Optimization of the Extraction of Flavonoids from Grape Leaves by Response Surface Methodology

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Summary: The extraction of flavonoids from grape leaves was optimized to maximize flavonoids yield in this study. A central composite design of response surface methodology involving extracting time, power, liquid-solid ratio, and concentration was used, and second-order model for Y was employed to generate the response surfaces. The optimum condition for flavonoids yield was determined as follows: extracting time 24.95 min, power 72.05, ethanol concentration 63.35%, liquid-solid ratio 10.04. Under the optimum condition, the flavonoids yield was 76.84 %.

Keywords: Optimization, Response surface methodology, Flavonoids, Extraction, Grape leaves.

Introduction

Many researchers have studied the beneficial effects of flavonoids in grapes and wines on human health. These benefits include antioxidant activity, prevention of coronary heart disease, anticancer activity, and others [1]. Flavonoids, abundant in fruits, teas, vegetables, and medicinal plants, have received the greatest attention and have been investigated extensively, since they are highly effective free radical scavengers and are assumed to be less toxic than synthetic antioxidants such as BHA and BHT, which are suspected of being carcinogenic and causing liver damage [2-4]. In this study, the extraction of flavonoids from grape Leaves was studied.

When many factors and interactions affect desired responses, response surface methodology (RSM) is an effective tool for optimizing the process. RSM uses an experimental design such as the central composite design (CCD) to fit a model by least squares technique. If the proposed model is adequate, as revealed by the diagnostic checking provide by an analysis of variance (ANOVA) and residual plots, contour plots can be usefully employed to study the response surface and located the optimum [5]. The purpose of our current work was to optimize the extraction of flavonoids from grape leaves by Response Surface Methodology (RSM).

Results and Discussion

Diagnostic Checking of the Fitted Model

Response surface methodology (RSM) has successfully been used to model and optimize biochemical and biotechnological processes [6, 7]. It is a powerful technique for testing multiple process variables because fewer experimental trials are used compared with the study of one variable at a time. Interactions between variables can also be identified and quantified [8]. RSM has been successfully applied to the optimization of the extraction yield of anthocyanin from sunflower hulls [9]. It has also been used for the selection of the appropriate ethanol content in the extraction medium, the extraction temperature and the extraction time when maximizing antioxidant activity of defatted borage meal [10]. In this study, RSM was used to determine the effect of extraction parameters such as extracting time, power, ethanol concentration, liquid-solid ratio on the flavonoid. ANOVA for the regression was performed to assess the "goodness of fit". The model for flavonoid yield was:

$$Y=+0.063436-0.00089\times X_1+0.00772\times X_2+0.01849\times X_3-0.03666\times X_4+0.00001\times X_1X_2-0.00002\times X_1X_3+0.00012\times X_1X_4+0.00002\times X_2X_3-0.000004\times X_2X_4+0.00001\times X_3X_4+0.00013\times X_1^2-0.00006\times X_2^2-0.00015\times X_3^2+0.00069\times X_4^2$$

The result of ANOVA was shown in Table-1. The Model F-value of 5.44 implies the model was significant. There was only a 0.16 % chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.05 indicated model terms were significant. In this study, X_1, X_3, X_2^2, X_3^2, X_4^2were significant model terms.

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Table-1: ANOVA for the fitted model.

Source	Sum of squares	d_f	Mean square	F value	Prob > F
Model	**0.14**	**14**	**0.010**	**5.44**	**0.0016**
X_1	0.040	1	0.040	21.27	0.0004
X_2	0.001	1	0.001	0.67	0.4258
X_3	0.026	1	0.026	13.65	0.0024
X_4	0.004	1	0.004	1.90	0.1893
X_1X_2	0.0002	1	0.0002	0.097	0.7603
X_1X_3	0.0004	1	0.0004	0.23	0.6359
X_1X_4	0.002	1	0.002	1.17	0.2970
X_2X_3	0.004	1	0.004	2.11	0.1685
X_2X_4	0.00002	1	0.00002	0.013	0.9099
X_3X_4	0.007	1	0.007	3.62	0.0780
X_1^2	0.0005	1	0.0005	0.25	0.6252
X_2^2	0.009	1	0.009	4.70	0.0478
X_3^2	0.023	1	0.023	12.40	0.0034
X_4^2	0.012	1	0.012	6.54	0.0228
Residual	**0.026**	**14**	**0.002**		
Cor Total	**0.17**	**28**			

Response Surface Plotting

Variables giving quadratic and interaction terms with the largest absolute coefficients in the fitted models were chosen for the axes of contour plots to account for curvature of the surfaces. In Figure 1, power and ethanol concentration were selected for the vertical and horizontal axes respectively for the contour plot and 3D-surface of flavonoids yield.

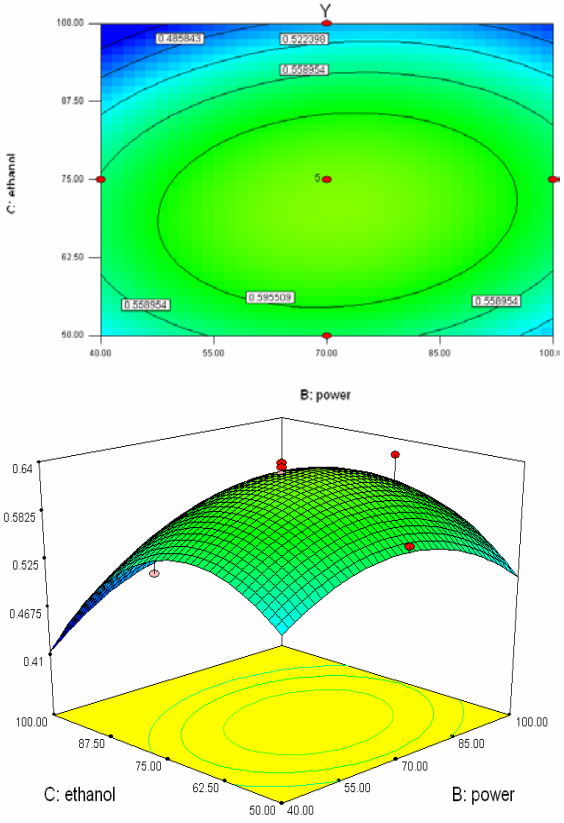

Fig. 1: Effect of power and ethanol concentration on flavonoids yield. Extracting time = 12.18min; liquid-solid ratio 12.34

Optimization

The model is useful in indicating the direction in which to change variables in order to maximize the flavonoids content (Y). By using Design Expert 7.0 software, the point at extracting time 12.18 min, power 40.60, and ethanol concentration 76.21%, liquid-solid ratio 12.34 could be recommended as a practical optimum. The estimated values for Y were 70.18%. A verification experiment at the optimum condition, consisting of 3 runs, was performed and the practical Y was 76.48%.

Experimental

Materials

The grape leaves for this study was collected in Yining. Rutin was from Sigma. Other chemicals were of analytical grade and used as received.

Experiment Design

One response was used: flavonoids yield Y, defined as the ratio of flavonoids in the extract to total amount of raw material expressed as percentage. Each of variables to be optimized was coded at 3 levels: -1, 0, and 1. Table-2 showed the variables, their symbols and levels. The selection of variable levels was based on our preliminary study.

Table-2: Variables and their level for central composite design.

Variable	Symbol	Code-variable level		
		-1	0	1
Extracting time (min)	X_1	5	15	25
Power (%)	X_2	40	70	100
Ethanol Concentration (%)	X_3	50	75	100
Liquid-solid ratio (mL/g)	X_4	10	20	30

A central composite design (CCD), shown on Table-3, was arranged to allow for fitting of a second-order model. The CCD combined the vertices of a hypercube whose coordinates are given by the 2^n factorial design with the "star" points. The star points were added to the factorial design to provide for estimation of curvature of the model. Five replicates (run 3, 6, 9, 17 and 21) at the center of the design were used to allow for estimation of "pure error" sum of squares. Experiments were randomized in order to minimize the effects of unexplained variability in the observed response due to extraneous factors.

Extraction of Flavonoids

The grape leaves (1 g) were extracted according to the experiment design. After filtered, the

supernatant was diluted to determine the content of flavonoids.

Table-3: Central composite design arrangement and response.

Run	Variable level				Response
	X_1	X_2	X_3	X_4	Y
1	25.00	100.00	50.00	10.00	66.20
2	25.00	40.00	50.00	10.00	67.20
3	15.00	70.00	75.00	20.00	63.90
4	15.00	100.00	75.00	20.00	62.00
5	5.00	40.00	100.00	30.00	44.20
6	15.00	70.00	75.00	20.00	62.60
7	25.00	40.00	50.00	30.00	66.40
8	25.00	100.00	100.00	30.00	58.90
9	15.00	70.00	75.00	20.00	63.40
10	5.00	70.00	75.00	20.00	58.40
11	5.00	100.00	100.00	30.00	50.20
12	5.00	40.00	100.00	10.00	51.30
13	5.00	100.00	100.00	10.00	45.40
14	5.00	100.00	50.00	10.00	57.30
15	15.00	70.00	100.00	20.00	51.70
16	25.00	100.00	100.00	10.00	60.40
17	15.00	70.00	75.00	20.00	60.40
18	15.00	70.00	50.00	20.00	57.70
19	5.00	100.00	50.00	30.00	52.20
20	25.00	40.00	100.00	30.00	56.90
21	15.00	70.00	75.00	20.00	55.50
22	5.00	40.00	50.00	30.00	48.50
23	25.00	40.00	100.00	10.00	46.00
24	15.00	70.00	75.00	30.00	67.80
25	5.00	40.00	50.00	10.00	59.90
26	25.00	100.00	50.00	30.00	57.60
27	15.00	40.00	50.00	10.00	54.70
28	15.00	70.00	75.00	10.00	74.40
29	25.00	70.00	75.00	20.00	72.70

Determination of the Content of Flavonoids

The content of flavonoids was measured as rutin quivalents from a rutin standard curve. One ml of the sample extract was transferred to a test tube, the solution was redissolved in 30% ethanol to 12.5 ml and 0.7ml of 5% $NaNO_2$ reagent was added. After an incubation period of 5 min , 0.7 mL of $Al(NO_3)_3$ was added, mixed well and kept for 6 min at room temperature,5ml of 1 M NaOH reagent was added. The solution was redissolved in 30% ethanol to 25 ml .The above solution was incubated for 10 min, and then the absorbance was readed at 500 nm using a spectrophotometer.

Statistical Analysis

A software package (Design Expert 7.0) was used to fit the second-order models and generate response surface plots. The model proposed for the response (Y) was:

$$Y = b_0 + \sum_{n=1}^{4} b_n x_n + \sum_{n=1}^{4} b_{nn} x_n^2 + \sum_{n \neq m-1}^{4} b_{tm} x_n x_m$$

where b_0 is the value of the fitted response at the center point of the design, which is point (0, 0, 0, 0). b_n, b_{nn} and b_{nm} are the linear, quadratic and cross-product regression terms, respectively.

Conclusions

Optimum extraction of flavonoids from grape leaves with ethanol extraction could be achieved by 1 part of grape leaves with 76.21% ethanol concentration, liquid-solid ratio 12.34, at power 40.60 for 12.18 min. Such conditions resulted in extraction of 0.7648 flavonoids from grape leaves.

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