned} \tag{1}$$

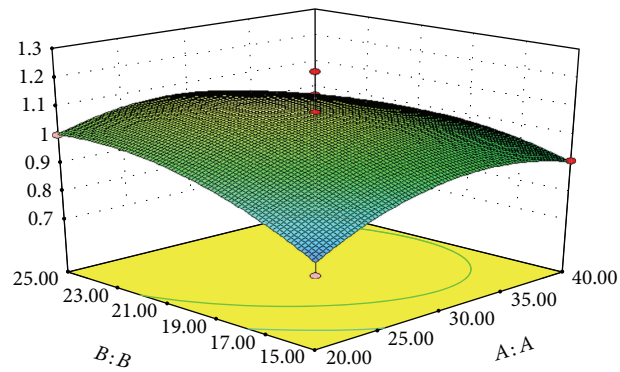

Figure 4: Response surface for effect of the extracting time and ethanol concentration on yield.

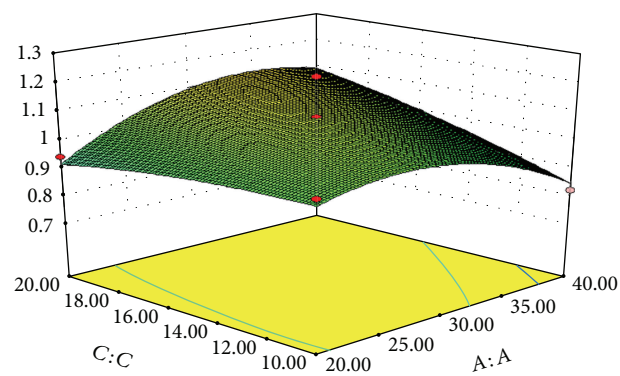

Figure 5: Response surface for effect of the material solvent ratio and ethanol concentration on yield.

Variance analysis results suggested that the effect of the factors in flavonoids extraction yield were B, C, and A in turn. While material solvent ratio and extracting time had significant effects on the total flavonoids extraction rate, ethanol concentration showed little effect. Effect of cross-term AC was significant, indicating the significant interaction of ethanol concentration and material solvent ratio. From Table 2, the relationship of the regression curve line with the actual statistical data was well described and used to determine the optimum conditions.

3.2.3. Response Surface Analysis. Comparison of Figures 4–6 showed that extracting time played a critical role for achieving higher extraction yields, observed as steep curve line with optimum value of 21 min. This may due to the strengthened medium motion caused by ultrasound and increased effect of vibrating homogenization and cavitation, which accelerated the dissolution of the total flavonoids and improved the extraction rate. The flat curve line and nonsignificant response value change of the other two factors (ratio of material solvent ratio and ethanol concentration) indicated their little influence on the flavonoids yield. Their optimum values were about 1 : 20 and 20 min, respectively.

Table 2: Variance analysis of the regression model.

Sources of variance	Sum of squares	Degrees of freedom	The mean square	F	Pr > F	Significant
Model	0.15	9	0.016	3.29	0.0655	*
A-Ethanol concentration	6.125×10^{-4}	1	6.125×10^{-4}	0.12	0.7371	
B-extracting time	0.026	1	0.026	5.27	0.0553	*
C-material solvent ratio	0.015	1	0.015	3.05	0.1242	
AB	9.025×10^{-3}	1	9.025×10^{-3}	1.80	0.2218	
AC	0.026	1	0.026	5.10	0.0484	*
BC	2.500×10^{-5}	1	2.500×10^{-5}	4.982×10^{-3}	0.9457	
A_2	0.042	1	0.042	8.39	0.0231	*
B_2	0.022	1	0.022	4.41	0.0739	*
C_2	1.684×10^{-3}	1	1.684×10^{-3}	0.34	0.5805	
The residual error	0.035	7	5.018×10^{-3}			
Loss of quasi item	7.725×10^{-3}	3	2.575×10^{-4}	0.38	0.7762	
Pure error	0.027	4	6.850×10^{-3}			
Sum	0.18	16				

* means the difference is significant at the 0.05 level.

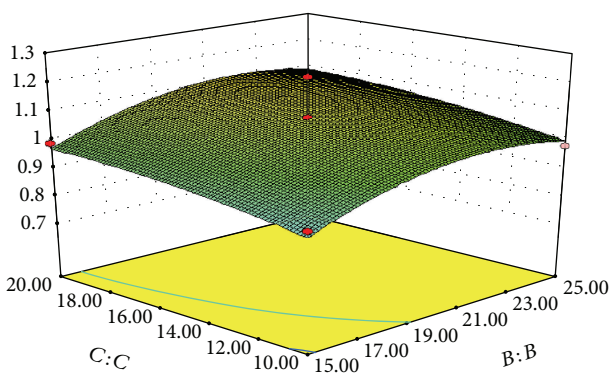

Figure 6: Response surface for effect of the extracting time and ethanol concentration on yield.

3.2.4. The Determination of the Optimal Process Conditions and Validation. The optimal conditions were determined by Design-Expert as follows: extraction time 21.45 min, material solvent ratio 1 : 20, and ethanol concentration 33.75%. Under the optimum conditions, the extraction yield of total flavonoids was 1.129%, which well fits the theoretical value, proving the reliability of extraction parameters obtained from response surface methodology.

3.3. To Generate the Determination Results of Flavonoid Antioxidant Capacity. The FRAP value is 467.59 μmol/L, which indicated that the total flavonoids of corn silk have good antioxidant activity.

4. Conclusions

In this study, the optimization of ultrasound-assisted extraction of total flavonoids from corn silk and evaluation of their antioxidant activity were conducted. On the basis of single-factor test and Box-Behnken experimental design, the quadratic regression model was established to fit the experiment data in good effect. Through response surface methodology (RSM) of yield, the optimal conditions were determined as follows: extraction time 20 min, solid-liquid ratio 1 : 20, and ethanol concentration 30%. Under the optimum conditions, the extraction yield of total flavonoids was 1.13%, which allowed higher extraction yields with lower temperature and extraction time when compared with conventional solvent extraction methods. With stable results, this method offered a theoretical basis for industrial and experimental extraction of total flavonoids from corn silk. FRAP value of the total flavonoids extracted from corn silk was determined as 467.59 μmol/L, which indicated good antioxidant activities of the total flavonoids from corn silk.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Ling-Li Zheng and Guan Wen contributed equally.

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