# Optimization of free radical scavenging capacity and pH of *Hylocereus polyrhizus* peel by Response Surface Methodology

**A W Putranto[1], S R Dewi[1], Y Puspitasari[2] and F A Nuriah[2]**

[1] Department of Agricultural Engineering, University of Brawijaya, Malang, ZIP 65145, Indonesia

[2] Department of Food Science and Technology, University of Brawijaya, Malang, ZIP 65145, Indonesia

E-mail : angkywahyu@ub.ac.id

**Abstract.** Red dragon fruit (*Hylocereus polyrhizus*) peel, a by-product of juice processing, contains a high antioxidant that can be used for nutraceuticals. Hence, it is important to extract and investigate its antioxidant stability. The aim of this study was to optimize the free radical scavenging capacity and pH of *H. polyrhizus* peel extract using Central Composite Design (CCD) under Response Surface Methodology (RSM). The extraction of *H. polyrhizus* peel was done by using green-Pulsed Electric Field (PEF)-assisted extraction method. Factors optimized were electric field strength (kV/cm) and extraction time (seconds). The result showed that the correlation between responses (free radical-scavenging capacity and pH) and two factors was quadratic model. The optimum conditions was obtained at the electric field strength of 3.96 kV/cm, and treatment time of 31.9 seconds. Under these conditions, the actual free radical-scavenging capacity and pH were 75.86 ± 0.2 % and 4.8, respectively. The verification model showed that the actual values are in accordance with the predicted values, and have error rate values of free radical-scavenging capacity and pH responses were 0.1% and 3.98%, respectively. We suggest to extract the *H. polyrhizus* peel using a green and non-thermal extraction technology, PEF-assisted extraction, for research, food applications and nutraceuticals industry.

## 1. Introduction

The anthocyanin pigment is one of the water-soluble pigments that is widely used as a natural ingredient for consumption because it is easily absorbed by the body. In addition, it has the capability of capturing free radicals and high antioxidant capacity and shows the inhibitory effect on the growth of some cancer cells [1]. Recently, the research focussed on utilization of waste material from the industrial processing of fruits and vegetables as a good source of bioactive compounds. The types and amounts of phytochemicals retained in the discarded material are important to be considered for deriving value-added ingredients [2]. Red dragon fruit (*Hylocereus polyrhizus*) peel was potentially to be extracted since it has a high anthocyanin pigment that can provide natural dyes to the food, high antioxidant activity 10 times more than flesh [2], high flavonoids and betacyanins content, also a strong inhibitor of the growth of melanoma cells than the flesh [3].

The extraction technology to extract *H. polyrhizus* peel that are commonly used are batch maceration and Microvawe Assisted Extraction (MAE). However, the technology still has many

shortcomings including: long extraction process, more solvents needed [4], MAE needs heat. The heating process can cause degraded pigments during the process, decreasing color quality, and affecting antioxidant activity [5].

In recent years, non-thermal PEF-assisted extraction with low electric field strength has been reported in several studies to improve the high extraction rate of some antioxidants such as total carotenoid from carrot pomace (2.5 kV/ cm) [6], anthocyanin from purple fleshed potatoes and grape pomace using 1.5 kV/cm and 3 kV/cm of electric field strength respectively [7]. The non thermal extraction using PEF technology has many advantages such as short extraction time consequently the energy used is very low [8], maintaining antioxidant qualities in heat sensitive materials [9], as well as high extraction yield due to damage to cell membrane and improved extraction characteristics [10].

PEF on food processing cannot be separated from the cell electroporation that inhibit the synthesis or formation on the membrane phospholipids. The formation of these pores depends on the electric field strength and the treatment time [11]. The studies of cell division to retrieve the valuable substances in waste product have not been determined, moreover the extraction of *H. polyrhizus* peel using non-thermal method, PEF-assisted extraction has not been fully explored. The present study is expected to explore the *H. polyrhizus* peel as a by-product juice processing to be a source of antioxidants and a natural colorant substitute for synthetic dyes. Therefore, the aim of the present study was to optimize the free radical scavenging capacity and pH of *H. polyrhizus* peel extract using Central Composite Design (CCD) under Response Surface Methodology (RSM).

## 2. Materials and Methods
### 2.1. Plant material, chemical and reagent
Fresh red dragon fruit (*Hylocereus polyrhizus*), a local type, were purchased in a market in Malang City, East Java, Indonesia. The chemicals used such as potassium chloride, hydrochloric acid 0.2 N, Na-acetate, distilled water and 2,2'-diphenyl-b-picrylhydrazyl (DPPH) reagent 0.2 mM were purchased from Sigma-Aldrich Chemicals.

### 2.2. Apparatus and instruments
Pulsed Electric Field (PEF) batch system used in this study was the main apparatus. In present study, the PEF was applied in green extraction of *H. polyrhizus* using low electric field strength. The treatment chamber (capacity of 9.1 L) was made of stainless steel as well as food grade material and configuration of electrodes inside adopts from coaxial models. PEF is also being equipped with control systems such as input voltage regulator, frequency regulator and timer. The PEF apparatus is specifically designed for the improvement of mass transfer, with electric field ranged from 1.3 to 6.45 kV/cm. The frequency from 0.5 to 31 kHz, the timer (Omron AT-8N) ranged from 0.05 second to 100 hours and pulse width produced of 78 μs. While the other instruments which were used in this study such as pH meter, Spectrophotometer UV-Visible (Genesys 10), analytical scale (Denver Instrument M-310), vortex, aluminum foil, filter paper and cuvette.

### 2.3. Green-PEF-assisted extraction of H. polyrhizus
The fresh red dragon fruit (*H. polyrhizus*) separated from the flesh and from the green fruit petals. Then, the sample were cut into dimension of 2x12x1 mm to enlarge the surface area of *H. polyrhizus* peel. In line with a green extraction concept, PEF assisted extraction using distilled-water as a solvent, since it was a green and safe solvent for food and also was a polar solvent to dissolve the anthocyanin. The sample and solvent with ratio of 1: 5 (w/v) were blended and filling into treatment chamber. Then set the PEF frequency of 8 kHz. The electric field strength and extraction time was adjusted by following the actual variables on run order of central composite design matrix (Table 1). After the extraction, the *H. polyrhizus* peel extract was placed in a dark bottle to prevent degradation of the compounds contained in the extract due to exposure from the outside before analysis.

## 2.4. DPPH radical-scavenging measurement

The ability of anthocyanin in extracts and fractions from *H.Polyrhizus* peel to scavenge the 2,2'-diphenyl-b-picrylhydrazyl (DPPH) radical was measured using the method from [12]. Thus, an aliquot of extract (0.1 mL) was added to 3.9 mL of ethanolic DPPH (60 mM). The mixture was shaken vigorously and left to stand at room temperature for 30 min in the dark and absorbance was measured at 517 nm. The free radical scavenging capacity was calculated as follows:

$$\% \text{ Free radical-scavenging capacity} = \left[ \frac{(A_{blank} - A_{sample})}{A_{blank}} \right] \times 100\% \tag{1}$$

where $A_{blank}$ was the absorbance of the control reaction (containing all reagents except the test compound), and $A_{sample}$ was the absorbance of the test compound.

## 2.5. pH measurement

The *H. polyrhizus* peel extract measured by using pH meter. Measurements were performed by calibration stage of the apparatus, by using a pH 4 distilled water then continued with pH 7 distilled water. Furthermore, pH measurement were taken on *H. polyrhizus* peel extract.

## 2.6. Experimental design

The experimental design in this study was experimental design using central composite design (CCD). In previous study consisted of two factors: 1). electric field strength (three levels): 3.5; 4.0 and 4.5 kV/cm and treatment time (five levels): 15, 30, 45 seconds. The best results of the previous study will be used as a central point in the present study. The optimization of free radical-scavenging capacity and pH in from *H. polyrhizus* peel extract used by Response Surface Methodology (RSM) in Design Expert (DX) 7.1.6 software. A two-factor central composite design was adopted to optimize the total carotenoid in carrot juice. The second order second-order polynomial equation shown in Eq.2:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i \, X_i + \sum_{i=1}^{k} \beta_{ii} \; X_i X_i^2 + \cdots + \sum_{i<j} \sum \beta_{ij} \, X_i X_j + \varepsilon \tag{2}$$

Where Y for the reponses, $\beta_0$ denoted the model intercept, i and j were the linear and quadratic coefficients, respectively, $\beta_i$, $\beta_{ii}$ and $\beta_{ij}$ were the regression coefficient, k was the number of factors studied and optimized in the experiment and $\varepsilon$ was the random error. The goodness of fit of the model was evaluated by the coefficient of determination ($R^2$) and the analysis of variance (ANOVA).

## 2.7. Verification of model

Verification of the model is needed for testing the accuracy of the model in describing the actual conditions. Verification was done by comparing the results of optimal treatment (electric field strength and extraction time) based on predicted value from RSM with the results of actual or experimental study.

## 3. Results and Discussion

### 3.1. Optimization by response surface method

Central composite design (CCD) was used to further optimize the extraction conditions with respect to the free radical-scavenging capacity and pH of *H. polyrhizus* peel extracts. An electric field strength of 4 kV/cm and extraction time of 30 second, were chosen from previous study. The responses values free radical-scavenging capacity and pH of extracts obtained under various experimental conditions are shown in Table 1.

Based on Table 1, the maximum free radical-scavenging capacity (76.54%) and pH (5.1) was recorded during Run no. 5, while the lowest antioxidant capacity (71.89%) in Run no. 11 and the lowest pH (4.6) was detected in Run no. 4. The response surface analysis using CCD, has several statistical models offered for analyzing research data to obtain the most optimal response. Some of the statistical models offered include quadratic models, linear models, 2FI models or also called two-

factor interactions, and cubic models. The four models was analyzed each other based on Sequential Model Sum of Squares, and supported by Lack of Fit Tests, as well as Model Summary Statistics to determine the model corresponding to optimum response.

**Table 1.** Actual and Coded Variables Combinations uisng Central Composite Design (CCD) matrix

| Run Order | Actual Variables | | Coded Variables | | Responses | |
|---|---|---|---|---|---|---|
| | Electric Field Strength (kV/cm) | Extraction Time (seconds) | $X_1$ | $X_2$ | Free Radical-Scavenging capacity (%) | pH |
| 1 | 4.50 | 45.00 | 1.000 | 1.000 | 72.99 | 4.9 |
| 2 | 4.00 | 51.21 | 0.000 | 1.414 | 76.04 | 4.7 |
| 3 | 4.00 | 8.79 | 0.000 | -1.414 | 75.79 | 5.0 |
| 4 | 4.50 | 15.00 | 1.000 | -1.000 | 72.49 | 4.6 |
| 5 | 4.00 | 30.00 | 0.000 | 0.000 | 76.54 | 5.1 |
| 6 | 4.00 | 30.00 | 0.000 | 0.000 | 75.64 | 5.0 |
| 7 | 3.50 | 15.00 | -1.000 | -1.000 | 72.09 | 4.9 |
| 8 | 4.00 | 30.00 | 0.000 | 0.000 | 77.44 | 5.1 |
| 9 | 4.00 | 30.00 | 0.000 | 0.000 | 75.19 | 4.9 |
| 10 | 3.50 | 45.00 | -1.000 | 1.000 | 73.19 | 5.0 |
| 11 | 4.71 | 30.00 | 1.414 | 0.000 | 71.89 | 4.8 |
| 12 | 3.29 | 30.00 | -1.414 | 0.000 | 73.39 | 4.7 |
| 13 | 4.00 | 30.00 | 0.000 | 0.000 | 73.94 | 4.9 |

Based on the three statistical models analysis, the chosen (suggested) model to explain the relationship between electric field strength variable ($X_1$) and extraction time ($X_2$) to free radical-scavenging capacity and pH responses was quadratic model. This is clarified by the analysis of variance (F-test) for quadratic model. The DX 7.1.6 modeling results obtained second order polynomial models. The three-dimensional plots and contour plots of optimal free radical-scavenging capacity and pH of *H. polyrhizus* peel extract was presented in Figure 1. It indicates an increase in free radical-scavenging capacity and pH with increasing electric field strength variable ($X_1$) and extraction time variable ($X_2$), but when these variables is increased even further the both responses were decreased.

The electric field strength variable is a factor that strongly influences the extraction using PEF since it will determine the pore formation rate in the electro-permeabilization process. However, the increase of electric field strength in this study will actually decrease the antioxidant activity and pH (Figure 1a and 1b). This is expected due to the higher electric field strength, more pores are formed on the membrane cell since it exceeds the critical field of the cell (Ef > Ec), hence the pores are irreversible [13]. When the cell membrane is irreversible, mass transfer from within the cell will occur rapidly. Displacement of these compounds will not only dissolve the antioxidants into the solvent, but also other compounds contained in cells that can actually reduce the percentage inhibitor antioxidants capacity. The decreased of antioxidants with increasing electric field strength also reported by [14] study that the significant decrease in total carotenoid in orange juice using bipolar pulses of 30 kV/cm, and significant decrease in carotene content in the PEF-treated (35 kV/cm, 4 μs bipolar pulses at 100 Hz) in tomato juices [15]. Therefore, in this study, the suggested optimum condition of electric field strength is 3.96 kV/cm which can be extract the anthocyanin optimally.

On the other hand, the anthocyanin stability is influenced by several factors, one of which is pH. The pH is very important for the color of anthocyanins. Some anthocyanins are red in acid solutions, violet or purple in neutral solutions, and blue in alkaline pH [16]. In this study, the higher of electric field strength will actually decrease the pH to reach 4.6 (Figure 1b). The pH change was expected due to other compounds that followed extractable anthocyanin pigments thus making it pigment color changes.
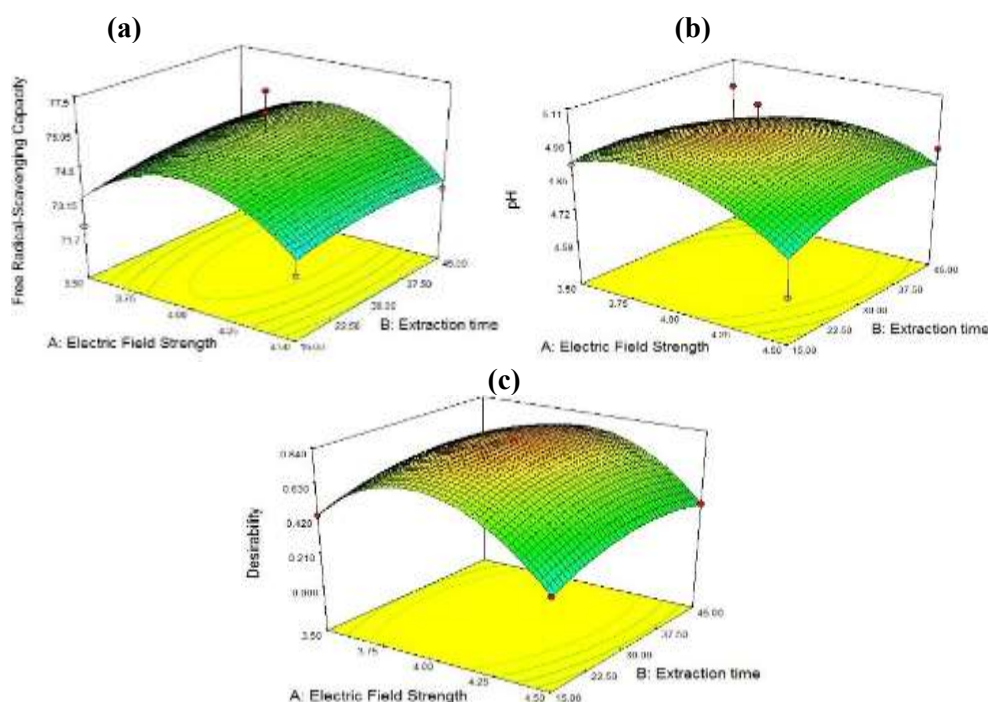
**Figure. 1** Three dimensional graph between combination of electric field strength and extraction time on optimal responses. (a) free radical-scavenging capacity response, (b) pH response, (c) desirability value of optimum condition

The optimum treatment is based on suggested model are shown in Figure 1c, where the optimum point is indicated by the desirability value of 0.84. The desirability value (ranged from 0 to 1.0) is the function value of optimization objective which indicates the program's ability to satisfy the desire based on the criteria specified on the final product. The desirability value closer to the 1.0 value, indicates the ability of the program to produce the desired product more perfect. The results of computerized prediction DX 7.1.6 software, the optimum point of electric field strength variable was 3.96 kV/cm, whereas the optimum extraction time variable was 31.9 second. Under these condition, the predicted optimum response values of free radical-scavenging capacity and pH of *H. polyrhizus* peel extract was 75.784% and 4.9, respectively.

### 3.2. Verification of optimal conditions

Verification of the model were conducted under optimal conditions compared with actual values of dependent variables. The comparison between predicted value and actual values or experimental study are shown in Table 2.

**Table 2.** Predicted and Actual Values of Response Under Optimal Conditions

| Responses | Model equations | Predicted value | Actual value (experimental)[a] | Error rate value |
|---|---|---|---|---|
| Free radical-scavenging Capacity (%) | $Y = -51.39457 + 62.57967\ X_1 + 0.18011\ X_2 - 0.02\ X_1X_2 - 7.8075\ X_1^2 - 0.0014\ X_2^2$ | 75.784 | $75.86 \pm 0.2$ | 0.10 % |
| pH | $Y = -1.338536 + 3.33536\ X_1 - 0.010202\ X_2 + 0.0067\ X_1X_2 - 0.45\ X_1^2 - 0.00027\ X_2^2$ | 4.9 | 4.8 | 3.98% |

Notes: [a] experimental using electric field strength : 3.96 kV/cm, extraction time : 31.9 second

The actual value or experimental results were in good agreement with the predicted values. The difference of free radical-scavenging capacity and pH between the predicted value and actual value has an error rate of 0.1% and 3.98%, respectively. The confidence level of the predicted values with the actual value more than 95%, which indicates that the estimation models are reasonable and accurately in predicting an optimal response. In this study, the *H. polyrhizus* peel extraction with PEF using optimal conditions have an antioxidant capacity of 75.86 ± 0.2% and higher than extraction by using maceration with a ratio of 1:50 (w/v) using a water solvent, which is having an antioxidant capacity of 51.35 ± 0.87% [17]. Whereas the experimental value based on optimal conditions obtained pH of 4.8 (red-purple color). The anthocyanin belongs to the group of natural dyes responsible for some colors in the red-blue range, this spectrum depends on an abundance of anthocyanin in the natural source. As a result anthocyanin can be food colorant with variation pH [16].

## 4. Conclusion

The green extraction technology of *H. polyrhizus* peel extract was well implemented using PEF. Factors that affect the improvement of mass transfer in *H. polyrhizus* peel extract using PEF is electric field strength and extraction time. By using RSM method, the correlation between responses and two variables was quadratic model. At this model, the optimum conditions was obtained at the electric field strength of 3.96 kV/cm, and treatment time of 31.9 seconds. Under these conditions, the actual free radical-scavenging capacity and pH were 75.86 ± 0.2 % and 4.8, respectively. The verification of the model showed that the actual value are in good agreement with the predicted values and has an error rate value of free radical-scavenging capacity and pH of 0.1% and 3.98%, respectively. The green PEF-assisted extraction method of *H. polyrhizus* peel extract not only has a sufficiently low electric field strength but also had a rapid extraction time. Therefore we suggest to extract the *H. polyrhizus* peel using a green and non-thermal extraction technology, PEF-assisted extraction, for research, food applications and nutraceuticals industry.

## References

[1]    Santoso W E A and Estiasih T 2014 *Jurnal Pangan dan Agroindustri* **2** 121-127
[2]    Tenore G C, Novellino E and Basile A 2012 *J. Func. Foods* **4** 129 – 136
[3]    Wu L C, Hsu H W, Chen Y C, Chiu C C, Lin Y I, Ho A 2006 *Food Chem.* **95** 319-327
[4]    Safdar N M, Tusneem K, Saqib J, Amer M, Karam A, Ambreen A S 2017 *J. Food & Drug Analysis* **25** 488-500
[5]    Patras A, Nigel P B, Colm O D, B K Tiwari 2010 *Trends. Food Sci. & Technol.* **21** 3-11
[6]    Putranto A W, Argo B D, Wijana S 2014 *Indonesian Green Technol. J.* **3** 1-9
[7]    Toepfl S 2006 Pulsed electric fields (PEF) for permeabilization of cell membranes in food and bioprocessing – applications, process and equipment design and cost analysis Prozesswissen-schaften der Technischen Universität Berlin Jerman
[8]    Zderic A and Edwin Z 2016 *J. Chem. Eng.* **109** 586-592
[9]    Zhang Z, Xin-An Z, Charles S B, Margaret B, Zhong H, Xia-Yu X 2015 *J. Mol. Sci.* **16** 24159-24173
[10]   Maskooki A and Mohammad N E 2012 *J. Food & Bioprod. Proc.* **90** 377-384
[11]   Raso J and Heinz V 2006 Pulsed Electric Fields Technology For The Food Industry - Fundamental and Applications Springer Science Business Media USA
[12]   Jiao Y, Jiang Y, Zhai W, Yang Z 2012 *J. Biotechol.* **11** 7046-7054
[13]   Shamsi K 2008 Effects of Pulsed Electric Field Processing on Microbial, Enzymatic and Physical Attributes of Milk and the Rennet-Induced Milk Gels RMIT University, Melbourne
[14]   Cortés C, Torregrossa F, Esteve M J, Frígola A 2006 *J. Agri. and Food Chem.* **54** 6247-6254
[15]   Odriozola-Serrano I, Aguilo-Aguayo I, Soliva-Fortuny R, Gimeno-Ano V and Martin-Belloso O 2007 *J. Agri. & Food Chem.* **55** 9036–9042
[16]   Wahyuningsih S, Wulandari L, Wartono M W, Munawaroh H, Ramelan A H 2017 *IOP Conf. Ser.: Mater. Sci. Eng.* **193** 012047

[17]    Manihuruk F M, Suryati T, Arief I 2016 *Media Peternakan* **40** 47-54