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Optimization of the Microwave-Assisted Extraction of Polyphenols from Red Pitaya Peel using Response Surface Methodology

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In order to maximizing extraction yield of polyphenols from red pitaya (*Hylocereus undatus*) peel, an agro-industrial byproduct in pitaya juice processing, the present work firstly focused on optimization of the microwave-assisted extraction (MAE) using response surface methodology (RSM). A Box-Behnken design (BBD) was used to monitor effects of five MAE factors on the polyphenols yield, including extraction time, ratio of solvent to raw material, microwave power, extraction temperature, and ethanol concentration. The optimal conditions were extraction time of 20.3 min, ratio of solvent to raw material 33.4:1 (mL:g), microwave power 497 W, extraction temperature 43.3°C and ethanol concentration 64.9%. Validation tests suggested that the actual yield of polyphenols was (463.8±1.1) mg gallic acid equivalents (GAE) per 100 g dry pitaya peel with the relative standard deviation (RSD) of 2.15% (*n* = 5) under the optimized conditions, which was in good agreement with the predicated yield. Antioxidant assays suggested that the ethanol extract from red pitaya peel had stronger DPPH, hydroxyl and superoxide free-radical scavenging capabilities than vitamin C at 1.0 mg/mL. These results provide an alternative way to make good use of red pitaya peel to produce natural antioxidant.

Keywords: Red Pitaya Peel, Polyphenols, Microwave-Assisted Extraction, Optimization, Antioxidant Activity

Introduction

Pitaya, called dragon fruit, is part of the Cactaceae family and native to the tropical regions of Central and South America.[1] It are also being grown commercially in China, Vietnam, Israel, Australia and the USA. Pitaya has three cultivating varieties, *Hylocereus undatus* (Red pitaya), *H. megalanthus* (Yellow pitaya) and *H. polyrhizus*,[2] which are gained popularity all over the world because of their rich nutraceuticals, such as polyphenols,[3-5] dietary fibers,[6,7] pectin,[8] essential fatty acids,[9,10] ascorbic acid, vitamin A, vitamin E, and lycopene.[11] With the increasing output of pitaya fruit and its flesh juice, more and more pitaya peel as a by-product is produced and causes environmental problem. Recently, more attention has been payed on the waste material resulting from the industrial food processing as a good source of bioactive compounds, such as polyphenols from industrial apple pomace,[12] phytic acid from rice bran.[13] A growing evidence indicated that pitaya peel has rich bioactive polyphenols, such as betalains, flavonoids, and phenolic acids.[4,14,15] So, the recovery of natural polyphenols from pitaya peel is alternative important application. Till now, however, investigation of extracting polyphenols from pitaya fruit peel has not been reported. Microwave-assisted extraction (MAE) of bioactive compounds has been widely applied in food and pharmaceutical industries due to its more rapid rate and similar or higher yields with reducing extraction time and solvent consumption. Response surface methodology (RSM) is a collection of statistical and mathematical techniques, which is effective for responses that are influenced by many factors and their interactions and had been widely applied in optimization of polyphenols extraction.[12,15,16] The present study focused on optimization of MAE of polyphenols from red pitaya peel using RSM. Preliminary trials in our laboratory indicated that five MAE conditions, extraction time (min), ratio of solvent to raw material (mL/g), microwave power (W), extraction temperature (°C), and ethanol concentration (%), might have significantly affect on the yield of polyphenol extraction. These effects were carefully investigated and discussed in this work.

Material and methods

Raw material

Red pitaya (*H. undatus*) used in this study was purchased from local fruit market (Hangzhou, China), and washed before peeling. The fresh peel was cut into

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small pieces (1×1 cm^2) followed by lyophilization (Labconco Freezone 2.5 plus; MO, USA). After finely milled with a muller (HX-200; Zhejiang Yongkang Xi`an Hardware Officinal Instrument Factory, Yongkang, China) and sieved through a 20-mesh (0.84 mm) sieve, all the samples were packed in plastic bags and stored at -20°C.

Chemicals

Gallic acid and Folin-Ciocalteu reagent were purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Water was purified with a Milli-Q system Millipore (Bedford, MA, USA). Ethanol was obtained from Hangzhou Gaojing Chemical Co., Ltd (Hangzhou, China). Filter paper was from Hangzhou Xinhua Paper Industry Co., Ltd (Hangzhou, China). All other chemicals were of analytical or HPLC grade.

Determination of total polyphenols content

The total phenolic content of red pitaya peel was determined by the modified Folin-Ciocalteu method proposed by Wijngaard and Brunton.[17] Triplicate tests were conducted for each sample. The results were expressed as milligram gallic acid equivalents (GAE) per 100 g dry pitaya peel.

Experimental design

Box-Behnken design (BBD) was employed to statistically optimize the formulation parameters and to evaluate their major effects, interaction effects and quadratic effects of the formulation ingredients on MAE yields of polyphenols from red pitaya peel. According to the principle of BBD, five important MAE factors, extraction time, ratio of solvent to raw material, microwave power, extraction temperature, and ethanol concentration, were taken as the variables tested in a 46-run experiment. As shown in Table 1, five MAE factors chosen for this study were designated as X_1, X_2, X_3, X_4 and X_5, and were prescribed into three levels, coded +1, 0 and -1 for high, intermediate and low value, respectively. Test variables were coded according to the following equation:

$$x_i = (X_i—X_0)/\Delta X \quad ... (1)$$

where x_i was the coded value of an independent variable; X_i was the actual value of an independent variable; X_0 was the actual value of an independent variable at center point and ΔX was the step change value of an independent variable. All experiments were performed in triplicate and the averages of polyphenol yields were taken as response. For predicting the optimal point, a second-order polynomial model was fitted to correlate relationship between independent variables and response (polyphenol yield). For these five factors, the equation was

$$Y=A_0+\sum_{i=1}^{5} A_i X_i+\sum_{i=1}^{5} A_{ii} X_i^2+\sum_{i=1}^{4}\sum_{j=i+1}^{5} A_{ij}\ X_i\ X_j \quad ... (2)$$

where Y was the response variable (polyphenol yield in real value). A_0, A_i, A_j and A_{ij} were the regression coefficients of variables for intercept, linear, quadratic and interaction terms, respectively. X_i and X_j were independent variables ($i \neq j$). Analysis of the experimental design and data were carried out using Design-Expert (version 8). The models were predicted through statistical analysis and regression analysis (ANOVA), and the fitness of the polynomial model equation was expressed by the coefficient of determination R^2. Models and regression coefficients were considered significant when p-values were lower than 0.05. The optimal MAE conditions of extraction time, ratio of solvent to raw material, microwave power, extraction temperature and ethanol concentration were calculated by the math software MATLAB (Version 7).[18]

Table 1 — Code and level of independent variable selected for BBD

Variables	Symbol		Levels		
	Coded	Uncoded	-1	0	+1
Extraction time (min)	x_1	X_1	10	30	50
Ratio of solvent to raw material (mL/g)	x_2	X_2	20:1	30:1	40:1
Microwave power (W)	x_3	X_3	200	400	600
Extraction temperature (°C)	x_4	X_4	30	40	50
Ethanol concentration (%)	x_5	X_5	40	60	80

MAE

MAE was carried out on a microwave instrument (NJC 03-2; Nanjing, China) with adjustable power settings ranging from 100 to 800 W. It was equipped with one 500 mL closed PTFE vessel, a power sensor, a temperature sensor, a temperature controller and cooling system. About 5.0 g of freeze-dried powder of red pitaya peel was put into MAE vessel and extracted under different conditions. The ranges of the variables studied were listed in Table 1. At the end of MAE, the extracts were centrifuged for 15 min at 4°C and 10 000 rpm (CR21GII, Hitachi Koki, Japan). Anhydrous ethanol was added to bring the final volume of the extract to 200 mL. After filtration through a 0.45 mm membrane, all the samples were preserved at 4°C until analysis.

Results and Discussion

Extraction model and statistical analysis

There were a total of 46 runs for optimizing the five individual parameters in the current BBD, which was applied to the MAE of polyphenols from red pitaya peel. The response values at different experimental combination for coded variables were listed in Table 2. The response (polyphenol yield) ranged from 39.1 to 462.1 mg GAE/100 g dry pitaya peel. By applying multiple regression analysis on the experimental data, the response variable and the test variables were related by the following second-order polynomial equation:

Table 2 — BBD and the response values for the polyphenols yield

Run	X_1 (min)	X_2 (mL/g)	X_3 (W)	X_4 (°C)	X_5 (%)	Response (mg GAE/100 g)
1	-1	0	-1	0	0	205.3
2	0	1	-1	0	0	368.5
3	-1	0	1	0	0	318.3
4	0	1	1	0	0	342.9
5	0	0	0	1	-1	54.6
6	1	0	1	0	0	242.4
7	0	0	-1	-1	0	259.5
8	1	1	0	0	0	305.3
9	0	0	0	0	0	407.5
10	-1	0	0	-1	0	241.5
11	0	0	0	0	0	406.1
12	1	0	-1	0	0	354.1
13	0	-1	0	1	0	315.8
14	0	0	-1	0	1	280.1
15	0	0	0	0	0	390.4
16	-1	-1	0	0	0	296.5
17	0	-1	-1	0	0	293.1
18	0	-1	0	0	-1	39.1
19	0	0	0	-1	-1	144.3
20	0	0	0	0	0	401.3
21	1	-1	0	0	0	323.4
22	-1	0	0	0	1	252.6
23	0	0	-1	0	-1	109.1
24	0	0	0	1	1	241
25	1	0	0	1	0	351.7
26	1	0	0	-1	0	405.0
27	-1	1	0	0	0	374.5
28	0	0	1	0	1	287.6
29	0	0	1	0	-1	158.9
30	0	0	0	0	0	401.3
31	-1	0	0	1	0	350.1
32	0	0	0	0	0	401.3
33	0	1	0	-1	0	318.2
34	0	1	0	0	1	386.3
35	-1	0	0	0	-1	174.2
36	0	-1	0	0	1	229.8
37	1	0	0	0	-1	110.7
38	0	1	0	1	0	276.5
39	0	0	0	-1	1	328.9
40	0	0	-1	1	0	434.8
41	0	-1	0	-1	0	313.2
42	0	0	1	1	0	462.1
43	0	-1	1	0	0	327.8
44	1	0	0	0	1	199.2
45	0	0	1	-1	0	358.5
46	0	1	0	0	-1	117.9

$$Y = 401.3 - 1.3X_1 + 15.7X_2 + 12.1X_3 + 7.3X_4 + 81X_4 - 49X_1X_2 - 56.2X_1X_3 - 40.5X_1X_4 + 2.5X_1X_5 - 15.1X_2X_3 - 11.1X_2X_4 + 19.4X_2X_5 - 17.9X_3X_4 - 10.6X_3X_5 + 0.5X_4X_5 - 59.7X_1^2 - 49.4X_2^2 - 26.5X_3^2 - 22.1X_4^2 - 167.2X_5^2 \quad \ldots (3)$$

The ANOVA of the quadratic regression model showed that the *F*-value of 5.93 implies the model was significant. The values of the determination coefficient (R^2) and the adjusted determination coefficient (*Adj.* R^2) were 0.8259 and 0.6867, respectively, which suggested that a high degree of correlation between the observed and predicted values. Moreover, the ratio of signal to noise (*Adeq. Precision*) was 7.924, which indicated an adequate signal. Therefore, this model could be used to navigate the design space. The *P* values are used to check the significance of each coefficient, which in turn may indicate the pattern of the interaction between the variables. The coefficient estimate for the parameter optimization suggested that one independent variable (X_4) and two quadratic terms (X_1X_1, X_2X_2, X_5X_5) had significant effect on the polyphenol yield (P<0.05). The results also showed that ethanol concentration was the most significant single factor which influenced the polyphenol yield followed by extraction solvent/raw material, microwave power, extraction temperature and extraction time.

Optimization of the MAE procedure by RSM

Equation 3 allowed the prediction of the effects of the five factors on the polyphenol yield. Contour plots for three independent response surface were shown in Figure 1. Two variables within the experimental range were depicted while the other variable was kept constant at zero level. The shapes of the contour plots, circular or elliptical indicated whether the mutual interactions between the variables were significant or not.[19] As shown in Figure 1, all increased variables, extraction time (X_1), solvent/raw material (X_2), microwave power (X_3), extraction temperature (X_4) and ethanol concentration (X_5), up to a threshold level led to increased polyphenol yield. Beyond this level, the polyphenol yield gradually increased, which indicated that a greater yield could be achieved if the moderate MAE conditions were selected. Using the math software MATLAB (Version 7), the optimal

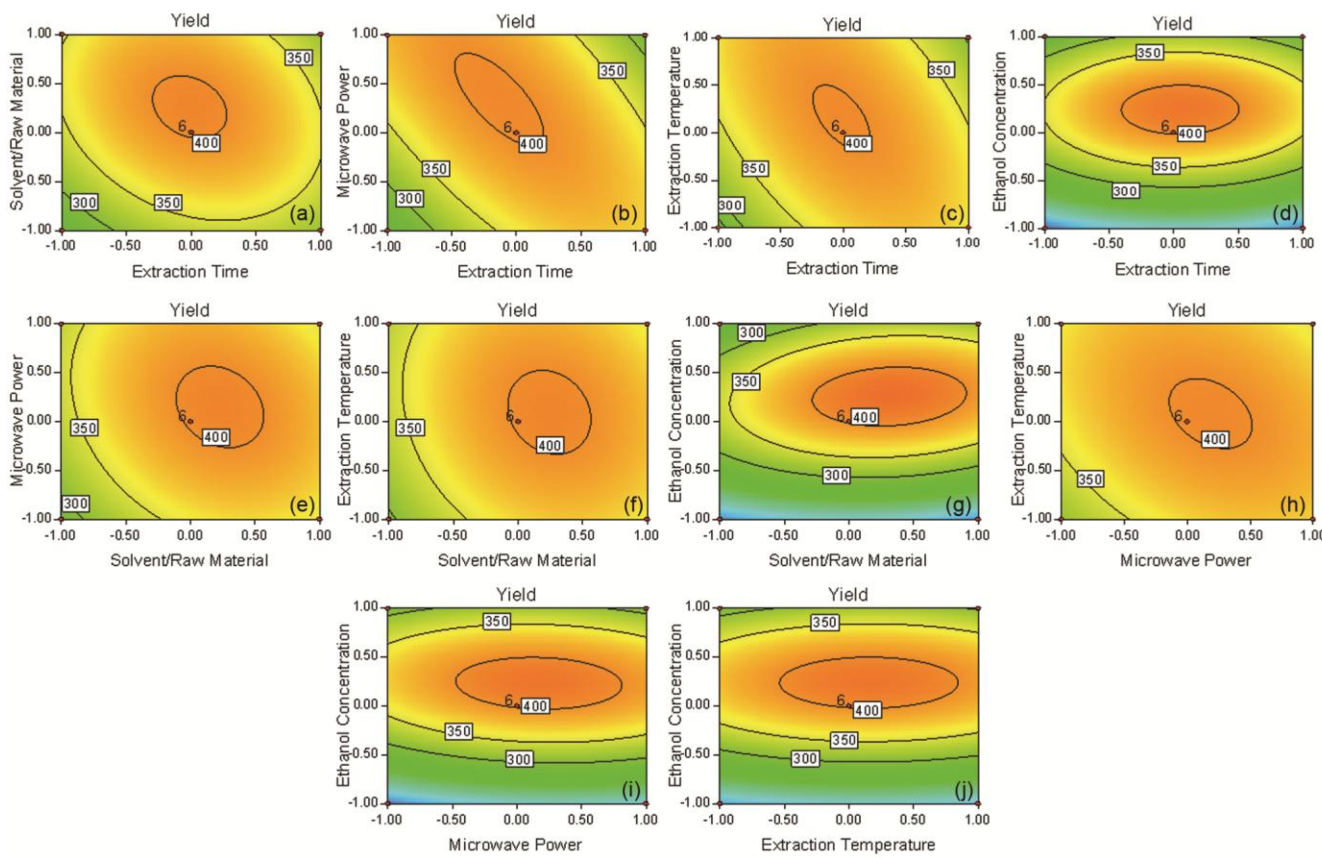

Fig.1 — Contour plots for the effects of two variables of MAE conditions on the polyphenol yield

conditions for MAE of polyphenols from red pitaya peel were theoretically calculated , which were as followed: extraction time 20.3 min, ratio of solvent to raw material 33.4:1 (mL/g), microwave power 497 W, extraction temperature 43.3°C and ethanol concentration 64.9%.

Validation of the model

In order to validate the adequacy of the model Equation (3), a verification experiment was carried out under the optimized conditions mentioned above. Under the optimal conditions, the model predicted a maximum response of 464.5 mg GAE/100 g dry pitaya peel. A mean value of (463.8±1.1) mg GAE/100 g dry pitaya peel with RSD = 2.15% (n=5), obtained from actual experiments, demonstrated the validation of the optimized extraction model. The good correlation between these results undoubtedly confirmed that the model was adequate for reflecting the predicted optimization.

Conclusions

An efficient process of solid-liquid MAE was developed for the extraction of polyphenols from red pitaya peel with enhanced yield. BBD was successfully employed to optimize MAE parameters in this work. The results indicated that extraction time 20.3 min, ratio of solvent to raw material 33.4:1 (mL/g), microwave power 497 W, extraction temperature 43.3 °C and ethanol concentration 64.9% were the best MAE conditions to extract polyphenols from red pitaya peel. The maximum polyphenol yield reached as much as (463.8±1.1) mg GAE/100 g red pitaya peel with RSD=2.15% under these optimal MAE conditions, which was in good agreement with the predicted yield (464.5 mg GAE/100 g dry pitaya peel). Till now, it was the first report on MAE of polyphenols from red pitaya peel using RSM. Polyphenols are the predominant ingredients in red pitaya (*Hylocereus undatus*) peel, an agro-industrial byproduct in pitaya juice processing, and were shown to have potential antioxidant and cytotoxic activities.[5,14,20] The recovery of polyphenols from red pitaya peel would be important way to produce natural bioactive nutraceuticals and bring a great benefit to pitaya juice processing plant and human health. The present work could be applied to develop industrial MAE process of polyphenols from red pitaya peel, including further studies concerning the optimal number of sequential steps to enhance the efficacy of a large-scale extraction system.

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