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Research Article

Optimization of Extraction of Flavonoids from Shaddock Peel by Response Surface Methodology

[1]Bin Li and [2]Jingjing Liu
[1]School of Food Science, Henan Institute of Science and Technology,
[2]Xinxiang University, Xinxiang, 453003, China

Abstract: Response Surface Methodology (RSM) was applied to predict optimum conditions for extraction of flavonoids from shaddock peel. A central composite design involving extracting temperature, extracting time and ethanol concentration was used and second-order model for extraction ratio of flavonoid was employed to generate the response surface. The optimized condition was as follows: extracting temperature 70°C, extracting time 127.2 min and ethanol concentration 92.16%. The predicted extraction ratio of flavonoid at condition was 81.4216%. Experimental verification gave the value of 80.35%.

Keywords: Extraction, falconoid, response surface methodology, shaddock peel

INTRODUCTION

Flavonoid, 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 (Liu and Zhu, 2007; Zou *et al*., 2004). The extraction from shaddock peel was ttraditionally known as rich in physiological activity and pharmacological action. It has shown a profound activity on antiphlogistic, reduce blood viscosity and reduce the formation of blood lipids, etc (Wang, 1999; Zhu *et al*., 2008; Wang *et al*., 2008). In food industry it is used as natural pigment, flavor modifying agent and bitter agent in food and beverage products etc.. And it can prepared rhamnose, citrine, acid azo dyes and semisynthetic flavonoids (Peng *et al*., 2009). In order to increase the flavonoid yield of the extract, the method extracting flavonoids from shaddock peel by using ethanol-water technology was studied in this study. In a preliminary study, many potential factors were screened for effects on the extraction of flavonoids. Extracting temperature extracting time and ethanol concentration were found to influence significantly the flavonoid yield. Optimization could be used to maximize flavonoid yield. 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 (Rustom *et al*., 1991). The purpose of our current work was to optimize the flavonoid purity of the extract by Response Surface Methodology (RSM).

MATERIALS AND METHODS

Materials: The shaddock peel (2012 production) for this study were purchased in Xinxiang, China. Rutin was from Shanghai Pharmaceutical (Group) Co., Ltd (Shanghai, China). Other chemicals were of analytical grade and used as received.

Experiment design: One response was measured: flavonoid yield (Y), defined as the amount of total flavonoids in 1 g of the extract expressed as mg/g. Each of variables to be optimized was coded at 3 levels: -1, 0 and 1. Table 1 shows the variables, their symbols and levels. The selection of variable levels was based on our preliminary study.

A Central Composite Design (CCD), shown on Table 2, 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 2n factorial design with the "star" points. The star points were added to the factorial design to provide for estimation of curvature of the model. Three replicates (run 1, 2 and 3) 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

Corresponding Author: Bin Li, School of Food Science, Henan Institute of Science and Technology, Xinxiang, 453003, China

Table 1: Variables and their levels for central composite design

		Coded-variable level		
Variable	Symbol	-1	0	1
Extracting temperature (°C)	X_1	30	50	70
Extracting time (h)	X_2	0.5	1.5	2.5
Ethanol concentration (%)	X_3	40	70	100

Table 2: Central composite design arrangement and response

	Variable level			
Run	X1	X2	X3	Response (Y)
1	0	0	0	71.58
2	1	0	-1	57.58
3	0	0	-1	59.11
4	0	-1	0	57.76
5	1	1	-1	78.95
6	-1	1	-1	64.72
7	0	0	-1	19.36
8	0	1	0	80.1
9	1	-1	1	45.06
10	1	0	0	33.62
11	-1	0	0	51.93
12	-1	-1	-1	53.49
13	1	-1	-1	19.41
14	0	0	0	54.79
15	0	0	0	25.94
16	0	0	0	30.85
17	-1	-1	1	52.1
18	-1	0	1	53.44

Table 3: ANOVA for the fitted model

Source	Sum of squares	df	Mean square	F value	Prob >F
Model	3954.82	9	439.42	1.92	0.1862
Residual	1834.97	8	229.37		
Lack of Fit	471.62	8	229.30	0.21	0.9384
Pure error	1363.35	3	454.45		
Cor total	5789.79	17			

effects of unexplained variability in the observed response due to extraneous factors.

Determination of total flavonoid content: A colorimetric method was used: 1 mL diluted solution containing the extract, 1 mL of 5% (w/w) NaNO2 and 10 mL of 30% (v/v) ethanol were mixed for 6 min and then 1 mL of 10% AlCl3 (w/w) was added and mixed. Six minutes later, 10 mL of 1 mol/l NaOH was added. Subsequently, the solution was diluted to 25 mL with 30% (v/v) ethanol prior to measurement. With 10 min standing, the absorbance of the solution was measured at 510 nm with a WFJ-7200 spectrophotometer (Unico, Shanghai, China). The flavonoid content was determined by comparison with rutin standard curve, which was made in the same condition.

Statistic analysis: A software package (Design Expert7.0) 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). Bn, bnm and bmn are the linear, quadratic and cross-product regression terms, respectively.

RESULTS AND DISCUSSION

Diagnostic checking of the fitted model: ANOVA for the regression was performed to assess the "goodness of fit". The model for Y was:

$$Y = 161.73092 - 0.28928X_1 - 5.38808X_2 + 21.25497X_3 + 0.012394X_1X_2 + 0.24871X_1X_3 - 0.08856X_2X_3 - 0.006X_1^2 + 0.051882X_2^2 - 8.94214X_3^2$$

The result of ANOVA was shown on Table 3. The Model F-value of 1.92 implies the model was significant. There was an 11.72 % chance that a "Model F-Value" this large could occur due to noise.

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 Fig. 1, extract time and ethanol concentration were selected for the vertical axes for the contour plot and 3D-surface of Y, while extracting temperature was measured at different levels.

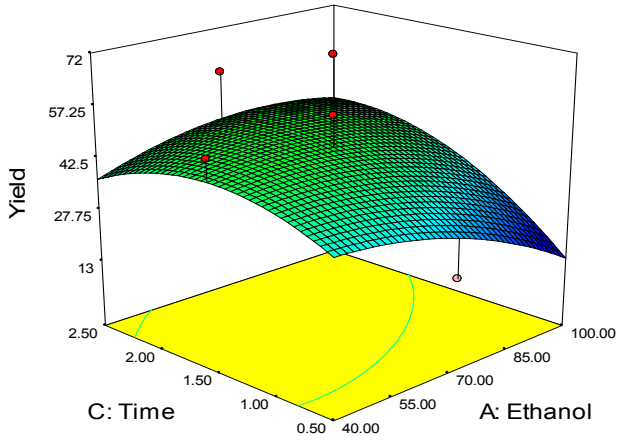

Fig. 1: Effect of extract time and ethanol concentration on flavonoid yield

Optimization based on flavonoid yield: By using Design Expert 7.0 software, the optimum condition was obtained as shown. The point at extracting temperature = 70°C, extracting time = 127.2 min and ethanol concentration = 92.16% could be recommended as a practical optimum. The estimated values for Y, 81.4216% was obtained at those conditions. A verification experiment at the optimum condition was performed and the practical yield of 80.35% was obtained.

CONCLUSION

The optimized condition for ultrasonic-assisted extraction of flavonoids from ginkgo leaves with ethanol was determined as following: extract time 127.2 min, extracting temperature 70°C and ethanol concentration 92.16%. The predicted flavonoid purity at condition was 81.4216%. Experimental verification gave the value of 80.35%.

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