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PREFACE

We would like to present the 4th volume and edition of our journal, Journal of Biobased Chemicals published by the Department of Chemical Engineering, University of Jember, Indonesia. This volume is expected to enhance the findings and research about natural product and their derivatives, mostly in energy, chemicals, and materials. We present articles related to the products, processes, and management of biobased chemicals.

This new journal was envisioned and founded to represent the growing needs of biobased chemicals research as an emerging and increasingly vital field, now widely recognized as an ideal substitution for fossil-based chemicals. The journal has an objective to deliver and provide notable and standardized research and findings through journal reporting. The journal is intended as a window or a library for practitioners and researchers to share their works, identify new issues, and organize further research, while industrial users could apply the invention for scale-up, problem-solving, and application.

Hopefully, this edition will contribute valuable thought for the readers and enhance future research related to biobased chemical products. Finally, we send gratitude to all participants including authors, reviewers, and editors for their contribution.

June 2024

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Extraction of Polyphenols from Horn Banana Peel (*Musa Paradisiaca var. Typica*) Using the Ultrasound-Assisted Extraction Method

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Abstract. Banana peel contains starch, protein, fat, total fiber, unsaturated fatty acids, pectin, amino acids, polyphenols, and micronutrients. Horn banana peel has many benefits, namely, it can be processed into a natural antioxidant material that can minimize banana skin waste. The banana peel extraction method used is Ultrasound Assisted Extraction. Ultrasound-assisted extraction at optimal conditions produces good levels of total polyphenols with a short extraction time. In the use of ultrasonic no additional chemicals or other materials are needed. This study aims to determine the effect of extraction time (10, 20, 30 minutes), extraction temperature (20, 30, 40 °C), and solvent ratio (gr/mL) (1:25, 1:30, 1:35) on polyphenols. The Design Expert V13 program with Response Surface Methodology (RSM) Box-Behnken Design (BBD) was used to determine the combination of extraction parameters that lead to optimal results for total polyphenol content. Based on the research that has been done, it can be stated that the extraction parameters affect the total polyphenol content. The highest total polyphenol content was 61,007 mg GAE/g sample with the extraction conditions at an extraction temperature of 30°C, an extraction time of 30 minutes, and a ratio of banana peel powder to a dissolution of 1:30 g/mL.

Keywords: *Banana Peel, Polyphenols, and Ultrasound-Assisted Extraction*

1. Introduction

Bananas are a fruit that contributes around 30% of fruit production in Indonesia [1]. Based on the Central Statistics Agency and the Directorate General of Horticulture in 2021, it was reported that the amount of banana production in East Java reached 2.116.974 tons with the highest number of banana producers in Indonesia [2]. Until now, the banana plant that is often used is still limited to the fruit part, while other parts of the banana plant are considered

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waste and there is little further processing of some of these parts [3]. The peel of the horned banana (Musa Paradisiaca var. Typica) is one of the by-products of using bananas [4]. Horned banana peels are not widely used by the public, banana peels can be processed into fertilizer, purifying water, and heavy metals, lead (Pb) and copper (Cu) [5–7]. However, banana peel processing in Indonesia has not been utilized optimally compared to the amount of banana production in Indonesia [8]. Banana peel contains starch (3%), protein (6-9%), fat (3.8% - 11%), total fiber (43.2% - 49.7%), and unsaturated fatty acids, pectin, amino acids, and micronutrients [9]. Based on research conducted by Idah (2017) on banana peel extraction, shows that the total polyphenol content is 3.50104 w/v or 35.0104 mg GAE/g extract.

Antioxidant behavior is related to anthocyanins and the constituents of antioxidants are anthocyanins [10]. Anthocyanins are polyphenolic derivative compounds contained in various types of plants and have many important physiological functions in every living organism. Anthocyanins in plants are widely used in the food, health, and cosmetic industries because they do not have harmful effects. Currently, there are ±700 types of anthocyanins isolated from various types of plants and they have been identified, including pelargonidin, cyanidin, peonidin, delphinidin, petunidin, malvidin, and anthocyanidin glycosides Banana peels are rich in several antioxidants [11]. Banana peels also contain active compounds, namely tannins, saponins, flavonoids, and phenols [12]. Phenolic compounds are bioactive secondary metabolites that are channeled by cicamic acid, pentose phosphate, and the phenylpropanoid pathway [13]. Phenolic compounds are divided into subgroups of phenolic acids, flavonoids, tannins, and stilbenes based on the number of attached phenolic hydroxyl groups and the structure that links the benzene ring [14]. Phenolic compounds are known to be able to prevent and treat several diseases such as arteriosclerosis, brain dysfunction, cancer, and diabetes [15].

Several extraction methods can be used to extract polyphenols from banana peels, one of which is UAE (Ultrasound-Assisted Extraction) [16]. The UAE method under optimal conditions produces a good amount of phenolic when compared with the heat reflux method [17]. The maceration method requires a long extraction time and produces a smaller yield compared to the UAE method [18]. When using ultrasonics, no additional chemicals or other ingredients are needed [19]. The UAE method also doesn't cost too much [20]. Extraction using the UAE method also produces high yields [21]. The use of ultrasonics in the process of extracting organic compounds in plants and grains with organic solvents occurs quickly [22 – 26]. The cell walls of the material can be broken down with ultrasonic vibrations so that the

contents can be released easily [27]. The use of conventional methods for banana peel extraction can be seen in Table 1.

Table 1. Research on Various Types of Ultrasound-Assisted Extraction Methods

Raw Material	Method	Solvent	Extraction condition	Target Compound	Yield
Cratoxylum cromossum ssp. Formosum [28]	*Ultrasonic Assisted Extraction* (UAE)	Ethanol	Temperature 30, 45, 60, 75 °C, Time 10, 20, 30, 40, 50 minutes, Ratio 1:10, 1:20, 1:30, 1:40, 1:50 and Ethanol concentration 0, 25, 50, 75, 100%	Polyphenol	40.00±1.00 mg/100 g gallic acid
Pumpkin and peach [29]	*Ultrasonic Assisted Extraction* (UAE)	Methanol	Temperature 30, 40, 50°C, Time 10, 20, 30 minutes, and Power 30, 50, 70%	Polyphenol	44.09±1.09 mg/100 g gallic acid
Dayak Onion [30]	*Ultrasonic Assisted Extraction* (UAE)	Aquadest	Temperature 30°C, Frequency 40 kHz, Power 100%, Time 30, 45, 60, 75 minutes, solvent volume 200, 240, 280, and 320 mL	Polyphenol	2.20 mg GAE/gram Dayak onion
Kaffir Lime [31]	*Ultrasonic Assisted Extraction* (UAE)	Ethanol	Temperature 10, 20, 30°C and preeliminary treatment of kaffir lime peel consisting of dried orange peel and fresh orange peel	Essential oil	11.730%
Mangosteen Skin [32]	*Ultrasonic Assisted Extraction* (UAE)	Ethanol 96%	Time 15, 30, 45 minutes, and ultrasonic amplitudes 35, 50, 65%	Antioxidant	6.71%
Red Dragon Fruit Skin [33]	*Ultrasonic Assisted Extraction* (UAE)	Aquadest and Citric Acid	Time 15, 30, 45 minutes and Amplitudes 65% and 95%	Anthocyanin	24.074 ppm
Cocoa Shell [34]	*Ultrasonic Assisted Extraction* (UAE)	Aquadest and Ethanol	Temperature 50 – 60°C which was carried out for 3 hours	Antioxidant	Without treatment (88.38%), aquadest solvent (90.61%), ethanol solvent (92.78%)
Cocoa Shell [35]	*Ultrasonic Assisted Extraction* (UAE)	Ethanol 80%	Ethanol solvent 80% with ratio 1:10, frequency 40 kHz, 296 W, 55°C for 45 minutes	Antibacterial	107.8±27.0 µm
Cocoa Shell [36]	*Ultrasonic Assisted Extraction* (UAE)	Ethanol 96%	Operation Time 2×30 minutes	Antibacterial	2%
Pomegranate Peel [37]	*Microwave Assisted Extraction* (MAE)	Aquadest, Ethanol 50 and 70%. Methanol 50 and 70%	Aquadest, Ethanol 50 and 70%. Methanol 50 and 70%. Ratio 60/1 mL/g. Power 600 W	Polyphenol	199.4 mg GAE/g dry skin

Raw Material	Method	Solvent	Extraction condition	Target Compound	Yield
Lime Peel [38]	*Ultrasonic Assisted Extraction* (UAE) and *Microwave Assisted Extraction* (MAE)	Ethanol 55%	MAE, ethanol 55%, Power 140 W for 45 seconds with 8 repetition of extraction steps. UAE, ethanol 55%, amplitudes 38%, and time 4 minutes	Polyphenol	54.4 mg GAE/g
Chayote [39]	Percolation	Ethanol	Time 2, 3, and 4 hours. Ratio 1:5, 1:10, and 1:15. Ethanol concentration 30, 50, 70%	Polyphenol	2.50 mg EAG/g
Cocoa Shell [40]	Maceration	Ethanol and Acetone:Water	Ethanol solvent 70% and acetone:water (7:3)	Phenol, Tannin, and Flavonoid	Ethanol 70% = 6.948%. Acetone:water (7:3) = 8.327%
Kepok Banana Peel [41]	Maceration	Alcohol 97%	Banana peel water concentration 20%, 40%, 60%, 80%, and 100%	Antioxidant	60.50 ppm
Green Tea [42]	Maceration	Aquadest	Stirring 110 and 200 rpm. Size 80 – 100 mesh, Temperature 80°C	Polyphenol	29.3%
Arabica and Robusta Coffee Skin [43]	Maceration	Ethanol	Temperature 75, 85, and 95°C. Ratio 1:100, 2:100, and 3:100. 2 types of coffee arabica and robusta	Polyphenol	8.089%

The solvent used in this research is 96% ethanol, ethanol is an organic solvent that contains polarity according to anthocyanin [33]. Choosing an extraction time that is too long to extract banana peel will require more energy and can damage the bioactive compounds being extracted [44]. The length of extraction time is also in line with the quantity of extract produced, this is because the length of contact between the material and the solvent is greater [45]. When the material reaches the saturation point, the increase in extract yield will stop or run out [46]. Too long sonication time can increase the temperature of the solution compared to the rate of temperature reduction by the extraction temperature controller so that it can degrade the extracted anthocyanin content [47]. An increase in temperature during the extraction process is too high along with a reduction in viscosity and surface tension can cause only a few bubbles to burst [48]. The temperature in the ultrasonic bath is controlled by the external circulation of the water bath thermostat and a predetermined frequency [49]. Temperature control is very necessary for maintaining consistency of extraction temperature with the heat exchanger

principle [50, 51].

This research aims to determine the effect of extraction temperature, extraction time, and solvent–material ratio on the extraction of polyphenolic compounds from horn banana peels. The ultrasonic-assisted extraction method was chosen as a conventional extraction method because it is cheap, simple, and efficient.

2. Material And Methods

2.1 Materials

Horn banana peel was obtained from Klakah Village, Klakah District, Lumajang Regency, East Java. Ethanol with a concentration of 96%, distilled water, Na_2CO_3 (p.a. Merck), folin ciocalteu reagent (p.a. Merck), and gallic acid (p.a. Merck). All reagents were analytical grade and were used without further purification.

2.2 Equipment

The tools used are Ultrasonic Batch (BAKU BK-1200 1.47L), oven (Envilife), UV-Vis Spectrophotometer 752AP, Analytical Balance (Pioneer), and Blender (Philips HR-2115), glass beaker, spatula, aluminum foil, and watch glass.

2.3 Methods

2.3.1 Determination of Extraction Parameters

The research formulation design and response analysis were carried out using the Design – Expert V13 program with Response Surface Methodology (RSM) Box – Behnken Design. In this study, the dependent variable was the concentration of extracted polyphenolic compounds. The independent variables are extraction time, ratio of horn banana peel powder to solvent, and temperature with limit values of 10 to 30 minutes, 0.285 to 0.4 gram/mL, and 30 to 50°C respectively. These values are introduced to the program to obtain the selected combination as in table 2.

Table 2. A combination of Extraction Parameters was Used in this Study

Run	Time Extraction (minutes)	Ratio Banana Peel with Solvent (g/mL)	Temperature (ºC)
1	20	1:30	40
2	20	1:30	40
3	20	1:30	40
4	20	1:35	30
5	10	1:30	30

Run	Time Extraction (minutes)	Ratio Banana Peel with Solvent (g/mL)	Temperature (ºC)
6	30	1:25	40
7	20	1:35	50
8	30	1:30	50
9	20	1:25	30
10	30	1:30	30
11	10	1:25	40
12	10	1:35	40
13	20	1:30	40
14	10	1:30	50
15	20	1:25	50
16	30	1:35	40
17	20	1:30	40

2.3.2 Extraction of Horned Banana Peel Waste

The banana peel is cut into small pieces and then dried using an oven at a temperature of 50°C [52]. The water content produced during this drying process is 8.4%. The dried fruit skin is mashed using a blender (1). The sample is then sieved to the desired particle size at 80 mesh [23]. Banana peel powder was dissolved in 96% ethanol with a predetermined ratio (Table 2). The mixture was put into an extraction container and subjected to ultrasonic treatment with varying times and temperatures (Table 2) according to the following scheme (Figure 1). The mixture was filtered with filter paper and the filtrate obtained was collected as an extract.

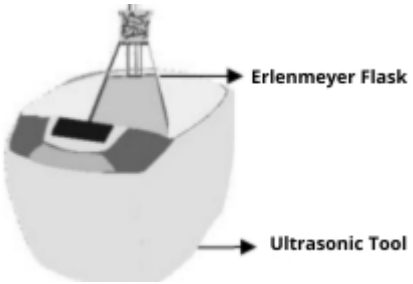


Figure 1. Schematic Representation of Ultrasonic Assisted Extraction of Horned Banana Peel Waste

2.3.3 Water Content Analysis

Determination of water content uses the drying method or thermogravimetric method, which is done by drying the material in an oven at a temperature of 105 – 110°C until a constant mass is obtained. Water content is defined as the mass ratio of the water phase to the solid phase, expressed as a percentage [53 – 55]. Calculation of the percentage of water content can be calculated using the formula:

$$\% \text{ Water content} = \frac{\text{Initial Mass} - \text{Final Mass (Constant)}}{\text{Initial Mass}} \text{ x } 100\ \% \qquad (1)$$

2.3.4 Determining Total Polyphenol Content

The total polyphenol content in each filtrate was determined using the Folin – Ciocalteu method of Norra *et al.* (2016) with slight modifications [60, 61]. This method is easy to perform, fast, and does not require expensive reagents [62]. The measurement begins by mixing 0.5 mL of 50% Folin – Ciocalteu reagent with 0,5 mL of sample filtrate. The mixture was stirred until homogeneous and left for 5 minutes, then 4 mL of 7.5% Na_2CO_3 and distilled water were added until the volume became 10 mL. samples were incubated for 30 minutes at room temperature. The absorbance of the filtrate was measured using a UV – Vis Spectrophotometer at a wavelength of 765 nm in triplicate [64, 64]. A Calibration curve for a standard solution of gallic acid with concentrations of 1, 2, 3, 4, and 5 ppm was created. Total polyphenol content was expressed as gallic acid equivalents in mg per gram dry weight of simplicial (mg GAE/g) [65]. Total polyphenol content can be determined using Equation 2 [66].

$$Total\ Polyphenol\ Content = \frac{Vs\ (ml) \times C\left(\frac{mg}{L}\right) \times Fp}{B} \qquad (2)$$

where Total polyphenol content in mg GAE/g, Vs is sample volume (mL), C is polyphenol concentration (mg/L), Fp is dilution factor, and B is mass of banana peel extract (g).

2.3.5 Data Analysis

This research used Design Expert 13 software and Response Surface Methodology (RSM) with Box Behnken type to determine the effect of parameters (extraction time, extraction temperature, and ratio between Horned powder and solvent) on total polyphenol content. A total of 17 combinations of extraction conditions will be applied to carry out an Analysis of Variance (ANOVA).

3. Results And Discussion

3.1 Extraction of Total Polyphenol Content from Horned Banana Peel

This research was carried out from November 2022 to March 2023 at the Basic and Process Laboratory, Chemical Engineering Study Program, Department of Mechanical Engineering, Faculty of Engineering, University of Jember. This research used Tanduk banana skin to be extracted and tested for total polyphenol content using the UAE extraction method. The water content value of the Banana Peel material is 8.4%. The total polyphenol content test in the extraction of horn banana peel

waste using the UAE method was carried out using the spectrophotometric method [67]. Spectrophotometric determination of polyphenol levels used Folin – Ciocalteu reagent. The hydroxyl group of the phenolic compound reacts with the Folin – Ciocalteu reagent to form a tungsten molybdenum complex blue which can be detected using a spectrophotometer. The addition of 7.5% Na_2CO_3 in the process of determining polyphenol levels aims to create alkaline solution conditions, this is because polyphenols and Folin – Ciocalteu can only react in alkaline conditions [68].

Determination of total polyphenol content using a standard solution, namely gallic acid (GAE). The use of gallic acid as a measurement standard is because gallic acid is a derivative of hydroxybenzoic acid which is a simple phenolic acid. The organic acid phenol content is pure and stable. The total polyphenol content is expressed in GAE (gallic acid equivalent), that is the equivalent number of milligrams of gallic acid in 1 gram sample [69]. Determination of total polyphenol levels in samples using the linear equation of the gallic acid standard curve, $y = 0.0863x + 0.0029$ ($R^2 = 0.9952$). An R-value that is close to 1 indicates the regression equation has approached linearity [70, 71]. The results of the analysis of the polyphenol content of Horned Banana Peel can be seen in Table 3

Table 3. Total Polyphenol Content in the Filtrate Resulting from the Extraction of Horned Banana Peels

Run	Extraction Time (minutes)	Ratio Banana Peel with Solvent (g/mL)	Temperature (ºC)	Polyphenol yield (mg GAE/g sample)
1	20	1:30	40	46.573
2	20	1:30	40	47.386
3	20	1:30	40	45.353
4	20	1:35	30	35.319
5	10	1:30	30	38.238
6	30	1:25	40	54.330
7	20	1:35	50	32.758
8	30	1:30	50	31.326
9	20	1:25	30	48.247
10	30	1:30	30	61.007
11	10	1:25	40	56.793
12	10	1:35	40	44.142
13	20	1:30	40	46.776
14	10	1:30	50	53.485
15	20	1:25	50	37.239
16	30	1:35	40	49.265
17	20	1:30	40	45.557

Based on the results of the research data in Table 3, it shows that the maximum total polyphenol content was obtained in the 10th running, 61.007 mg GAE/g sample, and the minimum total polyphenol content was obtained in the 8th running, that is 31.326 mg GAE/g sample. The 10th running obtained optimal results due to the maximum conditions in extracting polyphenols from horn banana peel waste, under the conditions of extraction time of 30 minutes, extraction temperature of 30°C, and the ratio of banana peel powder to solvent of 1:30. Phenolic compounds are the compounds most commonly extracted from horn banana peels. The total polyphenol content obtained from this research was greater when compared to research conducted by Ida (2018), the total polyphenol content of plantain peel extract had an average total phenolic content of 35 mg GAE/g [52]. In research conducted by Yulis (2020), the phenolic content of Muli banana peel extract was 100.016 mg GAE/g and Kepok banana extract was 32.176 mg GAE/g [12]. The difference in total levels of polyphenols produced is due to differences in extraction conditions and other factors that influence the extraction process, one of which is the type of banana peel extracted [72, 72].

3.2 Statistical Analysis

The results of research on polyphenol extraction from horned banana peel waste using the UAE method were analyzed by a Design Expert. Response surface analysis using Design Expert aims to determine appropriate data processing analysis. Data processing carried out included response analysis of total polyphenol content, Analysis of Variance (ANOVA) and also determining optimum extraction conditions [74]. Data on total polyphenol levels were then analyzed using Analysis of Variance (ANOVA) to prove that the parameters used in the extraction process could influence total polyphenol levels. ANOVA results are presented in Table 4.

Table 4. The Results of *Analysis of Variance* (ANOVA)

Source	Sum of Square	Df	Mean Square	F-Value	p-Value	
Model	1123.99	9	124.89	245.86	<0.0001	Significant
A-Temperature	98.02	1	98.02	192.97	<0.0001	
B-Times	1.34	1	1.34	2.63	0.1488	
C-Rasio d/p	154.22	1	154.22	303.60	<0.0001	
AB	504.63	1	504.63	993.43	<0.0001	
AC	17.84	1	17.84	35.12	0.0006	
BC	14.39	1	14.39	28.32	0.0011	
A^2	179.45	1	179.45	353.27	<0.0001	
B^2	162.55	1	162.55	320.00	<0.0001	

Source	Sum of Square	Df	Mean Square	F-Value	p-Value	
C^2	8.37	1	8.37	16.48	0.0048	
Residual	3.56	7	0.5080			
Lack of Fit	0.6306	3	0.2102	0.2874	0.8331	No significant
Pure Error	2.93	4	0.7313			
Cor Total	1127.54	16				

Based on the table above which was obtained using Design Expert software. The F Model value of 245.86 implies that the model is significant. There is only a 0.01% chance that an F value of this size could occur due to noise. The P – P-value of the model is <0.0001, this indicates that the model is significant. A significant model shows that the model has a real influence on the response to total polyphenol levels [75]. A model p-value of less than 0.05 indicates that the model is significant, while a model that isn't significant can be indicated by a p-value greater than 0,1. Variables said to be appropriate can be seen from the p-value. The results of the ANOVA data show that the temperature variable (A) and the ratio of banana peel powder to solvent (C) have significant values. This shows that the temperature variable and the ratio of banana peel powder to solvent have a real influence on the response of total polyphenol levels in the extraction process. Meanwhile, the time variable (B) has an insignificant value, this shows that the time variable doesn't have a real influence on the response of total polyphenol levels in the extraction process, this insignificant time variable is caused by the time range being too low compared to the optimum time.

The F-value of lack of fit is 0.2874, indicating that lack of fit isn't significant for pure error, the possibility of noise occurring from this value is 83.31. This shows that the model used to adjust the response variable is good at implying the relationship between the response value and the independent variable. Lack of fit which is not significant indicates that the model selection is appropriate because the appropriate model is lack of fit which is inversely proportional to the p-value [76]. Meanwhile, the value of the Sum of Square (SS) model is 1123.99. The sum of squares is a measure of the deviation of experimental data based on the average value of the data. This value is obtained from the deviation between the group average value and the overall average value. The SS value is used to calculate the Meam Square (MS) value. The MS value is obtained by dividing the SS value by df. The MS model value in the table above is 124,89 [77, 78].

Table 5. *Model Summary*

	R^2 *adjusted*	R^2 *predicted*
0.9968	0.9928	0.9870

Model analysis using design expert software shows that the model is appropriate. The criteria for a suitable model are shown by the difference between R^2 *adjusted* and R^2 *predicted.* Based on the table 5, the predicted R^2 of 0.987 is accordance with the adjusted R^2 of 0.9928. This is as expected because the corresponding difference value is less than 0.2. The research results can be declared according to the model if the resulting R^2 value exceeds 0.75 or is close to 1 [75, 79, 80]. The ANOVA results produce an R value of 0.9968, which indicates that the model is following the research results. The resulting R^2 *adjusted* value of 0.9928 shows that there is a strong relationship between the parameters of extraction time, extraction temperature of Horned Banana Peel powder, and the ratio of Horned Banana Peel powder to solvent on the response to total polyphenol levels [75]. Total polyphenol content in response to extraction parameters was modeled using Equation 3:

$$Total\ Polyphenol\ Content = 46.33 - 3.50A + 0.4088B + 4.39C - 11.23AB - 2.11AC - 1.90BC - 6.53A^2 + 6.21B^2 - 1.41\ C^2 \quad (3)$$

where,

A = temperature

B = time

C = ratio of banana peel powder to solvent

The values A, B, and C are respectively the parameters of extraction temperature, extraction time, and the ratio of Horn Banana Peel powder to solvent. If the parameter coefficient is negative then there is a decrease in the value of the total polyphenol content, and the opposite applies [75]. When the temperature decreases, the total polyphenol content also decreases, indicated by the temperature parameter (A) being negative. Meanwhile, when time and the ratio of horn banana peel powder to solvent increase, the total polyphenol content also increases as indicated by the time parameter (B) and the ratio of horn banana peel powder to solvent (C) is positive. Based on Equation 3, all extraction parameters statistically influence the total polyphenol content. The relationship between experimental data and model data is presented in Figure 2.

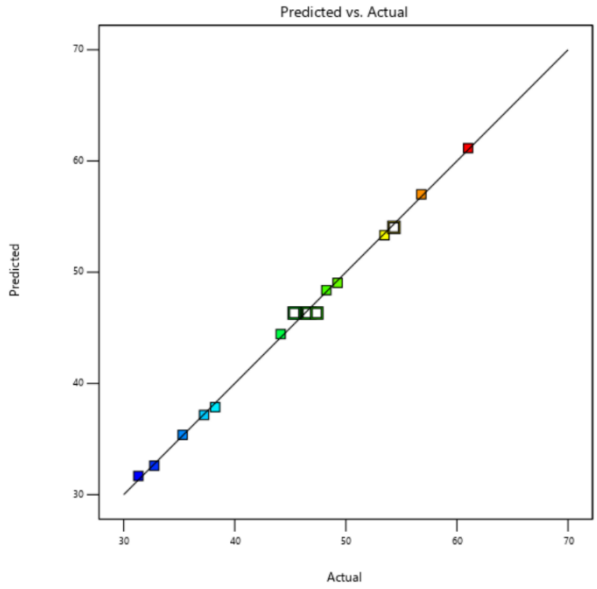
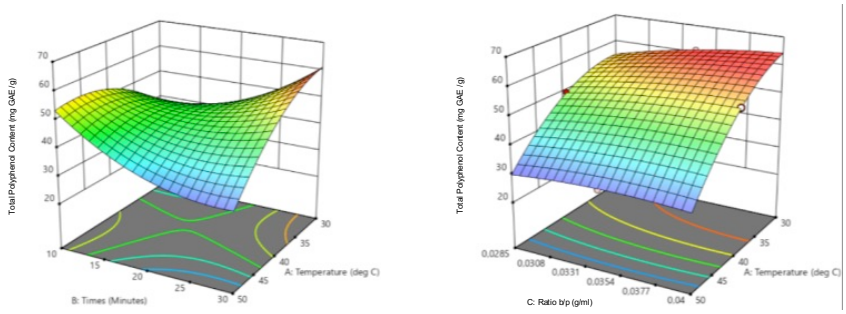


Figure 2. Graph of experimental data vs model data

Figure 2 shows that the graph of experimental data with model prediction data is very accurate, there is a strong correlation between experimental data and model data. The distance between the data and the trendline shows the accuracy of the data. The closer of the data is to the line, the more accurate the data will be [81]. Based on this research, the data plot touches a line which shows that the experimental data is close to the model data, supported by a R^2 value of 0.9968.

3.3 3D Surface Analysis of the Effect of Extraction Parameters on Total Polyphenol Content

a) b)



c)

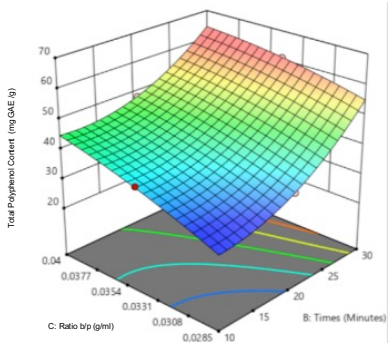


Figure 3. Respon Surface Analysis of Total Polypehenol Content as a Function of a) Extraction temperature and time, b) Extraction temperature and solvent ratio, and c) Extraction time and solvent ratio.

Figure 3 is a graph showing the effect of each extraction parameter (extraction time, extraction temperature, and ratio of Horned Banana Peel powder to solvent) on total polyphenol content. The figure shows that there is a combination of parameters that influence the response value through color differences. The optimum total polyphenol content resulting from this research was 61.007 mg GAE/g sample. Based on the total polyphenols obtained, it is estimated that the maximum total polyohenol extraction can be achieved when the combination of extraction temperature and extraction time is 30 °C and 30 minutes respectively.

The factor that influences extraction is extraction temperature. The increases temperature in the extraction process needs to be considered because high temperatures with long extraction times and exceeding the optimum limit can result in the loss of compound in the solution due to the evaporation process, and conversely if the extraction temperature is too low, it will result in not all active compounds being extracted from the material so that the results is low [86, 92]. Temperature conditions that are too high can thermally damage the target compound [84]. This is also in line with what was stated by Anal (2014), that at high

temperatures and longer extraction times, some phenolic compounds are likely to be oxidized and experience a decrease in extract yield [85]. Bioactive components such as polyphenols are not resistant to temperatures above 50°C, so they can changes in structure, physiochemical properties and the resulting extract is small, high heat results in the evaporation of some phenolic compounds resulting the decomposition of lycopenene, vitamins C and A [91]. Changes in the structure of polyphenols are characterized by not being detected when polyphenols are tested using a spectrophotometer. Low temperatures and fast extraction time also produce extracts that are not optimal so that the polyphenol extract obtained is low [87]. The higher extraction temperature ca result in greater solubility of phenol compounds in the ethanol solvent and also the higher temperature will result in the cell wall network of solid particles becoming softer, making it easier for teh solute to move into the solvent, but temperatures above 45°C will result in damage to the material being processed extract and undergo structural changes resulting in a decrease in total phenol [88, 89].

Winanta (2015) [90] explained that the longer the extraction time, the greater the quantity of material extracted due to the greater chance of contact between the material and the solvent, so that the resulting extract will increase to the saturation point of the solution [90]. Long extraction time produces a greater quantity of extract, this is because the contact time between the material and the solvent is longer [45]. However, when the material reaches the saturation point, the increase in extract yield will stop or run out [46]. Increasing the sonication time initially has a positive effect, but afterward the results tend to decrease over time [95]. In research conducted by Yilmaz and Tavman (2017), extraction time needs to be paid attention to in order to avoid the effects, because it can reduce yield due to decreased mass transfer due to cavitation bubbles [81, 94]. This condition caused by the maximum mass transfer that has been achieved and the degradation effect is caused by cavitaton which results in degradation of the solute [95, 96]. Cavitation is the formation of bubbles in a fluid flow due to a decrease in pressure in the fluid to below the saturated vapor pressure. Cavitation bubbles in the material are created from ultrasonic bubbles. When the bubble burst close to the cell wall, it results in the formation of a shock wave and liquid jets which cause the cell wall to rupture. This rupture of the cell wall causes components inside the cell to mix with the solution [93]. Extraction times that are too long result in the extract being hydrolyzed, while short extraction times result in not all active compounds being extracted from the material [33].

The ratio of ingredients to solvent is crucial because the amount of solvent used must be sufficient to dissolve the desired compound [97]. According to Figure 3, the ratio parameters of Horned Banana Peel powder to solvent show that the higher the ratio of horned banana peel powder to solvent, the higher the total level of polyphenols produced. These results are in accordance with the research conducted by Bancha *et al.* (2014) which stated that there was an increase in total polyphenol levels when using a ratio of ingredients to solvent from 1:30 to 1:50 g/mL [82]. This is also supported by the statement of Aulia & Wijanarko (2018), that the increase in total polyphenols increases as the solvent ratio increases [98]. However, according to Zhang *et al.* (2018) using a ratio of ingredients to solvent that is too high will cause a long extraction time [84]. Low total polyphenol yields from the extraction process can also caused by the presence of undesirable components [81]. The greater the liquid – solid ratio, the higher the mass transfer in the solvent due to the favorable concentration gradient during diffusion and the lower viscosity of the system increasing the cavitation effect [95].

4. Conclusions

Based on the research results, it can be concluded that the extraction parameters (extraction time, extraction temperature, and ratio of horned banana peel powder to solvent) influence the total polyphenol content. The optimum total polyphenol content from the results of this study was 61.007 mg GAE/g which was obtained under extraction conditions with an extraction temperature of 30°C, extraction time of 30 minutes, and a ratio of horned banana peel powder to solvent of 1:30 g/mL.

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Turmeric Leaves Extraction (*Curcuma Longa L.*) as a Natural Preservative Using Ultrasound-Assisted Extraction (UAE) Method

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Abstract. Turmeric leaves (*Curcuma longa L.*) contain phytochemical compounds that can be utilized as natural food preservatives or bioformalin due to their antibacterial properties. The extraction method used in this study is Ultrasound-Assisted Extraction (UAE), to determine the effect of variable sample-solvent ratio, time, and particle size on total flavonoid, tannin, and alkaloid compounds, as well as to determine the optimal shelf life of fresh tuna. This study used sample-solvent ratio variables of 1:10, 1:15, and 1:20; time variables of 10, 20, and 30 minutes; and particle size variables of 60, 80, and 100 mesh. The highest total flavonoid compound was obtained in the variable sample-solvent ratio of 1:20, the particle size of 100 mesh, and the time of 20 minutes at 98.076 mg/L. The highest total tannin compound was obtained in the variable sample-solvent ratio of 1:15, the particle size of 60 mesh, and the time of 10 minutes at 41.697 mg/L. The highest total alkaloid compound was obtained in the variable sample-solvent ratio of 1:10, the particle size of 100 mesh, and the time of 20 minutes at 10.092 mg/L. The optimum curing time for tuna is 36 hours at room temperature with variable sample-solvent ratio, time, and particle size of 1:20 g/mL, 20 minutes, and 100 mesh with 20% concentration. The running has the highest flavonoid compounds, so it can be concluded that flavonoid compounds have a major effect on the preservation process of tuna.

Keywords: *Turmeric leaves, phytochemical compounds, ultrasound-assisted extraction, and natural food preservatives.*

1. Introduction

Tuna is a type of fish that has a relatively high protein content and water content as well as dense fish meat. Tuna is prone to damage because it has a high-water content, so microorganisms multiply easily. The chemical content contained in tuna is 71-76.7% water

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content; 21.6-26.3% protein; and 1.45-3.4% ash content. Efforts made to inhibit the decay of tuna are smoking with conventional methods and the use of liquid smoke. Conventional smoking can lead to uneven flavor and concentration in the smoked part of the tuna. The time and temperature that are used are not uniform, so the smoking of the tuna is not perfect. The second method, smoking tuna using liquid smoke, provides a distinctive flavor and extends the shelf life of tuna. However, the liquid smoke used requires a purification process first because most of the materials used are waste [1]. Along with the development of technology and science, there is a recent method that is safer and more effective in preserving tuna, which is bioformalin. Bioformalin is a natural preservative derived from nature as a substitute for formalin, so it is safe to use to preserve fresh fish. The materials used in the manufacture of formalin come from plants, so the cost of bio-formalin production is relatively affordable. Natural compounds that can be utilized as antibacterials are flavonoids, tannins, alkaloids, saponins, and triterpenoids found in plants [2].

Turmeric (*Curcuma longa L.*) is a spice plant that can be used as herbal medicine in handling circulatory problems, anti-inflammatory, antimicrobial, and antibacterial. Research on turmeric plants currently only focuses on turmeric rhizomes, even though turmeric leaves also contain high phenolic compounds. Turmeric leaves extracted in this experiment are shown in Figure 1. Turmeric leaves can be utilized as an alternative to bioformalin which is safe for consumption by the human body because it has high antibacterial compounds. The mechanism of antibacterial compounds is to damage the cytoplasmic wall so that it can inhibit bacterial metabolism and denature the proteins contained in bacterial cells, causing the leakage of nutrients from the cells. Thus, bacterial cells will be inhibited from growing and then die [3]. The chemical composition contained in turmeric plants is 69.4% carbohydrates consisting of flour; 6.3% protein; 5.1% fat; 3.5% minerals; 13.1% water; and 3-5% curcuminoids [4]. Curcumin is classified as a phytochemical compound consisting of phenolic compounds including flavonoids, tannins, and alkaloids [5]. The percentage of phytochemical compounds contained in turmeric plants is flavonoid content of 2.71%; tannin content of 2.58%; and alkaloid content of 1.47% [6]. Antibacterial compounds found in turmeric leaves with the highest percentage are flavonoids.

Figure 1. *Curcuma longa L* [7].

The antibacterial ability of total flavonoid compounds is greater than the antibacterial ability of total tannin compounds. The strength of the antibacterial ability of a compound is due to the relation between the structure of the compound and the antibacterial ability. The structure of the tannin compound is larger than the structure of the flavonoid compound, so the tannin compound will be more difficult to bind to the active group because there are steric hindrances in the structure of the tannin compound. Tannin compounds have a working mechanism of antibacterial activity that involves disrupting protein transport in bacterial cells [8]. Alkaloids are phytochemical compounds with the lowest concentration in turmeric leaves. The ability of alkaloids as antibacterial is due to the ability of alkaloids to infiltrate DNA so that the constituent components of peptidoglycan in bacterial cells are disrupted, this causes the wall layer in bacterial cells to form imperfectly and results in cell death. Flavonoid compounds are considered more effective as antibacterials because flavonoids can directly interfere with the work functions of microorganisms by breaking down bacterial cell membranes so that bacteria cannot grow and survive [9].

Various extraction methods of phytochemical compounds have been improved from conventional methods to modern methods, including Ultrasound Assisted Extraction (UAE). The advantages of the UAE method are that it can increase the penetration speed of the solvent through the cell wall, the mass can move quickly, increase the yield obtained, uses low operating temperatures, requires a small amount of solvent, and short extraction time [10]. The use of the UAE method in this study is because some phytochemical compounds will be damaged when extracted at high temperatures. The high temperature will cause structural changes in flavonoid compounds resulting in a small yield [11].

Factors that affect the UAE extraction process are time, particle size, temperature, type, and amount of solvent. The extraction time is too long until it passes the optimum limit, which will cause the degradation of bioactive compounds and reduce the yield obtained [12]. According to Andriani *et al*, the use of temperature in the extraction process must be adjusted

to the material to be extracted. Extraction temperature and time that exceeds the optimum limit of the extracted material can result in bioactive compounds experiencing changes in chemical structure due to the oxidation process so that the yield produced is reduced [13]. According to Fachri *et al*, particle size is a variable that affects the yield of extracts obtained. The larger the particle size, the more surface area of the material in contact with the solvent so that the diffusion process will run quickly. The larger particle size will also cause a decrease in yield due to aggregation in the extraction process [14]. The more amount of solvent, the higher the yield produced. The more volume of solvent used, the less bioactive compounds will be extracted because the volume of solvent can cause microwaves to be focused on the solvent [12]. Table 1 represents previous research on the extraction of phytochemical compounds.

Table 1. Research on Phytochemical Compound Extraction

Materials	Methods	Results	Ref
Turmeric Leaves	Microwave Assisted Extraction (MAE)	This study used aquabidest solvent with variable power of 10%, sample-solvent ratio of 1:20 and time of 10 minutes to obtain total flavonoid compounds of 4.025 µg/g. Bioformalin was applied to meatballs with an optimum preservation time of 48 hours at room temperature.	[12]
Black turmeric rhizomes	Maceration	This study used 80% ethanol solvent. Maceration was carried out for 48 hours. The total flavonoid content was 2,775.650 mg/100-gram, total tannin was 2,578.140 mg/100-gram, alkaloid was 1,466.290 mg/100 gram.	[15]
Ruta graveolens Linn	Maceration	This study used 96% ethanol solvent. Flavonoid levels were produced at 1.67% and tannins at 7.04% maceration time for 24 hours.	[16]
Papaya leaves	Maceration	This study used 96% ethanol solvent with a sample-solvent ratio of 1:15, maceration time for 3 x 24 hours. The total flavonoid content was 9.41% and alkaloid content were 16.56%.	[17]
Palm fruit peels	Maceration	This study used 96% ethanol solvent and maceration was carried out for 4 days. The tannin content was 1.16%bb and alkaloid content were 930.120 mg/g extract.	[18]
Muntingia calabura L.	Microwave Assisted Extraction (MAE) and Ultrasound Assisted Extraction (UAE)	This study used 70% ethanol solvent. The use of MAE at 50% power, sample-solvent ratio of 1:25 produced flavonoid concentration of 132.410 mg/mL and the most optimal time was 5 minutes to produce flavonoid concentration of 91.669 mg/mL. The use of UAE with a frequency of 40kHz with a variable sample-solvent ratio of 1:10 obtained flavonoid concentration of 47.589 mg/mL and the optimal time was 10 minutes to produce flavonoid concentration of 56.777 mg/mL.	[19]
Tectona grandis	Ultrasound Assisted Extraction (UAE)	This study used an ultrasonic bath with a frequency of 40 kHz with 70% ethanol solvent. The most optimal total flavonoid compound content was obtained when the solvent ratio was 1:5 and the extraction time was 30 minutes as much as 6.688%	[20]

Materials	Methods	Results	Ref
Muntingia calabura L.	Soxhletation	This study used ethanol solvent with a sample-solvent ratio of 1:5 and an extraction temperature of 70°C. The tannin compound produced was 13.715 mg/L.	[21]
Avocado leaves	Ultrasound Assisted Extraction (UAE)	This study used ethanol solvent with a sample-solvent ratio of 1:10 and an extraction time of 40 minutes. The resulting tannin compound was 4.8 mg/L.	[22]
Etlingera elatior	Maceration	This study used ethanol solvent with an extraction time of 4 days. The alkaloid compound produced was 0.536 mg/L.	[23]

Phytochemical compounds can be obtained through an extraction process. The effectiveness of the extraction process depends on the type of solvent used [24]. The use of conventional extraction methods, such as maceration, takes longer and requires more solvent volume [25]. In addition, the MAE extraction process uses high temperatures so it will cause problems with the phytochemical compounds contained in turmeric leaves because they have thermolabile properties [26]. Based on these problems, an extraction method is needed that can extract phytochemical compounds in turmeric leaves by maintaining good extract quality, short extraction time, and producing high yields. However, it still maintains the concept of energy efficiency by using a small amount of solvent.

The novelty of this research is the extraction method, namely Ultrasound-Assisted Extraction (UAE). This study aims to determine the effect of sample-solvent ratio, time, and particle size on total flavonoid, tannin, and alkaloid compounds, as well as to determine the optimal storage time of fresh tuna. Aquabidest is used as a solvent in the extraction process because aquabidest is the result of a multistage distillation process (through 2 distillation processes) so its content is purer and safer as a solvent for food bioformalin. Based on the research of Susanti *et al*., showed that aquabidest solvent can produce the highest percent yield when compared to ethanol and methanol solvents. This is because aquabidest solvent has a higher solubility than ethanol and methanol solvents. The solubility of the solvent used in the extraction process can affect the percentage yield obtained. The high percentage of extract yield is because the aquabidest solvent can dissolve organic compounds contained in the plant optimally [27]. This extraction method utilizes ultrasonic waves that cause the formation of bubbles in the solvent to accelerate the rupture of cell walls and the release of bioactive compounds into the solvent more easily and quickly [28].

2. Metode

2.1 Materials

The materials used are aluminum chloride ($AlCl_3$) 10% Merck; aluminum foil Klin Pak; aquabidest solvent Merck; BCG solution Merck; caffeine Sigma-Aldrich; chloroform grade Pa

Smart-Lab; Folin-Ciocalteu reagent Merck; phosphate buffer pH 4.7 Supelco; quercetin Sigma-Aldrich; saturated sodium carbonate (Na_2CO_3) Merck; sodium hydroxide (NaOH) 1 M Supelco; sodium nitrite ($NaNO_2$) Merck; tannic acid Merck; tuna; and turmeric leaves from Bangsalsari, Jember.

2.2 Equipment

This research requires equipment, namely 60, 80, and 100 mesh test sieves; basin; BK-1200 digital ultrasonic bath; cuvette; filter paper; IKA rotary evaporator; Ohaus analytical balance; Pyrex glassware; Pyrex pipette; sharp oven; spatula; spoon; thermo scientific cimarec hot plate; UV-VIS spectrophotometric apparatus model 752AP; and vial bottle.

2.3 Research Variables

The independent variables used are the sample-solvent ratio of 1:10, 1:15, and 1:20; time of 10, 20, and 30 minutes; particle size of 60, 80, and 100 mesh, the fixed variables used are UAE frequency of 40 kHz, ultrasound temperature of 45℃, and water content of 4.5%, and the dependent variables used are total flavonoid compounds, total tannin compounds, and total alkaloid compounds.

2.4 Methods

2.4.1 Preparation of Equipment and Materials

Turmeric leaves were cut into pieces and dried using sunlight for 3 days to reduce the water content to obtain a water content of 4.5%. The dried turmeric leaves were then pulverized and sieved with 60, 80, and 100 mesh sieves.

2.4.2 Extraction Process

Turmeric leaves simplicial will be extracted using the UAE equipment to obtain phytochemical compounds with the help of aquabidest solvent. The extraction process uses three different variables to obtain optimal results. The extraction process using the UAE method is that 5 grams of turmeric leave simplicial mass is weighed three times for each particle size variable and put into a beaker glass, then aquabidest solvent is added with a sample-solvent ratio of 1:10, 1:15, and 1:20. The extraction process was carried out with an ultrasonic bath at a frequency of 40 kHz and a temperature of 45℃ for 10, 20, and 30 minutes. The filtrate obtained was filtered using filter paper and evaporated using a rotary evaporator at 75℃. The filtrate obtained was heated using an oven at 70℃ until the mass was constant. The turmeric leaf extract obtained will be tested for phytochemical compounds using UV-Vis

spectrophotometry [20]. The experiment was conducted in accordance with the results of the Design Expert Version 13 analysis using the Box Behnken Design method. The methods contained in Design Expert Version 13 consist of Composite Central Design (CCD), Box Behnken Design (BBD), and full three-level factorial design. The advantage of Box Behnken Design over the full three-level factorial design and CCD is that when using the same number of variables BBD produces a smaller number of experiments so it is more efficient and can reduce the cost of the experiment. Experiments using CCD, have the potential for failure in the process of forming nanoemulsion systems because there are extreme points of the test [29]. The selection of the BBD method in this study is because BBD is suitable for further research, while the CCD method will reveal new variables whose values are very complicated.

2.4.3 Measurement of Total Flavonoid Compounds

2.4.3.1 Preparation of Quercetin Standard Solution

Quercetin weighed as much as 10 mg and dissolved into 100 mL of aquabidest to obtain 100 ppm standard quercetin solution. A standard quercetin solution was prepared using several variables, such as 0, 20, 40, 60, and 80 ppm. The quercetin standard solution was taken as much as 0.5 mL using a pipette and then added 0.1 mL $NaNO_2$ 5%; 0.1 mL aluminum (III) chloride 10%; and 0.2 mL NaOH 1 M at minutes 0, 5, and 6. In the next stage, aquabidest was added until the total volume of the solution was 2.5 mL. The absorbance values of all standard solution concentrations were measured using a Uv-Vis spectrophotometer with a wavelength of 435 nm [12].

2.4.3.2 Preparation of Quercetin Standard Curve

The standard curve was made by correlating the concentration of standard quercetin solution with the absorbance shown in the measurement by UV-Vis spectrophotometry using a wavelength of 435 nm [12]. After making a standard curve, a linear regression equation will be obtained as Equation 1 as follows:

$$y = mx + c \quad (1)$$

2.4.3.3 Analysis of Total Flavonoid Compounds

Turmeric leaf extract of 100 mg was dissolved in 50 mL aquabidest solvent to obtain a solution concentration of 2000 ppm. 0.5 mL of the sample to be tested and then added 0.1 mL $NaNO_2$ 5%; 0.1 mL aluminum (III) chloride 10%; and 0.2 mL NaOH 1 M at minutes 0, 5, and 6. The next step, add aquabidest to a total solution volume of 2.5 mL. The solution was then

rested for 30 minutes. The absorbance of the calibration of the quercetin standard solution was measured by UV-Vis spectrophotometry using a wavelength of 435 nm. The total flavonoid compound content can be calculated by substituting the average absorbance value of the sample in Equation 1. The linear regression equation is obtained from the calibration curve of the quercetin standard solution that has previously been measured so that the total flavonoid compound is obtained [30].

2.4.4 Measurement of Total Tannin Compounds

2.4.4.1 Preparation of Tannic Acid Standard Solution

Tannic acid with an amount of 0.1 g was dissolved into 50 mL of aquabidest in a beaker glass, then the solution was poured into a 100 mL volumetric flask and aquabidest solvent until the limit mark. The tannic acid standard solution was made dilution variations of 0, 10, 20, 30, and 40 ppm. Each dilution variation was taken as much as 1 mL and put into a 10 mL volumetric flask which already contained 7.5 mL of aquabidest. Folin-Ciocalteau reagent was added to the flask as much as 0.5 mL. The solution was rested for 3 minutes, then 1 mL saturated Na_2CO_3 solution was added and the solution was rested for 15 minutes [31].

2.4.4.2 Determination of Maximum Wavelength

The maximum absorption wavelength can be determined by measuring the absorption of one of the standard solutions at a wavelength of 400-800 nm. The maximum wavelength is obtained if the wavelength produces the highest absorbance value [31].

2.4.4.3 Preparation of Tannic Acid Standard Curve

Various dilutions of the standard solution were read for tannin absorbance with a UV-Vis Spectrophotometer at the optimum wavelength of 640 nm. A standard curve was made by correlating the concentration of the standard solution with the absorbance resulting from measurements using a UV-Vis Spectrophotometer [31].

2.4.4.4 Analysis of Total Tannin Compounds

Turmeric leaf extract was weighed as much as 0.5 g, then dissolved in aquabidest until it reached a volume of 10 mL. Folin-Ciocalteau reagent was added to the flask as much as 0.5 mL. The solution was rested for 3 minutes, then 1 mL saturated Na_2CO_3 solution was added and the solution was rested for 15 minutes. The absorbance of the solution was measured using a UV-Vis Spectrophotometer at the maximum wavelength [31].

2.4.5 Measurement of Total Alkaloid Compounds

2.4.5.1 Preparation of Caffeine Standard Solution

Caffeine was weighed as 250 mg and dissolved into hot aquabidest, then diluted into a 250 mL volumetric flask to obtain a solution concentration of 1000 ppm. The solution was pipetted 2.5 mL and dissolved into aquabidest in a 25 mL volumetric flask to obtain a solution concentration of 100 ppm [32].

2.4.5.2 Determination of Maximum Wavelength

The absorbance of the caffeine solution was measured with a UV-Vis Spectrophotometer at a wavelength of 200-400 nm to determine the maximum wavelength. The maximum wavelength is obtained if the wavelength produces the highest absorbance value [32].

2.4.5.3 Preparation of Caffeine Standard Curve

A standard solution of 100 ppm caffeine was taken as much as 0; 0,3; 0,6; 0,9; 1,2; and 1.5 mL then diluted until the volume of the solution reached 10 mL so that the concentration of the standard solution was 0, 3, 6, 9, and 12 ppm. The standard solution was pipetted as much as 1 mL then added 1 mL phosphate buffer pH 4.7 and added 1 mL BCG solution, then the solution was extracted using 1.5 mL chloroform three times using vortex and separated chloroform phase. The extracted solution was then collected in a 10 mL volumetric flask and added chloroform to the limit mark [33]. The absorbance was measured using a UV-Vis Spectrophotometer at the optimum wavelength of 273 nm. A standard curve was created by correlating the concentration of the standard solution with the absorbance resulting from measurements using a UV-Vis Spectrophotometer [32].

2.4.5.4 Analysis of Total Alkaloid Compounds

Turmeric leaf extract was weighed as much as 10 mg and dissolved in 10 mL aquabidest to produce a solution concentration of 1000 ppm. The sample solution was pipetted as much as 1 mL and then added 1 mL phosphate buffer pH 4.7 and 1 mL BCG solution, then the solution was extracted using 1.5 mL chloroform three times using a vortex and separated the chloroform phase. The extracted solution was then collected in a 10 mL volumetric flask and added chloroform to the limit mark and the absorbance was measured using a UV-Vis Spectrophotometer at the maximum wavelength [33]. The absorbance that has been obtained is entered into the regression equation of the caffeine standard solution so that the total alkaloid compounds in turmeric leaves are known.

2.4.6 Determination of Optimum Shelf Time Based on Organoleptic Test

The extracted phytochemical compounds were then made into concentrations of 10% and 20%. Apply to fresh tuna. Tuna that has been washed with running water, then soaked in each treatment concentration of turmeric leaves extract (10% and 20%) for 2 hours. Soaking aims to allow the turmeric leaf extract to be absorbed by the flesh of fresh tuna (*E. affinis*). Fish that had been soaked with turmeric leaf extract was left in the open room for the specified time limit of 12 hours, 24 hours, and 36 hours at room temperature (25-28℃) and compared with fresh tuna without natural preservatives. Determination of the optimum shelf life was done by organoleptic test including aroma, texture, and color [34]. Analysis of the quality of tuna meat was carried out based on (SNI 01-2729.1-2006) by referring to the fresh fish organoleptic score sheet. Assessment includes meat (color and appearance), aroma, and texture. Fresh tuna has characteristics, such as color or appearance of brightly colored meat with no redness, a very fresh smell typical of tuna, and a solid texture and not slimy. The following is the assessment standard based on SNI 01-2729.1-2006:

a. Fresh: organoleptic score 7-9
b. Slightly fresh: organoleptic score 5-6
c. Not fresh: organoleptic score 1-3

3. Result and Discussion

3.1 Effect of Variables on Total Flavonoids

Determination of total flavonoid compounds using a standard solution of quercetin. Curve preparation of quercetin standard solution with concentrations of 0, 20, 40, 60, and 80 ppm and measured using a wavelength of 435 nm resulted in a linear regression equation as follows:

$$y = 0.00013x + 0.0645 \quad (2)$$

with an R-value of 0.9753. The R-value that is close to 1 indicates that the regression equation has met the linearity requirement [35]. Table 2 shows the total analysis of phytochemical compounds contained in turmeric leaves.

Table 2. Total Phytochemical Compounds of Turmeric Leaves

Run	Sample-Solvent Ratio (g/mL)	Particle Size (mesh)	Time (minutes)	Total Flavonoids (mg/L)	Total Tannin (mg/L)	Total Alkaloids (mg/L)
1	5 g/ 50 mL	60	20	55	41.261	6.246
2	5 g/ 75 mL	80	20	81.923	40.329	9.015
3	5 g/ 100 mL	100	20	98.076	37.428	8.092
4	5 g/ 75 mL	80	20	80.385	40.442	9.477

Run	Sample-Solvent Ratio (g/mL)	Particle Size (mesh)	Time (minutes)	Total Flavonoids (mg/L)	Total Tannin (mg/L)	Total Alkaloids (mg/L)
5	5 g/ 75 mL	80	20	87.307	40.355	8.707
6	5 g/ 50 mL	100	20	71.153	39.693	10.092
7	5 g/ 75 mL	100	10	87.300	39.083	7.630
8	5 g/ 50 mL	80	10	57.500	41.017	6.400
9	5 g/ 75 mL	80	20	80.076	39.972	9.323
10	5 g/ 75 mL	60	10	65	41.697	7.323
11	5 g/ 100 mL	60	20	89.615	40.077	9.169
12	5 g/ 75 mL	80	20	89.610	40.146	9.631
13	5 g/ 100 mL	80	10	82.692	40.703	8.400
14	5 g/ 100 mL	80	30	93.461	37.463	6.707
15	5 g/ 75 mL	60	30	77.308	39.501	6.861
16	5 g/ 50 mL	80	30	75.769	39.606	7.476
17	5 g/ 75 mL	100	30	91.153	37.185	6.553

Table 2 shows that the lowest total flavonoid compound content was obtained when the research parameters were 1:10 sample-solvent ratio, 60 mesh turmeric leaves particle size, and 20 minutes extraction time, which amounted to 55 mg/L. The highest total flavonoid compound was obtained when the research parameters were 1:20 sample-solvent ratio, 100 mesh turmeric leaves particle size, and 20 minutes extraction time, which amounted to 98.076 mg/L. Based on Table 2, it can be concluded that the extraction variables greatly affect the extraction process.

3.1.1 Statistical Analysis

The optimum variables in this study were analyzed using Box-Bahnken Response Surface Methodology (RSM) with 17 running. The resulting data in the form of total flavonoid levels were then analyzed using analysis of variance (ANOVA) on design experts. This analysis was conducted to determine whether the variables used in the extraction process could have a significant effect on total flavonoids. ANOVA results of total flavonoid levels with a linear model can be seen in Table 3 as follows:

Table 3. ANOVA Analysis for Flavonoid Content

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2158.81	3	719.60	39.79	< 0.0001	significant
A-Sample-Solvent Ratio	1441.98	1	1441.98	79.73	< 0.0001	
B-Particle Size	461.46	1	461.46	25.51	0.0002	
C-Time	255.36	1	255.36	14.12	0.0024	
Residual	235.12	13	18.09			
Lack of Fit	160.03	9	17.78	0.9472	0.5685	not significant
Pure Error	75.09	4	18.77			
Cor Total	2393.93	16				

In this study, the p-value of the feed-solvent ratio was <0.0001; particle size was <0.0001; and extraction time was 0.0002. Variables can be said to have a significant effect if

the probability value (p-value) of the analysis results is <0.05 or 5%, while the p-value results on the lack of fit show> 0.05. The P-value can be interpreted as the error value of the statistical calculation results [36]. Based on Table 3, the three variables show a p-value <0.05; so the model from the analysis in this study is said to significantly influence the flavonoid levels produced. This model produces a coefficient of determination (R^2) of 0.9018 or 90.18% which indicates that the linear model is appropriate and can be used to analyze the flavonoid content produced from the UAE extraction method. The R^2 value of >70% indicates that the experimental value is quite precise with the value predicted by the design expert by giving results that are close to the 100% value [37]. The R^2 value of 0.9018 indicates that sample-solvent ratio, particle size, and extraction time have an influence of 90.18% on flavonoid content. The adjusted R^2 value is 0.8791, while the predicted R^2 value is 0.8316 so it can be said to be appropriate because the difference is <0.2.

The result of ANOVA analysis is a regression equation that describes the relationship between the predicted response, total flavonoids (Y), and the variables tested. This can be expressed using a linear equation model as follows:

$$Y = 77.98 – 13.08A + 7.59B + 5.65C \quad (3)$$

Description: Y = Total flavonoids (mg/L)

A = Sample-solvent ratio (g/mL)

B = Particle size (mesh)

C = Time (minute)

The regression equation can be used to calculate the total flavonoid value if the variable values of solvent ratio, particle size, and time are different from the known values. The coefficients of sample-solvent ratio, particle size, and time show the amount of increase or decrease in the total flavonoid concentration value. Negative values of sample-solvent ratio, particle size, and time will decrease the value of total flavonoid concentration, while positive coefficients will increase the value of total flavonoids [38].

The relationship between the data generated from the experiment and the data from the design expert model can be seen in Figure 2 as follows:

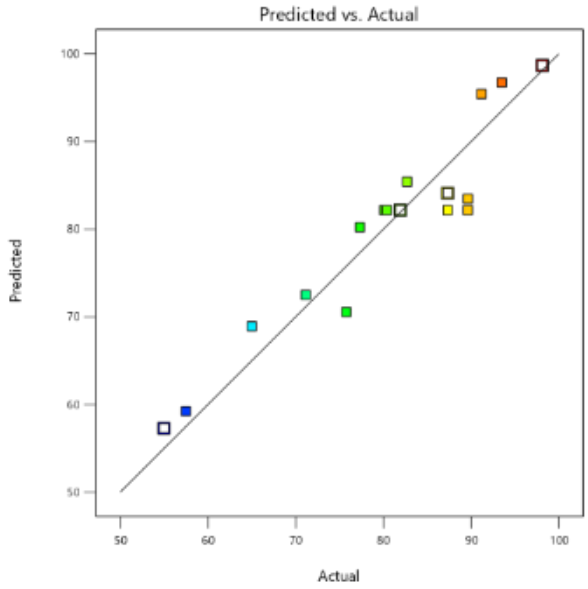
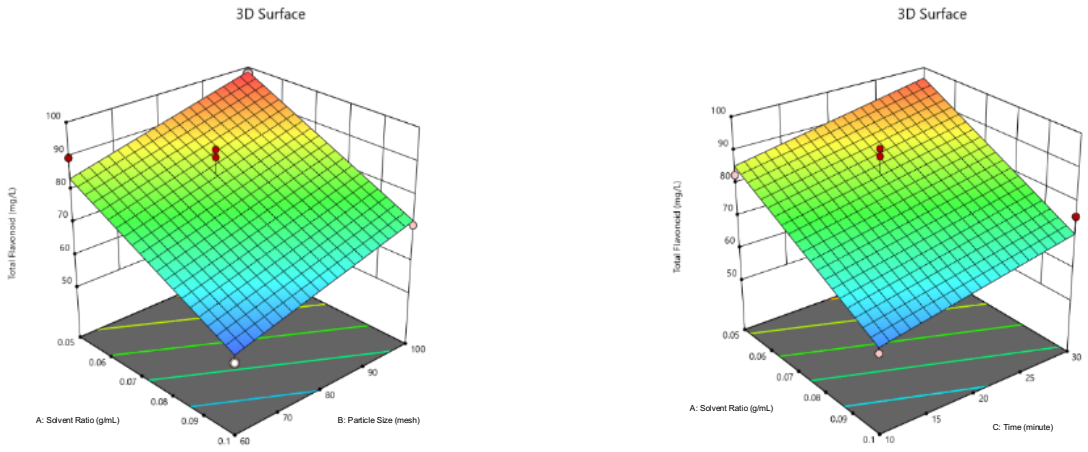


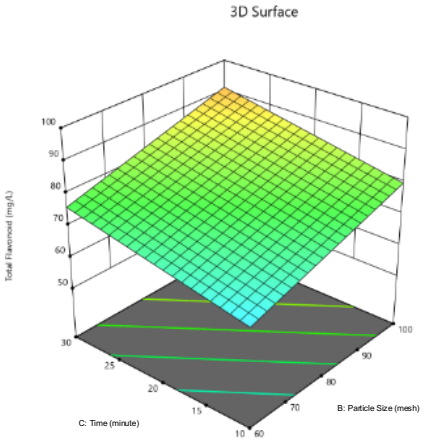
Figure 2. Relationship between model data and experimental data for flavonoid compound analysis

Figure 2 shows that the relationship between the experimental data that has been carried out and the predicted data is quite accurate, the layout distance between the experimental data and the tradeline shows fairly accurate data, this is characterized by the closer distance between the experimental data and the tradeline, the more accurate the data will be.

3.1.2 Effect of Extraction Parameters on Total Flavonoids



(a) (b)



(c)

Figure 3. Effect of extraction parameters on total flavonoid content (a) sample-solvent ratio with particle size, (b) sample-solvent ratio with time, (c) time with particle size

The effect of particle size on the extraction process is that the larger the mesh size or the smaller the particle diameter, the higher the flavonoid content produced, this is because the smaller the particle size in the sample, the solute will more easily reach the surface of the material to be extracted due to an increase in the contact surface area between the solid and the solvent. At smaller particle sizes, the solute diffusion path will be shorter on solid particles [39]. The next factor that can affect the flavonoid extraction process is the length of extraction time. The longer the extraction time, the higher the flavonoid content. This is because the longer the extraction time, the solvent can have a longer time to get into the particle cell wall and then remove the compounds in the particles so that the resulting flavonoid content will be higher. This is in line with research conducted by Isdiyanti *et al* [19]. The sample-solvent ratio can affect the flavonoid content produced, the greater the sample-solvent ratio used, the higher the flavonoid content produced. A higher sample-solvent ratio will increase the concentration gradient formed during the diffusion of solids into the solution, thereby increasing the extraction yield. This is in line with the research conducted by Mukti *et al* [12], that the optimum flavonoid content is produced at a sample-solvent ratio of 1:20.

Table 4. Comparison of Total Flavonoid Compound Results in Previous Studies

Raw material	Solvent	Method	Operating conditions	Total flavonoids (mg/L)	Reference
Turmeric Leaves	Methanol	Maceration	Extraction time 1x24 hours	48	[30]

Raw material	Solvent	Method	Operating conditions	Total flavonoids (mg/L)	Reference
Turmeric Leaves	Aquabidest	MAE	Sample-solvent ratio 1:20, power 10%, and extraction time 10 minutes	0.004	[12]
Turmeric Leaves	Aquabidest	UAE	Extraction time 20 minutes, particle size of turmeric leaves 100 mesh, and sample-solvent ratio 1:20	98.076	This research

Based on Table 4, the results of total flavonoid compounds of turmeric leaves from several literature are different. The total flavonoid compounds in this study showed higher results compared to the other two studies. This shows that the UAE extraction method is more optimal for extracting flavonoid compounds in turmeric leaves. The difference in results is due to differences in extraction conditions and methods used. In addition, extraction time and temperature also affect the total yield of flavonoid compounds obtained. The UAE method has the advantage of increasing the penetration speed of the solvent through the cell wall, the mass can move quickly, increasing the yield obtained, using low operating temperatures, requiring a small amount of solvent, and a short extraction time. Effendi's research [30] used the maceration method which was carried out at room temperature and did not use a benchmark for the amount of solvent used, so the solvent was added until the simplicial was submerged, while the research of Mukti *et al* [12], used the MAE method which was carried out with 199,5 watts of power. The use of a large wattage will also affect the total flavonoid compounds that can be extracted. This is because flavonoid compounds are susceptible to high temperatures.

3.2 Extraction of Total Tannin Content from Turmeric Leaves

Determination of total tannin compounds was carried out with a UV-Vis spectrophotometer using the Folin-Ciocalteu reagent. The standard solution used in the determination of total tannin compounds is tannic acid. The tannic acid standard solution curve was made with a solution concentration of 0, 10, 20, 30, and 40 and measured using an optimum wavelength of 640 nm to produce a linear regression equation as follows:

$$y = 0.0574x + 0.0946 \quad (4)$$

The equation is obtained by plotting the wavelength with the concentration of the solution. With the R-value of 0.9948. The R-value that is close to 1 indicates that the regression equation has met the linearity requirement [35].

Based on Table 2, the lowest total tannin content was obtained when the research parameters were 1:15 sample-solvent ratio, 100 mesh turmeric leaves particle size, and 30 minutes extraction time, which amounted to 37.1847 mg/L. The highest total tannin compound was obtained when the research parameters were 1:15 sample-solvent ratio, 60 mesh turmeric leaves particle size, and 10 minutes extraction time, which amounted to 41.6968 mg/L. The difference in the results of total tannin compounds in each experiment is due to the parameters used.

3.2.1 Statistical Analysis

Analysis of variance (ANOVA) with a quadratic model was used to analyze the data of total tannin compounds. In this study, the p-value of the sample-solvent ratio was <0.0001; particle size was <0.0001; and extraction time was <0.0001. Variables can be said to have a significant effect if the probability value (p-value) of the analysis results is <0.05 or 5%, while the p-value results in the lack of fit show >0.05. Based on Table 5, the three variables show a p-value <0.05; so, the model from the analysis in this study is said to significantly influence the tannin content produced. This model produces a coefficient of determination (R^2) of 0.9843 or 98.43% which indicates that the model is suitable and can be used to analyze the tannin content produced from the UAE extraction method. The R^2 value of >70% indicates that the experimental value is quite precise with the value predicted by the design expert by giving results that are close to 100%. The R^2 value of 0.9843 indicates that sample-solvent ratio, particle size, and extraction time have an influence of 98.43% on flavonoid content. The adjusted R^2 value is 0.9641, while the predicted R^2 value is 0.8079, so it can be said to be appropriate because the difference is <0.2.

Table 5. ANOVA analysis for tannin content

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	27.37	9	3.04	48.78	< 0.0001	significant
A-Sample-solvent ratio	4.36	1	4.36	69.93	< 0.0001	
B-Particle size	9.19	1	9.19	147.46	< 0.0001	
C-Time	8.04	1	8.04	129.03	< 0.0001	
AB	0.3393	1	0.3393	5.44	0.0524	
AC	0.7200	1	0.7200	11.55	0.0115	
BC	0.0221	1	0.0221	0.3548	0.5702	
A^2	0.6008	1	0.6008	9.64	0.0172	
B^2	0.9800	1	0.9800	15.72	0.0054	
C^2	0.6736	1	0.6736	10.80	0.0134	
Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Residual	0.4364	7	0.0623			
Lack of Fit	0.2942	3	0.0981	2.76	0.1759	not significant

Source	Sum of Squares	df	Mean Square	F-value	p-value
Pure Error	0.1422	4	0.0356		
Cor Total	27.81	16			

The amount of total tannin compounds (Y) as the response of the tested variables can be described in the equation model as follows:

$$Y = 40.53 + 0.7382A - 1.10B - 1.03C + 0.2841AB + 0.4138AC + 0.0744BC - 0.432A^2 - 0.4824B^2 - 0.4C^2 \quad (5)$$

Description: Y = Total tannin (mg/L)

A = Sample-solvent ratio (g/mL)

B = Particle size (mesh)

C = Time (minute)

The regression equation can be used to calculate the total tannin value if the variable values of the sample-solvent ratio, particle size, and time are different from the known values. The relationship between the data generated from the experiment and the data from the design expert model can be seen in Figure 4 as follows:

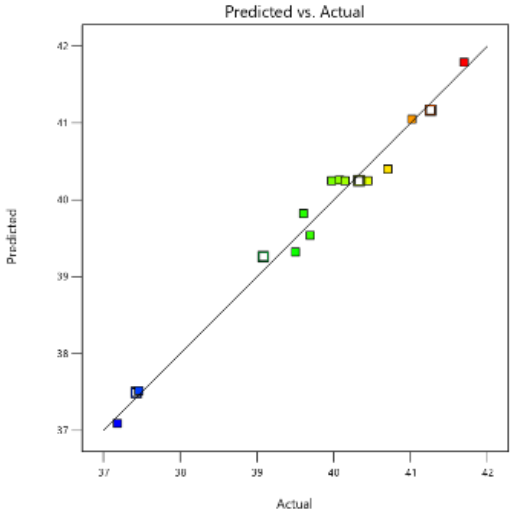


Figure 4. Relationship between model data and experimental data for tannin compound analysis

Figure 4 shows that the relationship between the experimental data that has been carried out and the predicted data is quite accurate, the layout distance between the experimental data and the tradeline shows fairly accurate data, this is characterized by the closer distance between the experimental data and the tradeline, the more accurate the data will be.

3.2.2 Effect of Extraction Parameters on Total Tannins

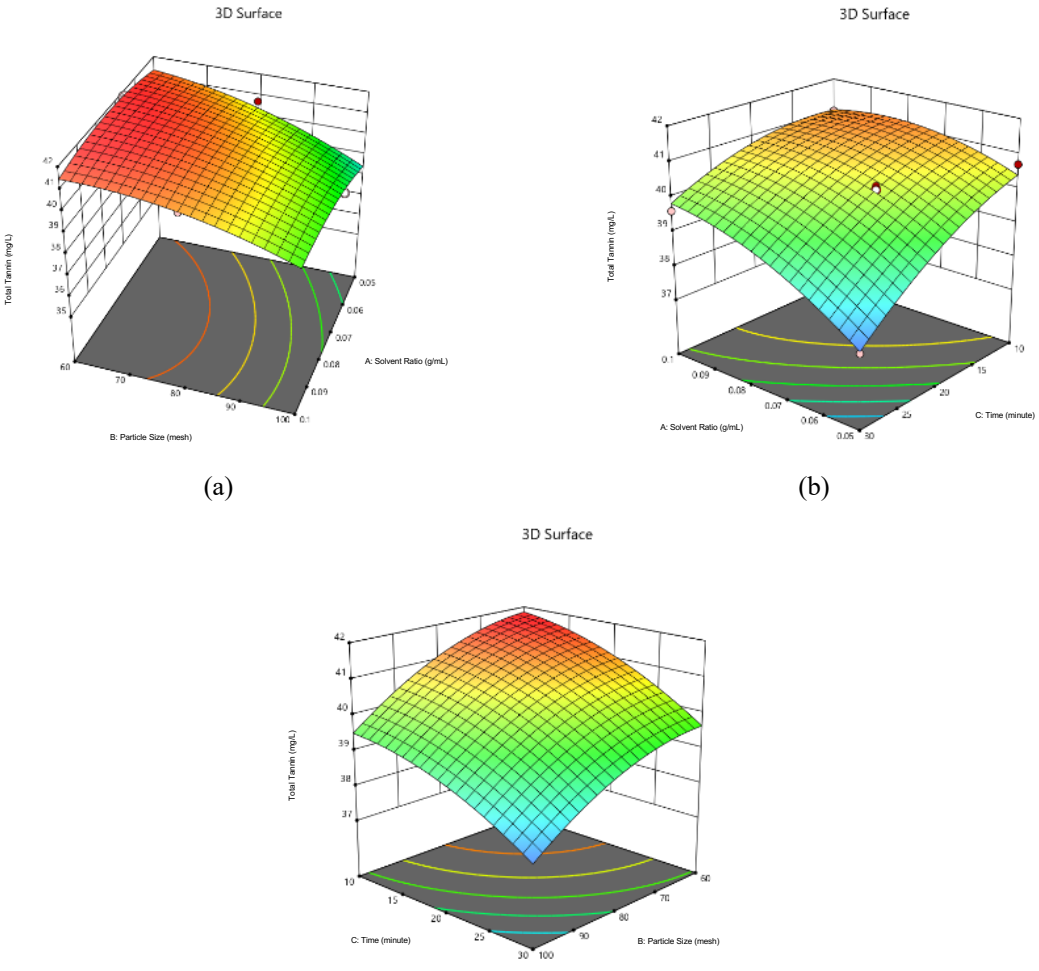


Figure 5. Effect of extraction parameters on total tannins (a) sample-solvent ratio with particle size, (b) sample-solvent ratio with time, (c) time with particle size

Based on Figure 5, it can be concluded that the total tannin content will decrease along with the longer the extraction time and the smaller the particle size (increasing mesh). This is in line with the research of Suharti, *et al*., that the longer time will cause tannin compounds to experience damage due to hydrolysis during the extraction process accompanied by a continuous heating process [40]. Based on the research of Rosalina, *et al*., the use of extraction time below 15 minutes aims to ensure that the media temperature during extraction does not exceed 45°C. The smaller the particle size, the greater the density between particles and the smaller the distance between particles. In this study, it can be seen that a particle size of 70 mesh produces tannin compounds that are less than optimal. This is because tannins are polar so in particle sizes below 70 mesh and short extraction times, tannin compounds will be extracted optimally. However, it is different from flavonoid compounds which require a particle

size of 100 mesh and a longer extraction time to extract flavonoid compounds optimally because flavonoids are semi-polar. The particle density can cause large obstacles to the ultrasonic waves during the propagation process towards the material. Thus, the higher the density of the material, the lower the propagation ability of the ultrasound waves [41]. The amount of tannin compounds will increase as the sample-solvent ratio increases, but the amount of tannin compounds will decrease when the volume of solvent used is excessive. This is in line with research conducted by Buanasari, that the more solvent used will inhibit the energy transfer process from ultrasonic waves to the solvent [42]. The solvent used in the extraction of tannin compounds is less than the extraction of flavonoids because the percentage of tannin compounds in turmeric leaves is lower at 2.58% compared to the percentage of flavonoids at 2.71%.

3.3 Extraction of Total Alkaloid Content from Turmeric Leaves

Determination of the total alkaloid compounds of turmeric leaves is done with two stages, including liquid extraction and UV-Vis spectrophotometer. The standard solution used in the determination of total alkaloid compounds is caffeine because caffeine is a xanthine group alkaloid compound with a crystalline form, easily soluble in water, and has a distinctive aroma and bitter taste [43]. Curve preparation of caffeine standard solution was carried out with solution concentrations of 0, 3, 6, 9, and 12 ppm and measured using an optimum wavelength of 273 nm to produce a linear regression equation as follows:

$$y = 0.0065x + 2.2984 \quad (6)$$

with an R-value of 0.9712. The R-value that is close to 1 indicates that the regression equation has met the linearity requirement [35].

Based on Table 2, the lowest total alkaloid compound content was obtained when the research parameters were 1:10 sample-solvent ratio, 60 mesh turmeric leaves particle size, and 20 minutes extraction time, which amounted to 6.246 mg/L. The highest total alkaloid compound was obtained when the research parameters were 1:10 sample-solvent ratio, 100 mesh turmeric leaves particle size, and 20 minutes extraction time, which amounted to 10.092 mg/L. Based on these data, it can be concluded that the extraction variables greatly affect the extraction results.

3.3.1 Statistical Analysis

Analysis of variance (ANOVA) with a quadratic model was used to analyze data on total alkaloid compounds. This study shows the resulting p-value is 0.0001; so the model of

analysis gives a real influence on the total alkaloid content. The variables of sample-solvent ratio, particle size, and extraction time have a p-value analysis result of 0.0507; 0.0022; and 0.2178 respectively. P-value <0.05 indicates that the model used is significant, while the insignificant model is characterized by a p-value greater than 0.1. From these data, it can be interpreted that the sample-solvent ratio and particle size variables have a real influence on the total alkaloids produced. While the time variable is insignificant it can be interpreted that the time variable has no real influence on the total alkaloids produced. This is expected because when alkaloid extraction uses ultrasonics, the time required is shorter, in contrast to the maceration extraction method. Ultrasonication produces mechanical agitation, cavitation, and thermal effects that can enhance the extraction process and the release of bioactive compounds. However, long ultrasonic times can cause degradation of the compounds present in the extract [44]. The results of the ANOVA analysis of tannin compounds are presented in Table 7 below:

Table 7. ANOVA analysis for alkaloid content

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	24.80	9	2.76	26.35	0.0001	significant
A-Sample-solvent ratio	0.5800	1	0.5800	5.55	0.0507	
B-Particle size	2.31	1	2.31	22.08	0.0022	
C-Time	0.1917	1	0.1917	1.83	0.2178	
AB	6.85	1	6.85	65.50	< 0.0001	
AC	2.02	1	2.02	19.35	0.0032	
BC	0.0946	1	0.0946	0.9043	0.3733	
A^2	0.2542	1	0.2542	2.43	0.1629	
B^2	1.02	1	1.02	9.76	0.0167	
C^2	11.41	1	11.41	109.16	< 0.0001	
Residual	0.7319	7	0.1046			
Lack of Fit	0.1817	3	0.0606	0.4404	0.7368	not significant
Pure Error	0.5502	4	0.1376			
Cor Total	25.53	16				

This model produces a coefficient of determination (R^2) of 0.9713 or 97.13% which indicates that the model is suitable and can be used to analyze the alkaloid content resulting from the UAE extraction method. The R^2 value of >70% indicates that the experimental value is quite precise with the value predicted by the design expert by providing results that are close to 100%. The adjusted R^2 value is 0.935, while the predicted R^2 value is 0.854 so it can be said to be appropriate because the difference is <0.2.

The total amount of alkaloid compounds (Y) as the response of the tested variables can be described in the Equation 7 model as follows:

$$Y = 9.17 - 0.2692A + 0.5502B - 0.1585C + 1.28AB + 0.6936AC - 0.1537BC - 0.2810A^2 - 0.4924B^2 - 1.65C^2 \quad (7)$$

Description: Y = Total alkaloids (mg/L)

A = Sample-solvent ratio (g/mL)

B = Particle size (mesh)

C = Time (minute)

The regression equation can be used to calculate the total alkaloid value if the variable values of the sample-solvent ratio, particle size, and time are different from the known values. The relationship between the data generated from the experiment and the data from the design expert model can be seen in Figure 6 as follows:

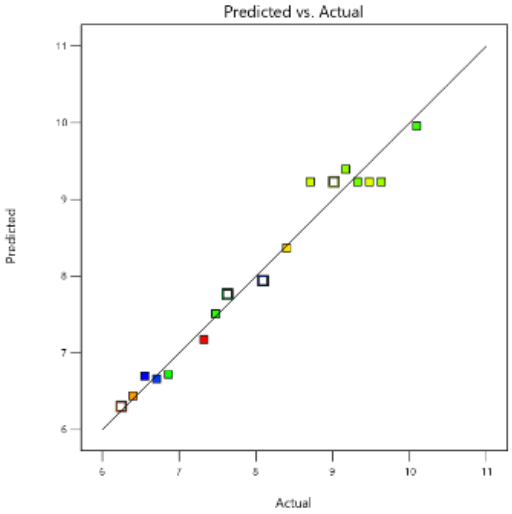


Figure 6. Relationship between model data and experimental data for analysis of alkaloid compounds

Figure 6 shows that the relationship between the experimental data that has been carried out and the predicted data is quite accurate, the layout distance between the experimental data and the tradeline shows fairly accurate data, this is characterized by the closer distance between the experimental data and the tradeline, the more accurate the data will be.

3.3.2 Effect of Extraction Parameters on Total Alkaloids

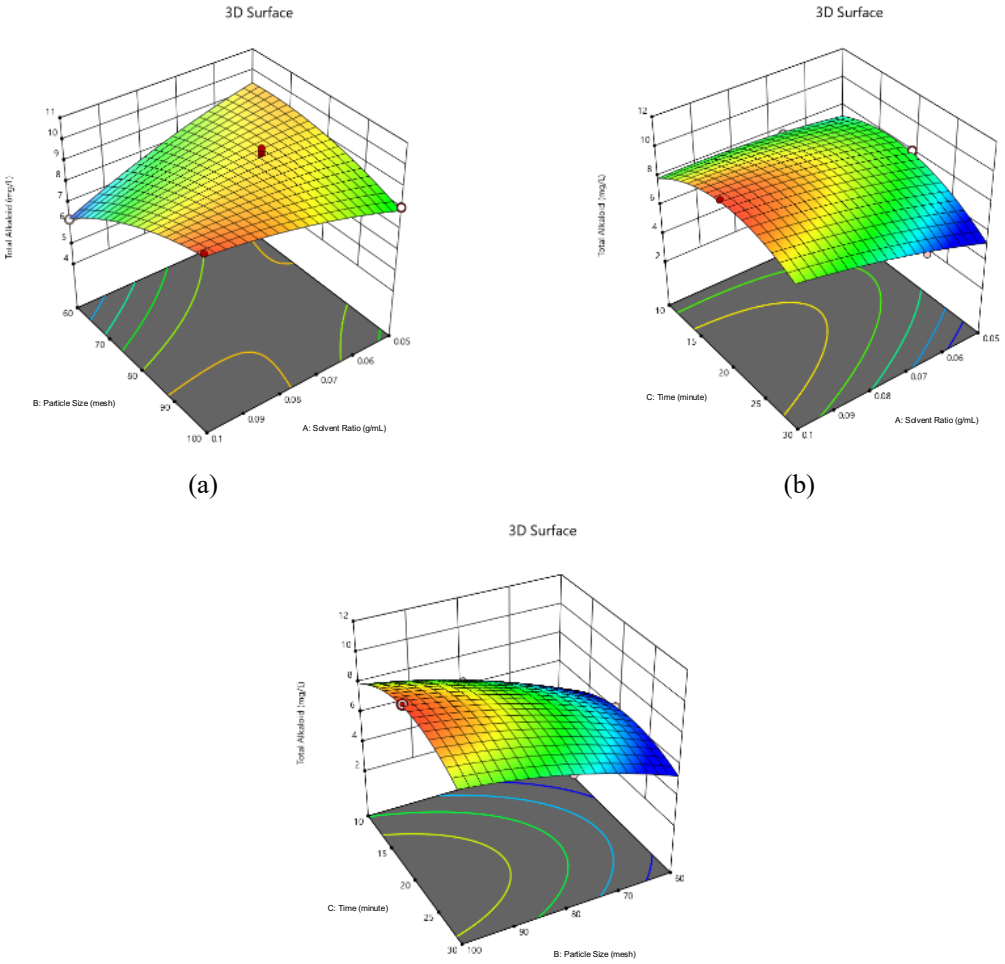


Figure 7. Effect of extraction parameters on total alkaloids (a) sample-solvent ratio with particle size, (b) sample-solvent ratio with time, (c) time with particle size

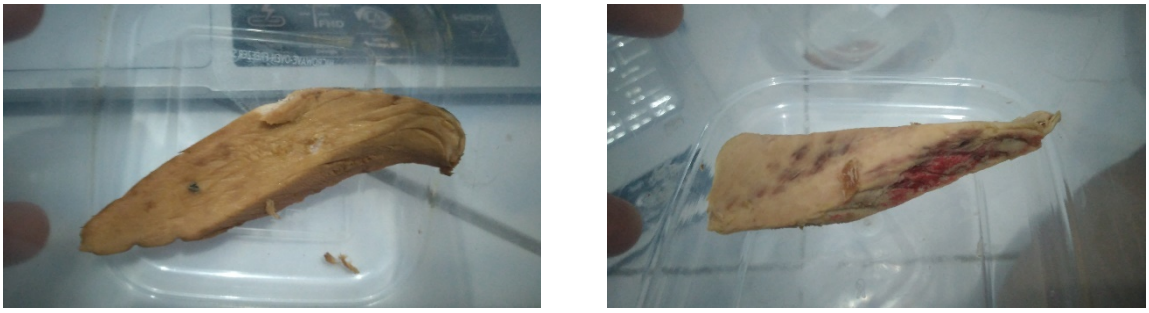
Based on Figure 7, the longer the extraction time, the more the obtained alkaloid compounds from a material. However, the longer extraction time will cause the extraction temperature to increase. Increased extraction time can provide an opportunity for the solvent to absorb ultrasonic waves for a longer time so that the heat generated from the process will increase the diffusion process of chemical compounds into the solvent. In this study, the total yield of alkaloid compounds increased from 10 minutes to 20 minutes of extraction time but decreased at 30 minutes of extraction time. This is because the percentage of alkaloids in turmeric leaves is small, so at an extraction time of 20 minutes the alkaloid compounds have been extracted perfectly. The use of a high sample-solvent ratio can increase the solvent's ability to absorb ultrasonic waves and convert them into heat energy so that it will easily extract

alkaloid compounds. However, the use of high solvent volumes can result in excessive swelling of the simplicial so that phytochemical compounds cannot be extracted optimally. The solvent used in the extraction of alkaloid compounds is less than the extraction of tannins because the percentage of alkaloid compounds in turmeric leaves is lower at 1.47% compared to the percentage of tannins which is 2.58%. Therefore, in this study the sample-solvent ratio of 1:10 produced the highest alkaloid compounds compared to the sample-solvent ratio of 1:15 and 1:20. This is consistent with the research of Mukhaimin *et al*. which states that the temperature of alkaloid degradation varies with each extraction method, if the extraction method used is direct heating of the material, then high temperatures can damage the alkaloid compounds contained in the material. In addition, modern extraction methods using high volumes of solvents can cause low yields of alkaloid compounds [45]. Extraction with the UAE method using a particle size of 100 mesh produces higher alkaloid levels of 10.092 mg/L compared to using a particle size of 60 mesh which is 6.246 mg/L. The opportunity to get high extraction results is by reducing the particle size, because the smaller the particle size (the larger the mesh), the surface area of a component increases so that the solvent can easily penetrate the wall of the material and bind the alkaloid compounds in it. This is following research conducted by Damanik *et al*., that the extraction process using a particle size of 100 mesh can produce a larger extract than with a particle size of 60 mesh. The smaller particle size will increase the number of pores formed in the sample powder so that the solubility of a substance can increase and the amount of solvent absorbed in the sample increases [46].

3.4 Analysis of Organoleptic Test

According to the results of smell, texture, and color analysis on tuna, it can be concluded that all treatments obtained the same results in the form of a decrease in quality value every 12 hours. Without the addition of bioformalin, tuna only lasts for 12 hours at room temperature. If more than 12 hours, tuna without bioformalin has characteristics such as slimy, moldy, rotten aroma, hard texture, and the meat turns red. Meanwhile, tuna with the addition of bioformalin can last for 36 hours at room temperature. If more than 36 hours, tuna with bioformalin starts to experience brownish discoloration, slimy texture, and slightly rotten aroma. The addition of bioformalin extract with a concentration of 20% is considered more optimal in the process of storing tuna compared to the addition of bioformalin with a concentration of 10%. The variables of sample-solvent ratio, time, and particle size respectively that produce optimum preservation time are 1:20 g/mL, 20 minutes, and 100 mesh. The highest rating was obtained for tuna added

with 20% turmeric leaf extract with variable ingredient ratios of 1:20, 100 mesh, and 20 minutes, namely a score of 9 for color, 8 for smell, and 8 for texture. The running has the highest flavonoid compounds, so it can be concluded that flavonoid compounds have a major effect on the preservation process of tuna. This is in line with research conducted by Rumayar *et al*. that the higher the concentration of extract used, the more optimal the storage time because it has good stability [47].



(a) (b)

Figure 8. Color difference in tuna with the addition of natural preservatives (a) and without the addition of natural preservatives (b)

4. Conclusions

Based on the results of this study, it can be concluded that the extraction variables sample-solvent ratio, time, and particle size greatly affect the total flavonoid, tannin, and alkaloid compounds. The highest total flavonoid compounds were obtained in the research variables of 1:20 sample-solvent ratio, 100 mesh turmeric leaves particle size, and 20 minutes extraction time, which amounted to 98.076 mg/L. The highest total tannin compound was obtained when the research variables were 1:15 sample-solvent ratio, 60 mesh turmeric leaves particle size, and 10 minutes extraction time, which amounted to 41.697 mg/L. The highest total alkaloid compound was obtained when the research variables were 1:10 sample-solvent ratio, 100 mesh turmeric leaves particle size, and 20 minutes extraction time, which amounted to 10.092 mg/L. The optimum preservation time was 36 hours at room temperature with variable sample-solvent ratio, time, and particle size of 1:20 g/mL, 20 minutes, and 100 mesh with 20% concentration. Running has the highest flavonoid compounds, so it can be concluded that flavonoid compounds have a major effect on the preservation process of tuna because flavonoids have the most important role in the preservation of tuna.

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Microwave-Assisted Hydrolysis of Robusta Coffee Parchment as a Reducing Sugar Feedstock

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Abstract. The hydrolysis process is a process of breaking down substances by reacting using water, the aim is to break down the substance. This research focuses on the hydrolysis process to determine the reducing sugar content of coffee parchment. The method used is microwave-assisted hydrolysis. This method can increase lignin release more effectively than conventional methods in the process of cellulose and hemicellulose hydrolysis. The most commonly used hydrolysis to hydrolyze cellulose is acid hydrolysis. This research uses coffee parchment raw material (horn skin) which contains cellulose and hemicellulose with HCl solvent. Hydrolysis with acid concentration is (1, 2, and 3%), microwave power (150, 300, and 450 W), and time (20, 25, and 30 minutes). In this study, the optimal reducing sugar yield was 8,054 g/mL under the operating conditions of 25 minutes, 3% HCl concentration, and 450 W microwave power.

Keywords: *Hydrolysis, coffee parchment, microwave-assisted hydrolysis, and reducing sugar.*

1. Introduction

Over time the need for fuel has increased, requiring alternative fuels. Fossil fuel reserves are dwindling, even though people's needs are increasing. In addition, as fuel oil becomes increasingly expensive, there is a growing need for alternative fuel sources such as biomass. Currently, Indonesia's petroleum reserves are only 1% of the world's oil reserves, therefore there is a need for other alternatives to replace the use of kerosene and LPG gas which are currently still widely used by the community [1]. Second-generation bioethanol with cellulose raw materials derived from various biomass materials is one form of alternative energy. The potential of cellulose waste from wood and non-wood materials is the most abundant natural resource, one of which is coffee parchment [2].

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Based on data from the Central Statistics Agency (BPS), coffee availability in Indonesia in 2020 reached 99.33% when combined with state-owned and private plantations. This shows the amount of coffee production that is exported both domestically and abroad. The availability of 45% coffee parchment in East Java, especially in the area around Bondowoso [3]. Coffee parchment as raw material can produce bioethanol with 38.68% content after going through hydrolysis and fermentation processes. The advantage of cellulose conversion into various products can also reduce environmental pollution problems due to the accumulation of agricultural biomass waste that is not optimally utilized [4]. Bioethanol is an organic fuel derived from plant materials through the natural process. However, there are various methods for producing bioethanol, such as the fermentation process involving microorganisms [5]. Bioethanol includes wood waste, agricultural waste, plantations, forest products, and organic components from industry and households [6].

As one of the alternative fuel sources processed from plants, bioethanol has the advantage of being able to reduce CO_2 emissions [7]. According to Wusnah et al, 2019 [8], sources of bioethanol are starch-containing plants (such as cassava, oil palm, tengkawang, coconut, kapok, jatropha, rambutan, soursop, malapari, and nyamplung), sugary (such as molasses, palm sap, sugarcane sap, and sweet sorghum sap) and cellulose fibers (such as sorghum stems, banana stems, straw, wood, and bagasse) [9]. Bioethanol has advantages compared to fuel, including having a higher oxygen content of about (35%) so that it burns more completely, a higher octane value (118), and is more environmentally friendly because it has a lower CO gas emission content of 19-25% [10]. One example of bioethanol raw material is coffee parchment, the availability of coffee parchment is quite large, in coffee processing will produce 65% coffee beans and 35% coffee parchment [11]. The structure of the coffee fruit can be seen in Figure 1 and the constituent content of robusta coffee can be seen in Table 1.

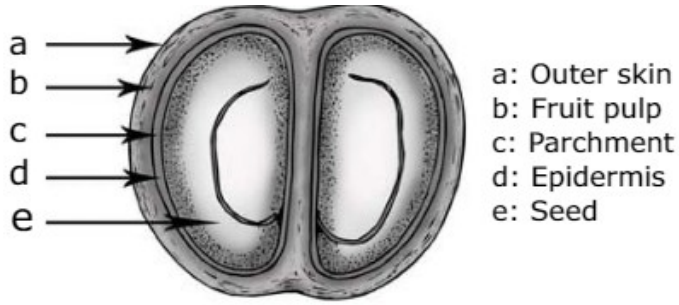

Figure 1. Structure of Coffee Fruit

Parchment has a fairly high cellulose content, making it possible to produce more glucose. According to Wardana et al, 2019 [4] the cellulose and hemicellulose content in Robusta coffee parchment after dry method treatment was found to be 27.26% cellulose and 11.65% hemicellulose, where the highest levels in the Bondowoso region were greater than other regions. The advantage of using Robusta coffee parchment as a raw material is that the fruit is widely grown around the Bondowoso district and has a high enough cellulose content so it can produce high glucose.

Table 1. Ingredients of Robusta Coffee

Outer skin	**Fruit pulp**	**Parchment**	**Epidermis**	**Seed**
Crude protein (91.7%)	Pectin (38.70%)	Crude protein (2.20%)	Chlorogenic acid (7.21%)	Crude fiber (27.2%)
Fat (2%)	Total sugar (45.80%)	Crude fiber (60.24%)	Fat (10.19%)	fat (10.6-12.6%)
Fiber (27.65%)	water (15.15%)	Cellulose (63%)		Crude protein (3-13.5%)
Reducing sugar (12.4%)		Hemicellulose (7.58%)		Reducing sugar (6-10%)
Non-reducing sugar (2.02%)		Ash (3.30%)		Non-reducing sugar (0.32-1.08%)
Tannin (7.47%)				Carbohydrates (4.47%)
Total pectin (6.52%)				Ash (3.38%)
Ash (3.36%)				Caffeine (9%)
N free (57.85%)				Chlorogenic acid (6-10%)

Based on Table 1, coffee skin contains 63% cellulose and 7.58% hemicellulose. Coffee parchment is an abundant agro-industrial cellulosic and lignocellulosic material that can be used to produce reducing sugars. The composition of coffee parchment consists of cellulose, hemicellulose, and lignin. However, the high lignin content requires pretreatment [12]. With its considerable cellulose content, the utilization of coffee skin as a raw material for reducing sugar with the application of bioethanol can be an opportunity for non-conventional fuels.

Bioethanol can be made from various agricultural materials, including materials containing sugar derivatives (saccharin), starch-containing materials, and cellulose-containing materials such as wood, and some other agricultural waste [10]. Materials containing saccharin can be directly fermented, but materials containing starch and cellulose must first be hydrolyzed into simple components, although they can be fermented directly using enzymes, currently the fermentation industry still utilizes microorganisms, because this method is much easier and cheaper, microbes that are widely used in the fermentation process are yeasts, molds, and bacteria [13]. The hydrolysis process is a process of breaking down substances by reacting using water, the aim is to break down these substances [12]. The purpose of this study is to optimize the hydrolysis process of coffee parchment using a microwave to determine the highest level of reducing sugar. Box–Behnken design (BBD) and response surface methodology (RSM) are employed to obtain statistical models for glucose stability optimization and identification of their stability regions. Factors including the HCL concentrations, hydrolysis time, and power microwave are subjected to sensitivity analyses, highlighting their ascendancy and interactional effect on the formulation stability.

The novelty carried out in this study is to vary the time, acid concentration, and power in the hydrolysis process of parchment, it is expected to obtain more optimal reducing sugar to produce the best bioethanol. According to Maryanti et al, 2019 [14], the most used hydrolysis to hydrolyze cellulose is acid hydrolysis. Some acids commonly used for acid hydrolysis include sulfuric acid (H_2SO_4), perchloric acid ($HClO_4$), and hydrochloric acid (HCl). The use of concentrated acid in the cellulose hydrolysis process is carried out at a lower temperature than dilute acid. The concentration of concentrated acid used is 1-3%. The reaction temperature is 100˚C and requires a reaction time between 20-40 minutes [15].

Microwaves are electromagnetic waves that have frequencies from 0.3 to 300 GHz. Lignin can be broken into smaller particles and detached from cellulose at 200ºC, pretreatment using microwave can be achieved within 60 minutes [16]. The use of microwaves enhances the release of protein bodies from starch more effectively than traditional methods, not only in the pretreatment process but also in the cellulose and hemicellulose hydrolysis process. In the hydrolysis process of these two materials in an acidic solution using the microwave method, starch is directly converted into simple sugars in a relatively short time. Compared with traditional heating, the reaction rate of starch hydrolysis to glucose is increased by 50-100 times [5]. The advantage of using this acid is that it contains sugar conversion up to 90% conversion

[17]. The initial moisture content of robusta coffee itself is around 48.7% and according to SNI, the maximum moisture content of dried coffee bean skin is 12.5% [18]. The results of glucose hydrolysis of robusta coffee obtained 6.73% and the results of glucose fermentation of robusta coffee skin for 7 days obtained a bioethanol content of 60%. The conversion process of glucose to produce bioethanol occurs maximally [19]. Previous research data can be seen in Table 2.

Some of the research results in the table obtained the highest reducing sugar yield of 42 g/mL using the acid hydrolysis method. The novelty of this research is that it uses the parameters of hydrolysis time, HCl concentration, and microwave power so that it influences the results of reducing sugar levels.

Table 2. Previous Research Data of Bioethanol Reducing Sugar Hydrolysis

Raw Material	Hydrolysis Method	Operating Conditions and Result	Reference
Robusta coffee parchment	Acid hydrolysis	The results of reducing water content obtained optimal results for 2 hours with water content lost by 12.056%, with a ratio of 8% hydrolysis starter obtained the highest reducing sugar of 673.765 mg/100 mL.	[10]
Water hyacinth	Acid hydrolysis	The best water hyacinth hydrolysis process conditions obtained were the use of 1 NH_2SO_4 catalyst and 600 W microwave power with a final reducing sugar content of 486 mg/L.	[16]
Rice straw	Acid hydrolysis using hydrolysis flask	Under optimal conditions, the highest reducing sugar yield from hydrolysis for 25 hours with 40% enzyme concentration was obtained at 8.75 mg.	[20]
Coffee skin	Acid hydrolysis	The highest reducing sugar content was obtained at 42.6 g/mL.	[13]
Arabica coffee parchment	Acid hydrolysis	Based on the 42% cellulose yield, the highest reducing sugar was obtained from the hydrolysis with 10% H_2SO_4 at 100°C by 8%.	[21]
Corn cob	Enzymatic hydrolysis with autoclave heating	Hydrolysis with cellulase enzyme, in alkaline treatment and repetition of 3 times obtained reducing sugar content of 9.96%.	[22]
Sorghum dregs	Microwave aqueous acid hydrolysis	By varying the concentration for 30 and 40 minutes, it was found that the reducing sugar content increased with increasing acid concentration at 30 minutes of hydrolysis time, namely 2.0-8.5 mg/L and experienced a significant increase when extending the hydrolysis time to 40 minutes, namely 19.1-42.7 mg/L. The largest reducing sugar concentration for ethanol production using 150˚C temperature with 2% acid concentration at 40 minutes hydrolysis time is 34.3 mg/L.	[5]
Sorghum Dregs	Acid hydrolysis using autoclave and microwave	The highest glucose level from the hydrolysis process using autoclaving obtained the highest reducing sugar of 30.86 g/L, with hydrolysis using microwave obtained reducing sugar of 44.97 g/L.	[23]
Cassava peel	Acid hydrolysis	From the hydrolysis process, the reducing sugar content was 9.9%.	[24]
Pineapple peel	Acid hydrolysis with stirring rate	From the results of this study, the optimal conditions of hydrolysis obtained the highest glucose occurred from the addition of HCl at 2 M and 300 RPM at 12.6%.	[9]

Raw Material	Hydrolysis Method	Operating Conditions and Result	Reference
Seaweed	Acid Hydrolysis using microwave	The highest reducing sugar content was 33.43 mg/L with an optimum temperature of 150°C, 2% acid concentration at an optimum hydrolysis time of 30 minutes.	[25]

2. Materials and Methods

2.1 Materials

The materials used in this study were robusta coffee parchment obtained from Sumber Wringin Village, Bondowoso District, East Java, distilled water, HCl 32%, NaOH 48%, anhydrous glucose, KNa tartrate, and 3.5-Dinitrosalicylic Acid.

2.2 Equipment

Blender (Miyako), 100 mesh sieve (RSL stainless steel 304), microwave (Samsung MS23K3515AS/SE), water bath (Prio WB-2-6), UV-Vis spectrophotometer (Tungsten 6mm).

2.3 Method

In this study, it was determined that: The control variable is the mass of coffee parchment of 100 grams with a size of 100 mesh [11]. The dependent variable is the reduced sugar content in the hydrolysis process. The independent variables used are hydrolysis time (20, 25, and 30 minutes) [15], acid concentration (1, 2, and 3%) [5], and Microwave power (150, 300, and 450 W) [26]. The analytical method used in this research is Box-Behnken Design (BBD) and response surface methodology (RSM) to obtain a statistical model for optimizing glucose stability and identifying areas of stability.

2.3.1 Raw Material Preparation

Robusta coffee parchment was cleaned first and then dried using an oven at 100˚C for 15 minutes and obtained a sample with a moisture content of 9.998%. Then, the coffee skin is pulverized using a blender so that the results are obtained in powder form. Furthermore, the coffee skin powder is sieved using a sieve with a size of 100 mesh [11].

2.3.2 Hydrolysis of Parchment

The coffee powder material weighed as much as 10 g with HCl catalyst concentration (1, 2, and 3%) as much as 100 mL then put into the flask to be hydrolyzed using microwave heating for (20, 25, and 30 minutes) with microwave power (150, 300, and 450 W) and then allowed to stand until the temperature of the hydrolysis results dropped to room temperature (25). This study carried out data analysis using Design Expert 11 Version Software with

Response Surface Methodology (RSM) BBD (Box Behnken Design) model 17 times shown in Table 3, then analyzed the reducing sugar content by DNS method.

Table 3. Variation of Box-Behnken Design (BBD) Acid Hydrolysis with 3 Variables Data

Run	Time (minutes)	Concentration (g/mL)	Power (W)
1	20	2	450
2	30	2	450
3	25	2	300
4	30	1	300
5	20	1	300
6	25	2	300
7	25	3	450
8	25	2	300
9	20	2	150
10	25	1	150
11	25	1	450
12	20	3	300
13	25	3	150
14	30	2	150
15	25	2	300
16	30	3	300
17	25	2	300

2.3.3 Reducing Sugar Analysis

Analysis of reducing sugars based on preliminary research on analyzing glucose levels using a UV-Vis Spectrophotometer using DNS reagents conducted by Kolo [5], Wardani [27], and Santi [28].

2.3.4 Preparation of DNSA Reagent

A total of 1 g of 3.5-Dinitrosalisilic was added to 20 mL of NaOH and homogenized. Separately dissolved KNa Tartrate (KNa $C_4H_4O_6.4H_2O$) as much as 30 g using distilled water and homogenized both solutions. Then put into a 100 mL volumetric flask, added distilled water until it reached the limit, homogenized, and transferred into a bottle [23].

2.3.5 Glucose Standard Curve Preparation

Standard glucose solutions were made with concentrations of 200, 400, 600, 800, and 1000 ppm, respectively. The preparation begins with making a glucose mother solution by dissolving 100 mg of anhydrous glucose and adding 100 mL of distilled water. Each standard glucose solution was taken 1 mL and placed in each test tube. Then added 1 mL of 3.5-Dinitrosalicylic Acid reagent and 1 mL of distilled water, then homogenized and heated in a water bath with boiling water for 5 minutes. Next, the solution was cooled to room temperature and added distilled water to a volume of 5 mL, and homogenized again. Absorbance was

measured using a UV-Vis Spectrophotometer with a wavelength of 540 nm. Absorbance is the ratio of the intensity of the light fired to the intensity of the absorbed light, the absorbance value will increase with the results obtained. Absorbance measurements were taken at each concentration of glucose solution and then a standard curve plot was made with glucose content (mg) abscissa (x) and absorbance as ordinate (y) [29].

2.3.6 Determination of Reducing Sugar

1 mL of sample was taken to the test tube, 0.5 mL of DNSA reagent was added, 2 mL of distilled water was homogenized, and the sample solution was heated in a water bath for 5 minutes. Then, the solution was cooled to room temperature, and added distilled water until the final volume of 5 mL, then tested using a UV-Vis spectrophotometer with a wavelength of 540 nm.

3. Result and Discussion

3.1 Analysis of Reducing Sugar Content

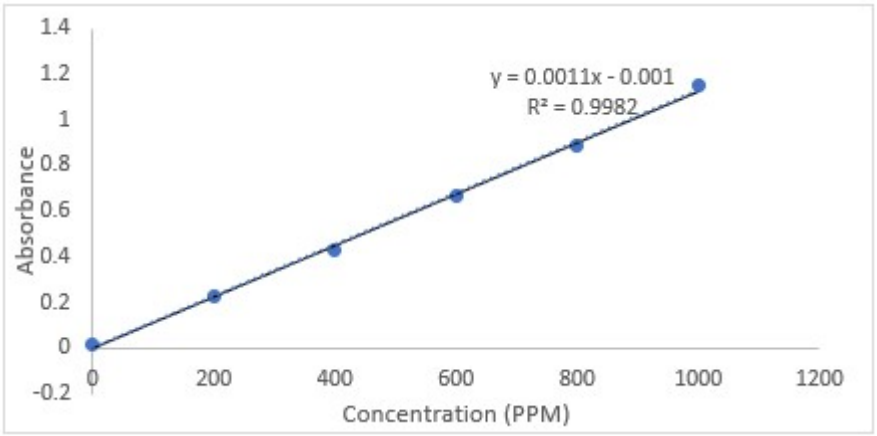

Figure 2. Glucose Standard of Solution Curve

The determination of reducing sugar content in this study was carried out first by determining the glucose standard curve to determine the linear regression equation. This curve was used to determine the concentration of reducing sugar in the samples. The glucose standard curve was obtained by measuring the absorbance of glucose standard solution with various concentrations of 200, 400, 600, 800, and 1000 ppm. Figure 2 shows that the curve is obtained from the linear regression equation at y = 0.0011x - 0.001, with an R^2 value of 0.9982. The results of reducing sugar can be seen in Table 4.

Table 4. Results of reducing sugar content of robusta parchment

Run	A Time (minutes)	B Concentration (%)	C Power (W)	Reduced Sugar Content (g/mL)
1	20	2	450	4.554
2	30	2	450	7.059
3	25	2	300	6.436
4	30	1	300	4.190
5	20	1	300	4.050
6	25	2	300	6.927
7	25	3	450	8.054
8	25	2	300	7.286
9	20	2	150	3.950
10	25	1	150	3.731
11	25	1	450	3.977
12	20	3	300	5.095
13	25	3	150	4.840
14	30	2	150	5.004
15	25	2	300	6.150
16	30	3	300	7.254
17	25	2	300	7.163

Based on Table 4, measured using a UV-Vis spectrophotometer, the highest reducing sugar content was 8.054 g/mL in the operating conditions of 25 minutes, 3% HCl concentration, and 450 W microwave power. Research with the microwave hydrolysis method conducted by Putera et al, 2019 [10], had lower results than this study, which amounted to 6.737 g/mL with an HCl concentration of 20% within 2 hours with an absorbance range of 3.22%.

3.2 *Analysis of Variance* (ANOVA)

The ANOVA result for this study can be seen in Table 5.

Table 5. Analysis of Variance (ANOVA) Results

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	33.08	9	3.68	18.88	0.0004	significant
A-Time	4.29	1	4.29	22.04	0.0022	
B-Concentration	10.80	1	10.80	55.48	0.0001	
C-Power	4.68	1	4.68	24.04	0.0017	
AB	1.02	1	1.02	5.23	0.0560	
AC	0.5256	1	0.5256	2.70	0.1443	
BC	2.20	1	2.20	11.31	0.0120	

Source	Sum of Squares	df	Mean Square	F-value	p-value	
A^2	2.88	1	2.88	14.79	0.0063	
B^2	2.82	1	2.82	14.47	0.0067	
C^2	2.86	1	2.86	14.67	0.0065	
Residual	1.36	7	0.1947	0.6007	0.6479	
Lack of Fit	0.4232	3	0.1411	0.6007	0.6479	not significant
Pure Error	0.9394	4	0.2349			
Cor Total	34.44	16				

Analysis of variance (ANOVA) is to determine whether these factors have a significant effect on variables with a form of statistical hypothesis testing where conclusions are made based on data or inferential statistical groups [30]. Based on Table 5, the variables of time (A), concentration (B), and power (C) used in this study have a significant effect on reducing sugar. This can be seen from the p-value (probability value) for time 0.0022, HCl concentration 0.0001, and power 0.0017 showing that the results of the analysis are smaller than the probability value which is <0.05 (5%).

Based on Table 5, the lack of fit value at the p-value is 0.6479, which represents that the ANOVA model produced passes the principle of fit. The lack of fit value is insignificant, lack of fit can be used if the value is ≥ 0.05. Lack of fit is a deviation or inaccuracy of independent variables against a model [31]. Fit statistics for this study can be seen in Table 6.

Table 6. *Fit Statistic*

R^2	Adjusted R^2	Predicted R^2	Adeq Precision
0.9604	0.9096	0.7608	12.0036

The values of R^2, adj-$R^{2,}$ and pred-R^2 are important to note. These values provide an expectation of the accuracy of an independent variable in the model. A good model has a minimum R^2 value of 0.8 or 80%. The value of R^2 is closer to 1, the better the resulting model [33]. Based on Table 6, the Fit Statistic data was obtained in this study. It is known that the value of R^2 is 0.9604, this indicates that hydrolysis time, HCl concentration, and microwave power have an influence of 0.9604 or 96.04% on reducing sugar. The adjusted R^2 value (adjusted) is 0.9096, then the predicted R^2 value is 0.7608 so that it can be accepted as appropriate because it has a difference < 0.2 [32]

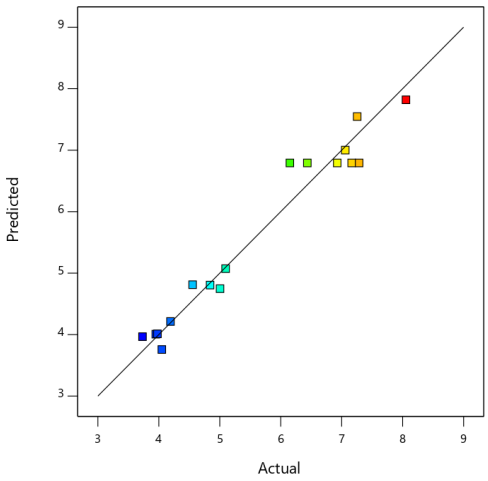

Figure 3. Comparison Chart of Model Data (Predicted) with Experiment (Actual)

Figure 3 shows the fit of the model data to the experimental data can be depicted on the pority plot graph. The straight line on the graph is the prediction data, while the actual data for each experimental data is depicted as a point on the graph. It can be seen from the resulting graph that there are some actual data obtained close to the predicted data.

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. The mathematical equation between the independent variable and the dependent variable to determine the reducing sugar content is shown in equation (2). The positive value in the equation is the response of reducing sugar content will increase directly proportional to the heating time with HCl concentration, the interaction between heating time and microwave power, the interaction between concentration and microwave power, and vice versa. The response of reducing sugar content will decrease as the HCl concentration decreases, this is indicated by the negative coefficient of the equation.

$$y = 1.4537A + 0.4269B + 0.005C + 0.1009AB + 0.00048AC + 0.00049BC - 0.3308A^2 - 0.8179B^2 - 0.000037C^2 \quad (2)$$

where,

Y = Reducing sugar content (g/mL)

A = Heating time (minutes)

B = HCl concentration (%)

C = Microwave power (W)

3.3 Effect of Hydrolysis Variables on Reducing Sugar Content

3.3.1 The effect of the relationship between time and HCl concentration on reducing sugar content response.

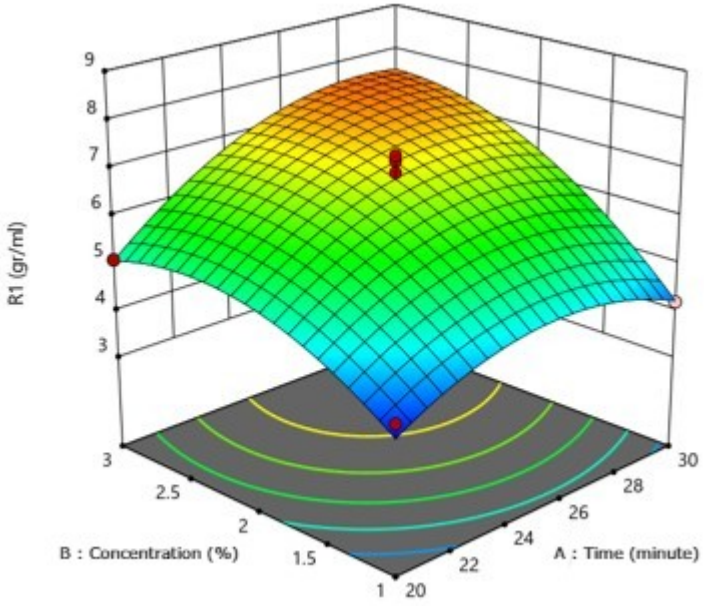

Figure 4. Effect of Hydrolysis Time (A) on HCl Concentration (B)

Figure 4 shows the reduced sugar content as affected by time (A) and HCl concentration (B). The reduced sugar content influenced by hydrolysis time is shown in Table 4. At a hydrolysis time of 20 minutes with 1% HCl concentration and 300 W of power, the reducing sugar content was 4.05 g/mL. If the time is increased to 25 minutes with 2% HCl concentration and 300 W of power, the result of reducing sugar is 7.16364 g/mL. the longer the hydrolysis time, the reduced sugar content. However, if it exceeds the optimum time, the reducing sugar content will also decrease due to inhibitors usually formed by hydroxymethylfurfural (HMF) compounds, too much acid concentration or too high hydrolysis temperature can increase the amount of inhibitors formed during the process so that it can inhibit glucose formation [10]. This is following with the research of Rifa'I et al, 2019 [6], so the time and HCl concentration variation has a significant effect on the response of reducing sugar content

3.3.2 The effect of the relationship between time and microwave power on reducing sugar content response

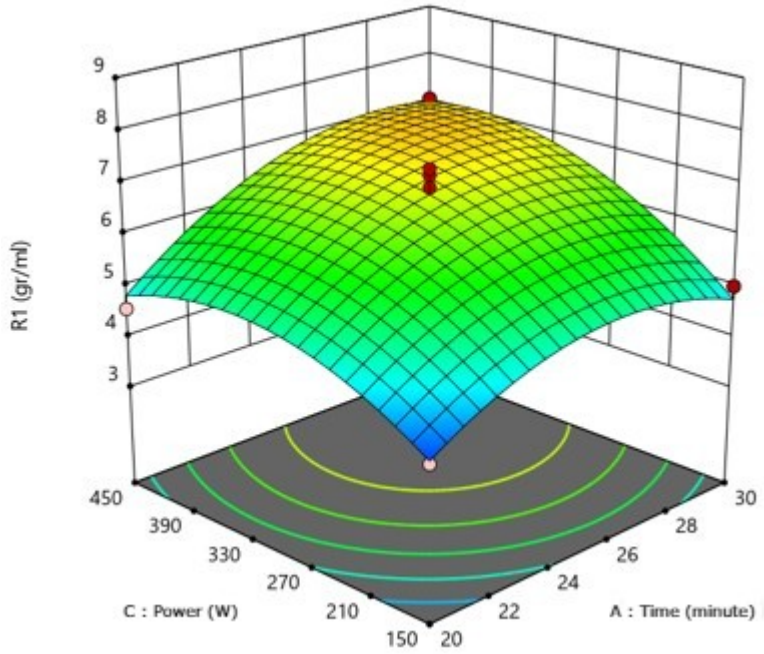

Figure 5. Effect of Hydrolysis Time (A) and Microwave Power (C)

Figure 5 shows the graph of the relationship between time (A) and power (C) to the response of reducing sugar content, optimal condition was obtained in the 7th experiment. It is known that at a time of 20 minutes and a concentration of 2% HCl with 150 W of power, the result of reducing sugar is 3.95 g/mL. when increased to 25 minutes and a concentration of 2% HCl with 300 W of power, the result of reducing sugar is 7.28636 g/mL. In this case, as the microwave power increases, the reduced sugar content will also increase. So, it can be said that if the time variable is increased with the experiment, it also accelerates the hydrolysis time in accordance with previous research conducted by Amini et al, 2022 [3].

3.3.3 The effect of the relationship between HCl concentration and microwave power on reducing sugar content response.

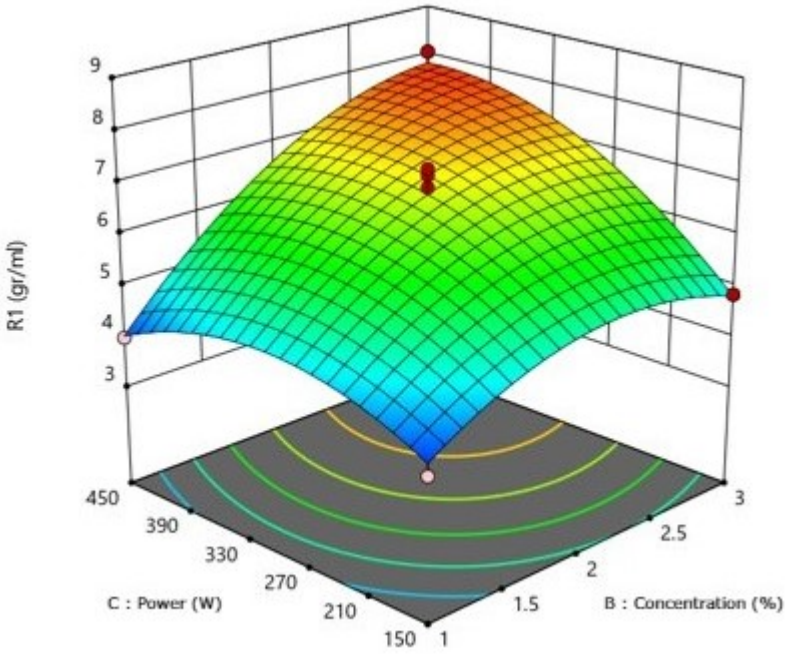

Figure 6. Effect of HCl Concentration (B) and Microwave Power (C)

Figure 6 shows the graph of the effect of the relationship between HCl concentration (B) and microwave power (B) to reducing sugar, obtained optimal results that occurred in the 7th experiment with a reducing sugar yield of 8.05455 g/mL. At a time of 25 minutes and a concentration of 1% HCl with a microwave power of 150 W, the reducing sugar was obtained at 3.73182 g/mL. If the HCl concentration is enlarged in the 25-minute experiment and 2% HCl concentration with 300 W of microwave power, the result of reducing sugar is 7.28636 g/mL, from these results it can be concluded that the addition of HCl has a significant effect on the increase in reducing sugar. This is comparable to the statement of Ahmad et al, 2020 [34]. It is known that the variation in HCl concentration has a linear effect along with the increase in H^+ ions on the glucose content produced. However, if the addition of acid concentration is increased beyond the optimum point, it will undergo decomposition into HMF compounds [33].

Based on Table 7, the optimal result on the response of one experiment on reducing sugar content is 8.021 g/mL when the hydrolysis time is 29.4, HCl concentration is 2.6%, microwave power is 370.8 W and also the desirability value reaches 1.000. The suitability of the model to the optimization value is obtained if the desirability value is close to one [34].

Table 7. Maximum optimization of reducing sugar content design expert

Hydrolysis Time	HCL Concentration	Microwave Power	Reduced Sugar Contet	Desirability
29.4	2.6	370.8	8.021	1.000

3.4 Comparison of Reducing Sugar with Previous Research

The following Table 8 provides data on the results of previous studies as a reference for comparison with this study.

Table 8. Comparison of Reducing Sugar Levels with Previous Studies

No.	Material	Methods	Reduced Sugar Content Result	Reference
1.	Robusta coffee	HCl, Agitation	6.73 g/mL	[10]
2.	Arabica coffee	H_2SO_4, Hot Plate	4.86 g/mL	[21]
3.	Robusta coffee	HCl, *Water Bath Shaker*	20.85 g/mL	[4]
4.	Robusta coffee	Kapang Pestalotiopsis sp. VM 9, Inokulasi	3.92 g/mL	[35]
5.	Kopi Robusta	HCl, *Microwave-Assisted hydrolysis*	8.05 g/mL	This study

4. Conclusions

Based on the results of the study, it can be concluded that the highest reducing sugar content of 8.054 g/mL is in the 7th run with 3% HCl concentration, 25 minutes hydrolysis time, and 450 W microwave power. Determination analysis using Analysis of variance (ANOVA) obtained the results that a significant effect occurred on all variables, namely hydrolysis time, HCl concentration, and microwave power with an R-value of 0.9096 where the value is close to 1, the better the experimental results. If the hydrolysis process passes the optimal conditions, the increase in reducing sugar will decrease because other compounds are formed, namely HMF.

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RESEARCH ARTICLE

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Extraction of Anthocyanins from Dragon Fruit Peel Using Solvent Extraction Method

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Abstract. Dragon fruit skin contains 26.4587 mg/L anthocyanins. Anthocyanins have benefits such as natural coloring agents in the food sector and are used as an alternative to synthetic dyes which are of course also safer for health. The purpose of this study was to determine the effect of extraction variables (time, solvent concentration, and particle size) on the anthocyanin content of dragon fruit skin from the extraction results with the Solvent Extraction method. The definition of Solvent Extraction is the separation of materials from a solid or liquid with the help of a solvent. The extraction process starts from the agglomeration of the extract with the solvent then contact occurs between the material and the solvent so that on the flat plane of the interface of the extraction material and the solvent there is mass deposition by diffusion. The extraction process starts with 25 grams of dragon fruit peel powder with a variety of particle sizes (30, 60, and 80 mesh) then put into an Erlenmeyer tube. Then, the citric acid solution with various concentrations (0.1 M; 0.2 M; and 0.3 M) was added as much as 250 ml. After that, the Erlenmeyer was placed on a stirrer to stir for (90, 120, and 150 minutes). After that, the extraction results were filtered using filter paper to produce a filtrate. Then the filtrate was precipitated to obtain anthocyanin extract. After that, it was analyzed using the spectrophotometric method to calculate anthocyanin content. In this study, the best results were obtained at 11.439 mg/L in conditions without repetition. The optimum conditions of extraction were obtained at a particle size of 60 mesh, a time of 150 minutes, and a solvent concentration of 0.5 M citric acid.

Keywords: *Dragon Fruit Peel, Anthocyanins, Solvent Extraction*

1. Introduction

Red dragon fruit (*Hylocereus polyrhizus*) also called pitaya fruit is a plant that comes from dry tropical climates. This fruit comes from Mexico, the red dragon fruit skin weighs 30-35% of the weight of the whole fruit which is only discarded as waste, so it can cause environmental pollution [1]. There are several types of dragon fruit, in this study using the type

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of red fleshy dragon fruit (*Hylocereus polyrhizus*). Red dragon fruit itself is one of the fruits that has potential as a natural colorant in food because it contains anthocyanin pigments. Red dragon fruit skin can usually be processed to be used as food products, as a base for cosmetics, and as natural dyes [2].

The content in the dragon fruit skin includes betalain compounds, anthocyanins, vitamin C, vitamin E, vitamin A, alkaloids, terpenoids, flavonoids, thiamine, niacin, pyridoxine, cobalamin, phenolics, carotene, and Phyto albumin [3]. Dragon fruit that has red flesh (*Hylocereus costaricensis*) has potential as a source of anthocyanins. Anthocyanins can be used as an alternative to synthetic dyes. In addition, it can also be used as an active ingredient in making cosmetics, because anthocyanins also act as antioxidants [4].

Anthocyanins are one of the pigments found in plants that have the potential to be used as food coloring and can replace synthetic dyes. Apart from being used as a dye, anthocyanins are also included in flavonoid compounds which have a function as natural antioxidants [5]. Apart from being a colorant, anthocyanins are antioxidants that are good for the body, including reducing the risk of degenerative diseases, such as cancer, and heart disease [6].

Antioxidants sourced from anthocyanins function as absorbers or traps where the molecules can react to free radicals and neutralize free radicals. Excessive oxidation reactions in our body can cause the formation of highly active free radicals that damage the structure and function of cells in our body [7]. Consuming foods that contain antioxidants such as anthocyanins can help the body's defense system return to normal [8].

The reason for choosing dragon fruit skin in this study is to reduce food waste in dragon fruit, especially in Banyuwangi regency which produces 82.544 tons per year. To get economic value, it is necessary to process the dragon fruit. One of them is extracting the substances contained in the dragon fruit skin. Factors that can maximize efficiency and selectivity in the extraction process such as the combination of solvents, temperature, pH, and extraction time.

In general, the definition of Solvent Extraction is the technique of separating one or more ingredients from a solid or liquid with the help of a solvent. So, Solvent Extraction is the transfer of solute between two solvents that do not mix [9]. This method will cause some constituents to move from the first solvent to the second solvent. The advantage of the Solvent Extraction method is the quality of the substance that will be produced because the dissolution technique uses a relatively low temperature so that the denaturation process of anthocyanin substances can be avoided compared to using the Microwave Assisted Extraction method so

that anthocyanin substances are not degraded due to the heating process in the extraction process. There are also fewer solvents used compared to using other methods.

Below is the difference in the results of anthocyanin levels of dragon fruit skin obtained by several methods in the research that has been done:

Table 1 Anthocyanin Yield of Dragon Fruit Peel in Several Methods

Method	Time	Anthocyanin Yield	Reference
Microwave Assisted Extraction (MAE)	6 minutes	25.031 mg/L	[10]
Microwave-Assisted Hydro distillation (MAHD)	4 minutes	52.184 mg/L	[11]
Microwave Assisted Extraction (MAE)	6 minutes	28.11 mg/L	[12]
Maceration	24 hours	8.355 mg/L	[13]
Maceration	72 hours	20.81 mg/L	[14]
Maceration	96 hours	4.73 mg/L	[4]
Ultrasound Assisted Extraction (UAE)	45 minutes	29.640 mg/L	[15]

The purpose of this study is to reduce the level of skin waste produced from dragon fruit and to determine the best variable to extract anthocyanins in dragon fruit skin.

2. Research Method

2.1 Materials

The raw materials in this study used red dragon fruit skin obtained from dragon fruit farmers around Jember Regency that had been dried, 10% Citric Acid, Aquadest, Acetic Acid, and Nitric Acid.

The tools used in this study of dragon fruit peel extraction are a set of tools for cutting, a set of tools for drying, a sieve (30, 60, 80 Mesh), a blender, a UV-Vis spectrophotometer, and a set of extraction tools.

2.2 *Sample Preparation*

Samples were obtained from red dragon fruit which was taken and sorted to select dragon fruit skin that was still suitable for sample material. The dragon fruit skin was washed first and then cut into small pieces. Then dried using an oven with a temperature of 50^{o}C for 7 hours and obtained a moisture content of 11.08%. To test the water content should not use a temperature of more than 50^{o}C because it can cause damage to the simplicial that will be used. After the drying process is complete, the dried dragon fruit skin is then pulverized using a blender for 5 minutes/10 grams. After that, the grinding results are sieved with particle sizes

(60, 80, and 100 mesh) and then put into plastic clips which can later be used for the extraction process [4].

2.3 Determination of Water Content

The moisture content of fresh dragon fruit peel and dragon fruit peel powder was determined using the oven method. A total of 5 grams of sample was put in a cup and then dried in an oven at 105ºC. Drying was carried out until a constant weight was obtained. The determination of water content is calculated with the following equation [4].

$$\text{Water Content} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100\% \quad (1)$$

2.4 Anthocyanin Extraction

A total of 25 grams of dragon fruit peel powder with various particle sizes (30, 60, and 80 mesh) was wrapped with filter paper and then put into an Erlenmeyer flask. Then, the citric acid solution with various concentrations (0.3 M; 0.4 M; and 0.5 M) was added as much as 250 ml.

After that, the Erlenmeyer was placed on a stirrer to stir for (90, 120, and 150 minutes). After that, the extraction results were filtered using filter paper to produce a filtrate. Then the filtrate was put into a test tube and then centrifuged to get anthocyanin extract. After that, it was analyzed using the spectrophotometric method to calculate the anthocyanin content Essential Oil Composition Analysis

2.5 Anthocyanin Content Analysis

2.5.1 Stages of Making Buffer

The first step is to make an acetate buffer solution (pH 4.5). The sodium acetate buffer solution should be stabilized at room temperature, but the pH should always be checked before use. The spectrophotometer was switched on and allowed to stand for 10 minutes before being used for measurements. The appropriate dilution factor was determined by dissolving 0.01 ml of the sample in a 25 ml volumetric flask with acetate buffer solution (pH 4.5) set to the limit mark and shaken. So that the absorbance of the sample can be obtained compared to the initial volume to obtain the dilution volume. Sodium acetate buffer solution was added to the cuvette, then the cuvette was inserted into the spectrophotometer to be measured at λ 510 and 700 nm so that the spectrophotometer could be zeroed. Each sample was dissolved in sodium acetate buffer solution (pH 4.5) with a predetermined dilution factor. Samples dissolved using buffer

solution were allowed to stand for 5 minutes before measurement [4].

$$\text{Anthcyanins yield} = \frac{A \times BM \times FP \times 1000}{\varepsilon \times b} \quad (2)$$

Description:

A : Absorbance

BM : Molecular weight cyaniding 3-glucoside (449.2 g/mol)

FP : Dilution factor

ε : Absorbance coefficient 26900 L/mol.cm^{-1} expressed by cyaniding-3-glucoside

b : thickness cuvette (1 cm)

3. Results and Discussion

Determination of anthocyanin levels or concentrations in red dragon fruit skin has the aim of obtaining maximum anthocyanin levels. Several treatments have been carried out in this study, namely variations in citric acid concentration, variations in particle size, and variations in extraction time, and measured using a UV-vis spectrophotometer.

Table 2. Yield Results on Red Dragon Fruit Peel Extraction Treatment

Run	Factors 1 A: Particle size (Mesh)	Factors 2 B: Time (Menit)	Factors 3 C: Concentration (M)	Yield (mg/L)
1	80	90	0.4	11.105
2	60	120	0.4	9.348
3	80	120	0.5	10.353
4	60	120	0.4	9.684
5	60	90	0.3	9.683
6	60	120	0.3	9.683
7	80	90	0.5	9.184
8	30	150	0.4	11.188
9	30	120	0.5	11.105
10	30	90	0.4	10.270
11	30	120	0.3	11.188
12	60	120	0.4	9.573
13	**60**	**150**	**0.5**	**11.439**
14	60	120	0.4	9.132
15	60	120	0.4	9.278
16	60	150	0.3	9.101

Run	Factors 1 A: Particle size (Mesh)	Factors 2 B: Time (Menit)	Factors 3 C: Concentration (M)	Yield (mg/L)
17	40	150	0.4	9.099

The value of total anthocyanin content was calculated using a UV-Vis spectrophotometer to measure the absorbance value. Absorbance is the ratio of the intensity of the incident light to the intensity of the absorbed light. The highest value of total anthocyanin content was obtained in the sample (60 mesh, 150 minutes, 0.5 M citric acid) which resulted in 11.439 g/L. Meanwhile, the lowest total anthocyanin content value was obtained in the sample (60 mesh, 120 minutes, 0.4 M citric acid) which amounted to 7.348 mg/L.

Table 3. Comparison of Anthocyanin Level Results

Methods	Time	Content	Description
Microwave-Assisted Hydrodistillation (MAHD)	45 minutes	52.184 mg/L	[11]
Solvent Extraction	150 minutes	11.439 mg/L	-

This study shows that the extraction of anthocyanins from red dragon fruit using the solvent extraction method obtained a total anthocyanin content of 11.439 mg/L with optimal conditions of particle size 60 mesh, time 150 minutes, and solvent concentration 0.5 M by extracting according to Table 3.2 obtained with the help of Design Expert. While in some studies, the highest anthocyanin content was achieved using the MAHD method, which was 52.184 mg/L. The difference in results is influenced by the hydro distillation process.

According to Shiddiqi [11] it is said that the hydro distillation process maximizes the separation between the solvent and the extract which causes the concentration of the solution and results in higher anthocyanin content values.

The anthocyanin content obtained from the extraction of red dragon fruit peel depends on the extraction time, particle size, and solvent concentration. These variables are important factors that affect the efficiency of anthocyanin extraction from red dragon fruit skin.

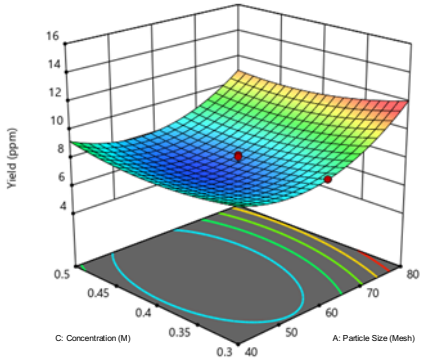

Figure 1. Effect of Concentration & Particle Size Variables on Yield

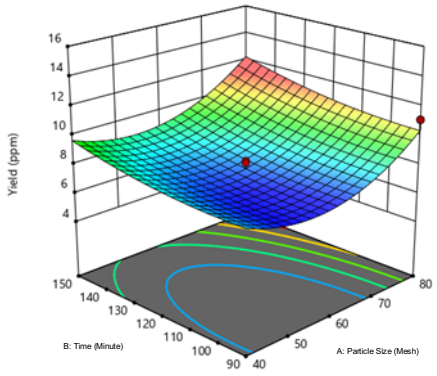

Figure 2. Effect of Time & Particle Size Variables on Yield

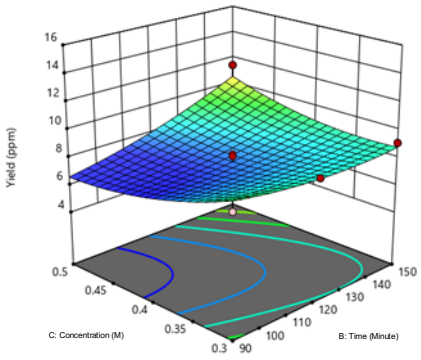

Figure 3. Effect of Concentration & Time Variables on Yield

There are several factors that can affect the increase in anthocyanin levels:

In Figure 1 & Figure 3 particle size in red dragon fruit peel yield also influence anthocyanin yield. This is because the smaller the particle, the ratio between surface area and volume increases so that it can more easily enter the dragon fruit peel powder network. According to Guntarti & Maulida [16] it is said that different particle sizes have different surface areas. The large surface between the simplisa and the solvent will provide a greater

opportunity to extract anthocyanins, which triggers the results of the 60 mesh particle size better than the 80 mesh size. So, anthocyanins will be extracted more if the solvent can interact more widely with simplisa.

In Figure 1 & Figure 3 the increase in citric acid solvent concentration in this study showed an increase in yield, while the use of citric acid solvents with lower concentrations resulted in lower yields. In this study, the extraction was carried out using a variable concentration of citric acid solvent variation of 0.3 M; 0.4 M; and 0.5 M. In this study, the highest yield was obtained when using 0.5 M citric acid solvent concentration.

This is because the more acidic conditions will cause the anthocyanin pigment to get bigger. This is because the greater the concentration of solvents, the greater the collision between solvents, thus accelerating the reaction process.

In Figure 2 & Figure 3, the extraction time influences the increasing anthocyanin yield, this is because the material has a long interaction with the solvent. The time variable itself is needed for anthocyanin exposure. Where the citric acid solvent takes time to penetrate the sample. According to Winata & Yunianta [17] it is said that the longer the extraction time, the quantity of extracted compounds will also increase, this is because the opportunity for contact between the material and the solvent is greater.

Analysis of Variance (ANOVA) is a statistical test used to estimate which variable from the data is more dominant based on the relationship between other variables [18]. The purpose of analysis using ANOVA is to test statistical hypotheses and to determine data optimization. A parameter can be said to be significant if the analysis results in a probability value ≤ 0.05 or 5% for the p-value and a lack of fit value for the p-value ≥ 0.05. Other parameters are the R^2 value greater than 0.7 and the precision value greater than 4 [19]. The research model produces a p-value of 0.0151, so the research analysis model has a significant effect on the extraction of results. ANOVA analysis results can be seen in Table 4.

Table 4. ANOVA Analysis Results

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	30.19	9	3.35	5.81	0.0151	Significant
A- Particle size	2.04	1	2.04	3.53	0.0025	
B-Time	2.61	1	2.61	4.52	0.0017	
C-Concentration	0.2439	1	0.2439	0.4221	0.0066	
AB	0.0497	1	0.0497	0.0859	0.7779	
AC	0.3617	1	0.3617	0.6260	0.4548	
BC	4.00	1	4.00	6.92	0.0338	
A^2	8.51	1	8.51	14.72	0.0064	
B^2	0.7390	1	0.7390	1.28	0.2954	

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Source	Sum of Squares	df	Mean Square	F-value	p-value	
C^2	1.63	1	1.63	2.82	0.1370	
Residual	4.05	7	0.5779			
Lack of Fit	3.44	3	1.15	7.54	0.0602	Not Significant
Pure error	0.6080	4	0.1520			

The analysis results show that the model formed is significant where the f-value is 5.81 and the p-value is 0.0151 < 0.05. Lack of fit formed 0.0602 > 0.05. Lack of fit that is not significant states that the test model used is appropriate so that it can explain the problem being studied. The variables of particle size, time, and solvent concentration have a value of less than 0.05, which means that they have a significant effect on yield results.

R-squared (R^2) is the coefficient of determination with a value between 0 and 1. If R^2 is close to 1, then the relationship between one variable and another variable is getting stronger. Conversely, if R^2 is getting smaller, the relationship between one variable and another variable is getting weaker. The results of the fit statistics that have been carried out can be seen in Table 5.

Table 5. Fit Statistic

Std. Dev.	0.7602	**R^2**	0.8819
Mean	9.38	**Adjusted R^2**	0.8653
C.V.%	8.11	**Predicted R^2**	0.6254
		Adeq Precision	6.4520

The R^2 value from the analysis results obtained 0.8819 or 88.19%, so it can be declared appropriate because the value is more than 75%. The adjusted R-value obtained is 0.8653, indicating that there is a significant relationship between the parameters of particle size, time, and solvent concentration on anthocyanin extraction results. The total anthocyanin yield as a response to the extraction parameters in the ANOVA model can be modeled using the following quadratic equation:

$$\text{Yield} = 9.36 + 0.5686\ A + 0.4459\ B - 0.1442\ C - 0.1095\ AB - 0.1891\ AC + 1.10\ BC + 1.07\ A^2 - 0.0974\ B^2 + 0.1440\ C^2$$

where A is the particle size of the simplisa, B is the extraction time, and C is the solvent concentration. The regression equation can be used to determine the response value of the total yield concentration obtained when the particle size, extraction time, and solvent concentration are different.

4. Conclusion

In this study, anthocyanins can be extracted using the Solvent Extraction method. From the experimental results, the highest yields were obtained at 11.439 mg/L. Optimal conditions were achieved when the process parameters were 60 mesh particle size, 0.5 M solvent concentration, and an extraction time of 150 minutes. For further research, it is recommended to use the Microwave-Assisted Hydrodistillation (MAHD) method because the yield of anthocyanins produced is greater than the Solvent Extraction method.

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RESEARCH ARTICLE

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Optimization Transesterification Reaction in the Synthesis of Biodiesel from Household Catering Waste Cooking Oil

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Abstract. Biodiesel is an alternative energy source for diesel engines or diesel fuel which is carried out using the transesterification method. Used cooking oil is reacted with methanol which will produce methyl ester and glycerol with the help of a base catalyst in the form of NaOH. The research aims to optimize the biodiesel obtained. The synthesis reaction for biodiesel was carried out at temperatures of 55°C, 60°C, 65°C and with varying times of 60, 90, and 120 minutes, with a volume ratio of used cooking oil-methanol, namely 1:4, 1:5, and 1; 6. The highest yield was 61.9404% and ANOVA (*Analysis of Variance*) was proven to be significant and fulfilled as in the *Design Expert 13*.

Keywords*: Used cooking oil, Biodiesel, Transesterification, Optimization, ANOVA*

1. Introduction

The supply of fossil diesel fuel is decreasing by the day due to continuous use. Alternative fuels are a solution that can be used to minimize the use of fossil fuels. Biodiesel has the opportunity to replace diesel fuel because the materials used come from renewable materials [1]. Biodiesel is the name for a type of *fatty ester* and usually comes from vegetable oil or comes from living creatures. Biodiesel itself has much smaller emissions than diesel emissions from petroleum [2].

Biodiesel which is a mixture of mono-alkyl esters of long-chain fatty acids, is a simple fuel used in diesel engines [3]. The benefits of biodiesel itself are as a lubricant to increase engine life and have a high selling price. Biodiesel as an environmentally friendly alternative fuel has the benefit of reducing and overcoming the impact of global warming [4].

The main ingredient for making biodiesel is used cooking oil. Used cooking oil is waste from frying, where the oil contains many dangerous compounds that can pollute the environment and cause disease [5]. Used cooking oil that is used continuously contains a lot of

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free fatty acids which are caused by the high temperature frying process. The formation of free fatty acids results from the hydrolysis process of triglycerides during frying. If the value of free fatty acids in used cooking oil increases, the quality of the used cooking oil will become worse. The free fatty acid content in used cooking oil is generally more than 1% [6]. The chemical content of used cooking oil can be seen in Table 1 below:

Table 1. Chemical Content of Waste Cooking Oil

Parameter	Waste Cooking Oil
Density (gram/cm^3)	0.8989
FFA (%)	9.3
Viscosity (40°C)	46.5
Glyseride Component (%)	56.5
Non-Glyseride Component (%)	43.5

Source: Yozanna, 2016 [7].

The process of making biodiesel from used cooking oil is carried out through several processes, one of which is the transesterification method (conversion of triglycerides into methyl esters) with the help of a catalyst to speed up the reaction [8]. The process of processing used cooking oil into biodiesel, not only requires a catalyst but also requires alcohol as a reactant. The reactant chosen was methanol, because methanol has a cheaper price, is more stable, and has the highest reactivity. The resulting product (if methanol is used) is more often referred to as *fatty acid methyl ester* [9]. The catalysts commonly used are homogeneous acid-base catalysts, such as NaOH and HCl. Homogeneous catalysts tend to have many disadvantages, namely that they can react with *free fatty acids* (FFA) and will form soap, making purification difficult [10].

To maintain and develop the quality of biodiesel production, provisions have been made for biodiesel quality standards. Several countries have set their biodiesel standards or have differences with other countries, one of which is Indonesia the biodiesel standard is set in SNI 7182:2015 with some quality requirements [11], the biodiesel standard can be seen in Table 2 below:

Table 2. Biodiesel Quality Standart

Test Parameters	Unit (min/max)	Standard	Test Method
Acid Number	mg KOH/ gram	0.5	SNI 01-2901-2006
Density at 40°C	kg/m^3	850-890	ASTMD 1298/ASTMD 4052
Cetane Number	Min	51	ASTMD 613

Test Parameters	Unit (min/max)	Standard	Test Method
Flash Point (Bowl Closed)	°C, min	100	ASTMD 7094
Pour Point	°C, max	18	ASTMD9704

Source: SNI 7182: 2015

Table 3. Previous Research

No.	Method	Variable	Catalyst	Result Obtains	Reference
1.	Transesterification	Variations in the ratio of used cooking oil : methanol (1:9, 1:12, and 1;15). The temperature variations used were 30°C, 45°C, and 60°C with time variations of 90, 120, and 150 minutes. The variations of catalyst used are (1.2, and 3%).	CaO	The results of the biodiesel quality test were that the yield was 53% under conditions of a ratio of 1:15, 3% catalyst, reaction temperature of 60°C with a time of 120 minutes.	[12]
2.	Transesterification	The variation in the ratio of used cooking oil:methanol is 1:2. The time variations used are 90 and 120 minutes. The temperature used is 65°C.	NaOH and KOH	The best biodiesel yield of all variations was shown at a time variation of 120 minutes using a KOH catalyst. The test results obtained a yield value of 76.7%, a density of 0.8669 gr/ml, and a viscosity of 5.15 Cst.	[13]
3.	Transesterification	Temperature variations (50°C, 55°C, 60°C, 65°C, and 70°C. Time variations used were 30, 60, 90, and 120 minutes. Transesterification reaction with a used cooking oil:methanol ratio of 1: 5.	KOH	The results obtained were that the amount of biodiesel yield produced in 30 minutes reached 80% and 60 minutes reached 90%. The most optimal temperature is between 60°C to 65°C. The FFA level obtained was 9.67%.	[14]
4.	Transesterification	The ratio of waste cooking oil:methanol is 1:6. The transes-terification process was carried out at varying temperatures of 60°C, 65°C, and 70°C and varying catalysts of 1%, 2%, and 3% for 60 minutes.	CaO-NaOH	The results obtained were the highest yield of 85%, density of 0.857 gr/ml, viscosity of 0.65 Cst at a temperature of 65°C and 1% catalyst.	[15]
5.	Transesterification	The temperature used was 65°C for 60 minutes. The ratio of used cooking oil :methanol is 1:6.	NaOH	The results obtained were biodiesel with a yield of 19%, an acid number of 0.4 and a viscosity of 3.35 Cst	[16]

The main aim of this research is to reduce used cooking oil waste and then reuse it into alternative fuels namely biodiesel [17]. Analysis needs to be carried out to find out the reaction results at a certain temperature and time that are most optimal for biodiesel and apply the BBD (Box-Behnken Design) approach, where the Box-Behnken Design (BBD) is one of the designs that can be accessed in RSM which has been widely used to increase biodiesel production. This is because BBD can be more efficient in parameter tuning, and requires fewer trials than other designs [18].

2. Methods

2.1 Tools and Materials

2.1.1 Tools

The equipment used in this research is a set of transesterification tools consisting of a three-neck flask, heating mantle, magnetic stirrer, thermometer, water bath, reflux condenser, stand and clamp, separating funnel, Erlenmeyer, beaker glass, and measuring cup.

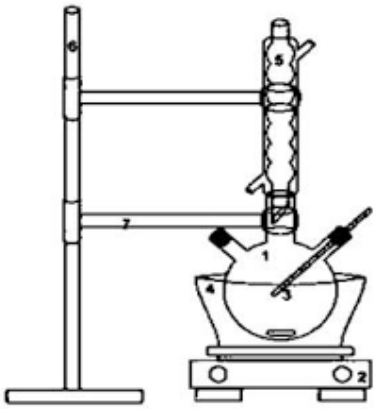

Figure 1. Series of Transesterification Equipment in a Batch Reactor
Source: Mirzayanti et al., 2022 [19]

2.1.2 Materials

The materials used in this research include Aquadest, PP Indicator, NaOH, NaOH 0.025N, Methanol 98%, and Waste Cooking Oil.

2.2 Methodology

2.2.1 Biodiesel Manufacturing Process

2.2.1.1 Filtering Wasted Cooking Oil

The used cooking oil is filtered using a vacuum pump and filter paper. The purpose of this filtering is to remove impurities remaining during frying.

2.2.1.2 Waste Cooking Oil Testing

After the used cooking oil has been filtered, the free fatty acid content of the used cooking oil is tested, namely by calculating the density, viscosity, and also FFA (Free Fatty Acid).

2.2.1.3 Reflux Process

Then 40 ml of used cooking oil was weighed, then put into a three-neck flask. Methanol was added with a volume ratio of 1:4, 1:5, 1;6 (used cooking oil: methanol), and 1% NaOH catalyst was added. Next, all the mixtures were refluxed using temperatures of 55°C, 60°C, and 65°C with stirring using a magnetic stirrer for 60, 90, and 120 minutes.

2.2.1.4 Solution Separation

The resulting mixture from the transesterification method is then cooled, then deposited for 1-2 hours in a separating funnel, until several phase layers are formed. The results obtained are 2 layers, namely pure biodiesel solution at the top and glycerol at the bottom.

2.2.1.5 Washing Solution

The top layer of biodiesel is taken, then the washing process is carried out with hot distilled water, namely at a temperature of 80°C. This washing aims to remove residual glycerol and soap from the transesterification reaction. Washing is carried out with a ratio of distilled water to the oil phase, namely 1:1. Then it was deposited for 3 days, the biodiesel obtained was taken and then FFA testing was carried out and the yield, density, and viscosity were calculated.

2.3 FFA

FFA testing is carried out using the titration method to determine the quality and usability of the oil. The titration is carried out using a 0.025 N NaOH solution which functions to measure several free fatty acids from the oil to be titrated as well as determining the levels of several acidic compounds using an alkaline solution [20].

$$FFA = \frac{V\ NaOH \times N\ NaOH \times BM\ fatty\ acid}{sample\ weight \times 1000} \times 100\% \quad (1)$$

Source: Hadrah et.al., 2018 [5].

2.4 Yield

Yield is used to determine the percentage of results obtained from a process. Yield is a comparison between the weight of biodiesel and the initial weight of oil. Yield can be influenced by many factors, namely temperature, settling time, and stirring [18]. The yield in the solution can be found using Equation 2. The following:

$$yield = \frac{biodiesel\ weight}{waste\ cooking\ oil\ weight} \times 100\% \quad (2)$$

Source: Prihanto and Irawan, 2017 [21].

3. Result and Discussion

3.1 Optimation

This research was designed using *Response Surface Methodology* (RSM) with Design Expert 13 Software. Based on research carried out using used cooking oil, the experimental data is known as shown in Table 4. The following:

Table 4. Design RSM (*Response Surface Methodology)*

Std	Run	Factor 1 A: Volume Ratio (mL)	Factor 2 B: Time (min)	Factor 3 C: Temperature (°C)	Response Yield (%)
3	1	160	120	60	51.0639
4	2	240	120	60	38.3784
12	3	200	120	65	41.9037
1	4	160	60	60	61.293
17	5	200	90	60	47.3298
7	6	160	90	65	45.6449
5	7	160	90	55	61.9404
8	8	240	90	65	43.9593
13	9	200	90	60	49.4191
15	10	200	90	60	42.9918
10	11	200	120	55	28.8011
9	12	200	60	55	58.6227
16	13	200	90	60	45.0757
11	14	200	60	65	26.7472
6	15	240	90	55	38.053
14	16	200	90	60	48.9123
2	17	240	60	60	47.1447

This model will optimize according to the variable data and response measurement data entered. Optimization is carried out by determining the desired response criteria (goal) with a range that is possible to achieve. The most optimal formula is the formula with the maximum desirability value. The desirability value is a function value for optimization purposes that shows the program's ability to fulfill desires based on the criteria set in the final product. A desirability value that is getting closer to 1.0 indicates the program's ability to produce the desired product is increasingly perfect [22].

Each response from the experimental results is then subjected to an ANOVA (Analysis of Variance) test to determine the significance of the response analysis between variables and to find out the model suggested by the Design Expert 13 Software. Table 5 shows the ANOVA (Analysis of Variance) of the results obtained with the Design Expert 13. The model can be declared to have a significant influence if it has a P value < 0.05. However, if the probability value (P > F) is greater than 0.1 then the model shown is not significant.

Table 5. ANOVA *(Analysis of Variance)* of *Yield*

Source	Sum of Squares	Df	Mean Square	F-value	P-value	
Model	1473.19	9	163.69	28.37	0.0001	Significant
A	343.31	1	343.31	59.50	0.0001	
B	141.63	1	141.63	24.55	0.0016	
C	106.30	1	106.30	18.42	0.0036	
AB	0.5349	1	0.5349	0.0927	0.7696	
AC	123.23	1	123.23	21.36	0.0024	
BC	505.76	1	505.76	87.65	< 0.0001	
A^2	129.81	1	129.81	22.50	0.0021	
B^2	33.68	1	33.68	5.84	0.0464	
C^2	101.05	1	101.05	17.51	0.0041	
Residual	40.39	7	5.77			
Lack of Fit	11.33	3	3.78	0.5197	0.6911	not significant
Pure Error	29.06	4	7.27			
Cor Total	1513.58	16				

The ANOVA results selected are those with the largest R^2 value. The largest R^2 shows that the model is recommended and the variable component values have a real (significant) effect on the conversion response [23]. The following is Table 6 of the ANOVA results on Fit Statistics:

Table 6. ANOVA *(Analysis of Variance)* Fit Statistics

Std. Dev.	= 2.40	**R^2**	= 0,9733
Mean	= 45,72	**Adjusted R^2**	= 0,9390
C.V. %	= 5,25	**Predicted R^2**	= 0,8502
		Adeq Precision	= 19,5045

The accuracy of the model can be seen in Figure 2. And the R-squared (R^2) obtained is 0.9733. In the sum of the square test, a model is declared appropriate if the Adjusted R^2 and Predicted R^2 values have a difference in values smaller than 0.2. If the research results are obtained, the value of Adjusted R^2 is 0.9390 and the value of Predicted R^2 is 0.8502, which shows that this model is significant.

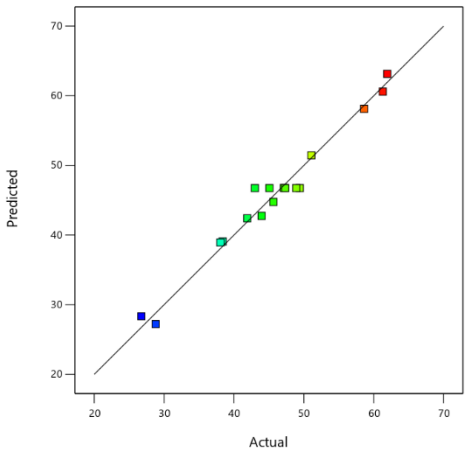

Fig 2. Predicted to Actual

3.2 Yield

The use of high methanol will cause the reaction to shift to the right and will produce maximum conversion [24]. The higher the methanol volume ratio used in the transesterification process, the resulting yield will decrease. This is because using a lot of methanol will form a layer of methanol on the top of the oil because not all methanol can react with the triglycerides contained in used cooking oil [25]. Figure 3 shows that a small reactant ratio (methanol) will produce a greater yield. The optimal used cooking oil: methanol ratio is based on Figure 3. namely at a ratio of 1:4 with 60 minutes the yield obtained is 61.9404%. The decrease in yield was very drastic, up to 26.7472%, which could be caused by the high volume of methanol during the transesterification process.

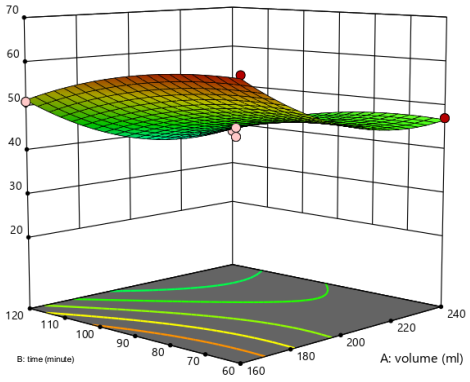

Fig 3. The Effect of Yield on Time and Ratio

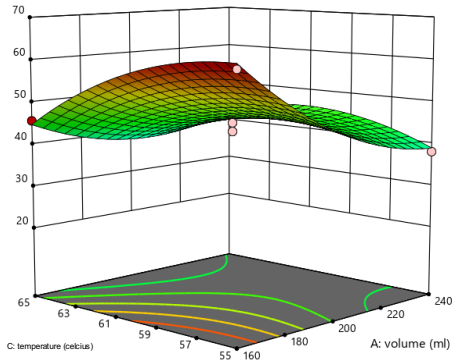

Fig 4. The Effect of Yield on Temperature and Ratio

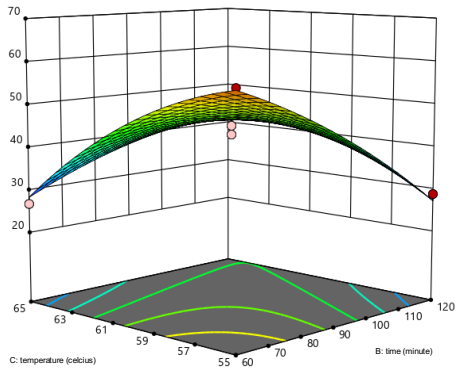

Fig 5. The Effect of Yield of Temperature and Temperature

In the transesterification process, changing temperatures can cause the molecules to move faster or the energy possessed by the molecules can overcome their activation energy [27]. Each temperature used in the transesterification process will produce a different yield. Based on Figure 4. The highest yield was obtained at a temperature of 55°C. Low yield results can be caused by several things, such as the temperature used during the mixing process. The temperature used during the process must not exceed the boiling point of methanol, because it will cause the methanol to evaporate and reduce. When the optimal heating temperature is reached, the yield obtained will increase. When the process temperature is 55°C, the raw material from used cooking oil receives perfect heat to react compared to other temperatures.

In Figure 5, it can be seen that the reaction time influences the biodiesel yield obtained. When equilibrium has been reached, the synthesis process should be stopped so that the energy is used more efficiently [26]. This can be proven that the largest yield is obtained at the lowest time, namely 60 minutes, however, if the reaction is continued for up to 90 minutes, 120 minutes the yield conversion in biodiesel decreases. The effect of time on the yield can be said to be a reversible reaction because the equilibrium reaction leads to the formation of fatty acid esters and glycerol. Triglycerides are converted to diglycerides, monoglycerides, and finally to glycerol.

3.3 FFA

Determining the quality of biodiesel can be done by knowing the level of free fatty acids in the biodiesel. The FFA levels in biodiesel must be low because if the FFA levels are high it will reduce the yield and viscosity of the biodiesel [28]. The size of the FFA content is influenced by several factors such as the ratio of used cooking oil: methanol, temperature, and time during the process. The results obtained were FFA values with the highest level of 0.0448 in running 12, with a ratio of 1:5, time 60 minutes, and temperature 55°C. The lowest level of FFA produced was 0.01536 in running 7, with a ratio of 1:4, time of 90 minutes, and temperature of 55°C. The size of the FFA is also inversely proportional to the yield obtained, if the resulting yield value is high then the resulting FFA value will be low and have a greater yield. It is proven in Table 7 that running 12 with the highest FFA content has a smaller yield compared to running 7 which has a low FFA content. Biodiesel which has low FFA content is a good biodiesel to use as fuel because it is non-corrosive and does not cause scale in diesel engines [29].

Based on research by Irepia Refa Dona [30], it is said that good quality biodiesel that is suitable for use is biodiesel that has low FFA levels. The longer the transesterification time will provide greater opportunities for compound molecules to react which can reduce FFA levels [31]. The decrease in FFA occurs as the process time increases, which means that the longer the time required for the transesterification process, the smaller the FFA produced. In the experiments that have been carried out, it was proven that the lowest FFA levels were obtained at 90 minutes at a temperature of 55°C, with a result of 0.01536, and the highest FFA levels were obtained at 60 minutes at a temperature of 55°C, with a result of 0.0448.

Table 7. Biodiesel Test Result

Run	Volume Ratio (ml)	Time (min)	Temperature (°C)	Density (gr/cm^3)	Viscosity (cSt)	FFA
1	1:4	120	60	0.8164	2.67444	0.027495
2	1:6	120	60	0.8196	2.31142	0.03264
3	1:5	120	65	0.8145	2.60282	0.02048
4	1:4	60	60	0.82	2.60185	0.0224
5	1:5	90	60	0.81648	2.45219	0.02752
6	1:4	90	65	0.8178	2.63464	0.01664
7	1:4	90	55	0.8123	2.53517	0.01536
8	1:6	90	65	0.8115	2.4455	0.01728
9	1:5	90	60	0.8135	2.49476	0.0192
10	1:5	90	60	0.8133	2.42793	0.01664
11	1:5	120	55	0.8213	2.5744	0.03136
12	1:5	60	55	0.8188	2.75359	0.0448
13	1:5	90	60	0.8157	2.61588	0.0384
14	1:5	60	65	0.82102	2.56424	0.02688
15	1:6	90	55	0.8216	2.60321	0.04352
16	1:5	90	60	0.8167	2.57845	0.04032

The use of high temperatures in transesterification reactions can result in accelerated movement of compound molecules which will also increase collisions between reactant molecules. The relationship between reaction rate and temperature is directly proportional, the higher the reaction temperature, the higher the reaction rate [32]. In the experiments carried out, the temperature that produced low FFA levels was found to be 55°C, at this temperature the reaction process occurred perfectly and optimally.

3.4 Density and Viscosity

The size of the density is proportional to the FFA content and viscosity, if the FFA content and viscosity are high then the density value will also be high [33]. It was proven in the experiment that the highest FFA content was carried out in running 12, producing a higher density of 0.8188 gr/cm3 compared to running 7, which had the lowest FFA content producing a density of 0.8123 gr/cm3. In Table 7, it can be seen that the density of biodiesel produced in the experiment does not meet the biodiesel quality requirements based on SNI 7182-2015. This is due to the influence of the composition of the mixture used when making biodiesel, the higher the composition of the mixture of used cooking oil and methanol, the higher the density it will produce [34]. The used cooking oil used in the transesterification process has a density of 0.8465 with a viscosity of 32.5733 cSt and an FFA content of 0.5056.

Table 8. Comparison of characteristics of test results with SNI

Testing	Result	Unit	Standart SNI
Rate of Free Fatty Acid	0.01536-0.0448	-	Max 0.2
Density	0.8115-0.8241	gr/cm^3	0.85-0.89
Viscosity	2.31142-2.75359	cSt (mm^2/s)	2.3-6.0

Source: SNI 7182-2015

The longer the transesterification time and increasing temperature, the shorter the methyl ester chain, so the viscosity value will also decrease [35]. The data obtained shows that all samples meet the biodiesel viscosity quality standards. The highest viscosity is 2.75359 cSt, while the lowest viscosity value is 2.31142 cSt. The viscosity value of biodiesel in each sample decreased as the reaction time increased. It was proven in research that the lowest viscosity value was in running 2 with the highest time of 120 minutes. The viscosity value is also proportional to the FFA content, if the FFA content is high then the viscosity is also high. In the research conducted, it was proven that the highest viscosity value in running 12 was 2.75359 cSt with the highest FFA content, namely 0.0448, at the lowest time is 60 minutes.

4. Conclusions

It can be concluded that the Design Expert 13. Software is useful in formulation to make it easier to determine optimal results. The R-squared (R^2), Adjusted $R^{2,}$ and Predicted R^2 values meet the requirements of ANOVA (Analysis of Variance). The yield obtained depends on the mole ratio of the reactants mixed. Optimal yield results were obtained at a ratio of 1:4, with a time of 60 minutes, at a temperature of 55°C, a yield of 61.9404% was obtained. To determine the quality of the biodiesel obtained, it is also necessary to test FFA, density, and viscosity, to ensure the suitability of the biodiesel samples obtained.

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