

Optimization of Antioxidant Extraction in *Hylocereus Polyrhizus* Seed using Response Surface Methodology

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Abstract: Seeds of red pitaya fruit (*Hylocereus polyrhizus*) are commonly left underutilized in the food industry. Thus, the objective of this present study was to optimize the extraction condition for the maximum DPPH radical scavenging activity of red pitaya fruit seed extract using response surface methodology (RSM). A Central Composite design was employed to determine the effects of the selected variables, comprising extraction time (30-90 min), extraction temperature (40-80 °C) and ethanol concentration (60-80 %) on the DPPH scavenging activities. Data were analyzed by using Design Expert (version 10.0.1, Stat-Ease, Inc., Minneapolis, MN, USA) statistical analysis software. The optimal extraction conditions for the highest antioxidant capacity were derived at 45 mins of extraction time, 70°C extraction temperature and 80 % ethanol concentration that resulted in 92.89 % of scavenging activity. The optimized model developed was verified by comparing the predicted and experimental value of response. The result of measured response agreed well with the predicted values, demonstrating that the model can be used for optimizing the conditions of RSP extract that ensure high recovery of DPPH radical scavenging activity.

Index Terms: red pitaya fruit seed, extraction condition, antioxidant, DPPH radical scavenging activity, response surface methodology.

I. INTRODUCTION

Pitaya or dragon fruit belongs to a botanical family known as, *Cactaceae* with genus name of *Hylocereus* and is classified as a climbing plant with aerial roots [1]. Pitaya is in the shape of an oblong with scaly structure on its outer peel [2]. The flesh of the fruit is juicy and sweet with numerous small and grainy black seeds. Previous studies discovered the significant amount of polyunsaturated fatty acids (PUFA) particularly linoleic acid (C18:2) in pitaya seed oil. This essential fatty acid is reported to help relieve rough skin and keep the skin moisture [3]. As one of the grainy seeds rich in polyunsaturated fatty acid, pitaya seed is generally used to extract the oil in pharmaceutical and cosmetic industries. Nevertheless, the extraction residual which is the defatted

seed is often thrown away.

The exposure of biological system to air pollution, ionization radiation and smoking becomes the main source of free radical formation. Free radical formation such as reactive oxygen species (ROS) is very harmful and considered an unstable molecule that contributes to the beginning of biomolecules oxidation [4] which later on can lead to the cell damages such as cardiovascular disease, cataracts, and brain dysfunction [5]. Plant-based antioxidant is often reported as a radical scavengers that provides protection to the human body by inhibiting the oxidizing chain reactions [6].

Fruits and vegetables represent a major part in our diet due to their undoubted nutritional values for our health. Besides, they display a diverse range of pharmacological properties such as antioxidant capacity, antimicrobial, and anti-inflammatory activities [7]. This defensive action is generally assigned to the effects of the dietary fibers and the phenolic compounds. These compounds are bioactive substances commonly found in plants. Plant phenolic consists of a great diversity of compounds such as flavonoids (anthocyanins, flavanols, flavones) and several classes of non-flavonoids (phenolic acids, lignins, stilbenes) that contribute to the great antioxidant activity [8]. Therefore, the aim of this study has been focused toward exploring natural antioxidants from plant sources. Thus, defatted pitaya seed residual was utilized as experiment material to determine the antioxidant properties using response surface methodology (RSM).

RSM is a statistical method frequently used to optimize the process involving several parameters that can affect the response by determining the mutual interactions between the response values and independent variables. To the best of the authors' knowledge, there has not been a study reported on the antioxidant activity of defatted pitaya seed. Thus, the objective of the present study was to examine the antioxidant capacity of extracts, obtained under optimized conditions by the means of DPPH radical scavenging assay.

II. MATERIALS AND METHOD

A. Materials and Reagent

DPPH (1,1-diphenyl-2-picrylhydrazyl) and ethanol were purchased from Sigma Aldrich. Only analytical grade of chemicals was used throughout the experiment.

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Fruits of red pitaya, *H. polyrhizus*, were collected from an orchard located in Sepang, Malaysia. For the pitaya seed preparation, the seeds were cleaned and washed under running water until all the flesh and pulp were removed. Seeds were then dried for 24 hrs in an oven at 60 °C and ground into smaller particles in a grinder. Then, the seeds were defatted by maceration method using n-hexane as the solvent. The defatted seeds were then left overnight in fume hood to let residual n-hexane evaporate before being used for antioxidants extraction.

B. Extraction of Antioxidants Compounds

3g of defatted seeds was mixed with ethanol of different concentrations in conical flask and placed in thermostatic water bath with constant shaking at 90 rpm. Parafilm and aluminum foils were used to cover the conical flask to prevent solvent loss during the extraction process. Then, the mixture was centrifuged at 10,000 rpm at 25 °C for 5 min to separate the insoluble material. The supernatant was filtered using the Whatman No. 1 filter paper and vacuum-dried in a rotary evaporator at 60 °C until the solvent was completely removed. All the samples were stored at -20 °C until further analysis.

C. DPPH Radical Scavenging Activity

The radical scavenging activity of each sample was carried out following a modified method described by [9]. Briefly, 10 mg of dried extract was weighed and dissolved in 10 mL ethanol (1 mg/ml). 10 mg of DPPH was dissolved in ethanol and made up to 100 mL in a volumetric flask (0.1 mM). Then 50 µL of the sample extracts was mixed with 150 µL ethanolic solution of DPPH in 96-well plate microliter plate. The solution mixture was incubated in the dark at room temperature and the absorbance changes at 515 nm were measured 30 min later using a Microplate Reader (Spectra

Max Plus 384, Molecular Devices Co., Ltd., San Jose, CA, USA). The sample extract without DPPH was used as blank test. All the analyses were conducted in triplicate. The DPPH scavenging activities (DPPHsc) of the extracts were calculated using the following equation:

$$DPPHsc (\%) = ((A_0 - A_1) / A_0) \times 100 \%, \tag{1}$$

Where A_0 is the absorbance of the blank sample and A_1 is the absorbance of the sample.

D. Experimental Design

RSM was performed using Design Expert Software (Version 10.0.1, Stat-Ease, Inc Minneapolis, MN, USA) program. Five-level-factor, central composite design (CCD) was utilized to determine the optimized extraction condition for better antioxidant activity. The design comprised 20 experimental points, including six replications of the centre points. Three independent parameters were studied including extraction time (A), extraction temperature (B) and ethanol concentration (C). Table I represents the coded and uncoded forms of five levels values of the experimental variables. The method of least squares regression was used by CCD to fit the data to a quadratic model. The quadratic model for each response is as follows:

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^k \beta_{ij} X_i X_j + e \tag{2}$$

Where Y is the predicted response, β_0 a constant, β_i the linear coefficient, β_{ii} the quadratic coefficient, β_{ij} the interaction coefficient of variables i and j, and X_i and X_j are independent variables. k is the number of variables and e is the error.

Table- I: Independent variables and their levels for CCD design

Variable	Units	Coded & uncoded level of variables				
		-α	-1	0	1	+α
A	Min	9.55	30	60	90	110.46
B	°C	26.36	40	60	80	93.64
C	%	53.18	60	70	80	86.82

E. Verification of the Models

The actual experimental values for optimal extraction conditions for DPPH in terms of independent variable (time, temperature and ethanol concentration) were determined by correlating with predicted value from the final response regression equations.

III. RESULTS AND DISCUSSION

A. Model Fitting

The experimental data for the response DPPH radical scavenging activity of red pitaya seed extracts are displayed in Table II. The result obtained showed that the DPPH radical scavenging activity of red pitaya seed extract ranged from 89.84 – 93.53 %. The least square method was used to

calculate the regression coefficients of the intercept, linear, quadratic and interactive terms of the model [10] and revealed in Table III. It was revealed that two linear parameters, temperature (B) and ethanol concentration (C), interaction parameters, (BC) and quadratic parameters (B2) were significant at (p < 0.05). The software generated the final predictive equation in terms of coded factors as shown in Equation 3, where empirical correlation between extraction time (A), temperature (B) and ethanol concentration (C) was established. The analysis of variance (ANOVA) for the experimental results obtained in Table II discovered that the quadratic model was significant (p < 0.05) with no significance in the lack of fit (p > 0.05). The coefficient of determination (R2) acquired for this model was 0.9325. This shows that



only 6.75 % of the total variations for DPPH radical scavenging activity could not be explained by the model.

$$Y_{(DPPH)} = + 92.89 - 0.1639A + 0.5042B + 0.5095C - 0.1487AB + 0.0963AC - 0.7113BC - 0.0777A^2 - 0.6982B^2 + 0.0531C^2 \quad (3)$$

B. Optimum Extraction Condition based on DPPH

The DPPH radical scavenging activity of pitaya seed extract ranged from 89.84 – 93.53 %. The mean recorded value was 92.40 %. The highest value was recorded for run No. 18 whereas the lowest value recorded was discovered at experiments No. 3. The recovery of DPPH radical scavenging activity was estimated based on the varied value of the tested factor using response surface. The three-dimensional response surface and two-dimensional contours designed by the fitted model are demonstrated in Figures 1-3. Each diagram shows the effects of mutual interaction between the extraction parameters on the yielded DPPH radical scavenging activity by holding the other variables at their zero level.

Fig. 1 illustrates the interactions between the extraction time and temperature on DPPH radical scavenging activity at concentration of the solvent which fixed at 70 %. It was observed that the scavenging activity was increased as extraction.

Table- II: The experimental results of DPPH radical scavenging activity from red pitaya seed extract.

Ru n	Time (A)	Temperatur e (B)	Ethanol concentratio n (C)	DPPH (%)
1	-α	0	0	92.97
2	0	0	0	92.35
3	0	-α	0	89.84
4	1	1	1	92.28
5	α	0	0	92.18
6	-1	1	-1	92.98
7	-1	-1	-1	90.66
8	1	-1	1	93.4
9	0	0	0	92.97
10	0	0	0	93.1
11	0	0	-α	92.36
12	0	0	0	93.36
13	0	α	0	91.8
14	0	0	0	92.51
15	-1	1	1	92.91
16	1	-1	-1	90.24
17	0	0	0	93.08
18	0	0	A	93.53
19	-1	-1	1	92.84
20	1	1	-1	92.56

temperature ascended over time. The results marked that an increase in temperature led to an increase in antioxidant

activity. This phenomenon was probably because of higher temperature can improve the mass transfer as well as increase the extracted molecules. However, immoderate increase of temperature during the extraction process may affect the stability of the phenolic compounds due to chemical and thermal degradations of some phenolic compounds accounting for decreased antioxidant activity which were previously mobilized at low temperature [11]. This was supported by the outcome obtained showing the scavenging activity was decreased after the temperature was elevated from 70 °C to 80 °C. This similar type of occurrence was also revealed by [12] that the antioxidant capacity of *C. asiatica* extract was stable up to 50 °C. Thus, it is believed that the polyphenol compounds extracted under high temperature had lower antioxidant activity as compared to those extracted under moderate temperature.

Extraction time is an important factor to be considered in minimizing energy and cost of the extraction procedure. The extraction time can either be as short as few minutes or very long as up to 24 hours [13], [14]. The influence of interactive effect between extraction time and ethanol concentration on DPPH radical scavenging activity is exhibited in Fig. 2. The highest antioxidant activity observed extraction time increased from 30 – 70 min was accompanied by small increment in antioxidant capacity. Prolonging the process duration up to 90 min did not significantly improve the radical scavenging activity. This occurrence could be explained by Fick’s second law of diffusion, which states that a final equilibrium will be accomplished between the concentration of the solute in the solid matrix (plant matrix) and in bulk solution (solvent) after a certain time [15]. Moreover, continuing the extraction period might induce the risk of phenolic oxidation unless reducing agents are added to the solvent system [16].

Table- III: Analysis of variance (ANOVA)

Source	Sum of Squares	Mean Square	F-value	p-value
Model	18.93	2.1	15.35	< 0.0001
A	0.38	0.38	2.68	0.1328
B	3.47	3.47	25.33	0.0005
C	3.54	3.54	25.86	0.0005
AB	0.18	0.18	1.29	0.2823
AC	0.07	0.07	0.54	0.479
BC	4.05	4.05	29.53	0.0003
A ²	0.09	0.08	0.64	0.444
B ²	7.03	7.03	51.26	< 0.0001
C ²	0.04	0.04	0.3	0.5981
Residual	1.37	0.14		
Lack of Fit	0.63	0.13	0.8439	0.5716
Pure Error	0.7434	0.15		
Cor Total	20.3			
Std. Dev	0.3702	R ²	0.9325	
Mean	92.4	Adj. R ²	0.8717	
C.V %	0.4007			

Meanwhile, the effects of temperature versus ethanol concentration at constant extraction time 60 min revealed that DPPH radical scavenging activity increased with the increase of the ethanol concentration as displayed in Fig. 3.

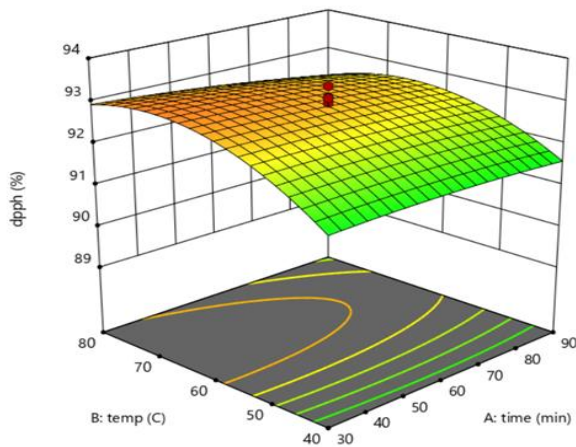


Fig. 1. Interactive effect of extraction time and temperature on DPPH radical scavenging activity

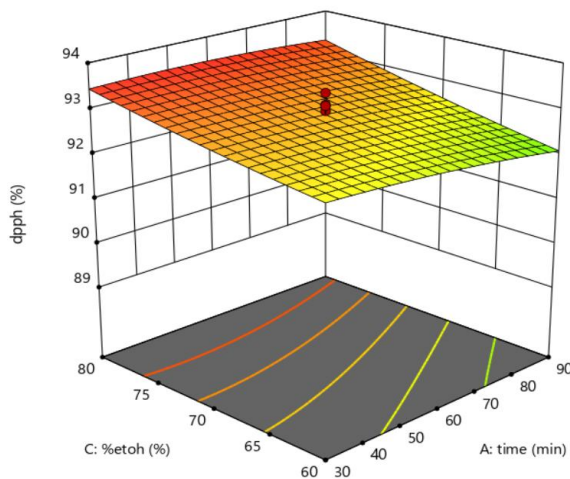


Fig. 2. Interactive effect of extraction time and ethanol concentration on DPPH radical scavenging activity

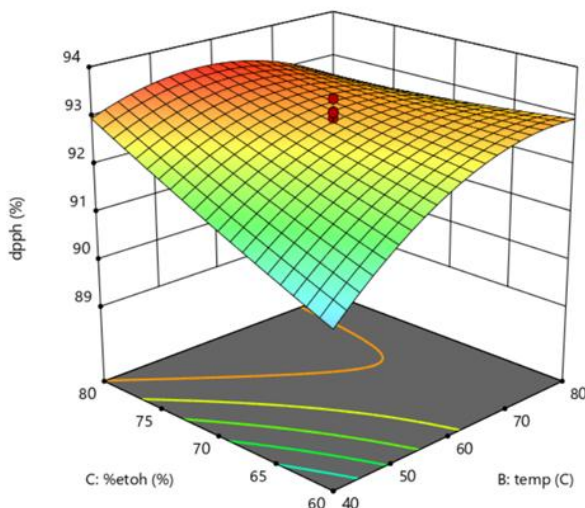


Fig. 3. Interactive effect of temperature and ethanol concentration on DPPH radical scavenging activity

Previous studies reported that binary-solvent system demonstrated higher yield of polyphenol compounds as compared to mono-solvent system [17]-[20]. This is because water is reported to act as a plant swelling agent, while ethanol can disrupt the bonding between the solutes and plants matrices [21] which therefore more phenolic compound accountable for antioxidant activity can be extracted.

C. Verification of the Models

The numerical optimization of extraction parameters for better DPPH radical scavenging activity was determined using Design Expert (version 10.0.1, Stat-Ease, Inc). The experiment was performed referring to the recommended optimum treatment conditions which were 45 mins of extraction time, 70°C extraction temperature and 80 % ethanol concentration.

The adequacy of the response surface models for predicting the optimum response value was verified by comparing the experimental and predicted value of response (Table IV). The obtained results from verification experiment were in close agreement with predicted value where deviation between the experimental and predicted values found to be insignificant ($p > 0.05$).

Table- IV: Response values under optimal conditions at 45 minutes of extraction time, 70°C extraction temperature and 80 % ethanol concentration.

Response	Predicted	Experimental
DPPH radical scavenging activity (%)	93.23	92.56 ± 0.21

IV. CONCLUSION

Response surface methodology was successfully employed in this study for optimization of extraction procedure that gives maximum DPPH radical scavenging activity. The result revealed that extraction temperature and ethanol concentration significantly influenced the measured response. The optimized condition identified was at 45 mins of extraction time, 70°C extraction temperature and 80 % ethanol concentration that ensure better recovery of response at 92.89 %. The results indicated that red pitaya seeds extract has a good antioxidant activity. Moreover, further studies should be conducted for the recovery of polyphenolic compounds before a large-scale production of red pitaya seed extract can be suggested.

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