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Multivariate optimization of ultrasound-assisted extraction rich polyphenol from robusta coffee fruit peel (*Coffea canephora*) using factorial design

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ABSTRACT Robusta coffee fruit peel contains many polyphenolic compounds, so the antioxidant activity is high. These compounds require special extraction techniques to obtain optimal results. This study aims to determine the optimal extraction process conditions for producing polyphenol compounds (flavonoids and anthocyanins) with strong antioxidant activity. The robusta fruit peel extraction process uses the ultrasound-assisted extraction (UAE) method and is analyzed using the full factorial design (FFD) application. Factors in determining the optimal extract consist of extraction time (10-45 minutes), extraction temperature (25-50°C), and solvent pH (2-5). The observed responses included percent yield, total flavonoids, anthocyanin levels, and antioxidant activity. The results of the extraction optimization yielded the optimal extract at an extraction time of 45 minutes, an extraction temperature of 50°C, and a pH of 2. The optimum extract has a yield value of 17.25%, TFC 242.26 mgCE/g, anthocyanins 367.04 mg/100 g, and IC_{50} 22.11 µg/mL. Extract optimization results show a good value with desirability of 0.997. The optimum extract contains 0.21% water content and 0.26% dry shrinkage. The optimum results of the phytochemical screening of the extract contained phenolic compounds, flavonoids, tannins, saponins, and alkaloids. The optimum extract verification results obtained yield percent values of 17.10%, TFC 250.28 mgCE/g, anthocyanins 391.20 mg/100 g, and antioxidant activity 24.54 µg/mL. The response results show values supporting verification, as evidenced by 95% CI and 95% TI range values.

KEYWORDS: Factorial design; polyphenol; antioxidant; robusta coffee fruit peel; ultrasound-assisted extraction

1. INTRODUCTION

Coffee fruit peel (cascara) is one of the most widely produced by-products, 45-50% of the crop. These by-products have not been used optimally, so most of them become waste that damages the environment [1]. Coffee pod waste contains phytochemical compounds that are beneficial to health. Using chemical and biotechnological approaches, coffee pod peel began to be developed as a substrate for extracting tannins, polyphenols, and caffeine [2]. Coffee berry peel has a red color caused by anthocyanin color pigments [3]. Anthocyanins have activity as natural antioxidants [3, 4]. Antioxidants in coffee peel include anthocyanins, tannins, flavonols, flavan 3-ols, and hydroxycinnamic acid [5]. The antioxidant activity of robusta coffee fruit peel is also contained in polyphenolic phenolic acids such as caffeine, chlorogenic acid, coumarin, ferulic and sinapic acid. The main phenolic compounds found in coffee berry peel include flavan-3-ols, hydroxycinnamic acid, flavonol 5-caffeoylquinic acid, epicatechin, catechin, caffeoylquinic acid, rutin, ferulic acid, and anthocyanins. Coffee peel contains carbohydrates (21-32%), pectic compounds (6.5%), protein (5-15%), minerals (9%), tannin compounds (3%), chlorogenic acid (2.4%), caffeic acid (1.6%) and caffeine (1.5%) [1]. Robusta coffee rind has the potential as an antioxidant [6], anti-cancer, anti-inflammatory [7],

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antibacterial [8], and antidiabetic [9]. The high content of active compounds in this waste has the potential to be processed into products that have efficacy and are economical, such as anti-aging supplements [10] and brightening masks. Therefore, optimizing the robusta coffee fruit peel extract is necessary to obtain the maximum phytochemical compounds.

Extraction optimization is carried out to get the best extraction method to produce the maximum target compound. The extraction process generally uses conventional methods such as maceration. Conventional extraction methods have drawbacks, requiring many solvents and long extraction times [11]. Sample preparation takes up more than 80% of the total time spent on research. The step to overcome these deficiencies is to use modern extraction techniques, one of which is the ultrasound-assisted extraction method (UAE) which uses a smaller volume of extraction and a shorter extraction time [12, 13]. UAE is used for various purposes related to sample preparation, especially for sample extraction with the help of ultrasonic waves [14, 15]. UEA extraction can shorten the time to efficiently extract phenolic and anthocyanin compounds from natural matrices [16, 17]. Extraction with the UAE method can increase the extraction results so that the quality of the extraction results also increases [18, 19]. Extraction with UAE is better for extracting flavonoid compounds than the maceration method. Extraction with the UAE method can increase the yield of the response used by producing a higher penetration of the Solvent into the target material, thereby increasing the antioxidant activity of the preparation [20], increasing the penetration of the solvent into the target material, accelerating the release of anthocyanin compounds [21], and preventing degradation anthocyanin [22].

Based on previous research by Myo and Udomkiri, optimization of coffee fruit skin using ultrasonic extraction was carried out using Arabica coffee samples with a phenolic compound content of 7.49 mg/g, flavonoids of 33.78 mg/g, and tannins of 7.11 mg/g [23]. Previous research by Fernandez conducting an analysis of the bioactive components of Robusta coffee fruit skin extract showed caffeine levels of 3.30 mg/g and DPPH antioxidant activity of 9.00 µg/mL [24]. The chlorogenic acid, anthocyanin, and flavonoid compounds in the skin of the coffee fruit act as antioxidants. In general, Robusta coffee has a greater caffeine and chlorogenic acid content than Arabica coffee; however, there has been no research on optimizing the extraction of Robusta coffee fruit skin to obtain the highest levels of polyphenolic compounds with the best antioxidant activity [25]. This research was designed to obtain the highest polyphenolic compounds with the best antioxidant activity using a factorial design and an ultrasonic extraction method.

Based on the description above, it is necessary to conduct further research on extraction optimization using the UAE method with the help of expert design software 13. Ultrasonic waves form UAE can form cavitation bubbles in the simplicia, causing the simplicia cells to break down and release more compounds. Extraction with UAE makes the optimization process easier with extraction time and temperature that can be set manually [26]. This research will produce extraction conditions for producing optimal antioxidant activity from robusta coffee berry peel extract. The antioxidant activity in this coffee by-product can be further utilized in the pharmaceutical field and is the best solution for dealing with environmental pollution due to coffee pod waste. Ultrasonic extraction was chosen because it is able to produce ultrasonic waves to form pores, which facilitate the dissolution of 70% ethanol to attract more phytochemical compounds. Extraction of flavonoid compounds with ultrasonic assistance showed surface changes that showed the formation of more cylindrical cavities in simplicia compared to conventional extraction. These results illustrate that the higher the ultrasonic waves emitted, the larger the cavity formed in the cell. The longer the extraction process with ultrasonic waves, the more the simplicia cells break and reduce the particle size so that the contact area with the solvent will increase and maximize the compound release process [27].

Extraction optimization using the UAE method takes advantage of the effects of temperature, time [28], and differences in pH. The upper and lower limit ranges are selected based on a literature review. This study used a temperature range of 25-50^{o}C with a time of 10-45 minutes and a pH range of 2-5. Temperatures that are too high cause damage to the extracted material [26]. Extraction time that is too long causes the extract to hydrolyze, while time that is too fast results in non-optimal extraction [29]. The use of low pH can increase the stability of anthocyanin pigments [30]. The observed responses included percent yield, total flavonoids, anthocyanin levels, and antioxidant activity. The research results were verified against data with a desirability value close to one using design expert software 13.

2. RESULTS

2.1. Optimization of Robusta Coffee Fruit Peel Extract with Full Factorial Design

Optimization of percent yield, total flavonoids, anthocyanins, and antioxidants was carried out to obtain the best conditions for extracting the peel of the Fruit Robusta coffee. Extraction optimization used time, temperature, and solvent pH variables. The results of the extraction optimization was observed in Table number 1.

Table 1. Yields of percent yield, flavonoids, anthocyanins, and antioxidant activity of extracts at each run.

Run	Time (Minutes)	Temperature (°C)	pH	Extract weight (g)	Yield (%)	Flavonoids (mgCE/g extract)	Anthocyanins (mg/100g)	IC_{50} Antioxidant (µg/mL)
1	10	25	5	20	12.40	129.83 ±1.79	140.66	100.21
2	10	50	2	20	14.30	186.21±0.98	241.97	59.49
3	10	50	5	20	13.80	168.81±1.55	203.39	71.00
4	45	25	2	20	16.50	235.14 ±1.74	329.64	34.13
5	45	25	5	20	14.85	202.03±0.59	295.74	58.02
6	10	25	2	20	12.85	152.66±1.28	179.62	90.28
7	45	50	2	20	17.25	242.26±1.09	367.04	22.10
8	45	50	5	20	15.65	217.51±0.52	317.95	46.75

Based on the research results in Table 1, the highest percentage yield was 17.25%, the highest flavonoid content was 242.26 ± 1.09 mgCE/g extract, the highest anthocyanin was 367.04 mg/100g, and IC_{50} was 22.10. These results are then analyzed against the Pareto chart, the interaction of each variable, and the 3D surface.

2.1.1. *Pareto chart analysis*

Pareto chart analysis was used to determine the factors that significantly influence the response to be generated. A factor must have a value above the *t*-value to significantly affect the response. The influence of each factor and factor interactions on yield response, flavonoids, anthocyanins, and antioxidants are presented in Figure 1.

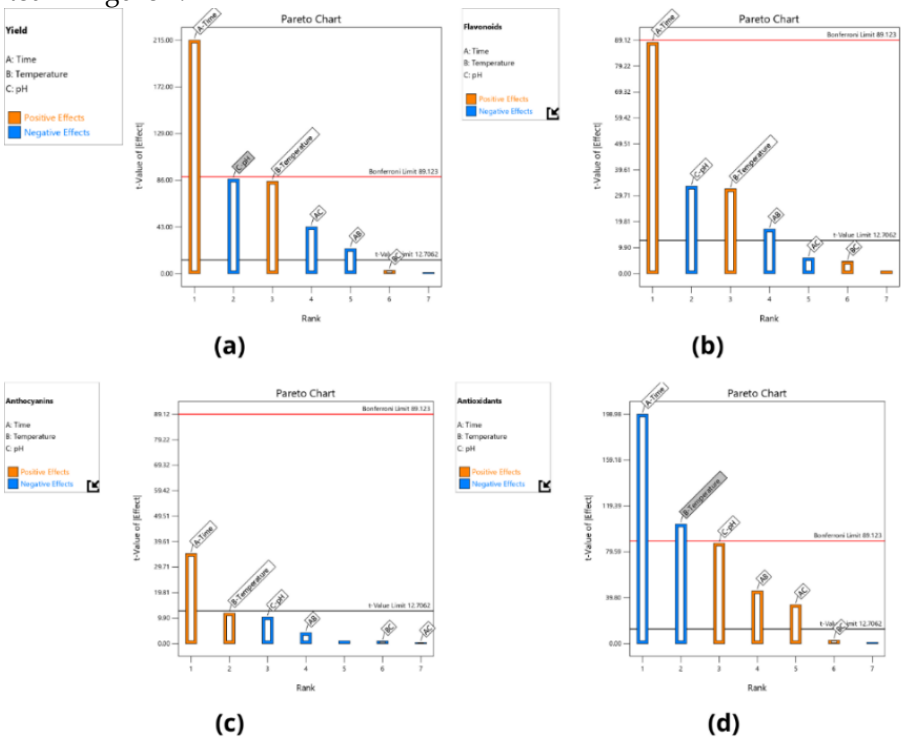


Figure 1. Pareto chart results of multivariate analysis in extraction process optimization modeling. (a) yield, (b) flavonoids, (c) anthocyanins, and (d) antioxidants.

Figure 1 (a) presents a Pareto graph of the yield response with all factors except the temperature-pH interaction above the t-value line so that these factors significantly influence the percent yield. *t*-value is a value that shows the proportion of contribution of a category to the total value. Figure 1 (b) presents a Pareto graph of the response of flavonoids with all factors except the time-pH and temperature-pH interactions being above the *t*-value line so that these factors significantly affect flavonoid levels. Figure 1 (c) presents a Pareto graph of the anthocyanin response with the factors of time, temperature, and pH above the *t*-value line so that these factors significantly affect anthocyanins. Meanwhile, the interaction between time-temperature, time-pH, and temperature-pH is below the line, which means it has no significant effect. Figure 1 (d) presents a Pareto graph of the antioxidant response with the factors of time, temperature, pH, time-temperature interaction, and time-pH interaction above the t-value line so that these factors significantly affect the IC_{50} value.

2.1.2. *Two-Factor interaction analysis*

Two-factor interaction analysis is used to determine the effect of the two-factor interaction. The interaction analyzed is in the form of temperature and time factors, with pH as the actual factor. The effect of each interaction of temperature and time factors on the response of yield, flavonoids, anthocyanins, and antioxidants is presented in Figure 2.

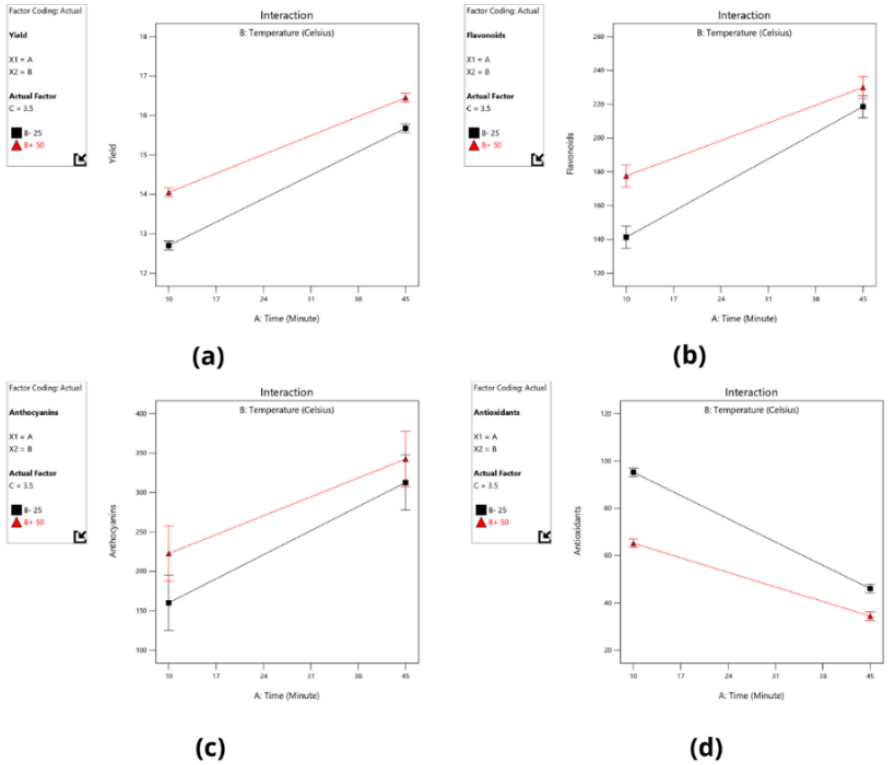


Figure 2. Two-factor interaction analysis. (a) yield, (b) flavonoids, (c) anthocyanins, and (d) antioxidants.

The slope of the regression line describes the relationship between two factors. The slope indicates the direction of the relationship between two factors, whether positive or negative. A positive slope relationship occurs when the value of one factor increases, the value of the other factor also increases. The interaction graph in Figure 2 (a) shows that the slope at the low-level temperature interaction has a sharp slope compared to the high level, which means that there is a relationship between the two factors that affect the yield. The interaction graph in Figure 3 (d) shows that the slope at high-temperature interaction has a sharper slope than at a low level, which means that there is a relationship between the two factors that affect TFC. The interaction graph in Figure 4 (d) shows the slope at low temperatures and level interactions. Height parallel, it was concluded that the interaction of the two factors did not affect anthocyanins. The interaction graph in Figure 5 (d) shows that the slope at low temperature interaction has a steeper slope than at a high level, so it can be concluded that the interaction of the two factors affects the IC_{50} value.

2.1.3. 3D Surface analysis actual factor pH 3.5

The 3D Surface analysis with an actual factor pH 3.5 determined the effect of all factors on the response. The influence of each factor can be observed by changing the color gradations in the image. Areas with a reddish color show a higher response result, while increasingly blue areas show a lower response result. The influence of factors on yield response, flavonoids, anthocyanins, and antioxidants is presented in Figure 3.

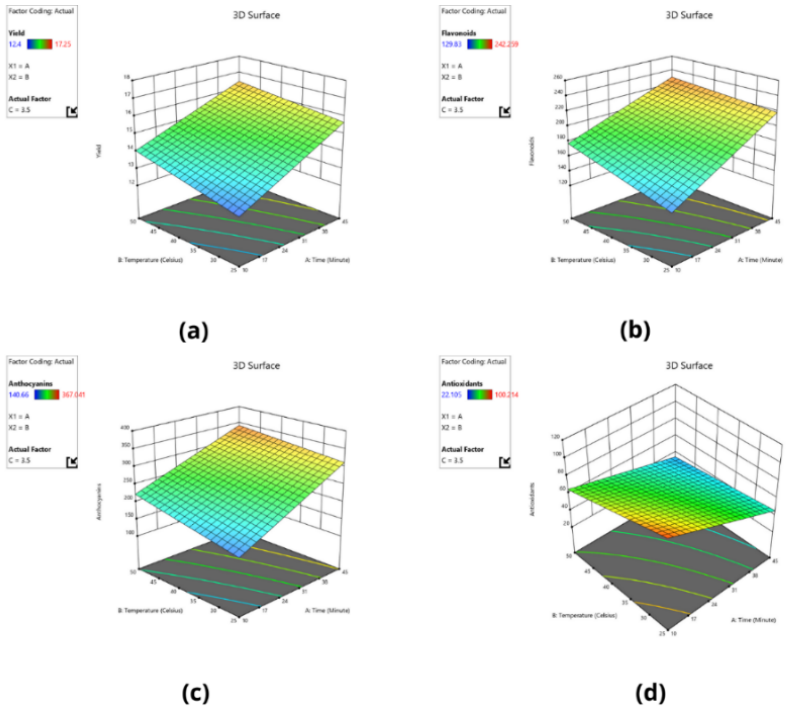


Figure 3. 3D Surface results from multivariate analysis of extraction process optimization using a full factorial design approach. (a) yield, (b) flavonoids, (c) anthocyanins, and (d) antioxidants.

Yield responses, flavonoids, and anthocyanins were selected for the results with the highest value, namely in the red area. Figures 3 (a), 3 (b), and 3 (c) present areas with increasingly red color gradations at high temperature and time levels. The red gradation shows the highest value for each response, yielding 17.25%, total flavonoids 242.259 mgCE/g, and anthocyanins 367.041 mg/100g. The bluer gradation showed the lowest value for each response, yielding 12.40%, total flavonoids 129.83 mgCE/g, and anthocyanins 140.66 mg/100g. The antioxidant response was chosen as the result with the lowest value, namely in the blue area. Figure 3 (d) presents areas with increasingly blue color gradations at low-level temperatures and times. The blue color gradation shows an antioxidant value of 22.105 µg/mL.

2.1.4. Anova and statistical parameters

The results of each response (yield, total flavonoids, anthocyanins, and antioxidant activity) were tested using ANOVA (Analysis of variance). The ANOVA test was carried out to determine the significance of the response analysis between variables so that the optimum model suggested by the application design expert 13 was obtained. ANOVA results and statistical parameters for each response are presented in Table 2.

The ANOVA percentage yield response results show a standard deviation (SD) value of 0.017 and a CV of 0.12. SD and CV values closer to 0 indicate a more even and uniform data distribution. The resulting R^2 value is 0.999 with the difference between adjusted R^2 and predicted R^2 which is 0.001 (<0.2), so it means significant data. The adequate precision value is 292.54 (> 4), which means the model has a good signal. A model has a good signal and can be used as a guideline for design space if the adequate precision value is greater than 4.

Table 1. ANOVA data for each response from full factorial design approach of extraction process.

Response	SD	CV (%)	*Adjusted* R^2	*Predicted* R^2	*adequate precision*	R^2	*p*-value
Yield	0.02	0.12	0.999	0.998	292.54	0.999	0.01
Flavonoids	1.04	0.54	0.993	0.999	116.46	0.999	0.02
Anthocyanins	5.51	2.12	0.995	0.957	43.16	0.999	0.05
Antioxidants	0.28	0.47	0.999	0.999	295.51	0.999	0.01

Analysis of variance (ANOVA) on the yield response of robusta coffee pod extract can be said to be significant if it has a *p*-value of less than 0.05 is 0.007. The ANOVA results for the total flavonoid response showed an SD value of 1.04 and a CV of 0.54, meaning the data was normally distributed. The resulting R^2 value is 0.999 with the difference between adjusted R^2 and predicted R^2 which is 0.006 (<0.2), so the data is declared significant. The adequate precision value is 116.46 (> 4), which means the model has a good signal.

The results of the ANOVA for anthocyanin responses showed an SD value of 5.51 and a CV value of 2.12. The resulting R^2 value is 0.999 with the difference between adjusted R^2 and predicted R^2 which is 0.038 (<0.2), so the data is declared significant. Adequate precision results are 43.16 (> 4), so it meets the requirements as an optimization model. The ANOVA results of antioxidant activity showed an SD value of 0.28 and a CV of 0.47. R^2 values close to 1 indicate a good correlation between experimental and predicted responses. The resulting R^2 value is 0.999 with the difference between adjusted R^2 and predicted R^2 which is 0.0009 (<0.2), so it is concluded that the predicted value is significant. Adequate precision results are 294.51, so it meets the requirements as an optimization model.

The results of each response (yield, total flavonoids, anthocyanins, and antioxidant activity) were then tested for statistical parameters. This test consists of the percent contribution and *p*-value of each factor. This test was conducted to determine the contribution and significance of a factor to the desired response. The results of the statistical parameters for each response are presented in Table 3.

Table 3. Statistical parameters for each response from full factorial design approach of extraction process.

Response	Observation	Intercept	A	B	C	AB	AC	BC	ABC
Yield	factor coefficient	14.72	1.34	0.53	-0.54	-0.14	-0.27	0.02	-0.01
	percent contribution		72.90	11.39	11.94	0.83	2.92	0.01	0.00
	p-value		0.01*	0.00*	0.01*	0.01*	0.03*	0.01*	0.80
Flavonoids	factor coefficient	191.81	32.43	11.89	-12.26	-6.24	-2.20	1.72	0.37
	percent contribution		75.64	10.17	10.81	2.80	0.35	0.21	0.01
	p-value		0.01*	0.02*	0.02*	0.04*	0.10	0.13	0.87
Anthocyanins	factor coefficient	259.50	68.09	23.09	-20.07	-8.18	-0.68	-1.85	-1.95
	percent contribution		82.11	9.44	7.13	1.18	0.01	0.06	0.07
	p-value		0.02*	0.05	0.06	0.14	0.78	0.52	0.48
Antioxidants	factor coefficient	60.25	-20.00	-10.41	8.75	4.59	3.39	0.29	-0.10
	percent contribution		64.77	17.56	12.39	3.41	1.86	0.01	0.00
	p-value		0.00*	0.01*	0.01*	0.01*	0.02*	0.21	0.79

Information: A: time, B: temperature, C: pH, AB: time-temperature, AC: time-pH, BC: temperature-pH, *Parameters with significant effect (*p*-value <0.05).

The results of the fitting equation for the percent yield model show that the influence of time, temperature, and temperature-pH interactions will increase the response because it has a positive value. The pH of the solvent, the time-temperature interaction, the time-pH interaction, and the time-temperature-pH interaction will decrease the response because they are negative. The ABC interaction shows a very low contribution value of 0.00, so it does not significantly affect the percent yield. Data on the percentage contribution of the yield percentage shows that extraction time has the highest influence on the yield

percentage yield, namely 72.90%. The percentage contribution of temperature and pH did not differ significantly with the values 11.39% and 11.94% respectively. The interaction of AB, AC, and BC has a very small contribution to the percent yield. Based on the *p*-value, ABC Interaction has no significant effect on the yield percentage, with a contribution of 0.00%. The factors of extraction time, temperature, solvent pH, time-temperature interaction, time-pH interaction, and temperature-pH interaction had a *p*-value below 0.05, so these factors increased the percent yield obtained. Based on the results of the statistical parameters, the total yield equation is obtained as follows:

Yield = 14.72 + 1.34A + 0.53B - 0.54C - 0.14AB - 0.27AC + 0.02BC
Information
A = Extraction Time
B = Extraction temperature
C = pH of the solvent
AB = time-temperature interaction
AC = time-pH interaction
BC = temperature-pH interaction

The results of the fitting equation for the total flavonoid model show that the influence of time, temperature, and temperature-pH interactions will increase the response because it has a positive value. The pH of the solvent, the time-temperature interaction, the time-pH interaction, and the time-temperature-pH interaction will decrease the response because they are negative. The ABC interaction shows a very low contribution value of 0.01, so it does not significantly affect the percentage yield. Data on the percentage contribution of total flavonoids showed that extraction time had the highest effect on the yield of total flavonoids, namely 32,43%. Percent contribution of temperature and pH did not differ significantly from the values respectively 10.17% and 10.81%. The interaction of AB, AC, and BC contributes very little to the total flavonoids. Based on the *p*-value, the ABC interaction did not significantly affect total flavonoids because it had a *p*-value above 0.05. Factors of extraction time, temperature, solvent pH, and time-temperature interaction had a *p*-value below 0.05, increasing the total flavonoids obtained. Based on the results of the statistical parameters, the total flavonoid equation was obtained as follows:

Total Flavonoids = 191.81 + 32.43A + 11.89B - 12.26C - 6.24AB
Information
A = Extraction Time
B = Extraction temperature
C = pH of the solvent
AB = time-temperature interaction

The anthocyanin fitting model equation results show that the effect of time and temperature factors will increase the response because it has a positive value. Solvent pH factor, time-temperature interaction, time-pH interaction, temperature-pH interaction, and time-temperature-pH interaction will decrease the response because it has a negative value. The time-pH interaction showed the lowest contribution value, namely 0.01, so it did not significantly affect anthocyanins. Data on the percentage of anthocyanin contribution showed that extraction time had the highest effect on the yield of total flavonoids, namely 82.11%. Percent contribution of temperature and pH, respectively, is 9.44% and 7.13%. Based on the *p*-value, the ABC interaction, the time-temperature interaction, the temperature-pH interaction, and the time-temperature interaction have no significant effect on anthocyanin because it has a *p*-value above 0.05. The extraction time and extraction temperature factors had a *p*-value below 0.05, so these factors had an effect on increasing the anthocyanin obtained. Based on the results of the statistical parameters, the anthocyanin equation is obtained as follows:

Anthocyanins=259.50 + 68.09A + 23.09B - 20.07C
Information
A = Extraction Time
B = Extraction temperature
C = pH of the solvent

The results of the fitting equation for the antioxidant model show that the effect of time, temperature, and time-temperature-pH interaction will decrease the response because it has a negative value. Solvent pH factor, time-temperature interaction, pH-time interaction, and temperature-pH interaction will increase the response because they are positive. The time-temperature-pH interaction showed the lowest contribution value, namely 0.00, so it did not significantly affect antioxidants. Data on the percent contribution of

antioxidants shows that extraction time has the highest effect on the antioxidant yield, 64.77%. Percent contribution of temperature, pH, time-temperature interaction, and time-pH interaction was 3.41%, 1.85%, and 0.01%, respectively. Based on the *p*-value, the ABC interaction has no significant effect on antioxidants because it has a *p*-value above 0.05. The factors of extraction time, extraction temperature, pH, time-temperature interaction, and pH-time interaction had a *p*-value below 0.05, so these factors had an effect on increasing antioxidants. Based on the results of statistical parameters, the antioxidant equation is obtained as follows:

Antioxidants = 60.25 - 20.00A - 10.41B + 8.75C + 4.59AB + 3.39AC
Information
A = Extraction Time
B = Extraction temperature
C = pH of the solvent
AB = time-temperature interaction
AC = time-pH interaction

2.1.5. Determination of optimum conditions

Determination of the optimum conditions of robusta coffee pod extract on extraction temperature, extraction time, and pH on yield response, total flavonoids, anthocyanin levels and antioxidant activity are determined based on the desired variable values. The variable criteria and the desired response are presented in Table 4. The optimum point solution is presented in Table 5.

Table 42. Variable criteria and desired response.

Variable	Goal	Lower Limit	Upper Limit	Importance
A: Tıme	*is in range*	10	45	3
B: Temperature	*is in range*	25	50	3
C: pH	*is in range*	2	5	3
Yield	*Maximize*	12.40	17.25	4
TFC	*Maximize*	129.83	242.26	5
Anthocyanins	*maximize*	140.66	367.04	5
Antioxidant	*minimize*	22.11	100.21	5

Table 5. Optimum point solution.

Number	Tıme (minute)	Temperature (°C)	pH	Yield	TFC	Anthocyanins	IC_{50}	Desirability
1	45.00	50.00	2.00	17.24	242.62	365.09	22.00	0.997
2	45.00	49.88	2.00	17.24	242.58	364.92	22.06	0.997
3	44.99	49.75	2.00	17.23	242.54	364.75	22.12	0.997

The target to be achieved in yield response is maximum, with a yield value ranging from 12.40% - 17.25%. The target to be achieved in the total flavonoid response is maximizing the total value of flavonoids ranging from 12.83 mgCE/g extract to 242.26 mgCE/g extract. The target in the anthocyanin response is maximizing it with anthocyanin values ranging from 140.66 mg/100g to 367.04 mg/100g. The target to be achieved in the antioxidant response is minimizing it with values ranging from 22.10 µg/mL to 100.21 µg/mL. The optimum condition is selected from the condition with the maximum desirability value, which is the closest value to 1.0. Based on Table 15, it can be predicted that the highest response value is based on a desirability value of 0.997, namely a yield of 17.24%, TFC 242.62 mg/g, anthocyanin 365.09 mg/100 g, and an antioxidant IC_{50} value of 22.00 µg/mL.

2.2. Optimum Extract Characterization and Verification

2.2.1. Extract characterization

Characterization and verification were carried out on the optimum extract. The characterization of the extract uses the parameters of water content and dry shrinkage under the conditions based on the 2011 Ministry of Health of the Republic Indonesia, less than 10%. The optimum extract characterization results are presented in Table 16. The optimum results for Robusta coffee peel extract's water content and dry shrinkage were 0.21 and 0.26, respectively, so they were declared feasible.

Table 6. Optimum extract characterization results.

Parameter %	Requirement*	Result ± SD	*Explanatiol*
water content	<10%	0.21	Good
drying shrinkage	<10%	0.26	Good

*According to Ministry of Health of the Republic Indonesia 2011

2.2.2. Phytochemical Screening

Optimum extract phytochemical screening was carried out to analyze the content of phenolic secondary metabolites, flavonoids, tannins, saponins, and alkaloids. The results of the phytochemical screening are presented in Table 7.

The optimum results of the phytochemical screening of robusta coffee fruit peel extract was positive for all the tested secondary metabolites.

2.2.3. Optimum extract verification

Software verification and prediction are carried out to prove that the verification results are within the prediction range determined by the application design expert. Extract verification results are presented in Table 18. Based on the verification results in Table 8, the extraction temperature of 50°C, extraction time of 45 minutes, and pH 2 resulted in a yield response of 17.1%, total Flavonoid content of 250.28 mgCE/g, Anthocyanin 391.20 mg/100 g and antioxidant activity of 24.54 µg/mL. The antioxidant activity of Robusta coffee pod peel under optimal conditions and catechins as a comparison test with 3 replications did not show a different absorbance value. The value of each response is in the range of 95% Confident Interval (CI) and 95% Tolerance Interval (TI).

3. DISCUSSION

Coffee fruit peel (cascara) is one of the most widely produced by-products, 45-50% of the crop [1]. Coffee pod peel which is known as a waste product contains many nutritious compounds such as chlorogenic acid (5-caffeoylquinic acid), 42.20%; epicatechin, 21.60%; isochlorogenic acid I, 5.70%; isochlorogenic acid II, 19.30%; isochlorogenic acid III, 4.40%; catechin, 2.20%; rutin, 2.10%; protocatechuic acid, 1.60%; and ferulic acid, 1.00%[31]. The peel of the extracted Robusta coffee fruit will produce a bright red to dark red colour. The coffee fruit peel contains quite high levels of flavonoids and anthocyanins, so it makes red. The riper the coffee cherries, the higher the anthocyanin content so that the colour of the cherries is redder [32, 33].

Table 7. Phytochemical screening results.

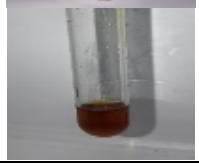
Secondary Metabolites	Result	Figure
Phenolic	+	
Flavonoids	+	
Tannins	+	
Saponins	+	
Alkaloids	+	

Table 8. Point prediction optimal results response of robusta coffee fruit peel extract.

Response	Predict	Observed	95% CI low	95% CI high	95% TI low	95% TI high
Yield	17.24	17.01	17.03	17.45	16.41	18.07
TFC	242.62	250.28	230.28	254.97	193.82	291.43
Anthocyanins	365.09	391.20	299.60	430.58	106.21	623.97
Antioxidant	22.00	24.54	18.62	25.38	8.64	35.35

Robusta coffee pod peel that has been extracted was then analyzed using four responses in the form of yield, TFC, anthocyanin and antioxidant activity. The model fitting data showed that the best extraction results were at 45 minutes extraction time at 50^{o}C and pH 2, it was because an increase in extraction temperature and extraction time would be increase the diffusion of the solvent into the simplicial tissue. So that it will increase the number of extracted compounds, including flavonoid compounds [34], an extraction time of 10 minutes tends to be too short, and not all compounds can be extracted, while extraction times of more than 50 minutes cause unstable compounds to degrade [35] easily. Extraction temperatures in the 20-30°C range are less effective in releasing the extracted content, while temperatures in the 60°C cause many compounds to be damaged [35, 36]. The best temperature for extracting compounds using ultrasonic waves is 50-52^{o}C [37]. Extract conditions with a pH of 2 produced the highest yield percent because the more acidic the solvent used, the more tissue cell walls were damaged, more bioactive compounds were extracted [38]. pH 2 is the best for extracting flavonoids and anthocyanins because flavonoids and anthocyanins are stable at acidic pH [39, 40]. Increasing the concentration of hydrogen ions in an acidic environment can suppress the loss of flavonoids due to oxidation. A low solvent pH will make the anthocyanins take the form of flavum cations, which make the Robusta Coffee Fruit Peel extract dark red so that the pH 2 solvent in the extraction produces a higher anthocyanin value [41].

The percent yield model fitting results vary between 17.25% and 12.40%. The UEA method can break down cells by forming pores (cavitation effect), making it easier for 70% ethanol solvent to attract more phytochemical compounds [42]. Ultrasonic-assisted extraction will cause cavitation in the simplicial, widening the contact area between the solvent and the sample. The extent of the contact area will increase

the number of secondary metabolites extracted [43]. Cavitation is the formation of bubbles due to the transmission of ultrasonic waves; this causes the release of energy, which can mechanically damage the cell walls. As a result, the solvent diffuses more effectively into the simplicial [42]. The response results were carried out by an Analysis of variance (ANOVA) test to determine the significance of the response analysis between variables to obtain the optimum model suggested by the Design Expert. The results of the ANOVA per cent yield test meet the requirements of the extraction model.

The fitting model for testing the flavonoids of robusta coffee fruit extract produces a linear regression y = 0.00118x-0.025 with a correlation coefficient of r = 0.999. Based on previous research, Robusta coffee fruit peel contains 132,61 mg of flavonoids/100 grams of extract [1]. These results indicate that extraction with UAE produces better flavonoid compounds. The antioxidant activity of robusta coffee peel extract using the percolation extraction method yielded a value of 52.55 µg/mL [44]. Extraction results using UAE have a higher antioxidant activity value than the maceration method, so it is proven to increase extraction efficiency [45].

The optimum condition is chosen from the conditions with the maximum desirability, the value closest to 1.0, which indicates the program's ability to produce the desired product more perfectly. Based on Table 5 it can be predicted that the highest response value is based on a desirability value of 0.997, namely a yield of 17.24%, TFC 242.62 mg/g, anthocyanin 365.09 mg/100 g, and an antioxidant IC_{50} value of 22.00 µg/mL. Characterization and verification were carried out on the optimum extract. The characterization of the extract uses the parameters of water content and dry shrinkage under the conditions based on the 2011 Ministry of Health of the Republic of Indonesia, less than 10%. Optimum extract phytochemical screening was carried out to analyze the content of phenolic secondary metabolites, flavonoids, tannins, saponins, and alkaloids with positive extract results containing all the tested secondary metabolites. The phytochemical screening will produce a different colour for each test compound. The results of the extract flavonoid test proved that the positive extract contained flavonoid compounds, as indicated by the formation of red colour in the extract. The formation of red colour in the extract is due to the metal Mg and HCl succeeding in reducing the benzopyran nucleus in the flavonoid structure to form red flavilium salts [46].

The software verification and prediction results are observed to determine whether they are within the prediction range determined by the application design expert. If the verification value is within the prediction range, then the predicted value supports verification. The 95% Confident Interval value is the range between two values; the mean value is the average of the optimum response results, which is right in the middle, while the 95% Tolerance Interval value is the range of tolerance values from observations and is limited by the upper and lower limit values [47].

4. CONCLUSION

Optimization using a multivariate analysis approach using full factorial design can be carried out well in the Ultrasound-assisted extraction process from robusta coffee fruit peel to produce polyphenol-rich material. The current results show that based on the factorial design analysis, the optimal conditions for robusta coffee pod extract were obtained at an extraction temperature of 50°C, an extraction time of 45 minutes, and a solvent pH of 2 with a percent yield of 17.10%, a total flavonoid content of 250.28 mgCE/g, anthocyanins 391.20 mg/100 g and antioxidant activity 24.54 µg/mL. The optimal characteristics of the robusta coffee berry peel extract had a moisture content of 0.21 and dry shrinkage of 0.26 with positive phytochemical screening results containing phenolic compounds, flavonoids, tannins, saponins and alkaloids.

5. MATERIALS AND METHODS

5.1. Chemicals and Reagents

Robusta coffee peel collected from PT Perkebunan Nusantara VII (PTPN 7), Pagar Alam, Sumatera Selatan (Indonesia). Chemicals such as ethanol and methanol are purchased from PT. Bratachem (Indonesia). Several reagents such as magnesium powder, potassium acetate, HCl, NaOH, FeCl3 were obtained from PT. Dira Sonita Palembang (Indonesia). Standard compounds of catechins and DPPH were purchased from Sigma-Aldrich (Singapore).

5.2. Sample Preparation

The samples studied were coffee peels obtained from the PTPN area, Pagar Alam, South Sumatra. Coffee cherries were chosen red because they have a higher secondary metabolite content than when they were still green [48]. Robusta fruit peel coffee was dried at 40 °C by using an oven. Dry robusta fruit peel

coffee was used to grind into a fine powder using a grinder. The powder was stored at room temperature until further use.

5.3. Extraction

Coffee peel extraction was carried out using ethanol as a solvent with a ratio of 1:5, namely 20 g of simplicia in 100 mL of ethanol. The sample is put into a beaker glass covered with aluminium foil. Samples were put into the UAE tool according to the treatment of each process.

5.4. Extract Yield Calculation

The yield value indicated the effectiveness of a raw material or simplicia so that it can be converted into the final product as an extract. The units used to show the value of the results are percent (%). The formula for calculating the total yield is based on the following equation:

Yield (%) = $\frac{Me}{Ms}$ x 100%

Information :

Me : Extract Mass

Ms : Simplisia Mass

5.5. Determination of Total Flavonoid Levels [49]

5.5.1. Making catechin solutions

Catechins were weighed as much as 10 mg and dissolved in 10 mL of ethanol p.a to obtain a catechin concentration of 1000 µg/mL. A 1000 µg/mL catechin solution was taken as much as 1 mL and put into a 10 mL volumetric flask, and then the volume was adjusted up to the boundary mark to obtain a concentration of 100 µg/mL.

5.5.2. Determination of the maximum wavelength of catechins (λ max)

A total of 100 µg/mL catechin solution was made into 30 µg/mL, then 1 mL was taken and reacted with 0.3 mL 5% $NaNO_2$ and 0.5 mL 2% $AlCl_3$, then incubated for 30 minutes, then 0.25 mL 2M NaOH was added. Wavelength was carried out at 500 nm.

5.5.3. Catechin curve standard loading

A standard 100 µg/mL catechin solution was prepared in 5 series of concentrations, namely 30 µg/mL, 40 µg/mL, 50 µg/mL, 60 µg/mL and 70 µg/mL. 1 mL of each concentration series was reacted with 0.3 mL of 5% $NaNO_2$ and 0.5 mL of 2% $AlCl_3$. The solution was incubated for 30 minutes, and 0.25 mL of 2M NaOH was added.

5.5.4. Measurement of flavonoid levels in extracts

The extract was weighed as much as 10 mg and then dissolved in 10 mL of ethanol p.a until a concentration of 1000 µg/mL was obtained. 1000 µg/mL concentrated solution is diluted to 250 µg/mL. A total of 1 mL of the 250 µg/mL extract solution was pipetted and reacted with 0.3 mL 5% $NaNO_2$ and 0.5 mL 2% $AlCl_3$. The solution was incubated for 30 minutes, and added 0.25 mL of 2M NaOH. Samples were replicated three times for each analysis, and the average absorbance value was obtained.

TFC=$\frac{X(\mu g/mL) \times V\ (mL)}{m}$ x Dilution Factor

TFC = Number of total flavonoids

V = Total volume of the extract

m = Sample weight (g)

X = Concentration

5.6. Determination of Anthocyanin Content [50]

5.6.1. Preparation of 30 µg/mL DPPH reagent solution

DPPH has weighed as much as 10 mg and dissolved with methanol in a 100 mL volumetric flask to obtain a concentration of 100 µg/mL. The solution was then diluted to obtain a DPPH concentration of 30 µg/mL.

5.6.2. Determination of DPPH maximum wavelength (λmax)

Pipette 3.8 mL (30 µg/mL) of DPPH solution and put it in a vial. Then 0.2 mL of methanol p.a was added and homogenized. The bottles were then covered with aluminium foil and incubated for 30 minutes. Absorption measurements were carried out using a UV-Vis spectrophotometer with a 400-800 nm wavelength.

5.6.3. Preparation of robusta coffee fruit bark extract sample solution

A total of 25 mg of Robusta coffee rind extract from each of the 8 UAE extraction conditions was put into a 25 mL volumetric flask and then dissolved with methanol p.a up to the mark of the volumetric flask. The results are in the form of a solution with a concentration of 1000 µg/mL. Furthermore, dilution was carried out with concentrations of 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL and 500 µg/mL.

5.6.4. Preparation of catechin reference stock solutions

Catechins were weighed as much as 1 mg and put into a 10 mL volumetric flask. Then it was dissolved with methanol p.a up to the mark in the volumetric flask. The results are in the form of a solution with a concentration of 100 µg/mL. Further dilution was carried out to obtain concentrations of 20 µg/mL, 25 µg/mL, 30 µg/mL, 35 µg/mL and 40 µg/mL.

5.6.5. Determination of antioxidant activity

Antioxidant activity was measured on sample solutions from 8 conditions of UAE extraction and reference solutions. 0.2 mL of each solution concentration was pipetted and put into a vial. Then 3.8 mL of 30 µg/mL DPPH solution was added using a micropipette, and the vial was closed using aluminium foil. The samples were homogenized and incubated for 30 minutes. The absorbance was measured using a UV-Vis spectrophotometer with the maximum wavelength of DPPH.

$$\% \text{ Inhibition} = \frac{blank\ absorbance - sample\ absorbance}{blank\ absorbance} \times 100\%$$

The calculation results are entered into the regression equation Y = bX+a with extract concentration (µg/mL) as the abscissa (X-axis) and the % inhibition (antioxidant) value as the ordinate (Y-axis). The IC_{50} value from the calculation when the % inhibition is 50%.

5.7. Calculation of Water Content

Robusta coffee fruit extract was weighed as much as 1 gram and put into the evaporation cup. Samples were dried at 105°C for 5 hours in an oven. The sample is cooled in a desiccator and then weighed. The drying process was repeated until a constant weight was obtained. The water content is calculated as a per cent of the initial sample weight.

$$\text{Water content} = \frac{w1-w2}{w1} \times 100\%$$

Information
W1 = sample weight before heating
W2 = sample weight after heating

5.8. Calculation of Drying Shrinkage

One gram of Robusta coffee peel extract was weighed and put into an evaporation cup previously heated at 105°C to a constant weight. Samples were dried at 105°C for 5 hours in an oven to obtain a constant weight. The sample is cooled in a desiccator to room temperature, and then the fixed weight is recorded.

$$\text{Drying Shrinkage} = \frac{w1-w2}{w1} \times 100\%$$

W1 = sample weight before heating
W2 = sample weight after heating

5.9. **Phytochemical Screening**

5.9.1. Phenolic identification

A total of 3 mL of robusta coffee fruit peel extract was reacted with 10% iron (III) chloride solution. The dark blue or greenish-black colour indicates the presence of phenols [51].

5.9.2. Flavonoids identification

A total of 1 mL of extract and add 0.5 g of Mg powder and 10 drops of concentrated HCl; if it reacts positively, it will produce an orange, pink or red solution [51].

5.9.3. Tannins identification

Take 1 ml of extract and add 10 drops of 1% $FeCl_3$ solution. If it reacts positively, it will produce a strong green, red, purple, blue or black [51].

5.9.4. Saponins identification

Robusta coffee peel extract was diluted with water (1:1) and shaken vertically for 15 minutes. A positive reaction indicated the formation of 1-10 cm high foam, which was stable for 15 minutes [52].

5.9.5. Alkaloids identification

A total of 1 mL of extract was mixed with 1 mL of 2N HCl and 9 mL of hot distilled water, then heated for 2 minutes and filtered. Dragendorf reagent was added to the extract mixture. The sample will produce a red colour for a positive result [52].

5.10. Data Analysis

Data analysis used the factorial design method in the Design Expert®13 application free trial. This method determines the factors' effect on the desired response [53,54]. The factors used were time, temperature and pH, while the responses were yield, TFC, anthocyanins and antioxidants.

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