Modeling and Optimization of Total Phenol of Tamarillo Seed Extract Using Response Surface Method

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Abstract. Tamarillo (Solanum betaceum) contains phenol compounds which can be used as antibacterial, these compounds can be obtained from the extraction process. Microwave assisted extraction (MAE) can accelerate the extraction process by utilizing microwave heating. The aim of this research is to find the optimum point of solvent ratio and extraction time to produce Tamarillo extract with maximum total phenol content. The modeling and optimization method used is response surface methodology (RSM). In the central composite design (CCD), the input used includes the ratio of solvent to low level ratio is 1:40 (w/v) and the high level is 1:60 (w/v), while the low level extraction time is 4 minutes and the high level is 6 minutes, so there are 13 experimental designs. The suggested model results are quadratic. The optimal value produced in maximizing total phenol in Tamarillo is 3.31 mg GAE/g extract from a variation of solvent ratio 1:56.30 (w/v) with extraction time of 5.93 minutes with desirability value of 1. The validation results obtained are 3.33 mg GAE/g extract, where this value is 0.60% different from the predicted results. Validation results can be accepted because the value is still within the acceptable error threshold or below 5%.

Keywords: Response surface method, solvent ratio, Tamarillo, total phenol

1. Introduction

Various studies of active compounds especially the extraction of phenol compounds from natural sources such as fruits and vegetables have increased rapidly in recent years [1]. Phenolic compounds are secondary metabolites with antioxidants and anti-poisons that are beneficial in human health [2]. Many studies have been conducted on the extraction of phenol compounds as antioxidants from various types of fruits and vegetables [3-6]. Various methods of extracting phenols from fruits and vegetables have also been widely reported [7][8]. Tamarillo (Solanum betaceum) is a fruit that originated in South America and was introduced in New Zealand in the late 1800s. It has an ovoid shape with a length of about 60-80 mm and a diameter of 40-50 mm. Tamarillo is commonly known as a fruit that contains hydro- and lipo-soluble bioactive compounds such as anthocyanins and carotenoids [9]. Mature fruits change color to orange or purple depending on the variety [10]. Tamarillo has been shown to have antioxidant capacity in vitro which is characterized by the presence of phenolic compounds [11]. Phenolic compounds in Tamarillo seeds are greater than other parts [12].

Extraction is usually done to get phenol compounds. Extraction is a very important stage in the isolation, identification, and use of phenolic compounds [13]. Important parameters that affect the extraction process are solvent concentration, extraction time, and temperature [14]. To obtain an environmentally friendly method, the consumption of solvents must be reduced, the extraction time...
must be shortened, and the yield and quality of extracts must be improved. Various new extraction techniques have been developed, one of which is microwave assisted extraction (MAE) [15]. MAE is a process that uses microwave energy and solvents to extract target compounds from several matrices. So far, there have been no studies that optimize the phenol extraction process using MAE in Taramillo seeds.

Optimization needs to be done to get the maximum total phenol value. The optimization process normally takes a long time with expensive costs [16]. Response surface method (RSM) is one of the statistical and mathematical modeling methods for building empirical models. This method can be used to predict, observe, and optimize a response (output) that is influenced by several independent variables (inputs) [17]. Research relating to the modeling and optimization of total phenols in fruits and vegetables using RSM has been widely carried out [18-20]. But there is no research that models and optimizes the total phenol in Taramillo seeds using RSM. The purpose of this study is to model and optimize the ratio of solvent and extraction time to the total phenols in Tamarillo seed extract.

2. Methods

The research was conducted at the Agrochemical Engineering Laboratory, Faculty of Agricultural Technology, Universitas Brawijaya, Malang, Indonesia. The tools used in this study i.e. microwave (Samsung MG2H3185) which is used as MAE; rotary vacuum evaporator (IKA RV10) to reduce the solvent extraction of Taramillo; and UV-Vis (Spectronic Genesys 10 S UV) spectrophotometer. The materials used in this study i.e. Taramillo seeds (age 3.5 months with a diameter of 50-70 mm); distilled water as extraction solvents; Folin Ciocalteu's solution, gallic acid, and sodium carbonate solution as phenol analysis material.

Before the extraction process, Taramillo seeds are mashed with a blender, then filtered with a size of 18 mesh. Then the Taramillo seeds were dissolved using distilled water at a ratio of 1:30, 1:50, and 1:70 (w/v) which were then homogenized with a blender for 2 minutes, then the materials are put in the microwave. The power in the microwave is set at 100 W and the time is adjusted according to the research variations. After the extraction process is complete, the next process is maceration for 4 hours. The liquid obtained in the extraction process is then continued to the refining process of the solvent. The purification process uses a rotary vacuum evaporator with a temperature of 55 °C, a rotational speed of 55 rpm, and a pressure of 100 mbar. The results of the extract will be tested for phenols.

For the modeling and optimization process, this study uses RSM with a central composite design (CCD) with two factors. These two factors are solvent ratio (mL) and extraction time (minutes). The lower and upper limits of the RSM-CCD set from preliminary research, the solvent ratio of 1:40 with the extraction time of 4 minutes as the lower limit, and the solvent ratio of 1:60 with the extraction time of 6 minutes for the upper limit as presented in Table 1. Determination total phenol levels were determined by the folin ciocalteu method [21].

| Table 1. RSM-CCD |
|------------------|
| Name             | Units | Low | High | -alpha | +alpha |
| A [Numeric]      | mL    | 40  | 60   | 35.85   | 64.14  |
| B [Numeric]      | minute| 4   | 6    | 3.58    | 6.41   |

3. Results and Discussion

The observation data is presented in Table 2. The total phenol results show that the lowest value of phenol is in column 10 with the solvent ratio and extraction time is 1:40 (w/v) and 6 minutes which results in a total phenol value of 1.89 mg GAE/g extract. Then the highest total phenol value in column 7 with a solvent ratio and extraction time of 1:50 (w/v) and 5 minutes resulted in a value of 3.40 mg GAE/g extract. Calculations based on Sequential Model Sum of Squares are presented in Table 3. Sequential models of sum of square consist of sum of square and mean of square which are the ratio of sum of square to degrees of freedom. In the process of selecting this model, a comparison of "prob> f" values is performed. Mathematical models that are considered suitable are those that have
a value of "prob > F" below 0.05. The model selection in Table 3 suggests the Quadratic model. While the Cubic model is a model that is not recommended. The selection of the appropriate Sequential Model Sum of Squares statistical model is one that has a p-value less than alpha (p < 5%) which means the model has an error of less than 5%. The p value also has a definition of statistical significance or the consistency of the results when repeated many times. From Table 3, the p value in Quadratic vs 2FI is <0.0001 which means less than 0.05 or 5% so it can be said that the Quadratic model is the best and recommended.

Table 2. Research results on Design Expert 10.0.3

| Std | Run | Factor 1 A: Solvent Ratio b/v | Factor 2 B: Extraction Time Minute | Response 1 Total Phenol mg |
|-----|-----|-------------------------------|------------------------------------|--------------------------|
| 7   | 1   | 50                            | 3.58                               | 2.31                     |
| 10  | 2   | 50                            | 5                                  | 3.37                     |
| 4   | 3   | 60                            | 6                                  | 3.09                     |
| 12  | 4   | 50                            | 5                                  | 3.14                     |
| 8   | 5   | 60                            | 6.41                               | 3.13                     |
| 6   | 6   | 64.14                         | 5                                  | 2.57                     |
| 9   | 7   | 50                            | 5                                  | 3.40                     |
| 1   | 8   | 40                            | 4                                  | 2.55                     |
| 2   | 9   | 60                            | 4                                  | 1.89                     |
| 3   | 10  | 50                            | 6                                  | 2.54                     |
| 13  | 11  | 50                            | 5                                  | 3.33                     |
| 5   | 12  | 35.85                         | 5                                  | 2.69                     |
| 11  | 13  | 50                            | 5                                  | 3.31                     |

Table 3. Model selection by Sequential Model Sum of Squares

| Source             | Sum of Squares | df | Mean Square | F-value | p-value |
|--------------------|----------------|----|-------------|---------|---------|
| Mean vs Total      | 107.14         | 1  | 107.14      |         |         |
| Linear vs Mean     | 0.69           | 2  | 0.3499      | 1.72    | 0.22    |
| 2FI vs Linear      | 0.36           | 1  | 0.3660      | 1.98    | 0.19    |
| Quadratic vs 2FI   | **1.58**       | 2  | **0.7878**  | **60.49** | < 0.0001 Suggested |
| Cubic vs Quadratic | 0.0006         | 2  | 0.0003      | 0.01    | 0.98    |
| Residual           | 0.09           | 5  | 0.0181      |         |         |
| Total              | **109.87**     | 13 | **8.45**    |         |         |

The second model selection is Lack of Fit Tests. A model that is considered good is a statistically insignificant model. In the Lack of Fit Tests, the model can be accepted if the p value is more than 5% (P > 5%). The Lack of Fit Tests data are presented in Table 4. Based on the results, the best p value suggested by RSM is 0.3164 where this value is owned by the quadratic model and meets the requirements for inaccuracy testing.

Table 4. Model selection using Lack of Fit Tests

| Source             | Sum of Squares | df | Mean Square | F-value | p-value |
|--------------------|----------------|----|-------------|---------|---------|
| Linear             | 1.99           | 6  | 0.33        | 32.39   | 0.0024  |
| 2FI                | 1.63           | 5  | 0.32        | 31.72   | 0.0026  |
| Quadratic          | **0.05**       | 3  | **0.01**    | **1.63** | **0.31** Suggested |
| Cubic              | 0.04           | 1  | 0.04        | 4.84    | 0.09    |
| Pure Error         | 0.04           | 4  | 0.01        |         |         |

The next model is Model Summary Statistics where the determination of the model used is based on the standard deviation and the maximum R² value. The parameters seen are the lowest standard deviation, the highest R-square, the highest adjusted R-square, the highest Predicted R-square, and the lowest PRESS value. The results of the analysis are presented in Table 5. In Table 5, the recommended RSM model is a quadratic model which has a standard deviation value of 0.11. This
value is the smallest. The quadratic R-square value is 0.96, a value greater than the linear R-square value and 2FI. R-square value of quadratic model is 0.9666 which shows that the two factors i.e. solvent ratio and extraction time, affect the diversity of responses by 96%, while the remaining 3.34% is influenced by other factors. The Adjusted R-square value serves as the generalization of R² in the population due to an element of population estimation in it. Quadratic model has an adjusted R² value of 0.94 which is the highest value. Next is the PRESS value used to indicate the error prediction of the sum of the squares of the value of 0.42 in the quadratic model to be the lowest value when compared to the linear model of 3.45 and the 2FI model of 4.33. In the Predicted R-square column the value of the quadratic model is 0.84 which is greater than the linear, 2FI, and cubic models. Because the standard deviation value of the lowest quadratic model is compared to other models, the R-square value is also high, and the Adjusted R-square value of the quadratic model shows the maximum value, then the quadratic model is the model suggested by RSM. Based on the entire model selection analysis i.e. the Sum of Square Sequential Model, Lack of Fit Tests, and Model Summary Statistics, RSM suggests a quadratic model.

| Source  | Std. Dev. R² | Adjusted R² | Predicted R² | PRESS |
|---------|--------------|-------------|--------------|-------|
| Linear  | 0.45         | 0.25        | 0.10         | -0.26 | 3.45 |
| 2FI     | 0.43         | 0.39        | 0.18         | -0.58 | 4.33 |
| Quadratic | 0.96       | 0.94        | 0.84         | 0.42  | Suggested |
| Cubic   | 0.13         | 0.96        | 0.92         | -0.18 | 3.24 |

Quadratic model is analyzed using Analysis of Variance (ANOVA) to determine the relationship between several variables. The results of ANOVA indicate whether the model has a significant value on the results of the study or not in describing the results of the study. The ANOVA results in this study are presented in Table 6.

| Source   | Sum of Squares | df | Mean Square | F-value | p-value |
|----------|---------------|----|-------------|---------|---------|
| Model    | 2.64          | 5  | 0.52        | 40.56   | < 0.0001 | significant |
| A-Solvent Ratio | 0.0098 | 1  | 0.0098     | 0.75    | 0.41    |
| B-Extraction Time | 0.69 | 1  | 0.69       | 52.98   | 0.0002  |
| AB       | 0.36          | 1  | 0.36       | 28.10   | 0.0011  |
| A²       | 1.00          | 1  | 1.00       | 76.87   | < 0.0001 |
| B²       | 0.77          | 1  | 0.77       | 59.72   | 0.0001  |
| Residual | 0.09          | 7  | 0.01       |         |         |
| Lack of Fit | 0.05   | 3  | 0.01       | 1.63    | 0.31    | not significant |
| Pure Error | 0.04    | 4  | 0.01       |         |         |
| Cor Total | 2.73        | 12 |             |         |         |

From the analysis conducted by RSM obtained polynomial equations in the form of coded and actual, RSM equation for the optimization of total phenols in coded factors:

\[ Y = 3.31 - 0.0350 X_1 + 0.2937 X_2 + 0.3025 X_1 X_2 - 0.3794 X_1^2 - 0.3344 X_2^2 \]

where \( Y \) is the total phenol response (coded), \( X_1 \) is the solvent ratio (coded), and \( X_2 \) is the extracted time (coded).

While the model in actual factors:

\[ Y_{(\text{total phenol})} \text{ (kgf/cm}^2) = -8.26497 + (0.224629 \times \text{solvent ratio}) + (2.12496 \times \text{extraction time}) + (0.030250 \times \text{solvent ratio} \times \text{extraction time}) - (0.003794 \times \text{solvent ratio}^2) - (0.334375 \times \text{extraction time}^2) \]
The accuracy of the model is known from the comparison of the actual value of the study with the predicted value of the model presented in Figure 1.

![Figure 1. Comparison of actual and predicted value](image)

Figure 1. Comparison of actual and predicted value

Figure 2 is a two-dimensional contour plot drawing which is a transverse slice of a three-dimensional curve. Contour plots are used to analyze the effect of interactions between factors on response. The contour of the plot shows that the solvent ratio and the extraction time have a significant effect on the total phenol value. The outermost line on the graph shows the lowest response value and inward shows the higher response value.

![Figure 2. (a) Response to total phenol 2D](image)

![Figure 2. (b) Response to total phenol 3D](image)

Figure 2. (a) Response to total phenol 2D  Figure 2. (b) Response to total phenol 3D

The optimal solution is presented in Table 7. The optimum point with the best response results is obtained at a solvent ratio of 1: 56.30 (w/v) and extraction time of 5.93 minutes, the total phenol value of 3.31 mg GAE/g extract with desirability is 1. From Figure 3, there is a red color which means that the high desirability value. The desirability graph presented in Figure 3 shows that the desirability value is in the red area which indicates that the more red the area is the better. The value of desirability produced is 1 which means that this study has a 100% accuracy rate.

The validation of the optimum model produced by RSM is needed to test the accuracy of the model in describing the actual situation. Validation is done by comparing the prediction results with the results of the study presented in Table 8. Using a solvent ratio of 1: 56.30 (w/v) and extraction time of 5.93 minutes produces a predicted total phenol value of 3.31 mg GAE/g extract and the actual value of 3.33 mg GAE/g extract. Results can be declared valid if the deviation is less than 5%. The validation results show the difference value of 0.02 mg GAE/g extract (0.6%). Because the deviation value is less than 5%, the model that has been produced by RSM can be accepted and considered valid.
Table 7. Optimal results using RSM

| Solvent Ratio (b/v) | Extraction Time (minute) | Total Phenol (mg) | Desirability |
|---------------------|--------------------------|-------------------|--------------|
| 56.30               | 5.93                     | 3.31              | 1.00 Selected |

Figure 3. (a) Desirability 2D  
Figure 3. (b) Desirability 3D

Table 8. Validation result

| Variabel                  | Optimum Value | Total Phenol (mg) | Prediction | Actual |
|---------------------------|---------------|-------------------|------------|--------|
| Solvent Ratio (b/v)       | 56.30         | 3.31              |            | 3.33   |
| Extraction Time (minute)  | 5.93          |                   |            |        |

4. Conclusion

In this study, a model that explains the relationship between the solvent ratio and extraction time to the total phenol of Taramillo seed extract has been obtained by using response surface methodology (RSM). Based on the analysis of three models i.e. Sequential Model Sum of Square, Lack of Fit Tests, and Summary Model Statistics, the best model suggested is the quadratic model. The results of optimization of total phenol values with RSM resulted in an optimal value of total phenols of 3.31 mg GAE/g extract from a variation of the solvent ratio of 1:56.30 (w/v) and extraction time of 5.93 minutes. From the validation results, the actual value is 3.33 mg GAE/g extract. The results of this validation have a difference value of 0.02 (0.6%). Therefore, the RSM model developed in this study can be accepted.

References

[1] Chang S K, Alasalvar C, Shahidi F 2016 Review of Dried Fruits: Phytochemicals, Antioxidant Efficacies, and Health Benefits Journal of Functional Foods 21 113–132
[2] Rodriguez-Mateos A, Vauzour D, Krueger C G, Shanmuganayagam D, Reed J, Calani L, Mena P, Del Rio D, Crozier A 2014 Bioavailability, Bioactivity and Impact on Health of Dietary Flavonoids and Related Compounds: an Update Archives of Toxicology 88 1803–1853
[3] Lim Y P, Pang S F, Yusoff M M, Mudalip S K A, Gimbun J 2019 Correlation between the Extraction Yield of Mangiferin to the Antioxidant Activity, Total Phenolic and Total Flavonoid Content of Phaleria macrocarpa Fruits Journal of Applied Research on Medicinal and Aromatic Plants 14 100224
[4] Orsavova J, Hlavacova I, Mleek J, Snopek L, Misurcova L 2019 Contribution of Phenolic Compounds, Ascorbic Acid and Vitamin E to Antioxidant Activity of Currant (Ribes L.) and Gooseberry (Ribes uva-crispa L.) Fruits Food Chemistry 284 323–333
[5] Muniyandi K, George E, Sathyanarayanan S, George B P, Abrahamse H, Thamburaj S, Thangaraj P 2019 Phenolics, Tannins, Flavonoids and Anthocyanins Contents Influenced Antioxidant and Anticancer Activities of Rubus fruits from Western Ghats, India Food Science and Human Wellness 8(1) 73–81
[6] Hendrawan Y, Widyaningtyas S, SuciptoS 2019 Computer Vision for Purity, Phenol, and pH Detection of Luwak Coffee Green Bean Telkomnika 17(6) 3073–3085

[7] Hendrawan Y, Sabrinauly S, Hawa L C, Argo B D, Rachmawati M 2019 Analysis of the Phenol and Flavonoid Content from Basil Leaves (Ocimum americanum L.) Extract Using Pulsed Electric Field (PEF) Pre-Treatment Agricultural Engineering International: CIGR Journal 21(2) 149–158

[8] Hendrawan Y, Larasati R, Wibisono Y, Umam C, Sutan S M, Hawa L C 2019 Extraction of Phenol and Antioxidant Compounds from Kepok Banana Skin with PEF Pre-Treatment. IOP Conference Series: Earth and Environmental Science 305 (1) 012065

[9] Ramakrishnan Y, Adzahan N M, Yusof Y A, Muhammad K 2018 Effect of Wall Materials on the Spray Drying Efficiency, Powder Properties and Stability of Bioactive Compounds in Tamarillo Juice Microencapsulation Powder Technology 328 406–414

[10] Correia S I and Canhoto J M 2012 Biotechnology of Tamarillo (Cyphomandra betacea): From In-vitro Cloning to Genetic Transformation Scientia Horticulturae 148 161–168

[11] Vasco C, Avila J, Ruales J, Svanberg U, Kamal-Eldin A 2009 Physical and Chemical Characteristics of Golden-yellow and Purple-red Varieties of Tamarillo Fruit (Solanum betaceum Cav.) International Journal of Food Science and Nutrition 60 278–288

[12] Gannasin S P, Adzahan N M, Hamzah M Y, Mustafa S, Muhammad K 2015 Physicochemical Properties of Tamarillo (Solanum betaceum Cav.) Hydrocolloid Fractions Food Chemistry 182 292–301

[13] Setyaningsih W, Saputro I E, Carrera C A, Palma M 2019 Optimisation of an Ultrasound-assisted Extraction Method for the Simultaneous Determination of Phenolics in Rice Grains Food Chemistry 288 221–227

[14] Zhou P, Wang X, Liu P, Huang J, Wang C, Pan M, Kuang Z 2018 Enhanced Phenolic Compounds Extraction from Morus alba L. Leaves by Deep Eutectic Solvents Combined with Ultrasonic-assisted Extraction Industrial Crops and Products 120 147–154

[15] Hendrawan Y, Pramesi D M, Rachmawati M, Susilo B, Wibisono Y, Dewi S R, Izza N 2018 Application of Microwave Assisted Extraction in Extracting Torbangun Leaves (Coleus ambonicus, L.) and Its Effects on Polyphenol and Flavonoids Content Advances in Food Science, Sustainable Agriculture and Agroindustrial Engineering, 1(2) 8–16

[16] Saravanakumar K 2010 Acoustic and Thermodynamic Properties of Binary Liquid Mixtures of Acetophenone and Benzene Journal of Applied Sciences 10 1616–1621

[17] Ghellab S E, Mu W, Li Q, Han X 2019 Prediction of the Size of Electroformed Giant Unilamellar Vesicle using Response Surface Methodology Biophysical Chemistry 253 106217

[18] Ameer K, Chun B S, Kwon J H 2017 Optimization of Supercritical Fluid Extraction of Steviol Glycosides and Total Phenolic Content from Stevia rebaudiana (Bertoni) Leaves using Response Surface Methodology and Artificial Neural Network Modelling Industrial Crops and Products 109 672–685

[19] Riciputi Y, Cerio E D, Akyol H, Capanoglu E, Cerretani L, Caboni M F, Verardo V 2018 Establishment of Ultrasound-assisted Extraction of Phenolic Compounds from Industrial Potato by-Products using Response Surface Methodology Food Chemistry 269 258–263

[20] Balli D, Bellumori M, Orlandini S, Cecchi L, Mani E, Pieraccini G, Mulinnaci N, Innocenti, M 2020 Optimized Hydrolytic Methods by Response Surface Methodology to Accurately Estimate the Phenols in Cereal by HPLC-DAD: The Case of Millet Food Chemistry 303 125393

[21] Roy M K, Juneja L R, Isobe S, Tsushida T 2009 Steam Processed Broccoli (Brassica Oleracea) has Higher Antioxidant Activity in Chemical and Cellular Assay Systems Food Chemistry 114 263–269