Optimum response of melinjo peel (gnetum gnemon) antioxidant activity using response surface methodology (RSM)

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Abstract. Melinjo (Gnetum gnemon) is one of plants provided in abundant numbers. Melinjo peel contained antioxidant compound which is beneficial for the human body health. This compound protects the body cells from free radicals. This research aims to optimize the antioxidant activity in the Melinjo peel tea through Response Surface Methodology with Central Composite Design (CCD). The design consisted of 13 combination treatments with variable of 0.05% - 0.1% citric acid concentration levels and 5-10 minutes of blanching duration levels. The result of antioxidant activity of the treatment design was 75.21-87.6%. The antioxidant activity of melinjo peel tea was analyzed through DPPH method with 518 nm of spectrophotometer wave length. The optimum point was obtained through 0.075% citric acid concentration and 7.50 minutes of blanching duration with 78.68% antioxidant activity while the verification result of antioxidant activity was 76.37%. This had fulfilled the fit requirement by not exceeding the 5% margin of error.

Keywords: antioxidant activity; blanching; citric acid; melinjo peel tea

1. Introduction

Melinjo (Gnetum gnemon) is one of plants provided in abundant numbers. Based on the horticultural plant statistics of 2014, Java Island supplied 148.762 tons of melinjo productivity. So far, melinjo is still utilized traditionally where the fruit was processed into melinjo chips (emping) while the peel was only utilized as side dish vegetables. Melinjo peel which was not optimally utilized will turn out merely as waste. Melinjo peel will decay if it is stored in fresh condition in an open space, while being stored in the refrigerator for a long time will decrease the sensory quality especially the texture and color. Whereas, melinjo peel has a development potential, which according to Santoso, et al [1] melinjo peel contains ascorbic acid, tocopherol and polyphenol which are beneficial for human body health in the form of antioxidant.

The research of Ardiansyah and Apriliyanti [2] and Khoriyah [3] which discussed the melinjo peel tea processing through enzymatic oxidation treatment obtained the best result where the water content was 6.48%, the ash content was 3.5%, total phenol 34.8% (titration method) and yield 56.9%. However, the melinjo peel tea had a weakness in its sensory characteristics. Then, Khodafi [4] fixed the sensory characteristics weakness on the melinjo peel tea through blanching as the initial treatment by using citric acid as the media (0-1%) and the blanching duration (5-10 minutes) that the melinjo peel tea was becoming acceptable to the consumer. Apriliyanti et al [5] mentioned that melinjo peel
tea with blanching as the initial treatment through citric acid as the media contained 2.02-2.52 mg/100 g total phenol with 32.47-48.47% antioxidant activity.

According to Pujimulyani et al [6] blanching is aimed to deactivate the polyphenol oxidase enzyme. Besides, blanching could help increasing antioxidant activity since the inactive compounds were supposed to be active during the process. It was mutual with the research of Kim et al [7] that tannin heating showed the increasing antioxidant activity compared to those without heating process. Antioxidant is a molecule which is able to slow or prevent other molecules oxidation. Oxidation is a chemical reaction which produces free radicals, that it will trigger a chain reaction which will damage the cells [7]. Antioxidant such as phenol compound is able to prevent the chain reaction.

Based on the explanation above, a continued effort was required to improve the melinjo peel tea quality and optimization by optimizing melinjo peel tea through Response Surface Methodology (RSM). RSM will help in obtaining response’s optimum condition and considering the interaction among reviewed factors, RSM is the most accurate, efficient, and economic approach. This research aims to determine the optimum response of melinjo peel tea antioxidant activity by using citric acid and blanching duration. RSM program was run through Design Expert v.7 (DX7.0) software. The output of this research will be information for potential utilization through producing (scale up) melinjo peel waste into melinjo peel tea with optimum antioxidant activity.

2. Method

2.1 Materials and Methods

The required material is the ripe melinjo (Gnetum gnemon) peel which has orange until reddish color range obtained from Kediri, East Java, DPPH (1,1-diphenyl-2-pycrilhydrazyl) (Sigma Aldrich), and methanol (pa, QREAC). The utilized tools are such as cabinet oven (Model R-5A Harvest Saver, USA) and spectrophotometer (Ultrospec 2100 Pro UV-Visible, Cambridge UK).

2.2 Melinjo Peel Tea Processing

Melinjo peel tea processing were selecting melinjo peel, cleaning and washing, weighing, blanching, drying, and grinding. The selected melinjo peel was the ripe, fresh, clean, intact and having orange until reddish color range. The melinjo peel was separated from the dirt and washed with running water. The melinjo peel was weighed according the treatment needs. Blanching was conducted by using citric acid as the media and the blanching duration setting. The concentration used was 0.05-0.1% and the duration was 5-10 minutes. Melinjo peel was dried in 105°C for 30 minutes inside the cabinet oven to conduct a thorough and quick drying process. After being dried, the melinjo peel tea would be finely ground by blender until it turned out into powder which later will be packed by using tea bag.

2.3 Experiment Design

Blanching is the initial treatment process to obtain sensory characteristics and optimum antioxidant activity. This process was conducted by using citric acid as the media and blanching duration setting. The research was designed based on the Response Surface Methodology (RSM) by using Central Composite Design (CCD) which consisted of two factors; the citric acid concentration (X1: 0.05-0.1%) and blanching duration (X2: 5-10 minutes) with one observed antioxidant activity response (Y1). The research treatment combination consisted of 13 combinations where each treatment followed the central composite design (CCD) as presented in the table 1.

2.4 Determining Antioxidant Activity

Antioxidant activity test was conducted by taking 0.1 ml of melinjo peel tea steeping which then will be mixed with 5 ml of methanol by using vortex and took the 4 ml portion. After that, added 1 ml of 1,1-diphenyl-2-pycrilhidrazil (DPPH) 20 ppm, mixed by using vortex, and incubated in the dark space for 30 minutes, then absorbed at 517 nm wavelength [8]. The antioxidant activity was calculated with the following equation (1):
Antioxidant Activity (%) = \frac{\text{Abs DPPH control} – \text{Abs DPPH remnants}}{\text{Abs DPPH control}} \times 100\% \quad (1)

Description:
Abs DPPH Control: DPPH absorbance before being reacted with the sample
Abs DPPH Remnants: DPPH absorbance after being reacted with the sample

Table 1. Central Composite Design of Antioxidant Activity Optimum Response Through Blanching Treatment with Citric Acid and Duration by using RSM

| Run | Citric Acid Concentration (%) | Blanching Duration (minute) | Antioxidant Activity Response (%) |
|-----|-------------------------------|-------------------------------|----------------------------------|
| 1   | 0.075                         | 3.96                          |                                  |
| 2   | 0.08                          | 7.50                          |                                  |
| 3   | 0.05                          | 10.00                         |                                  |
| 4   | 0.10                          | 10.00                         |                                  |
| 5   | 0.04                          | 7.50                          |                                  |
| 6   | 0.08                          | 7.50                          |                                  |
| 7   | 0.08                          | 7.50                          |                                  |
| 8   | 0.05                          | 5.00                          |                                  |
| 9   | 0.08                          | 7.50                          |                                  |
| 10  | 0.10                          | 5.00                          |                                  |
| 11  | 0.08                          | 11.04                         |                                  |
| 12  | 0.08                          | 7.50                          |                                  |
| 13  | 0.11                          | 7.50                          |                                  |

2.5 Data Analysis

2.5.1 Analyzing Antioxidant Activity Response and Optimizing Citric Acid concentration and Blanching Duration. The response data obtained from the citric acid concentration and blanching duration treatments was inputted to the Design Expert 7 program, then the program will analyze the tendency of the polynomial equation with the suitable order to the obtained result from each response. There were different polynomial equations such as mean, linear, 2FI, quadratic, and cubic. There were 3 ways to respond each polynomial equation which were based on Sequential Model of Sum of Square, Lock of Fit Tests and Model Summary Statistic. The Design Expert 7 program with CCD design will provide suitable math equation model suggestion for each variable response. The model equation suggestion will be displayed on the Fit summary to select the most suitable equation model.

Besides, Design Expert 7 program provided the analysis of variance (ANOVA) facility to show the significance of the recommended model. Next, the recommended model will be displayed on a contour plot, in the form of two or three dimensional figure or graphic. The ideal model identification shall have significance to the response, insignificant lack of fit value, mutual supportive Prediction R-squared and adjust R-squared values, and the Adequate Precision value which is not more than 4. In this step, Design Expert 7 program also provided normal plot residual facility which indicates whether the residue (the gap between actual response and the predicted response value) followed the normality line (straight line). Data points which are getting closer to the normality line indicates the data points are spread normally, which means the actual result is getting closer to the predicted result of Design Expert 7.

The next step is optimization, each analyzed response, the output of the optimization step is recommendation of several new optimum formulas based on the program. The most optimum design shall have the highest desirability, and then it will be continued to the verification step to ensure the validity of the obtained process and equation. The provided optimum process solution was also
accompanied by the prediction of each response’s value that the compatibility can be seen on the verification step.

2.5.2 Verifying the Optimum Response Condition of the Melinjo Peel Tea Antioxidant. The verification aims to prove the prediction and the value of optimum condition solution recommended by the Design Expert 7 program. The verification was replicated for 5 times with the same tea making process as well as the determined treatment. The result of actual response value verification test will be compared to the result predicted by the Design Expert 7 program. The program will also provide confident interval and prediction interval for each prediction response value at 5% significance. Confident Interval is an interval which shows the average expectancy of the next measurement result at 5% significance, while Prediction Interval is an interval which shows the expectancy of the next response measurement in the same condition of 5% significance.

3. Result and Discussion
3.1 Antioxidant Activity Response of Melinjo Peel Tea
The result of antioxidant activity response was displayed on the following table 2. Later, the result would be analyzed by using Design Expert 7 program to determine the suitable model as an effort to predict the relationship between treatment and response. Median, Modified, Quadratic, 2FI, Qubic, and Linear were several models provided by Design Expert 7 program.

| Run | Citric Acid Concentration (%) | Blanching Duration(minute) | Antioxidant Activity Response (%) |
|-----|------------------------------|----------------------------|----------------------------------|
| 1   | 0.10                         | 5.00                       | 87.603                           |
| 2   | 0.08                         | 7.50                       | 76.860                           |
| 3   | 0.08                         | 7.50                       | 79.029                           |
| 4   | 0.10                         | 10.00                      | 75.207                           |
| 5   | 0.08                         | 3.96                       | 85.641                           |
| 6   | 0.08                         | 7.50                       | 79.752                           |
| 7   | 0.08                         | 7.50                       | 78.409                           |
| 8   | 0.08                         | 7.50                       | 79.287                           |
| 9   | 0.11                         | 7.50                       | 78.151                           |
| 10  | 0.05                         | 7.50                       | 80.320                           |
| 11  | 0.08                         | 11.40                      | 81.043                           |
| 12  | 0.04                         | 7.50                       | 82.025                           |
| 13  | 0.05                         | 5.00                       | 80.579                           |

Table 3 were analysis of variance (ANOVA) for response surface quadratic model. The ANOVA showed that the polynomial model of antioxidant response was quadratic. Design Expert 7 also recommended quadratic as the suitable model. The result of ANOVA showed that the quadratic model was significant with p “prob>f” value of 0.0055 which was less than 0.05. The Lack of Fit F-Value of 0.1276 exceeded the p “prob>f” value of 0.05 showed that it was insignificant. The Lack of Fit insignificance is the ideal model requirement since it showed the compatibility of antioxidant response data and the model.

Design Expert 7 informed the R-Squared, Adjust R-Squared, and Predicted R-Squared antioxidant activity response values were 0.8684; 0.7744; and 0.2645. It also informed that the Adequate Precision value for antioxidant response was 10.096. It showed that the obtained value indicated the adequate model. The polynomial equation for the antioxidant response was as following equation (2)
Y = 78.68 - 0.45 X_1 - 2.39 X_2 - 3.03 X_1^2 + 0.51 X_1^2 + 2.14 X_2^2

(2)

The Internally Studentized Residuals normality graphic on figure 1 showed the response data extracted by the Design Expert 7 spread normally. It could be seen from the points surrounding the normality line. From the data spread around the normal line (spread no too far) showed that the test result was compatible with the assumption provided by ANOVA.

Table 3. ANOVA for Response Surface Quadratic Model

| Source             | Sum of Squares | df | Mean Square | F Value | P-Value | Prob > F |
|--------------------|----------------|----|-------------|---------|---------|----------|
| Model              | 116.38         | 5  | 23.28       | 9.24    | 0.0055  | Significant |
| X_1 - Citric Acid concentration | 1.59         | 1  | 1.59        | 0.63    | 0.4530  |           |
| X_2 - Blanching Duration | 45.88        | 1  | 45.88       | 18.20   | 0.0037  |           |
| X_1 X_2            | 36.83          | 1  | 36.83       | 14.61   | 0.0065  |           |
| X_1^2              | 1.80           | 1  | 1.80        | 0.71    | 0.4263  |           |
| X_2^2              | 31.72          | 1  | 31.72       | 12.59   | 0.0094  |           |
| Residual           | 17.64          | 1  | 2.52        |         |         |           |
| Lack of fit        | 12.80          | 3  | 4.27        | 3.52    | 0.1276  | Not Significant |
| Pure Error         | 4.84           | 4  | 1.21        |         |         |           |
| Cor Total          | 134.03         | 12 |             |         |         |           |

Figure 1. Internally Studentized Residual Normality Graphic of the antioxidant activity response

Figure 2 showed that the antioxidant response influenced the designed formulation components and vice versa. The color variation displayed on the two-dimensional contour plot graphic became the identic characteristics with the antioxidant response result of Design Expert 7 program. The designed formulation components might not influence each other, which will be displayed through the same two-dimensional graphic with similar color. Blue color showed that the lowest antioxidant response was 75.207%, while the highest was 87.603%.

Blanching duration significantly influenced the antioxidant activity change on the melinjo peel tea. The graphic below showed the longer blanching duration will tend to decrease the antioxidant activity. According to Kim et al [7], this was due to the influence of sample interaction duration with the condition during the blanching such as the high heat and steam which could cause over-moisture on the melinjo peel. In turns, phenol inside the melinjo peel will experience tannin degradation and turned it into simple phenol compound.
The three-dimensional graphic in figure 3 showed the interaction among independent variables, by showing the graphic with different heights. The lowest area of the graphic showed that the lowest antioxidant response value was 75.207% while the highest area showed that the highest antioxidant response value was 87.603%. Figure 3 also informed that the citric acid concentration was inversely proportional to the blanching duration where the more citric acid concentration used will increase the antioxidant activity obtained.

Citric acid was used as the blanching media to not only maintain the original color of the melinjo peel but also add the better taste. According to Pujimulyani et al [6] using 0.05% citric acid could increase the antioxidant activity rather than 0%. It was caused by the glycosides compound hydrolysis into aglycone and glycone. One of factors influenced the antioxidant activity of a food material was pH, while the citric acid could decrease the pH content. Antioxidant activity could increase during the acidic state. Lower pH indicates more free H+, H+ regenerates the antioxidant compound by bounding to the phenoxy radicals to reform the antioxidant compound [10].

3.2 Optimizing and Validating Melinjo Peel Tea Antioxidant Activity

Optimization is an activity to obtain the best treatment with minimum effort and maximum result as desired. Greater prediction accuracy value will decrease the obtained data errors. In RSM method, an optimum design will be determined by the desirability value which is closer to 1. Any value closer to 1 means the design is more optimum, while any value further from 1 means the design is not optimum yet. Antioxidant activity as the final result and the optimization measurement of a component was
determined by the importance level of each component (citric acid concentration and blanching duration).

The importance level of each component/response which criteria will be defined was measured by the value in the Design Expert program, with the interval value of 1 (+) until 5 (++++)+. Higher importance level of a component means higher priority on fulfilling the defined criteria. The priority value can be seen on the following table 4. The table also informed that there were other weighing value such as goal or the target to achieve, minimize or minimum lower limit, maximize or maximum upper limit.

| Component/ Response | Target/ Goal | Lower Limit | Upper Limit | Importance |
|---------------------|--------------|-------------|-------------|------------|
| Citric Acid Concentration | is target 0.08 | 0.05 | 0.1 | 3 (+++) |
| Blanching duration | is target 7.5 | 5 | 10 | 3 (+++) |
| Antioxidant Activity | is target 79 | 75.207 | 87.603 | 3 (+++) |

Design on the optimization process placed the concentration target to 0.08% with 7.50 minutes of blanching duration that the obtained response was predicted to be 78.677664% with 1.000 desirability, the obtained optimization result was then validated as suggested by Design Expert 7 program. The validation on antioxidant activity optimum result was aimed to find out the prediction accuracy or desirability such as in the following figure 4.

![Figure 4](image)

The validation result of melinjo peel tea antioxidant activity optimization was replicated five times with the provision the treatment variable was based on the program which recommend 7.50 minutes of blanching duration and 0.08% citric acid concentration as the most central treatment. Table 6 informed that the validation result was different to the prediction of Designer Expert 7 program which stated 78.6776%, while the obtained validation was 76.3740%. The percentage gap with margin of error was 2.29%, thus it was suitable to the data error limit, 5% for the maximum upper and lower limit of the program’s prediction percentage value [11].

| Component/Response | Prediction | Low Level | High Level |
|--------------------|------------|-----------|------------|
| Citric Acid Concentration | 0.075% | 0.057% | 0.100% |
### Table 6. Validation result of melinjo peel tea antioxidant activity

| Response | Replication | Mean     |
|----------|-------------|----------|
| Antioxidant Activity (%) | 1 | 2 | 3 | 4 | 5 |  
| 74.0186 | 77.5310 | 77.8409 | 75.5165 | 76.9628 | 76.3740 |

#### 4. Conclusion

Based on the research, the optimum response of melinjo peel tea antioxidant activity was 78.6776% with blanching through citric acid media 0.075% and 7.50 minutes blanching duration. The average result of the antioxidant activity was 76.3740%. It has fulfilled the fit requirement with 5% margin of error. In the future, there shall be an advance in vivo research of melinjo peel tea with experimental mice.

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