Optimization of Process on Extracting Flavonoids from Dry Onion Skins by Alkaline Method

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Abstract: The flavonoids in dry onion skins were extracted by alkaline solution method. On the basis of single factor combined with ternary quadratic regression general rotary unitized design, the effects of several extraction parameters on the extraction efficiency of total flavonoids were investigated, including extraction times, pH of the extraction solution, material / extraction solution ratio, extraction time, and temperature. $NaNO_2$-$Al(NO_3)_3$-$NaOH$ method was used for the determination of total flavonoids. The optimal extraction conditions were as follows: two times of extraction; pH, 11; material / extraction solution ratio, 30 : 1; extraction temperature, 83.0 ~ 84.0 ℃; extraction duration, 1.9 ~ 2.0 h. Under the above optimal conditions, the flavonoids extracted from dry onion skins yielded 2.82%.

1. INTRODUCTION

A growing body of evidence indicates that adequate fruit and vegetable consumption has a role in maintaining health and preventing disease [1-3]. Some of these protective effects may be attributed to flavonoids. It is now increasingly recognised that flavonoids may exert the health-promoting effects, including well-known antioxidant activity and anticancer properties [4-6]. Onions (Allium cepa), one of the most produced vegetable crop, are rich in flavonoids, mainly flavonols [7]. A large number of the outer dry layers of onion bulbs which are not edible and removed before consumption are usually discarded as solid by-product wastes [8]. They may have possible impact on the environment. It is reported that dry onion skins contain high levels of flavonoids, a major class of non-nutrient antioxidants [8-11]. Therefore, it is with economic value and practical significance to recover flavonoids from such onion solid wastes.

Flavonoids are weak acid and can dissolve in alkaline solution. Few authors have focused their attention on the extraction of total flavonoids from dry onion skins using the alkaline aqueous solution. In this study, the alkaline extraction of total flavonoids from dry onion skins was investigated to optimize various extraction conditions using a single factor combined with ternary quadratic regression general rotary unitized design and the mathematical model is set up.

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2. EXPERIMENTAL

2.1. Materials and Instruments

Dry skin waste of red onion was collected from Zhengzhou North Central Vegetable Wholesale Market. Onion skin powder was obtained through being cleaned, dried and ground. Quercetin ($\geq$ 98%), from National Institute for Food and Drug Control, China, was used as reference substance. The other reagents are of analytical grade.

SP752 UV-Vis spectrophotometer, Shanghai Spectrum Instruments Co., Ltd; SHZ-2000 multi-function vacuum pump of dual-supporting recycled water, Gongyi Yingyu Instrument Co.; RE-52A rotary evaporator, Shanghai Yarong Biochemical Instrument Co.; HH-2 digital-display thermostat water bath, Jintan Huafeng Instrument Co., Ltd..

2.2. Extraction of Flavonoids (EOF)

Several 500 mL round bottom flasks were added by 5.00 g onion skin powder, respectively, then added by a certain volume of NaOH solution with different pH values. The flasks were bathed under different temperatures for the different time according to experimental design. After that, the extraction liquid was filtered through paper filter. This procedure was repeated twice or more times. The filtrates were then combined in a 500 mL flask and the same NaOH solution was added to a final vlolume of 500 mL.

2.3. Preparation of Standard Solution (POS)

A standard solution (0.0101 mg/mL) of quercetin was prepared as follows: quercetin (20.2 mg) was accurately weighed and dissolved in ethanol-water (60 : 40, V / V) solution, and then the solution was diluted to 100 mL in a

volumetric flask (100 mL) with ethanol-water (60: 40, V/V) solvent. 5 mL of this solution was transferred to a volumetric flask (100 mL) and diluted to 100 mL with ethanol-water (60: 40, V/V) solution.

2.4. Determination of the Wavelength of Flavonoid Colorimetric Analysis

Firstly, 0.1 mL of the EOF or 5.0 mL of the POS was accurately transferred to a volumetric flask (10 mL), diluted to 5 mL with ethanol-water (60: 40, V/V) solution, and was added by 0.3 mL of NaNO2 (5%) solution, shaken up and then standed for 5 min. Secondly, 0.3 mL of the $Al(NO_3)_3$ (10%) solution was added to the volumetric flask, shaken, and was left to stand for 6 min. Finally, 4.0 mL of the NaOH (4.0%) solution was added to the volumetric flask, followed by addition of ethanol-water (60: 40, V/V) solution to the scale, shaken, and left to stand for 15 min before determination. Wavelength scanning was performed in a range of 200 ~ 600 nm using the solution without EOF or POS as reference solution.

The dilute solution of EOF and the quercetin were determinate simultaneously using ethanol-water (60: 40, V / V) solution as reference solution to identify the wavelength of colorimetric analysis.

2.5. Determination of Total Flavonoids

The chromogenic methods were often used to determine the content of total flavonoids in foods and herbs by ultraviolet–visible detector [12]. The principle is that most flavonoid molecules contain 3-hydroxyl/4-carbonyl, 5-hydroxy/4-carbonyl or ortho-substituting hydroxyl. Aluminum and other metal salts can be complexed by them to form colored complexs. $NaNO_2$-$Al(NO_3)_3$-NaOH colorimetric method was most commonly used among these chromogenic methods [13]. It is well known that the main components of the onion skin flavonoids are quercetin and its derivatives. Thus, quercetin was selected as standard in the present experiment. Wavelength scanning for the flavonoid extracts and quercetin before and after chromogenic reaction was performed in a range of 200 ~ 600 nm, as shown in Fig. (**1**).

It can be seen that in the range of 400 to 600 nm absorption of quercetin and flavonoid crude extracts was weak and there was not obvious absorption peak. But a very well defined peak at 318 nm was indicated in the range of 280 to 400 nm. Under less than 280nm, absorption peak was very messy. Therefore, the standard curve for quercetin was drawn at 318 nm.

2.6. Standard Curve

Six portions of the POS (0, 1.0, 2.0, 3.0, 4.0, and 5.0 mL) were accurately removed, respectively, in six volumetric flasks (10 mL), then chromogenic reaction was carried out as described in determination of the wavelength of flavonoid colorimetric analysis. Using the concentration of quercetin solution as abscissa and the absorbance as y-coordinate, the linear chart was constructed. The standard curve for quercetin is shown in Fig. (**2**).

Based on the data, linear regression was fitted using the least squares method. The regression equation of quercetin concentration (C) and absorbance (A) was as follows.
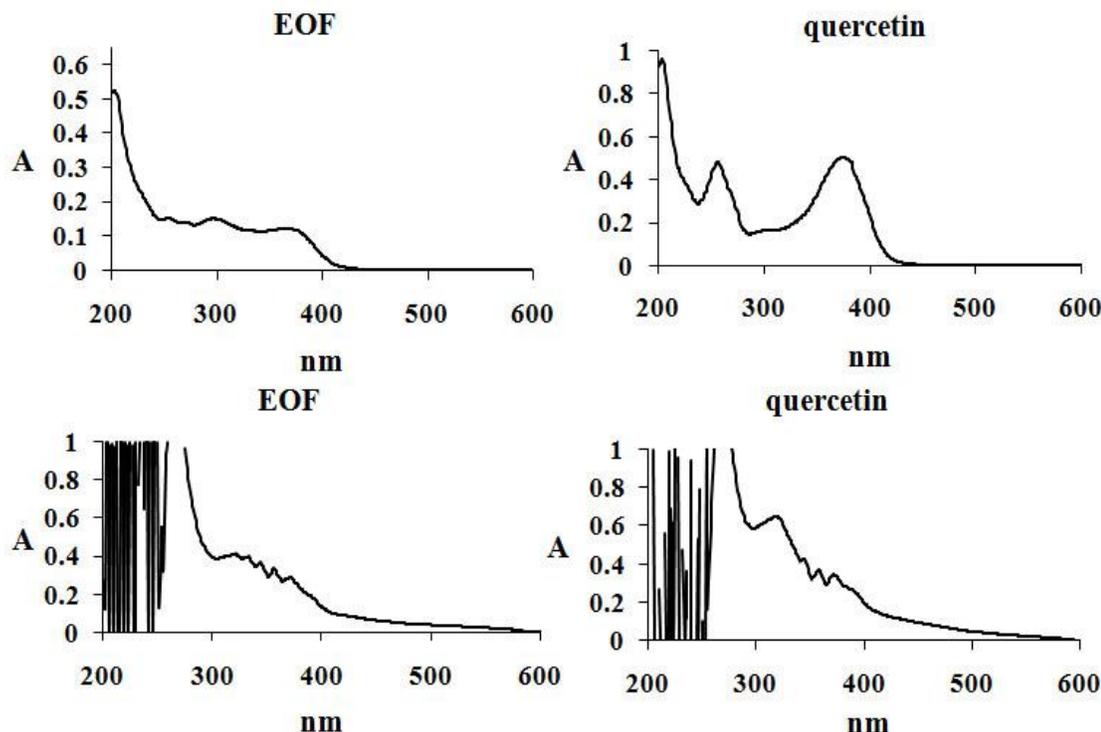
$$A = 114.23C + 0.0016 \qquad (1)$$

Fig. (1). The wavelength scan on the materials before (upper) and after colorimetric reaction (down).
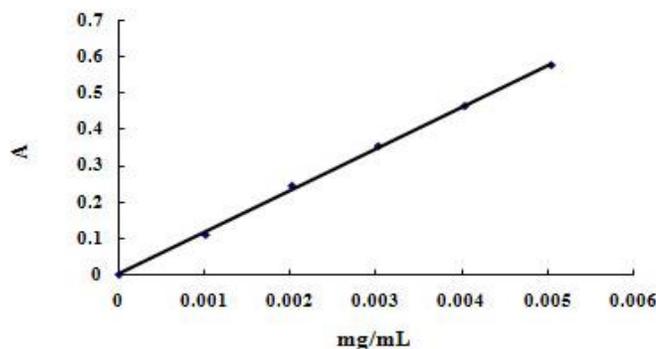
Fig. (2). The standard working curve of quercetin.

3. RESULTS AND DISCUSSION

3.1. Single Factor Test for Flavonoid Extraction from Onion Skins

3.1.1. Effect of Different Extraction Times on the Flavonoid Yield

Extracting flavonoids from dry onion skins was carried out under such conditions as temperature 85℃, NaOH extraction solution/material 30: 1, and NaOH solution pH 11. Flavonoids were extracted, respectively, through one time, twice, three times, and four times, and extractions were conducted for 2 h per time. The results are shown in Fig. (**3**).
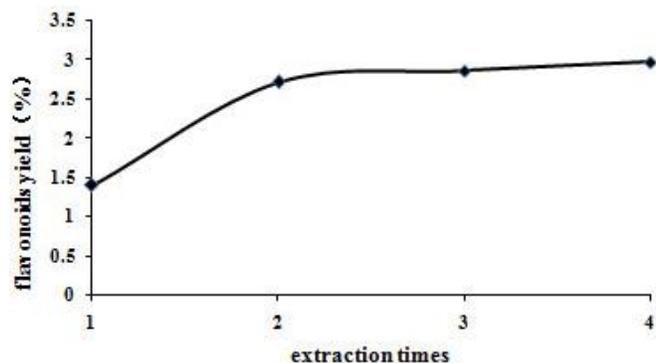

Fig. (3). Effect of extraction times on the flavonoid yield.

The flavonoid yields were 47.4%, 43.8%, 5.0%, respectively, when extractions were conducted one time, twice, and three times. The most of flavonoids were extracted from the materials after twice extractions. Flavonoid yields hardly increased with increasing extraction times to three times and more times. Moreover, increasing extraction times will increase the use of alkaline solution, which not only pollutes the environment but also increases difficulty in dealing with solvent and obtaining flavonoids. Taking into account cost, extraction times were optimized as twice.

3.1.2. Effect of Extraction Solution pH on the Flavonoid Yield

Flavonoid extraction conditions was temperature 85℃ and NaOH extraction solution/material 30:1. Extractions were conducted twice for 2 h per time. The pH of the extraction solution was adjusted, respectively, to 1, 3, 5, 7, 8,

9, 10, 11, 12, 13. Flavonoid yield obtained is shown in Fig. (**4**).

From Fig. (**4**), when pH < 7, the flavonoid yield was low and increased slowly with the increase of extraction solution pH value. When pH was 7 to 13, the flavonoid yield first increased and then decreased. The flavonoid yield was the biggest at pH 11. This is because the flavonoids show a weak acid, alkaline environment favors flavonoid extraction, but when alkali is too strong, flavonoids are oxidized easily.
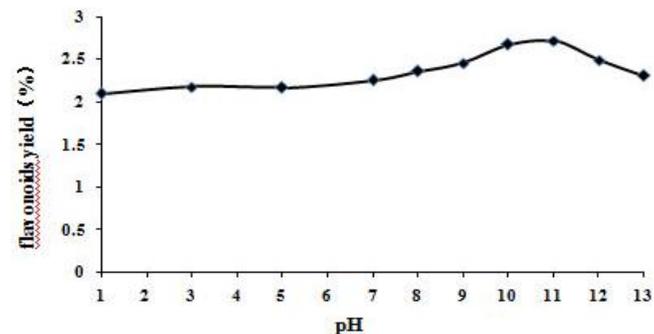

Fig. (4). Effect of extraction solution pH on flavonoid yield.

3.1.3. Effect of Ratio of NaOH Extraction Solution and Material on the Flavonoid Yield

Extracting flavonoids from dry onion skins was carried out under such conditions as temperature is 85℃ and NaOH solution pH 11. Ratios between NaOH extraction solution and the materials were 10 : 1, 20 : 1, 30 : 1, 40 : 1, 50 : 1, respectively, and extractions were conducted twice for 2 h per time.

As shown in Fig. (**5**), the yield of flavonoids gradually increased with the increasing of ratio of NaOH solution / material. When the ratio between NaOH solution and the materials increased to 30 : 1 and more, there was a little change in the flavonoids yield. Considering solvent usage, energy consumption and other factors, the ratio of 30 : 1 was optimum for the high yield of flavonoids.
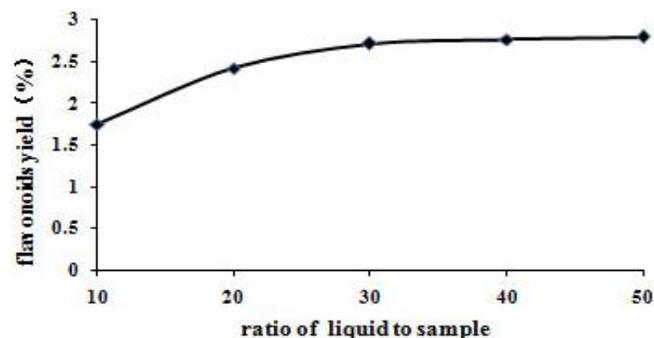
Fig. (5). Effect of material-extraction solution ratio on the flavonoid yield.

3.1.4. Effect of Extraction Duration on the Flavonoid Yield

Extracting flavonoids from dry onion skins was carried out under 85℃, NaOH extraction solution/material 30: 1, and NaOH solution pH 11. Extractions were conducted twice for 0.5 h, 1 h, 1.5 h, 2.0 h, 2.5 h, and 3 h per time, respectively. Flavonoid yield obtained is shown in Fig. (**6**).

It an be seen that flavonoid yield first increased with the increase of extraction time and then decreased over 2 h. This showed that flavonoids in onion skins can be fully dissolved and reach the dissolution equilibrium within two hours. The yield decrease after two hour is because flavonoids were damaged in alkaline and heating environment for a long time.
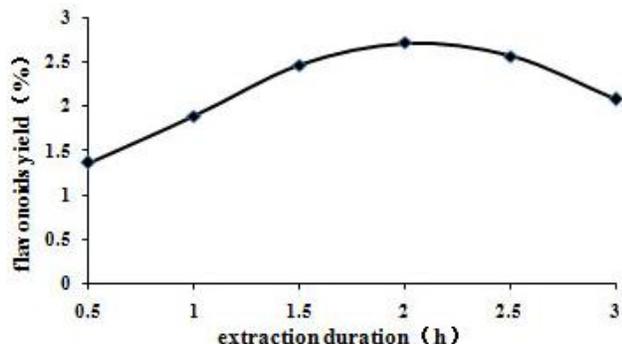

Fig. (6). Effect of extraction duration on the flavonoid obtained.

3.1.5. Effect of Extraction Temperature on the Flavonoid Yield

Extracting flavonoids from dry onion skins was carried out under NaOH extraction solution/material 30 : 1 and NaOH solution pH 11. Under the above conditions, extraction temperatures were 30℃, 40℃, 55℃, 70℃, 85℃, 100℃, respectively, and extractions were conducted twice for 2 h per time. It can be seen from Fig. (**7**) that the flavonoid yield increased at first and then decreased with extraction temperature increasing. The increasing rate of the flavonoid yield between 30℃~70℃ was low and the rate became very high between 70℃~85℃, the flavonoid yield was the highest at 85℃, and over 85℃, the flavonoid yield decreased rapidly. This may be due to the effect of temperature on the solubility of flavonoids, but too high temperature not only increased the solubility of impurities also led to the oxidation of flavonoids.
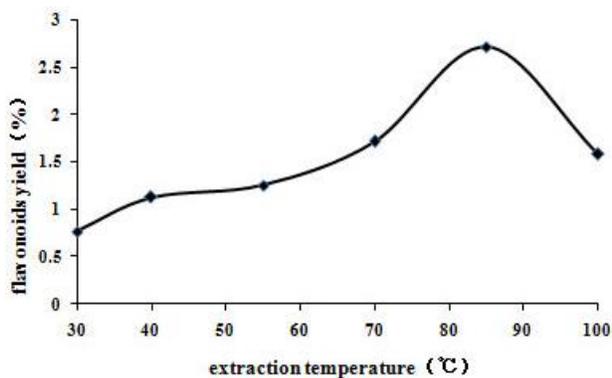

Fig. (7). Effect of extraction temperature on the flavonoid yield.

3.2. Ternary Quadratic Regression General Rotational Combinational Design

3.2.1. Experimental Design

The flavonoid yield extracted from dry onion skins was affected by extraction temperature, time, liquid/material ratio and other factors, furthermore, these factors were affected by each other. In order to find the optimal extraction conditions, the effects of these factors on the extraction rate were investigated comprehensively.

Orthogonal test is a very useful experimental design which helps obtain good results through less experimental number. However, the optimal conditions obtained by this design can only be limited to a given level of factors rather than all the levels of factors. Regression design is an effective data processing method. The experimental results may be predicted through determining the regression equation. But it can only passively analyze and process experimental data. Regression orthogonal design combines the advantages of these designs. It not only is reasonable and needs less experiment number, but also may make a mathematical model. The quadratic general rotary unitized design may partly eliminate the correlation between regression coefficients by sacrificing the orthogonal part, thus, the predicted variance is equal from the regression equation of various points on the spherical surface which has equal distance from the test central point. On the one hand, this design retains such advantages as less experimental number and easy calculation in orthogonal design, on the other hand, it helps overcome the shortcoming that the variance of the predictive values from quadratic regression in orthogonal design depends on the position of the test points in the factor space, and directly find the optimal region according to predictive values.

In the present experiment, the extraction process is optimized using ternary quadratic general rotary unitized design referring to the data of the single factor test in which the absorbance corresponding to the amount of total flavonoids in 5 g onion skin extract sets as the objective function, extraction temperature (°C), extraction time (h), and ratio of extraction solution and material (V/W) set as regulatory factors. Coded values of the treatment variables are shown in Table **1**.

Experiment design and the resultsobtained are shown Table **2**.

Regression analysis was conducted based on the absorbance using the response surface regression (SAS RSREG) procedure of statistical software SAS (9.1 SAS Institute Inc., Cary, NC, USA) [14]. The test results are shown in Table **3**.

The data in Table **3** were analysed in accordance with the standard quadratic polynomial regression. The regression equation of absorbance corresponding to the flavonoid extracts was obtained.

$$Y = 0.315000 - 0.009688X_1 + 0.0000635X_2 + 0.008687X_3 -$$
$$0.025750X_1^2 + 0.003625X_1X_2 - 0.005375X_2^2 - 0.001875X_1X_3 -$$
$$0.001625X_2X_3 - 0.003000X_3^2$$

$$(2)$$

It can be seen from the analysis of variance in quadratic regression model that $P < 0.0001$, the regression model is of significant level, and the correlation coefficient (R^2) is 0.9536, indicating that 95.36% of the experimental data can be explained using this equation. In this model, the correlation coefficients of the liner term, of the quadratic term, and of the interaction terms are 0.1193, 0.8274, 0.0068,

Table 1.`   **Coded values of the treatment variables.**

Coded values	Factors		
	Extraction temperature X_1 / ℃	Extraction time X_2 / h	Extraction solution : material X_3 / V / W
-2	70	1.5	20:1
-1	77.5	1.75	25:1
0	85	2	30:1
1	92.5	2.25	35:1
2	100	2.5	40:1
Changes in value（Δ）	7.5	0.25	5:1

Table 2.   **Experiment design and the results.**

The number of Experiment	X_1	X_2	X_3	Y(A)
1	1	1	1	0.233
2	1	1	-1	0.294
3	1	-1	1	0.317
4	1	-1	-1	0.279
5	-1	1	1	0.335
6	-1	1	-1	0.217
7	-1	-1	1	0.361
8	-1	-1	-1	0.341
9	2	0	0	0.193
10	-2	0	0	0.231
11	0	2	0	0.303
12	0	-2	0	0.294
13	0	0	2	0.327
14	0	0	-2	0.289
15	0	0	0	0.317
16	0	0	0	0.309
17	0	0	0	0.311
18	0	0	0	0.324
19	0	0	0	0.326
20	0	0	0	0.318
21	0	0	0	0.312
22	0	0	0	0.307
23	0	0	0	0.319

respectively. Significance test of the regression equation coefficients showed that the effects of the liner term and the quadratic term on the experimental results was of significant level, and the interaction of various factors was not significant. Rms (Root MSE) is 0.009003. The analysis of the residual showed that the lack of fit of regression model is not significant, indicating that the quadratic equation can better fit the actual extraction experiments.

3.2.2. t-Test of the Regression Model

t-Test showed that $P < 0.01$ in X_1, X_3, X_1X_1 and X_2X_2 of the equation (2), the interaction of various factors was not

Table 3. Regression analysis.

		Source	DF		Parameter Estimates	Standard error	t-Value	P- Value
		Variable intercept	1		0.315000	0.002955	106.61	<.0001
		X_1	1		-0.009688	0.002251	-4.30	0.0009
Linear		X_2	1		0.0000625	0.002251	0.03	0.9783
		X_3	1		0.008687	0.002251	3.86	0.0020
		X_1X_1	1		-0.025750	0.001698	-15.16	<.0001
Quadratic		X_2X_2	1		-0.005375	0.001698	-3.17	0.0075
		X_3X_3	1		-0.003000	0.001698	-1.77	0.1008
		X_1X_2	1		0.003625	0.003183	1.14	1.14
Crossproduct		X_1X_3	1		-0.001875	0.003183	-0.59	0.5659
		X_2X_3	1		-0.001625	0.003183	-0.51	0.6183
	Source		DF		Sum of squares	mean squares	F- Value	P- Value
	Model		9		0.02165	0.00241	29.67	<.0001
	Error		13		0.00105	0.00008106		
	Corrected Total		22		0.02270			
		Lack of Fit	5		0.000705	0.000141	3.23	0.0685
Residual		Pure Error	8		0.000349	0.00004361		
		Total Error	13		0.001054	0.00008106		
				Response Mean		0.291261		
				Root MSE		0.009003		
				R-Square		0.9536		
				Coefficient of Variation	3.0912			

significant. The term in P > 0.25 had little effect on the absorbance and can be negligible. Therefore, the regression equation can be abbreviated as follows:

$$Y = 0.315000 - 0.009688X_1 + 0.008687X_3 - 0.025750X_1^2 - 0.005375X_2^2 \tag{3}$$

3.2.2. Analysis of key factors

In order to analyze the separate impact of each single factor on the extraction rate of total flavonoids from onion skin, two other factors in the regression equation (3) fixed at zero level and the partial sub-regression equation of the third factor was obtained through the dimensionality reduction:

$$y_1 = 0.315000 - 0.009688X_1 - 0.025750X_1^2 \tag{4}$$

$$y_2 = 0.315000 - 0.005375X_2^2 \tag{5}$$

$$y_3 = 0.315000 + 0.008687X_3 \tag{6}$$

The derivative can be respectively obtained.

$$dy_1 / dx_1 = -0.009688 - 0.0515X_1 \tag{7}$$

$$dy_2 / dx_2 = -0.01075X_2 \tag{8}$$

$$dy_3 / dx_3 = 0.008687 \tag{9}$$

If dyi/dxi = 0 (i = 1,2), most appropriate amount of the main factors alone can be derived when the y_i is of great value: X_1 = -0.188, X_2 = 0. Equation (4 ') - (6') show that the effect orders of the main factors on the extraction rate are temperature (X_1) > liquid ratio (X_3) > extraction time (X_2).

According to these equations, the curve of the absorbance to the three factors is obtained and shown in Fig. (**8**).

Fig. (**8**) shows that the temperature is the most significant influencing factor. As the temperature changes, the parabola
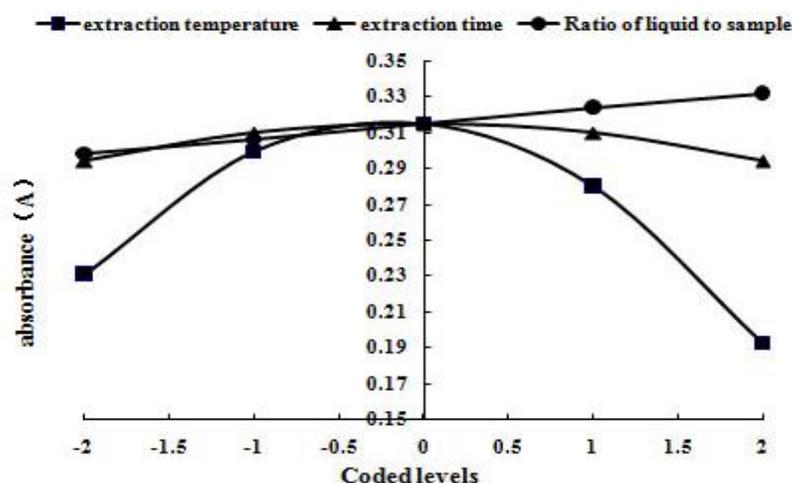
Fig. (8). Effect of single factors on absorbance.

Table 4.   The canonical parse of response surface based on coding (stable point).

Factor	Coded	Uncoded	Predicted value Y（A）	Eigen vectors			Eigen values
				X_1	X_2	X_3	
X_1	-0.135213	-0.270426		-0.063791	-0.324649	0.943681	-0.010628
X_2	-0.165277	-0.330555	0.323345	0.069174	0.941894	0.328711	-0.022102
X_3	0.810975	1.621950		0.995563	-0.086247	0.037627	-0.103770

changes of the absorbance are significant. At about -2 to -1, the absorbance increases rapidly, at -1~0, the absorbance increases slowly, the absorbance is maximum around 0, then decreases rapidly. The graphics of the extraction time effect on the absorbance is a gently downward and symmetrical parabola. The extraction rate did not rise but fell with time increase. It may be attributed to the idea that flavonoids degradated partly under relatively high temperature for a long time. The absorbance increased linearly with increaseing in the ratio of extraction solution to material, but the range of increase was small.

3.3. Optimization of Extraction Process

Principal component analysis was conducted to the equation (3') using the SAS RSREG procedure. A stable point was obtained, shown in Table **4**.

Its characteristic values are (-0.010628, -0.022102, 0.103770) and all negative. According to the design principle, Y should have a maximum value. The data was processed and optimized. The coded combination with maximum Y was obtained as: X_1 = -0.270426~-0.135213, X_2 =-0.330555~-0.165277, X_3 = 0.810975~1.621950, and namely, extraction temperature: 83.0 ~ 84.0℃, extraction time: 1.9~2.0 h, the ratio of extraction solution and material: 34.1~38.1 : 1, and the maximum absorbance is 0.323345. Based on the regression equation $A = 114.23C + 0.0016$, extraction concentration C is calculated, and the maximum flavonoid yield is 2.82% when C calculated is placed in the equation (1).

The average values of the extraction conditions are: temperature 83.5℃, time 1.9 h, the ratio of extraction solution and material 36 : 1. Under such experimental conditions, the flavonoid yield from dry onion skins reached 2.82%, and was consistent with the result by theoretical calculation.

CONCLUSION

Using quadratic regression rotary orthogonal unitized design, mathematical model was established as:

$$Y = 0.315000 - 0.009688X_1 + 0.008687X_3 - 0.025750X_1{}^2 - 0.005375X_2{}^2$$

the extraction rate (Y), temperature (X_1), time (X_2), the ratio of extraction solution and material (X_3).

The optimum conditions of flavonoids extracted from onion skins by the alkaline method are: temperature 83.0~84.0°C, duration 1.9~2.0 h, the ratio of extraction solution and material 34~38 : 1, and extraction is conducted twice under such conditions. The total onion flavonoid yield may be up to 2.82%. The relative size of the factors involved in rotary orthogonal experiments is extraction temperature (X_1) > the ratio of extraction solution and material (X_3) > extraction time (X_2).

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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