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Efficient Anthocyanin Extraction from Aqueous Mixture of Cocoa Peel using Microwave Assisted Extraction (MAE) Method

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Abstract. Anthocyanins are polyphenolic compounds that are abundant in nature, with diversity in various types of plants. This study aimed to optimize the anthocyanin extraction process from cocoa shell waste using the MAE method to obtain the optimum particle size, ingredient/solvent ratio, and extraction time to produce high anthocyanin concentrations. The optimized extraction process variables include the size of the particle (1.05; 1.25; 1.49 and 2.5 x 10^{-4} m), the ratio of substrate/solvent (4.5; 12.5; 62.5; 79.5 x 10^{-3} g/mL), time of extraction (2, 4, 10, 14 minutes) and microwave power (100, 275, 450, and 625 watts). The Design Expert vs11 program with the Box-Behnken Design Response Surface Methodology (RSM) was used in the research, and the selection of process conditions was carried out from a combination of factors that resulted in an optimal response. The relationship between variables on the modeled anthocyanin concentration response:
Y=0.000486-9.98637E-07A+0.026734B0.000041C-7.58240E-07D-0.000102AB+2.48606E-07AC+2.62878E09AD-0.000539BC+0.000012BD+9.71853E08CD
(A: particle size; B: cocoa shell: solvent ratio; C: extraction time; D: microwave power).
The optimal response value for anthocyanin concentration was 11.85.10-4 M, and the conditions of the extraction process were the particle size in the extraction process was 0.105 mm, the ratio of cocoa peel mass/ethanol was 0,03125 g/mL, the extraction time was 2 minutes, and the microwave power was 100 W.

Keywords: *anthocyanin, aqueous mixture, cocoa peel, MAE.*

1. Introduction

Indonesia is a cocoa-producing country after Ghana and Ivory Coast. In 2017, cocoa yields reached 590,684 tons, and will increase in 2022 to 728,046 tons [20]. The increase in Indonesian cocoa production has boosted the export value of Indonesian cocoa in solid form and processed forms such as cocoa butter and chocolate, which are consumed, generating the

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third largest foreign exchange in the plantation sub-sector under palm oil or rubber commodities [15]. Cocoa is a plant grown in several tropical areas and belongs to the Malvaceae family. The cacao plant has brownish-yellow fruit with broad leaves. The active compounds in cocoa can be antioxidant, anti-inflammatory, antiseptic, and diuretic. The efficacy of cocoa peel as a traditional medicine is a remedy for burns, fever, dry lips, snake bites, coughs, rheumatism, and fatigue [2, 5, 13]. Cocoa pods can be divided into four parts, namely peel, placenta, seeds, and fruit. The processing technology of cocoa beans produces the main waste of unused cocoa peels. The weight of the cocoa peel reaches 75% of the total weight of the cocoa pods. Cocoa pod shells are generally only used as animal feed, fuel, or disposed of as agricultural waste. The active substances in cocoa peels are phenolic compounds such as tannins, pyrogallol, quercetin, lignin, and resorcinol. The polyphenol compounds in cocoa shells are proanthocyanidins, catechins, and anthocyanins. The anthocyanin content in cocoa shells reaches 39.82% [8 17].

The maceration method is a simple extraction method carried out by soaking the material in a solvent for several days at room temperature and protected from light. This method uses a solvent that diffuses into the material cell, where the active compound comes out due to osmotic pressure. The advantages of the maceration method are that it is simple, easy, has a low cost, and guarantees that the active substance extracted will not be damaged [3 12]. More active compounds in cocoa shells will be produced if ethanol and water are used as solvents. Ethanol and water are polar, universal, and readily available. Polar compounds dissolve in water [11]. In a study conducted by Jessica [6], it was explained that using 96% ethanol solvent in the maceration method yielded anthocyanin levels of 30.22 mg/L, with an optimum temperature of 100 °C, and a degradation percentage of 61.97%. Another study stated that at 40 °C, an anthocyanin yield of 4.499% was obtained with a color intensity of 0.430 [1].

This study used the Design Expert v11 application with the Response Surface Methodology (RSM) Box-Behnken Design to create a research design to determine the effect of variables and combinations of variables on the extraction results [9]. This application makes it possible to obtain the results of the analysis and modeling of a problem with one or more treatments [10, 14, 23]. According to Liu, J, et al., [19] and Panax G, et al.,. RSM is a collection of statistical and mathematical techniques for developing, improving, and optimizing processes, which are influenced by several factors (independent variables), [22]. Response Surface Methodology (RSM) not only defines the effect of independent variables but also produces mathematical models, which explain chemical or biochemical processes. The main idea of this method is to determine the effect of independent variables on response, obtain a model of the relationship between independent variables and responses, and obtain process conditions that produce the best response [16 18]. In addition, the advantages of the RSM method include that it does not require large amounts of trial data and does not require a long time [7 21].

2. Materials and Methods

2.1 Materials

The materials used are cocoa shells from the Indonesian Coffee and Cocoa Research Center, citric acid 0.01 M (Merck, pa), and distilled water.

2.2 Equipment

The tools used in this study are a microwave (SAMSUNG MS23H3125FK-SE) and a UV-VIS spectrophotometer, a Merk Shimadzu from Japan.

2.3 Methods

The Design Expert vs11 program with the Box-Behnken Design Response Surface Methodology (RSM), four variables with a total of 30 runs. Design-Expert v11 is used to design research formulations and analyze responses. The dependent variable in this study was the concentration of anthocyanins while the independent variables were A. particle size (1.05; 1.25; 1.49 and 2.5 x 10-4 mm), B ratio of substrate/solvent (0.00625; 0/01875; 0.03125; 0.04375 g/mL), C time of extraction (2, 4, 10, 14 minutes) and D microwave power (100; 275; 450; 625 (W) design expert program were applied in the extraction process using an aqueous mixture solvent (aqueous solution: 10% citric acid ((1:6 v/v); ethanol)) shown in Table 1. The schematic of the extraction design is shown in Figure 1.

Table 1. Formulation design for anthocyanin extraction research using the MAE method

Run	Ratio (g/mL)	Particle size (mm)	Time (minute)	Power (W)
1	0.01875	0.125	6	100
2	0.01875	0.125	6	275
3	0.00625	0.149	10	100
4	0.01875	0.125	6	275
5	0.01875	0.125	2	275
6	0.01875	0.125	6	275
7	0.01875	0.125	6	275
8	0.00625	0.149	10	450
9	0.01875	0.125	6	275

Run	Ratio (g/mL)	Particle size (mm)	Time (minute)	Power (W)
10	0.04375	0.125	6	275
11	0.03125	0.105	2	100
12	0.00625	0.105	10	100
13	0.03125	0.149	2	100
14	0.03125	0.105	10	100
15	0.00625	0.149	2	100
16	0.01875	0.105	6	275
17	0.03125	0.105	10	450
18	0.00625	0.105	10	450
19	0.00625	0.149	2	450
20	0.03125	0.149	2	450
21	0.03125	0.105	2	450
22	0.00625	0.125	6	275
23	0.03125	0.149	10	100
24	0.01875	0.250	6	275
25	0.01875	0.125	6	625
26	0.01875	0.125	6	275
27	0.01875	0.125	14	275
28	0.03125	0.149	10	450
29	0.00625	0.105	2	100
30	0.00625	0.105	2	450

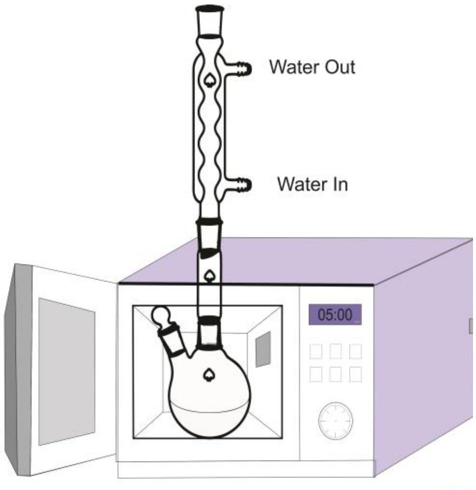

Figure 1. Design of an extraction tool using the MAE method

The extraction results obtained were filtered and centrifuged. The aqueous phase was evaporated at 40 °C. The supernatant was analyzed for anthocyanin content using UV-Vis spectrophotometry. 1 ml of cocoa shell extract supernatant and 5 ml of ethanol were put into a test tube. Each extraction result is subjected to the same steps. The absorbance of one of the extracts was measured at a wavelength of 400-700 nm with a UV-Visible spectrophotometer to determine the maximum wavelength. The maximum wavelength results are used to obtain the absorbance of the extract for each run. The maximum wavelength results are used to obtain the absorbance of the extract for each run. The maximum anthocyanin wavelength extracted from cocoa shell is 494 nm. The total anthocyanin concentration is determined using the equation:

A = Maximum absorbance; ε = molar extinction coefficient (26.900); MW Molecular weight is calculated as cyanidin 3-glucoside (MW = 449.2 g/mol) [30]; FP 10^3.

3. Result and Discussion

The results of the analysis of the concentration of cocoa bean extraction on the response can be seen in Table 2.

Table 2. Results of anthocyanin concentrations in MAE extraction

Run	Ratio (g/mL)	Particle size (mm)	Time (minute)	Power (W)	Yield (M)
1	0.01875	0.125	6	100	0.000635
2	0.01875	0.125	6	275	0.000727
3	0.00625	0.149	10	100	0.000382

Run	Ratio (g/mL)	Particle size (mm)	Time (minute)	Power (W)	Yield (M)
4	0.01875	0.125	6	275	0.000694
5	0.01875	0.125	2	275	0.000574
6	0.01875	0.125	6	275	0.000608
7	0.01875	0.125	6	275	0.000639
8	0.00625	0.149	10	450	0.000433
9	0.01875	0.125	6	275	0.000587
10	0.04375	0.125	6	275	0.000961
11	0.03125	0.105	2	100	0.001185
12	0.00625	0.105	10	100	0.000360
13	0.03125	0.149	2	100	0.000610
14	0.03125	0.105	10	100	0.000602
15	0.00625	0.149	2	100	0.000387
16	0.01875	0.105	6	275	0.000662
17	0.03125	0.105	10	450	0.000996
18	0.00625	0.105	10	450	0.000455
19	0.00625	0.149	2	450	0.000360
20	0.03125	0.149	2	450	0.000729
21	0.03125	0.105	2	450	0.000879
22	0.00625	0.125	6	275	0.000442
23	0.03125	0.149	10	100	0.000595

Run	Ratio (g/mL)	Particle size (mm)	Time (minute)	Power (W)	Yield (M)
24	0.01875	0.250	6	275	0.000697
25	0.01875	0.125	6	625	0.000830
26	0.01875	0.125	6	275	0.000686
27	0.01875	0.125	14	275	0.000644
28	0.03125	0.149	10	450	0.000977
29	0.00625	0.105	2	100	0.000318
30	0.00625	0.105	2	450	0.000366

The results showed that in the MAE method extraction, the highest anthocyanin concentration was obtained in the 11th formulation (0.00625 mm; 0.03125 b/v; 2 minutes; 100 W in citric acid: distilled water. The results of the anthocyanin concentrations of each run show that there is an effect of increasing the ratio on the extraction results where the greater the weight of the extracted cocoa peel powder indicates the higher amount of anthocyanin active substance so that an increase in the ratio of the mass of cocoa peel powder to the volume of solvent causes the anthocyanin extract yield to be obtained was increase. However, when the mass of the cocoa peel powder is increased significantly, a saturated condition is formed so that the process of transferring the mass of the extract back to the cocoa shell occurs, which results in a decrease in the anthocyanin concentration in the product. The extraction time also affects the anthocyanin concentration, where the longer the extraction time, the higher the anthocyanin concentration. The length of contact time between the anthocyanins in the cocoa peel and the solvent causes the yield of anthocyanin extract to be increased.

The particle size of cocoa peel is analysed in the extraction process. The smaller particle size of the cocoa shell causes an increase in the concentration of the extracted anthocyanins. The smaller the particle size, the higher the surface area of the cocoa shell particles, so that the probability of interaction between the anthocyanins contained in the cocoa peel and the solvent is increased, which encourages an increase in the extraction rate, so that the quantity of anthocyanin produced becomes large. The power of the MAE method is the last factor to be

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studied for its effect on the anthocyanin extraction process. The existence of power in the extraction process with the MAE method causes an increase in the energy possessed by the extracted substances and solvent molecules. Increasing the energy of the two molecules will accelerate the initiation of the contact process between the active substance and the solvent. This condition increased the amount of anthocyanin extract obtained during extraction using MAE. Giving high power results in an increased amount of anthocyanins and side reactions that interfere with the extraction process. This is because high power increases the molecules' energy, which causes the process of breaking chemical bonds to occur. So that in high power conditions, the amount of anthocyanin produced decreases.

The mathematical equation model to predict anthocyanin concentrations shows significant results with a p-value less than 0.05. Based on ANOVA analysis, each variable, namely particle size, the ratio of cocoa peel: solvent, extraction time, and microwave power, showed a significant effect on the response of anthocyanin concentrations. Lack of Fit F-Value yield response with a p-value greater than 0.05 indicates an insignificant Lack of fit. The lack of fit value, which is not significant, is a requirement for a good model because it suggests the suitability of the anthocyanin concentration-response data with the model equation. The value of R^2 (coefficient of determination) is 0.8539, indicating that the model supports 85.39%

Table 3. The relationship between the variables and the modeled anthocyanin concentration response

Mathematic Equation	Significant (p<0.05)	*Lack of fit* (p<0.05)	R^2
Y= 0.000486 – 9.98637E – 07A + 0.026734B0.000041C – 7.58240E – 07D – 0.000102AB + 2.48606E – 07AC + 2.62878E09AD – 0.000539BC + 0.000012BD + 9.71853E08CD	0.0472	0.0543	0.8539

(A: particle size; B: cocoa peel: solvent ratio; C: extraction time; D: microwave power)

This equation shows that the response to anthocyanin concentration will increase proportionately to the increase in the ratio of cocoa peel mass: solvent, particle size, and time of extraction, particle size and power; ratio and power; and time of extraction and power. The graph of the standard plot of residuals, which indicates the relationship between the actual value and the predicted value in Figure 2, is close to the standard line, showing that the yield response data is usually distributed. This means the actual results will be close to those predicted by the DE version 11 Program. The surface shape of the interaction relationship between variable components can be seen more clearly in the three-dimensional graph shown in Figure 3. The results show that the effect of the ratio can increase extraction, which allegedly occurs because the greater the amount of cocoa shells present, the higher the source of the active substance that can be extracted. So that the results of the anthocyanins obtained are also greater. The particle size of cocoa shell indicates an increase in anthocyanin concentration. A decrease in particle size will cause the surface area of the cocoa shell particles to increase, so the contact probability between the anthocyanins contained in the cocoa shell and the solvent increases. This encourages an increase in the extraction rate, so the quantity of anthocyanin produced becomes large.

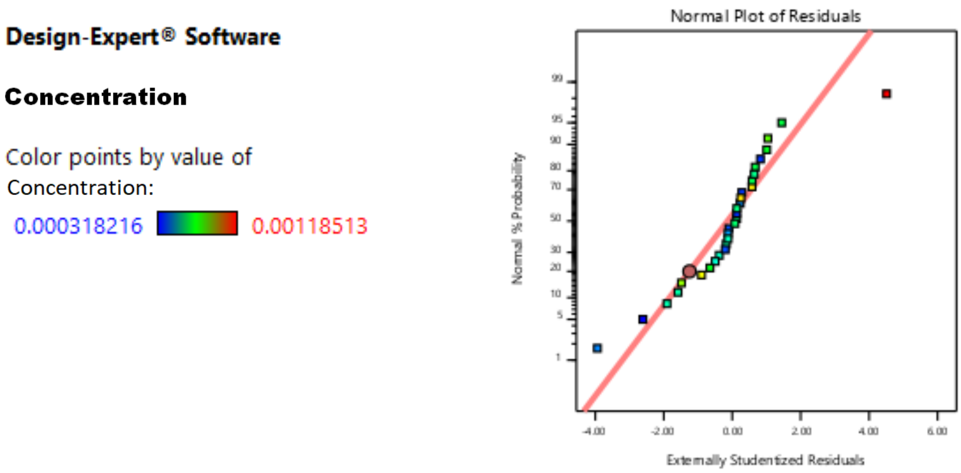

Figure 2. Plot of the normality of the residual anthocyanin concentration responses

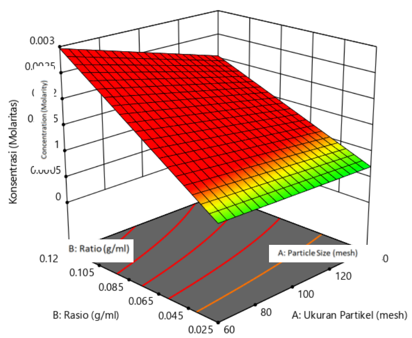

Figure 3. Three-dimensional graph of the results of the anthocyanin concentration response test

4. Conclusions

The optimal response value for anthocyanin concentration was 11.85.10-4 M, with the condition that the particle size in the extraction process was 0.105 mm, the ratio of cocoa shell/ethanol was 0.03125 g/mL, the extraction time was 2 minutes, and the microwave power

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was 100 W. The relationship between variables to the modeled anthocyanin concentration response:Y=0.000486-9.98637E-07A+0.026734B0.000041C-7.58240E-07D-0.000102AB+2.48606E-07AC+2.62878E09AD-0.000539BC+0.000012BD+9.71853E08CD

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