The Enhanced Extraction Conditions for Phenolic and Flavonoid Compounds from the Underutilized Red Pitaya Peels Using Response Surface Methodology

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Abstract

In this study, response surface methodology (RSM) was augmented to determine the effects of independent variables, namely extraction temperature (45-65°C), ethanol concentration (70-90%) and extraction time (80-120 min) to optimize the retained antioxidant compounds of the red pitaya peels through responses which were total phenolic content (TPC) and total flavonoid content (TFC). Regression analysis showed that more than 90% of the variation was explained by the second-order polynomial models of the different responses and the experimental values displayed that the extraction conditions had significant effect ($p<0.001$) on the TPC and TFC respectively. The optimized conditions were ethanol 82% for 103 min at 56°C with values of 172.01 mg/g for TPC and 7.45 mg/g for TFC respectively, which were in a good agreement with those predicted, thus indicating the suitability of the employed model in optimizing the extraction conditions of the red pitaya peels and similar natural functional product optimization.

Keywords : Response Surface Methodology, Red Pitaya Peels, Antioxidant, Total Phenolic Content, Total Flavonoid Content

I. Introduction

Pitaya or dragon fruit (*Hylocereus* spp) is a climbing vine cactus species which has successfully attained international recognition, both as an ornamental plant and as an economical fruit crop. There are three varieties of pitaya namely white flesh pitaya with yellow peel (*Selenicereus megalathus*), white flesh pitaya with red peel (*Hylocereus undatus*) and red flesh pitaya with red peel (*Hylocereus polyrhizus*) (Nerd, Sitrita, Kaushika, & Mizrahi, 2002; Hoa, Clark, Wadddell, & Woolf, 2006.).

Red pitaya (*Hylocereus polyrhizus*) is being extensively cultivated in Malaysia, Thailand, Vietnam, Australia, Taiwan and some other parts of the world. It is increasingly gaining attention in many countries due to their exotic aesthetic characteristics, high economic potential and exceptional tolerance to extreme drought.

Massive quantities of solid wastes produced annually by the food processing industry contain significant amount of biodegradable organic matter and their disposal led to severe environmental issues. However, in many instances there are still a rather significant lack of appropriate feasibility studies on the exploitation of such wastes and consequently their utilization is still in its infancy (Makris, Boskou, & Andrikopoulos, 2007). If the active ingredients are to be of therapeutic value or prophylactic in human subjects, it is vital to perform a proper extraction to retrieve them (Liyana-Pathirana and Shahidi 2005). Response Surface Methodology (RSM) is a powerful statistical tool which is widely employed for the optimization of complex processes and extraction technologies as it depicts the complete effects of the variables, evaluate the interactions between multiple parameters, reduce the number of experimental trials and shorten the trial time. The employment of antioxidants rich fruit residuals in the agribusiness based industries has a significant impact on the exploitation of plant by-products since they are one of the greater value alternatives. Therefore, the aim of the present work was to optimize the conditions including temperature, ethanol concentration and extraction time for the recovery of phenolic and flavonoid compounds from the underutilized red pitaya peels using RSM.

II. Materials and Methods

Materials and reagents

Freshly harvested red pitaya fruits (H. *polyrhizus*) were purchased from a fruit plantation located in Sepang, Selangor, Malaysia. The peels were separated from the pulp and left for sun drying at an ambient air temperature about 30°C from 8 am to 5 pm and packed in a plastic bag. The dried peels were then ground to fine powder and packed in an airtight polyethylene bag and stored in dark at room temperature for further experiments. All the solvents and chemical reagents used in this study were of analytical grade and purchased from Malaysia Sigma-Aldrich.

Experimental design

Central composite rotatable design (CCRD) was used to identify the optimized extraction conditions to obtain the antioxidants based on three selected variables and also their predicted values respectively. Three independent variables were studied namely extraction temperature (45-65°C), ethanol concentration (70-90 %) and extraction time (80-120 min) whereas the response variables were TPC and TFC. The data obtained were fitted to a second-order polynomial regression model, expressed by Equation (1):

$$Y = \sum A_0 + \sum_{i=1}^{k} A_{ij}\ X_i + \sum_{i=1}^{k} A_{ii}\ X_i^2 + \quad \sum_{i=1}^{k-1} \quad \sum_{j=i+1}^{k} A_{ij}\ X_i X_j \qquad (1)$$

Y is the response of the independent variable, while A_0, *Ai*, *Aii*, *and Aij* are the regression coefficients for intercept, linear, quadratic and interaction effect terms respectively. X_j and X_i are the encoded independent variables ($i \neq j$).

Analytical procedure

Total Phenolic Content (TPC)

The total phenolic content (TPC) of red pitaya peels was determined based on the method adapted from previous studies with slight modifications (Singleton et al. 1999). Ethanolic solution of the extract in the concentration of 10 mg/mL was used in the analysis. The reaction mixture was prepared by mixing 500 µL of the ethanolic solution of extract, 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 mL 7.5% $NaHCO_3$. Blank was concomitantly prepared, containing 500 µL ethanol, 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 mL 7.5% $NaHCO_3$. The samples were thereafter incubated in dark for 2 hours. The absorbance was determined at 765 nm on a Microplate Reader (Spectra Max Plus 384, Molecular Devices Co., Ltd., America). The same procedure was repeated for the standard solution of gallic acid and the calibration line was constructed. Based on the measured absorbance, the concentration of phenolics (mg/mL) was read from the calibration plot and the content of phenolic in the extract was expressed in terms of gallic acid equivalent (mg of gallic acid/g of extract).

Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of red pitaya peels was examined according to the method described in previous research with minor modifications (Quettier et al. 2000). The sample contained 100 µL of methanol solution of the extract in the concentration of 10 mg/mL and 100 µL of 2% $AlCl_3$ solution dissolved in ethanol. The sample was incubated in dark for 30 minutes at room temperature and the absorbance was measured at 406 nm. The same procedure was repeated for the standard solution of quercetin and the calibration line was constructed. Based on the measured absorbance, the concentration of flavonoids (mg/mL) was read from the calibration plot and the content of flavonoid in the extract was expressed in terms of quercetin equivalent (mg of quercetin/g of extract).

III. Statistical Analysis

The experimental design and the regression analysis of the experimental data exploited Design- Expert 7.0.0 (Trial version, Stat-Ease Inc., Minneapolis, MN, USA). Analysis of variance (ANOVA) was used in order to evaluate the statistic significances of the model, lack of fit of the model and also the regression terms. The goodness-of-fit of the regression model was evaluated by, the coefficient of determination (R^2), Fischer's *F*-test at a probability (*p*) of 0.001, 0.01 or 0.05 was used to determine the second-order model equation and lack of fit. In order to maximize the phenolic and flavonoid content, response optimizer was applied using the desirability function (Chan et al. 2009).

IV. Results and Discussion

Optimization of parameters

Design expert 7.0.0 software was used to get the optimum extraction conditions which included temperature, ethanol concentration and time to maximize

TPC and TFC respectively. Second-order model optimized the responses and the predicted values of the different responses to the optimum conditions (in the range constraint). When constraint in the range was selected, the optimum conditions obtained were 56.39 °C temperature, 82.22 % ethanol concentration and 103.55 min with the desirability of 90.5 %. However, practically it is difficult to maintain the recommended conditions during processing and some deviations are expected. Therefore, optimum conditions were targeted as 56 °C temperature, 82 % ethanol concentration and 103 min with a desirability of 93.9 %.

The experimental and predicted values for responses namely TPC and TFC under various combinations of extraction conditions are given in Table 1. The results showed that the phenolic and flavonoid content ranged from 157.78 to 176.77 mg of gallic acid/g of extract and 6.22 to 7.14 mg of quercetin/g of extract respectively for the samples treated under extraction conditions. The linearity and quadratic effect of the independent variables, their interactions and regression coefficients on the response variables are given in Table 2.

Verification of predictive model

According to the optimum conditions obtained from the target constraint, all the experiments were conducted to verify the variation in the value of the antioxidant potentials of red pitaya peels. The experimental values obtained for TPC and TFC assays were 172.01 mg/g and 7.45 mg/g respectively. The experimental values for both responses based on these optimum conditions were found to be very close to the predicted values which were 171.52 mg/g and 7.10 mg/g for TPC and TFC respectively.

This indicates high fit degree between the experimental values and the predicted values from the regression model. The predicted model of the TPC and TFC were obtained from the following second-order polynomial equations:

$$Y_{TPC} = 170.71 + 1.60\ A + 1.48\ B + 2.12\ C + 2.79\ AB + 2.87\ AC + 3.02\ BC - 2.75\ A^2 - 2.61\ B^2 - 2.46\ C^2 \quad (2)$$

$$Y_{TFC} = 7.12 + 0.011\ A + 0.024\ B - 0.011\ C + 0.043\ AB + 0.053\ AC + 1.500E\text{-}033\ BC - 0.26\ A^2 - 0.25\ B^2 - 2.46\ C^2 \quad (3)$$

Table 1. Experimental and predicted values for the responses TPC and TFC under different extraction conditions

Standard order	Ethanol concentration (%)	Temperature (°C)	Time (min)	TPC[1]	TPC[2]	TFC[1]	TFC[2]
1	70.00	45.00	80.00	166.41	166.38	6.40	6.40
2	70.00	65.00	80.00	158.27	158.25	6.23	6.23
3	80.00	55.00	100.00	157.78	157.73	6.36	6.36
4	63.18	55.00	100.00	160.74	160.76	6.36	6.36
5	90.00	45.00	120.00	158.87	158.83	6.26	6.26
6	80.00	31.18	100.00	162.17	162.20	6.31	6.31
7	80.00	55.00	100.00	162.26	162.26	6.23	6.23
8	96.82	55.00	100.00	176.77	176.78	6.44	6.44
9	70.00	45.00	120.00	160.17	160.24	6.37	6.37
10	70.00	65.00	120.00	165.65	165.61	6.41	6.41
11	80.00	71.82	100.00	160.82	160.84	6.37	6.37
12	80.00	55.00	100.00	165.83	165.83	6.45	6.45
13	90.00	65.00	80.00	160.16	160.20	6.33	6.33
14	90.00	65.00	120.00	167.34	167.33	6.29	6.29
15	80.00	55.00	100.00	170.78	170.71	7.12	7.12
16	80.00	55.00	100.00	170.65	170.71	7.12	7.12
17	80.00	55.00	100.00	170.99	170.71	7.12	7.12
18	90.00	45.00	80.00	170.42	170.71	7.12	7.12
19	80.00	55.00	133.64	170.99	170.71	7.12	7.12
20	80.00	55.00	66.36	170.45	170.71	7.12	7.12

[1]Experimental value; [2]Predicted value

Table 2. The linearity and quadratic effect of the independent variables, their interactions and regression coefficients on the response variables

	TPC			TFC		
Source	Mean Square	*F*-value	*p*-value	Mean square	*F*-value	*p*-value
Model	63.67	1915.03	<0.0001	0.29	1687.57	<0.0001
A – Temperature	34.88	1049.30	<0.0001	1.722E-003	10.07	0.0099
B – Time	30.05	903.74	0.0015	7.873E-003	46.05	<0.0001
C – Ethanol concentration	61.38	1846.31	<0.0001	1.519E-003	8.89	0.0138
AB	62.23	1871.95	<0.0001	0.015	85.52	<0.0001
AC	66.05	1986.75	0.0003	0.023	132.68	<0.0001
BC	72.90	2192.69	<0.0001	1.800E-003	0.11	0.7523*
A^2	109.22	3285.20	<0.0001	0.96	5629.17	<0.0001

B^2	97.97	2946.74	<0.0001	0.91	5295.41	<0.0001
C^2	86.86	2612.74	<0.0001	1.18	6920.31	<0.0001
Lack of fit	3.104E-003	0.049	0.9976*	4.526E-005	0.15	0.9701*

* Non-significant (p > 0.05)

Total Phenolic Content (TPC)

There was a rapid increase in the amount of phenolic compounds extracted with an increase in temperature and time from 45-55 °C and 80-100 mins respectively and declined beyond this range. It was evident that TPC increased with increasing extraction time when the extraction was conducted at a higher temperature. When the temperature increases, red pitaya peels and the surrounding solvent tend to interact vigorously and the weakened cell wall integrity favors the release of the strongly bounded antioxidant compounds in the mixture (Spigno et al. 2007). Therefore, prolonged extraction beyond 120 minutes as the temperature is approaching 65 °C decreases the phenolic content as a result of thermal degradation of the antioxidant compounds found in the plant extract.

TPC value also showed an increase with increasing ethanol concentration and temperature from 70-80 % and 45-55 °C respectively at a constant time of 100 min. The highest TPC was attained when the temperature was kept constant at 55 °C was in the range of time of 80-100 min and ethanol concentration of 70-80 %. The phenolic content began to decrease as the extraction duration approached 120 min because it was believed that long extraction time increases the exposure to oxygen which eventually led to oxidation of antioxidant compounds (Naczk and Shahidi 2004). The effects of extraction temperature, ethanol concentration and extraction time on the total phenolic content of the red pitaya peels are shown in Figure 1.

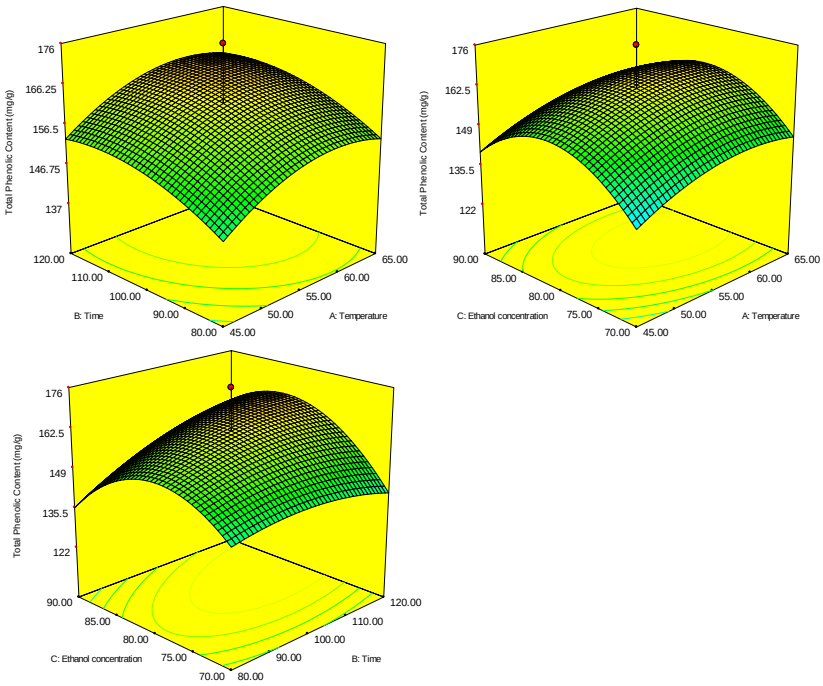

Fig. 1. Response surface (3D) and contour plots showing the effects of extraction temperature, ethanol concentration and extraction time on the total phenolic content of the red pitaya peels.

Total flavonoid content (TFC)

When the ethanol concentration was fixed at 80 %, the flavonoid content increased with the increasing temperature and time up to 55 °C and time of 100 min which then started to decline beyond these points. But, similar to TPC, the temperature range for the extraction of flavonoid compounds should also be meticulously regulated due to the high chances of thermal decomposition of flavonoid derivatives particularly those containing the hydroxyl groups (Davidov-Pardo et al. 2011; Biesaga and Pyrzynska 2013).

It can be seen that the maximum flavonoid of the red pitaya peels was attained around the range of temperature and ethanol concentration of 55-57 °C and 75-80 % respectively. Polar aqueous solvents tend to dissolve more polar plant-derived flavonoids at various ranges of extraction times. The optimal extraction yield may be achieved when the polarity of the fluid and its flavonoids coincide. (Kumar et al. 2008). Different concentrations of ethanol exhibit different effects on the fluid polarity and therefore had multiple effects on strengthening the flavonoids solubility (He et al. 2005).

The flavonoid content was observed to be positively influenced by the synergism between the ethanol concentration and extraction time. Red pitaya peels displayed maximum flavonoid content of 7.14 mg of quercetin/g of extract at the ethanol concentration of 80 % ethanol and 20 % water while the extraction time was 100

minutes. The effects of extraction temperature, ethanol concentration and extraction time on the total flavonoid content of the red pitaya peels are shown in Figure 2.

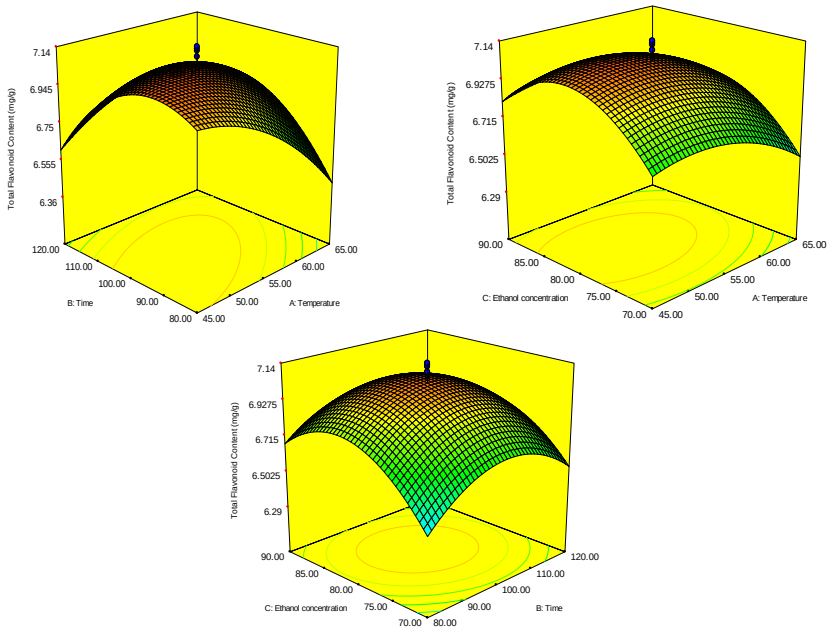

Fig. 2. Response surface (3D) and contour plots showing the effects of extraction temperature, ethanol concentration and extraction time on the total flavonoid content of the red pitaya peels

V. Conclusion

The present study also confirmed that RSM is successfully employed and more advantageous over classical method as its application enables us to attain detailed information regarding the interaction effects among the extraction factors. Thus, the temperature, solvent usage and extraction time can be greatly reduced as compared to single factor experiments. The optimal conditions for the phenolic and flavonoid extractions of red pitaya peels were found to be 56°C, 82%, and extraction duration of 103 min. Under the optimum conditions, the experimental values were in a good agreement with those predicted, therefore indicating the suitability of the model employed. Therefore, RSM is a reliable method which could be applied effectively to predict extraction of the antioxidant compounds from red pitaya peels to be utilized for industrial purposes.

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