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OPTIMIZATION OF ANTIOXIDANT COMPOUNDS IN COCOA POD HUSK (*Theobroma cacao* L.) WITH VARYING TEMPERATURE AND DRYING TIME

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ABSTRACT

The cocoa pod husk (CPH) was dried using a food dehydrator at several temperatures (varying between 55 to 65°C) and for different durations (varying between 3 to 5 hours). To guarantee optimal performance in the drying conditions, a central composite design was employed. The objective of the study was to use Response Surface Methodology (RSM) to enhance the drying conditions of cocoa pod husk (*Theobroma cacao* L.). The findings suggested that the regression model accurately represents every aspect of the data and successfully captures the actual correlation between the independent variable and the outcome. The moisture content varied between 6.01% and 22.78%. The antioxidant activity value ranged from 148.06 to 270.78 ppm, while the total phenols value ranged from 18.02 to 29.72 ppm. The analysis indicated that the maximum concentration of total phenols was 29.72 parts per million (ppm) under a temperature of 60°C and the duration of 4 hours. In contrast, the minimum moisture level recorded was 6.01% when the temperature was 60°C, and the time was 5.41 hours. Furthermore, the antioxidant activity was quantified to be 148.06 parts per million (ppm) when the temperature was 60°C and the duration was 4 hours. The optimal parameters for total phenolics and minimum moisture content and IC50 (showing high antioxidant activity) were determined to be a drying temperature of 60.162°C and a drying period of 4.177 hours. The moisture content, antioxidant activity and total phenols were determined to be 12.056%, 165.503 ppm and 25.536 ppm, respectively, at the most optimal conditions. The study variables such as moisture content, antioxidant activity and total phenols collectively influenced the outcomes. Therefore, this study shows the suitability of this model to increase antioxidant activity and total phenol content of cocoa pod husk. Consequently, it can be effectively applied in various industries, including food, beverages and cosmetics.

Key words: Cocoa Pod Husk, RSM, drying, moisture, antioxidant activity, total phenols, optimization

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INTRODUCTION

Cocoa, scientifically known as *Theobroma cacao* L., is endemic to the Amazon region and is grown extensively in tropical regions with temperatures ranging from 18°C to 32°C and ecosystems characterized by high humidity levels of 70–90% [1]. Cocoa (*Theobroma cacao* L.) has been an important plantation commodity that has contributed significantly to the Indonesian economy since 1930. The estimated global production of cocoa for the 2020–2021 period was 5.24 million metric tonnes [2]. According to Food and Agriculture Organization (FAO), Indonesia ranks as the third-largest cocoa producer globally, following Ghana and Cote D'Ivoire [3]. In 2017, Indonesia's estimated cocoa production was 659,776 metric tons. Indonesia's cocoa production amounts to 683 kilotonnes, cultivated on an area of 1.46 million hectares. The cocoa plantation area in Central Sulawesi Province is the largest in Indonesia, covering 278.3 thousand hectares, which accounts for 18.44% of the total cocoa plantation area in the country [4]. Furthermore, alongside the growing export prospects, the domestic market for cocoa beans remains substantial. The cocoa processing industries in Java are potential markets for cocoa bean marketing.

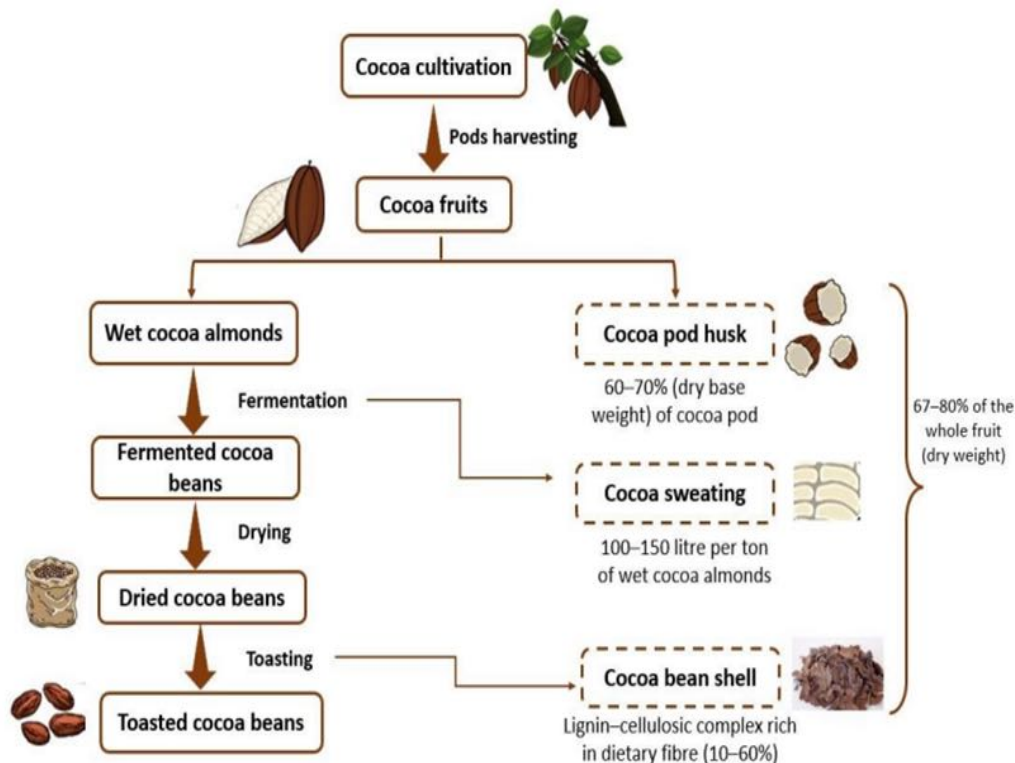


Figure 1: Stages of Processing Cocoa and By-products, Adapted from Azizah and Febrianto [6]

It is documented that 1 tonne of dried cocoa beans generates 10 tonnes of cocoa pod husk waste [5]. These findings indicate that around 90% of the overall mass of cocoa beans is disposed off as waste, while the remaining 10% is sold [6]. This not

only leads to environmental consequences stemming from garbage management, but also has economic repercussions. Figure 1 illustrates the various stages of cocoa processing and the resultant by-products. The weight of cocoa pods comprises approximately 60% to 70% of the total weight of the cocoa fruit. The community's utilization of cocoa pod husk is now restricted to animal feed and compost. In some cases, the pods are simply discarded, leading to environmental pollution, unpleasant odors and the spread of fruit rot disease in cocoa [7, 8].

Cocoa pod husks are known for containing bioactive substances that can operate as a natural source of antioxidants, which are helpful for human health [7, 9]. According to Vasquez [10], every 100g of cocoa fruit's dry mass includes 0.34g of theobromine, 4.6-6.9g of phenols and 5.2g of tannins. The cocoa beans contain polyphenolic components, which are precisely classified as flavonoids, including catechins and epicatechins. Catechins, including epicatechins, are a specific type of flavonoids which demonstrate significant antioxidant properties. Prior to extraction, to emphasize the substantial occurrence in cocoa pod, it is necessary to dry the material in order to decrease its moisture content, prevent decay and extend its storage duration [11]. Drying is a common way to preserve materials while minimizing the loss of nutrients. It is important to emphasize that an inadequate drying procedure might undermine the bioactive components in cocoa beans, resulting in a significant reduction in polyphenol content after drying [8].

The efficacy of the natural drying process, which entails exposure to direct sunlight, is diminished due to its potential to harm phenolic components and diminish their antioxidant activity. Flavonoid compounds are highly susceptible to breakdown when subjected to elevated temperatures and sun exposure [12, 13, 14]. An alternate strategy for drying involves the use of artificial techniques, and one specific solution is the utilization of a food dehydrator [15]. Food dehydrators are seen as more efficient because of their customizable drying control mechanism, which enables a significant reduction in moisture content within a short timeframe [16]. In addition, subjecting materials to high-temperature drying can expedite the process of phenolic breakdown and polymerization [6].

In addition, elevated drying temperatures can have an effect on the pH levels, resulting in the solidification of cocoa [17]. Harun [18] found that drying the mangosteen skin at a temperature of 85°C resulted in the production of herbal tea with the most favorable characteristics. This study found that the moisture content, yield and antioxidant capacity of the components are affected by the temperature and duration of the process of drying. Hence, it is imperative to carry out research to determine the impact of the ideal drying temperature and duration, as well as their interaction, on the properties of cocoa pod skin powder (*Theobroma cacao* L.). This



will enable the identification of the most suitable combination of drying temperature and duration to preserve the desired characteristics of cocoa pod husk powder. Process optimization can be achieved by utilizing a simulation model, or by optimizing a mathematical model based on simulation outcomes. Response Surface Methodology (RSM) utilizes mathematical and statistical methods to assess the most favorable conditions by minimizing the number of experiments required to analyze the relationship between factors and responses, and to describe the correlation between independent and dependent variables. Response Surface Methodology offers the benefit of not only determining the impact of independent variables but also producing a mathematical model that elucidates chemical or biological processes. Furthermore, the RSM method is not reliant on a substantial quantity of experimental data and does not necessitate a lengthy duration [19]. Therefore, this study aimed to obtain ideal results for moisture content, antioxidant activity and total phenols and, at the same time design an effective food dehydrator for cocoa pod husk.

MATERIALS AND METHODS

Materials

The husks of the cocoa pods (*Theobroma cacao* L.) were obtained from Bumi Mulyo Jati (BMJ) Mojopahit tourist village, Mojokerto Regency. This research used cacao pod husk from harvests that were 2-3 years old from the start of planting or fruit aged 5.5 to 6 months after flowering. Other ingredients were methanol, distilled water, gallic acid, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) crystals. The research was conducted at the Agroindustry Process Engineering Laboratory, Bioindustry Laboratory and Entrepreneurship Laboratory, Faculty of Agricultural Technology, Universitas Brawijaya.

Cocoa pod husk pretreatment and drying

The size of the CPH was initially reduced to accelerate the drying process. The collected CPH were sliced using an automated slicer to generate a consistent CPH size. The CPH pieces were then equally distributed on the tray, ensuring that they are not layered on preceding each other to optimize the dehydration or drying process using a food dehydrator (BioChef). Cocoa pod husks (CPH) were subjected to a drying process with a certain temperature and drying time, set at a temperature range of 55 - 65°C for a duration of 3 - 5 hours, as specified by the central composite design (CCD) outlined in Table 1. The dried cocoa pod husks were separated into two different parts for the parameter test of the study. A single part of the dried CPH sample was crushed into powder using a grinder (Shenzhen PQA) to determine antioxidant activity and total phenol. Moisture content measurement was conducted on dry CPH without grinding process.



Study design

The research employed an experimental design, specifically utilizing the Central Composite Design (CCD). The research utilized two independent factors, drying temperature and drying time, to determine three dependent variables including: moisture content, antioxidant activity and total phenols. The conceptual instructions for the modeling method adopt the previously released modeling with certain adjustments [20]. The drying temperature varied from 55°C and 65°C, while the drying time ranged from 3 to 5 hours. A final total of 13 experiments were carried out, all of which are shown in Table 1. The experimental process utilized the Design of Expert 13 software. The equations (Equations 1 and 2) given below illustrate the relationship between the dependent variable and the independent variable. Equation 1 includes, a basic model based on a linear function, while equation 2 results from converting the experimental data into a quadratic model.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_k X_k + \varepsilon \quad (1)$$

$$Y = \beta_0 \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i=1} \sum_{i < j} \beta_{ij} X_i X_j + \varepsilon \quad (2)$$

The equation represents the relationship between the value of the response variable (Y), a constant value (β_0), and several coefficients (β_i , β_{ii} and β_{ij}). The coefficient β_i indicates the linear term, while the coefficient β_{ii} indicates the quadratic term, and the coefficients β_{ij} indicate the interaction terms, X_i and X_{ij} denote numerical variables. Subsequently, an evaluation is conducted to analyze the degree of lack of fit, R-squared coefficient and sufficiency. An ANOVA was employed to compute accuracy, the sum of squared prediction error (PRESS), and the coefficient of variation.

Characterization of dried cocoa pod husks

In order to determine the moisture content, 2 grams of dried CPH samples were placed in a petri dish and dried in an oven (Mettler) at 105°C until their weight remained constant. The constant weight derived from the analytical instruments (Shimadzu Model) was subsequently contrasted with the weight of the sample before the drying process, with results expressed in g/100g or dry basis based on AOAC method [21].

The response and activity of antioxidants are determined using UV-Vis spectrophotometers according to the method described by Lateef [22], with certain modifications. A solution of absolute methanol was used to dissolve 0.5g of powder-dried CPH sample at concentrations of 50, 100, 150, 200, and 250 ppm. Each sample in a reaction tube received 4 mL of 0.1 mM DPPH in methanol solution. The sample was left for thirty minutes at room temperature. The absorption was measured by the UV-Vis spectrophotometer at a wavelength of 517 nm. The



percentage of inhibition was then determined by mixing four milliliters of pre-prepared DPPH in methanol with one milliliter of absolute methanol to serve as the blank. A linear regression equation was used to determine the value of IC50 based on the inhibition percentage.

The total phenol content was determined in this investigation by modifying the method described by Nofita [23]. A portion of 1 mL of 0.5 g of dried CPH powder dissolved in 50 mL of ethanol was transferred to a reaction tube. A sample solution was added with 0.4 mL of Folin-Ciocalteu in the tube, which was then homogenized and left for 7 minutes. Following adding 4 mL of a Na₂CO₃ and aquadest solution to the sample solution until the boundary mark of the tube was reached, the solution was re-homogenized and left for 2 hours. Following that, the absorption of the sample was measured at a wavelength of 765 nm utilizing a UV-Vis spectrophotometer. The values converted to grams of gallic acid per gram of CPH represent the total phenolic content of CPH.

RESULTS AND DISCUSSION

Statistical analysis

Statistical analysis was performed through fitting model.

The experimental outcomes regarding the response variable under different drying conditions are displayed in Table 1. The data collection was analyzed using the Design Expert 13 program. To determine the appropriate response range for the parameters being investigated, a regression examination was performed to calibrate a computational model using the data obtained from experiments. The predicted answers were implemented to assess the consequences of two independent variables on the responses that were provided.

The regression equations are as follows:

$$\text{Moisture content} = 73.6173 + -0.729504X_1 + -4.2308X_2 \quad (3)$$

$$\text{Antioxidant Activity} = 3,912.42 + -101.375X_1 + -394.196X_2 + 1.91775X_1X_2 + 0.796363X_1^2 + 36.3518X_2^2 \quad (4)$$

$$\text{Total Phenol} = -197.896 + 4.70349X_1 + 45.2622X_2 + -0.2805X_1X_2 + -0.03214X_1^2 + -3.541X_2^2 \quad (5)$$

The response was significantly influenced by temperature and drying time ($p < 0.05$) in terms of first-order linear effect (X_1 , X_2), second-order quadratic effect (X_1^2 and X_2^2), and interaction effect (X_1X_2). Moisture content, the coefficients regression of factors X_1 and X_2 show a negative notation. Antioxidant activity shows factors X_1 and X_2 are indicated by the same notation in both linear first order and quadratic second order relations. Total phenols have inverse relationships with factors X_1 and



X_2 , are indicated by the same notation, in both linear first order and quadratic second order relations.

Analysis of variance (ANOVA) was conducted to identify the significant impacts of the process factors on the different responses [24]. The results can be found in Table 2. Following the evaluation of the model's performance, various statistical measures were computed. The R^2 was 0.8369 for moisture content, 0.8369 for antioxidant activity, and 0.7590 for total phenol, which is higher than the adjusted R^2 values of 0.8043, 0.7203 and 0.5869, respectively. The R^2 moisture content was found to be 0.8369, suggesting that 83.68% of the total variances were effectively represented according to the statistical model. The lack of fit is not statistically significant, indicating the regression model is highly significant. The limited CV (coefficient of variation) seen in the duplicated measurements demonstrates the exceptional replication and precision of the results. Adequate precision can be determined by the degree of accuracy, and a value within the range of 4.96–14.87 shows a precision ratio that is higher than 4 indicating a better level of accuracy and reliability in this response [25]. Therefore, it is possible to enhance this variable by implementing the model.

Analysis of the model

When conducting an optimization process using RSM, it is critical to ensure that the model suggested aligns with actual or experimental data. If the model does not match the response data, the accuracy of point prediction tends to be undesirable. It involves fitting generic quadratic equations to the data for smoothing and prediction purposes, as well as, doing regression analysis [26]. Figures 2(a) - (c) show a regression line that demonstrates an adequate alignment between the actual value and the predicted value of moisture content, antioxidant activity, and total phenols responses. This indicates that the research established results that were favorable [27]. The close alignment between the actual and anticipated values is crucial for assessing the accuracy of the model and its capacity to accurately predict the optimal conditions required to achieve the desired results [28].



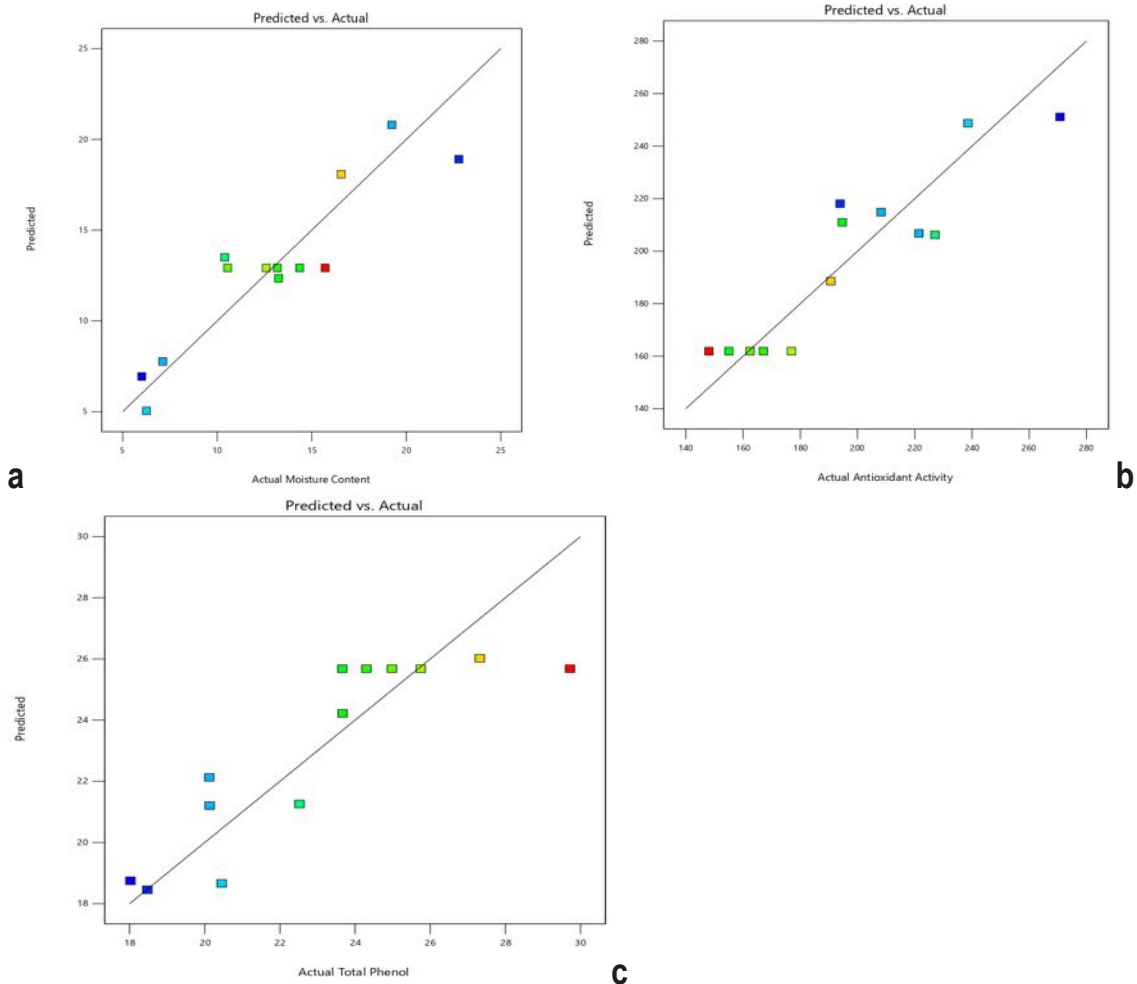


Figure 2: The fitted line plot for (a) MC, (b) AA, and (c) TPC

In addition, the adequacy of each response's fit impacts the coefficient of determination (R^2 value) displayed in Table 3. The optimization model for obtaining the optimal conditions for dried cocoa pods, with a desirability value of 0.706, indicated that the design expert's recommended model was sufficiently compatible to be applied to the study's results. Typically, R^2 values greater than 0.8 or 80% suggest a strong ability of the model to explain the variation in the response data relative to the average [29]. The R^2 value of 0.7% falls below the average, but it is not significantly less than 1, which is the most desirable value. Therefore, the model remains adjustable based on the level of significance that each factor has on the response.

Following the examination of model suitability with actual data, the process optimization occurs using response surface methods. These are followed by the analysis of contour 3D plots as shown by Figures 3(a) - 3(c), illustrating the value of moisture content (%), antioxidant activity (ppm), and total phenol (ppm) responses, respectively. The three-dimensional contour plot is formed by examining the



relationship between two independent variables, particularly the drying temperature and drying time, and how they affect each response, resulting in distinct curve shapes [30]. Fig. 3 (b) and (c) shows that the antioxidant activity and total phenol response had a quadratic plot contour result, while the moisture content response shows a linear relationship with both factors (Fig 3(a)).

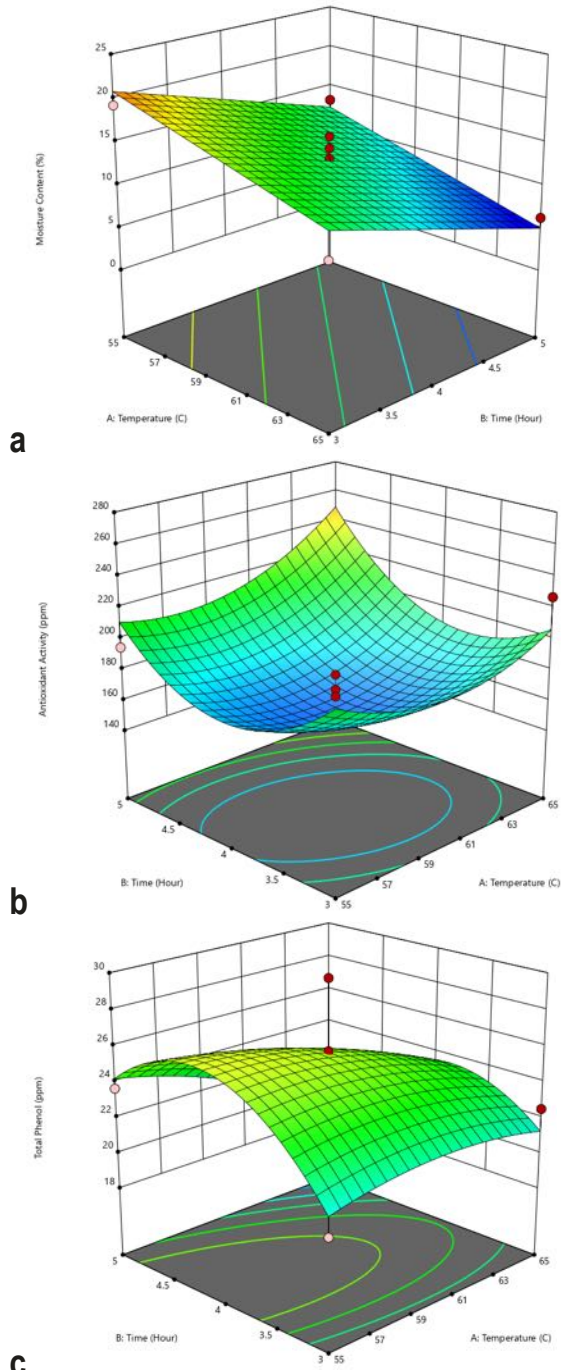


Figure 3: Contour plot of dried cocoa pod husk for (a) MC, (b) AA, and (c) TPC

The specific values of the experimental outcomes for each response in this research are shown in Table 1. The moisture content of dried cocoa pod husk (CPH) varies within the range of 6.01 – 22.78%. The lowest moisture content is obtained at the treatment of the 60°C drying temperature factor, which is the design midpoint and the drying time is 5.41 hours as the highest point. The antioxidant activity is characterized by an IC50 value ranging from 148.06 – 270.78 ppm. The lowest values produced involved drying the material at a temperature of 60°C for a duration of 4 hours, these values were considered to be at the midpoint of the research design. In the last response, the total phenol of the gallic acid in the study ranged from 18.02 - 29.72 ppm where the highest value was obtained from the same treatment point as the lowest IC50.

In the equation discussed in section 3.1, it is established that the coefficients of factors A and B, which represent the moisture content response, show a negative notation. This indicates that, when the values of the two factors are increased the result is a decrease in the moisture content value. The results of this study were in agreement with the results obtained by Aviara and Igbeka [30], indicating that the drying factors of temperature and duration have a linear relationship that is inversely correlated to the moisture content of the material. In the context of the antioxidant activity response (Section 3.1), it is established that factors A and B are indicated by the same notation in both linear first order and quadratic second order relations. The equation reveals that the IC50 value decreases as the temperature and time decrease. However, it is established in Fig. 3 that the augmentation of both components at a specific point leads to a decrease in the IC50 value, followed by an increase after reaching the maximum point. The significance of the IC50 value lies in its relevance to low antioxidant activity, as a higher IC50 value suggests lower antioxidant activity [31]. The total phenol response has an inverse relationship with the two components, as indicated by the regression equation. As the value of the two factors increase, the total phenol decreases. Réblová [32] and Kristianto [33] discovered that the temperature had an effect on the antioxidant activity of phenolic acids. Certain acids exhibited a more gradual decline in activity at elevated temperatures compared to others. However, based on the information shown in Table 1 and Fig. 3, it is evident that the total phenol content increases up to a certain point when the values of both factors are optimized.



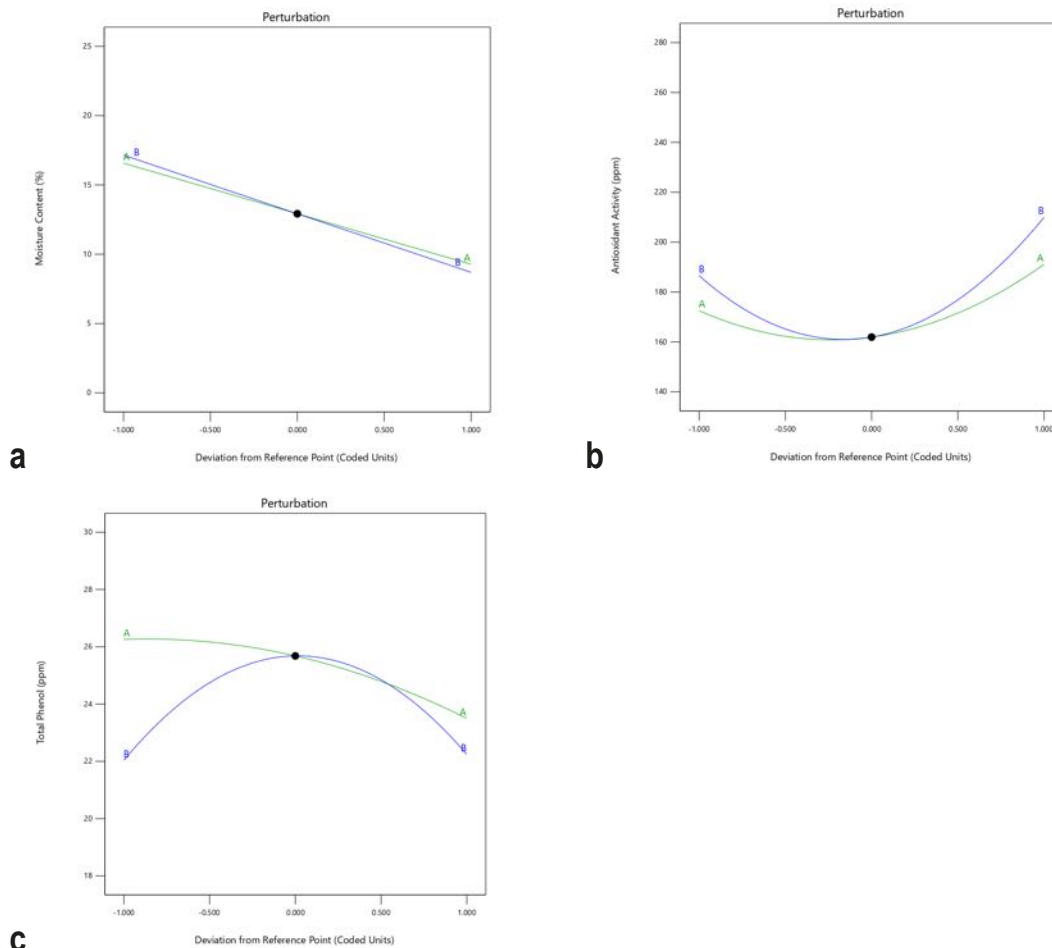


Figure 4: The deviation from the comparison for (a) MC, (b) AA, and (c) TPC

Response Surface Methodology optimization includes a perturbative analysis to assess the stability and sensitivity of response values to small modifications in variables, in addition to analyzing the regression equation and response surface contours [34]. Figure 4 displays a perturbative chart illustrating all three reactions to the investigation. The moisture content response has a strong linear relationship, as evidenced by the perturbation graph closely approximating a straight line. The perturbation graph for the last two responses exhibits curves, suggesting a quadratic correlation between the response and the factor. The perturbation graph obtained from the execution of RSM optimization utilizing Design Experts has distinct slopes, which serve as signs of variations in the sensitivity of each response to the factors [35].

MODEL VERIFICATION AND NUMERICAL OPTIMIZATION

Optimization is the subsequent and more advanced phase following the study of the response model on RSMs. Optimizations are conducted based on expected outcomes or the goal of the experiment, specifically targeting dry CPH with a low



moisture content, low IC50 values suggesting strong antioxidant activity, and high total phenol values. Thus, in this study, the moisture content and AA (Antioxidant Activity) response parameters were adjusted using the minimization function, whereas the total phenol value was optimized using the maximizing function with equal importance value. Design Experts use functional constraints and optimization objectives to produce the most accurate forecast of the optimal point for the CPH drying process [36]. The data in Table 3 indicates that when the drying temperature is 60.162°C and the drying time is 4.177 hours, the resulting dry CPH is expected to have a moisture content of 12.506%, an IC50 value of 165,503 ppm, and a total phenol concentration of 25,536 ppm. At the limits of the response value and the predetermined factor, the selected optimal point prediction has a good desirability value close to 1, of 0.706 or 70,6% [37].

Verification of the optimal point prediction came as the final step of optimization. Validation or verification in this work was achieved by experimental means, Eweama [38], wherein studies were conducted at the optimal prediction point with three sets of data to obtain a reliable model validity value. The average value represents the discrepancy between the actual value and the predicted value generated by the software. Table 4 presents a comparison between the actual values and the predicted values of the optimal position. According to the data in the table, there is a difference between the actual value of the experiment and the predicted value at the optimum point. However, the gap between the experimental values and the predicted optimal point for each reaction was minimal, with only a 3.18% difference for moisture content, a 4.34% difference for antioxidants and activity, and a 0.94% difference for total phenol. A difference score of less than 5% shows a high level of accuracy between the suggested model and ideal point prediction, compared to the actual values of the experimental results, which makes the model reliable [39].

CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

Cocoa pods husk have potential as a source of natural antioxidants. The optimal value of dried cocoa pod husk was achieved at the drying conditions of 60.162°C temperature and 4.177 hours of time. Additionally, the moisture content was determined to be 12.06%, antioxidant activity was measured at 165.503 ppm and the total phenol content was found to be 25.536 ppm. The results showed that as temperature and drying time increased, moisture content decreased, while IC50 values rose, indicating lower antioxidant activity. However, lower temperature and time led to an increase in phenol content. The findings demonstrate that the optimized drying conditions enhance antioxidant activity and phenol content in cocoa pod husks. Future research should explore multi-objective optimization to improve nutrient retentions further.



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Authors' Contributions

Imam Santoso conceptualized and conducted the study, interpreted findings, verified and corrected the original draft and finalized approval of the version to be published. Suprayogi contributed to analysis and interpretation of data, critical revision for important intellectual content, and approval of the final version of the manuscript to be published. Endrika Widyastuti contributed to analysis and interpretation of data, critical revision for important intellectual content, and approval of the final version of the manuscript to be published. Syairil A'yuniah contributed by collecting the data, drafting of the article and final approval of the version to be published. Khairunnisa Lestari contributed by collecting the data, drafting of the article and final approval of the version to be published. Octavia Widyastuti Kusumaningtyas contributed by collecting the data, drafting of the article and final approval of the version to be published. Srijoni Banerjee conceived and designed the analysis, analysis and interpretation of data and critical revision for important intellectual content.

Data availability

The data used to support the findings of this study are included within the article.

Conflict of interest

The author(s) acknowledges that there was a potential conflict arising from financial interests associated with the funding of the research. However, the authority declared that the financial support had no impact on research objectivity.

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Table 1: The experiment result conducted utilizing the central composite design (CCD)

Run	X_1 : Temp. (°C)	X_2 : Time (Hour)	Moisture Content (%)	Antioxidant Activity (ppm)	Total Phenols (ppm)
1	55	3	19.24	221.471	20.13
2	60	4	14.36	155.076	23.66
3	60	4	12.59	176.911	25.75
4	60	2.58579	22.78	193.906	18.47
5	65	3	10.4	227.057	22.52
6	60	5.41421	6.01	270.778	18.02
7	60	4	13.18	167.099	24.3
8	60	4	15.71	148.061	29.72
9	55	5	13.25	194.673	23.67
10	65	5	6.26	238.614	20.45
11	60	4	10.55	162.431	24.98
12	52.9289	4	16.56	190.672	27.32
13	67.0711	4	7.12	208.241	20.12

Table 2: Result Analysis of variance (ANOVA) of the various responses

Response	p-value	determinat ion (R ²)	Adjuste d R ²	Lack of Fit	CV	Adequate Precision
Moisture Content	0.0001	0.8369	0.8043	0.3636	17.06	14.8733
Antioxidant Activity	0.0111	0.8369	0.7203	0.0659	9.67	6.9099
Total Phenol	0.0391	0.7590	0.5869	0.5853	9.76	4.9615



Table 3: Optimum condition and predicted response value of dried cocoa pod husk

Temperature	Drying Time	Moisture Content	Antioxidant Activity	Total Phenol	Desirability	Comment
60.162	4.177	12.056	165.503	25.536	0.706	Selected

Table 4: Accuracy value of the responses at optimum condition

Response	Experimental	Predicted	Accuracy
Moisture Content (%)	9,333	12,506	96,82%
Antioxidant Activity (ppm)	169,839	165,503	95,66%
Total Phenol (ppm)	26,476	25,536	99,06%

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