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OPTIMIZATION OF PHENOLIC COMPOUNDS IN ROBUSTA GREEN BEANS COFFEE THROUGH THE WET FERMENTATION PROCESS WITH THE RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Coffee is a refreshing drink that has potential as an antioxidant shown by its total phenol content. Fermentation is used to increase the phenolic compound content in coffee. This study aimed to optimize the wet fermentation process to produce optimal phenolic compounds in green bean coffee. The process optimization was carried out using Response Surface Methodology with three independent variables, namely the addition of yeast, the addition of sugar, and fermentation time. The results of the quadratic model equation research to get the optimal process, namely $Y = 65.18 + 0.56X_1 + 2.66X_2 + 16.26X_3 + 1.51X_1X_2 + 0.18X_1X_3 + 0.66X_2X_3 + 9.29X_1^2 + 23.71X_2^2 + 6.08X_3^2$ with r2 of 0.8242 The optimum value of the wet fermentation process was based on the predictive value of the quadratic model, namely the addition of yeast 3.25%; the addition of sugar 21.38%; and 124.73 hours of fermentation time resulted in a total phenol of 10.22 mgGAE/g.

INTRODUCTION

Coffee is a drink that has a function as a refresher because it contains caffeine and phenolic compounds. Phenolic compounds are secondary metabolites produced by plants to prevent ultraviolet radiation and pathogenic pests (Farah and Donangelo, 2006). Phenolic compounds in coffee fruit consist of chlorogenic acid, quinic acid, and transhydroxy sinamit (Perdani et al., 2019). Phenolic compounds have the potential as antioxidant ingredients that can scavenge free radicals to protect against degenerative diseases, cancer, and cardiovascular disease (dePaula and Farah, 2019).

Phenolic compounds play an essential role in the quality of the coffee drinks they produce, they have an impact acidity, bitterness, and flavor (Farah and Donangelo, 2006). Phenolic compounds occurs during the Maillard reaction and Strecker degradation during the roasting process. However, it causes the phenolic compounds to drop by up to 50% during the roasting process, thereby reducing antioxidant activity. The initial phenolic compounds must be high to keep the phenolic compound content high during the disease process (Laukaleja and Kruma, 2019).

The increase in phenolic compounds can be obtained through the fermentation process (Balli et al., 2020). Fermentation is the process of breaking down complex organic compounds into simpler compounds using the help of microbes. In the coffee processing process, fermentation makes it easier to remove mucus that sticks to the coffee beans (Haile and Kang, 2019). The fermentation process is influenced by several factors, including the number of microbes, the availability of the substrate, and the duration of fermentation (Abdillah et al., 2014).

Haile and Kang (2019) reported that green beans coffee had been fermented using yeast produced a total phenol compound of 0.90 mgGAE/mL, which was higher than that without yeast added at 0.76 mgGAE/mL. Kwak et al. (2018) also reported the same results, the total phenolic compound using yeast was 1.30 mgGAE/mL higher than that without yeast was 0.72 mgGAE/mL. Total phenol compounds were also affected by fermentation time. Haile and Kang (2019) reported that 24-hour fermentation produced 0.81 mgGAE/mL of total phenol compounds, which was lower than 48 hours fermentation yielded 90 mgGAE/mL total phenols. Dewi et al. (2014) reported that the greater the addition of sugar on the sixth day, the total phenolic compounds decreased, where without the addition of sugar, it produced 0.90 mg/mL while the addition of 25% sugar produced 0.80 mg/mL.

To produce optimal phenolic compounds from the fermentation process, it is necessary to optimize the fermentation process. One of the optimization methods used in the food processing process is the Response Surface Methodology (RSM) (Wahyono et al., 2018). RSM is a statistical and mathematical method that functions to build, develop, and optimize the formulation process based on the correlation between the independent and response variables (Bezerra et al., 2008).

So far, there has not been any optimization of phenolic compounds in green bean robusta coffee based on RSM's wet fermentation process. Therefore, in this study, the optimization of phenolic compounds in green bean robusta coffee was carried out through a wet fermentation process using the RSM method.

METHODE

The materials used in this study were Robusta cherry beans on full red ripe from Kalipagu Village, distilled water, tape yeast (NA KOK LIONG), chloroform (MERCK Chloroform 1.02444.), calcium carbonate (MERCK Calcium Carbonate Precipitated for Analysis 1.02066.1000), Galic acid (MERCK), Ethanol (MERCK 1.00983.2500; Molar Mass:46.07g/mol), NaOH 0.1N (MERCK), Folin-Ciocalteu (MERCK).

The tools used in this research were fermentor, filter, petri dish (Iwaki), test tube (Pyrex), erlenmeyer (Pyrex), evaporator (BIBBY STERILIN RE200), UV-Vis spectrophotometer (SHIMADZU UV-1800), analytical scales (SARTORIUS MSA225S), filter paper (Whatman), funnel (Pyrex), dropper pipette (Pyrex), separating funnel (Pyrex), measuring cup (Pyrex), beaker glass (Pyrex), beaker (Pyrex), measuring flask (Pyrex), burette (Pyrex), and pH meter (HANNA HI 98107).

Making green beans coffee through a wet basis fermentation process

Peel the skin and flesh of the coffee cherries using a pounding method to separate the skin and flesh from the beans. The coffee bean fermentation process was adding yeast and sugar. The weight of coffee beans for each treatment is 1 kg, the amount of added sugar and yeast could be seen in table 2. Homogenization of green beans coffee, sugar, and yeast with stirring for 5 minutes before fermentation. Fermentation was carried out under aerobic conditions without agitation at room temperature. According to the treatment, the fermentation process stops at a certain time, which could be seen in table 2. Washing I is carried out when the coffee fermentation has finished stopping the coffee fermentation process. Soaking is carried out for 12 hours. Washing II is carried out to remove the remains of mucus and sugar. Drying was carried out using a cabinet drier at 50°C for 12 hours.

Optimization process

In this study, the research design was based on the Central Composite Design (CCD) construction consisting of 3 treatment factors, the addition of yeast (X_1), the addition of sugar (X_2), and fermentation time (X_3) shown in Table 2.

Table 1 Codes and variables of experimental design

Variable	Unit	Kode	-1	0	1
addition of yeast (X_1)	%	A	1.5	3.25	5
addition of sugar (X_2)	%	B	10	20	30
fermentation time (X_3)	Hour	C	120	180	240

Table 2 The treatment design was based on the Central Composite Design (CCD) construction for the variables of adding yeast, adding sugar, and fermentation time

Run	Addition Of Yeast (%)	Addition Of Sugar (%)	Fermentation Time (Hour)
1	3.25	20.00	180.00
2	0.30	20.00	180.00
3	1.50	30.00	240.00
4	5.00	10.00	120.00
5	1.50	10.00	120.00
6	3.25	20.00	180.00
7	3.25	20.00	180.00
8	1.50	10.00	240.00
9	3.25	20.00	79.09
10	3.25	20.00	180.00
11	5.00	30.00	120.00
12	1.50	30.00	120.00
13	3.25	20.00	280.90
14	6.19	20.00	180.00
15	3.25	3.18	180.00
16	5.00	30.00	240.00
17	3.25	36.81	180.00
18	3.25	20.00	180.00
19	3.25	20.00	180.00
20	5.00	10.00	240.00

Table 3 Effect of addition of yeast, the addition of sugar, and fermentation time to the total phenol of green bean coffee

run	Factor 1 A: Addition of yeast (%)	Factor 2 B: Addition of sugar (%)	Factor 3 C: Time of fermentation (Hour)	Response Total phenol (mgGAE/g)
1	3.25	20.00	180.00	8.54
2	0.30	20.00	180.00	9.35
3	1.50	30.00	240.00	8.31
4	5.00	10.00	120.00	9.15
5	1.50	10.00	120.00	8.97
6	3.25	20.00	180.00	7.14
7	3.25	20.00	180.00	8.38
8	1.50	10.00	240.00	6.69
9	3.25	20.00	79.09	11.59
10	3.25	20.00	180.00	8.83
11	5.00	30.00	120.00	10.19
12	1.50	30.00	120.00	12.19
13	3.25	20.00	280.90	9.33
14	6.19	20.00	180.00	12.44
15	3.25	3.18	180.00	5.05
16	5.00	30.00	240.00	7.36
17	3.25	36.81	180.00	4.94
18	3.25	20.00	180.00	10.23
19	3.25	20.00	180.00	8.41
20	5.00	10.00	240.00	7.02

The selection of independent variables was based on research from Tobing (2009), the concentration of yeast that produces coffee with the best aroma and organoleptic quality is at a yeast concentration of 1%. Then based on research from Kehek (2017), the initial concentration of added sugar in Batu banana fermented drink with the best aroma and organoleptic quality is at a concentration of 20%. Then based on research from Towaha and Rubiyo (2016), the length of time of fermentation that produces coffee with the best aroma and organoleptic quality and most liked by panelists was fermentation for 6 days. The codes for the experimental design variables are shown in Table 1. The primary response variable was total phenol. Wahyono et al. (2018) state that the following equation:

$$Y=\beta_0+\beta_1X_2+\beta_3X_3+\beta_{12}X_1X_2+\beta_{13}X_1X_3+\beta_{23}X_2X_3+\beta_{11}X_2^2+\beta_{33}X_3^3$$

It was a 2nd order polynomial equation where Y is the response variable while β_0, β_i, β_{ii}, β_{ij} were regression coefficients and k is the number of variables.

Data analysis

In the optimization stage, the data were analyzed using Dx12 software with the data processing method of central composite design, and to obtain the response surface shape, the Surfer 32 software was used. The optimization process and construction of predictive response curves are in 3 dimensions. The significant test of the response variable was carried out at $P < 0.05$. Furthermore, the verification and validation stages were carried out.

Phenol test

Measurement of phenol levels refers to Jayasri et al. (2009), the research of using the

Folin-Ciocalteu method. The first was to prepare a coffee sample that has been dissolved with ethanol first. Then 5 mL of foline reagent was taken and dissolved using 45 ml distilled water so that 10 times the dilution of the foline solution was then prepared 7.5 % Sodium Carbonate by taking 7.5 g of sodium carbonate and dissolve in 100 ml of distilled water. Then 0.5 ml of the sample was taken and then reacted with 2.5 mL of foline reagent, incubated for 2 minutes, then reacted with 2 ml of 7.5 % Sodium Carbonate and incubated for 30 minutes. The absorbance of the solution was measured using a spectrophotometer at a wavelength of 725 nm. The standard used is gallic acid.

RESULTS AND DISCUSSION
Development of a model to predict total phenol

The determination of the model to predict the relationship between treatment and response was based on the data in Table 3. The high coefficient of determination (R^2) indicates the closeness of the relationship between treatments and responses, which is the basis for determining model selection. Table 4 showed that based on the quadratic model, the variable time fermentation has a significant effect (P <0.05)yeast and addition of sugar did not have a significant effect. The addition of yeast and sugar did not significantly affect the total phenol of green coffee beans.

Based on the quadratic model, the variable yeast addition sugar and fermentation time with the total phenol of green bean robusta coffee has a high closeness indicated by an R^2 of 0.8242. The high coefficient of determination indicated that the model could explain the relationship between experimental data and predictions well (Wahyono et al., 2018). The insignificant Lack of fit value (P> 0.05) of 0.264 showed that the model is very suitable for the response variable.

The following was a quadratic model equation:
$$Y=65.18+0.56X_1+2.66X_2+16.26X_3+1.51X_1X_2+0.18X_1X_3+0.66X_2X_3+9.29X_1^2+23.71X_2^2+6.08X_3^2$$
Noted:
Y= Total phenol (mgGAE/g)
X_1= Addition of yeast (%)
X_2= Addition of sugar (%)
X_3= Time of fermentation (Hour)
R^2= 0.8242

Effect of addition of yeast, the addition of sugar, and fermentation time to the total phenol of green beans coffee

Figure 1 showed a 3D profile of addingsugar and fermentation time to green beans coffee. Based on Figure 1, it was known that the time of fermentation has a significant effect on the total phenol of green bean coffee with a significance value of 0.0065 less than 0.05, while the addition of yeast and addition of sugar has no significant effect.

Table 4 Analysis of variance based on response surface with the quadratic model for total phenol

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	65.18	9	7.24	5.21	0.0083	significant
A-Addition of yeast	0.56	1	0.56	0.40	0.5397	
B-Addiition of sugar	2.66	1	2.66	1.91	0.1968	
C-Time of fermentation	16.26	1	16.26	11.70	0.0065	
AB	1.51	1	1.51	1.08	0.3225	
AC	0.18	1	0.18	0.13	0.7257	
BC	0.66	1	0.66	0.47	0.5069	
A^2	9.29	1	9.29	6.69	0.0272	
B^2	23.71	1	23.71	17.06	0.0020	
C^2	6.08	1	6.08	4.38	0.0629	
Residual	13.90	10	1.39			
Lack of Fit	8.96	5	1.79	1.82	0.2643	not significant
Pure Error	4.94	5	0.99			
Cor Total	79.08	19				

The total phenol has increased with the longer the fermentation time to the optimal point. Kwak et al. (2018) reported that the total phenol of green coffee beans fermented without yeast (control) was significantly lower than green coffee beans fermented using yeast. This was also supported by the results of research from Haile and Kang (2019), which states that green coffee beans with yeast fermentation produce a higher total phenol than without fermentation. During the fermentation process, yeast produces metabolites in proteolytic enzymes, which hydrolyze the complex phenolic bonds to become simpler, more active, and quickly absorbed. Also, during the fermentation process, the bond strength of phenol compounds becomes weaker, making it easier to extract. The phenolic compounds in coffee are chlorogenic, caffeic, ferulic, p-coumaric, and caffeoylquinic acid.

Optimization and validation of total phenol from green beans coffee using response surface methodology

Determination of the optimum value of the addition of yeast (X_1), the addition of sugar (X_2), and time of fermentation (X_3) using the regression model equation that has been obtained. Referring to the 3D contour in Figure 1, a picture of the surface response in the form of a saddle was obtained, where the optimum point was indicated by a red dot at the top of the saddle. Based on the optimization results, the optimum value of the addition of yeast was 3.25%; added sugar 21.38%; and 124.73 hours of fermentation time resulted in a total phenol of 10.22 mgGAE/g with a desirability value of 0.81, which means that the optimum value was suitable because the desirability value was close to 1.
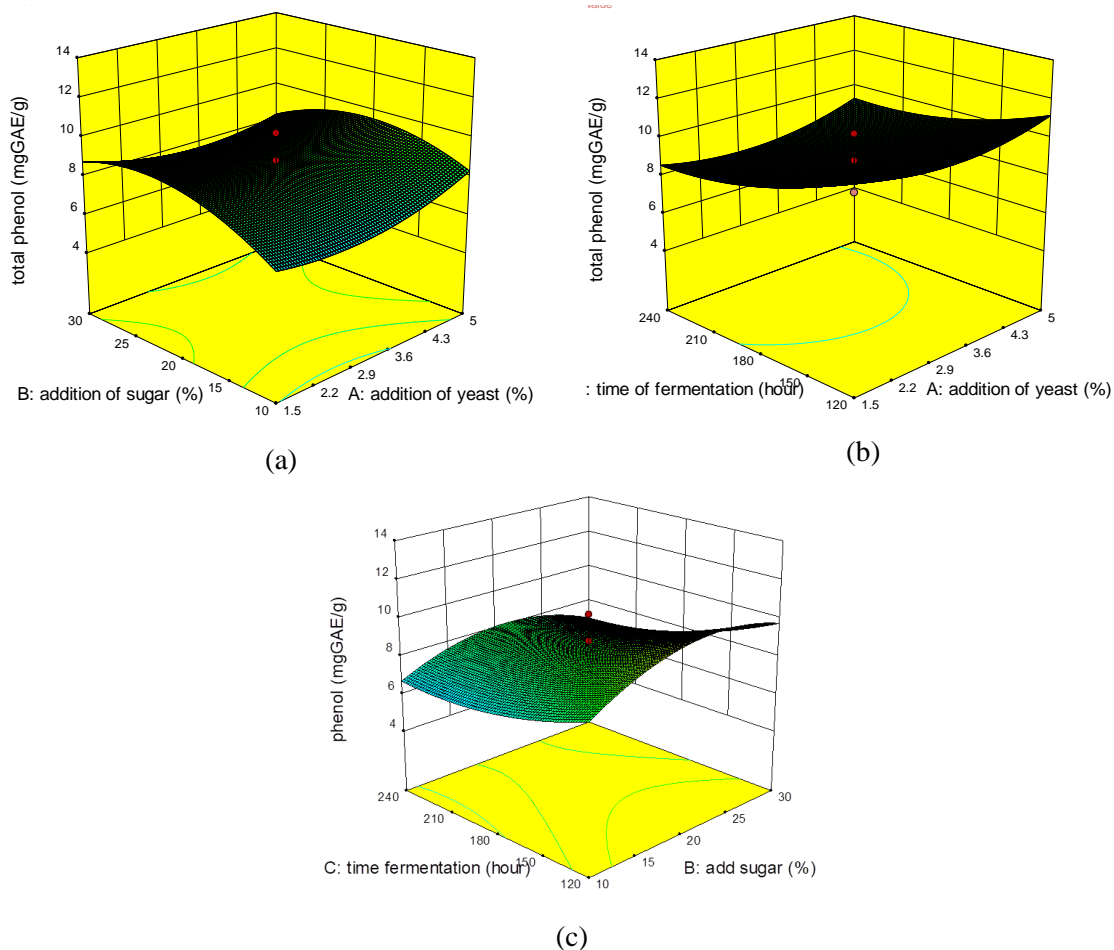

(a)

(b)

(c)

Figure 1 (a) The effect of addition of sugar and addition of yeast, (b) The effect of time fermentation time and addition of yeast, (c) The effect of time fermentation time and addition of sugar on the total phenol of green beans coffee

The high amount of yeast in the fermentation process affects the speed of fermentation because yeast activity increased with the amount of yeast added so that it produces high metabolites to break down the phenolic compounds present in coffee. Fermentation speed was also supported by the addition of sugar given. Sugar acts as a source of substrate for microbial reproduction and growth. The more sugar added, the greater the substrate available for reproduction and growth microbial (Abdillah et al., 2014).

The length of fermentation has an impact on the number of microbes produced. The optimum fermentation time was 135.48 hours because when the yeast produces maximum proteolytic enzymes, it will produce phenolic compounds inactive form. Naturally, phenolic compounds in plants act as secondary metabolites for plant self-defense from microbial attack so that the phenolic compounds produced during fermentation could inhibit the reproduction and growth of microbial (Hui et al., 2012).

The optimization results require a validation test to determine the accuracy of the resulting equation model. Based on the validation results, the total phenol value was 10.76 mgGAE/g, while the optimization results were 10.22 mgGAE/g. The validation results showed the accuracy of the optimization result equation model was 94.71%, which means that the resulting prediction model was able to estimate the actual results with high accuracy (Wahyono et al., 2018).

CONCLUSION

The optimum value of the wet fermentation process was based on the predictive value of the quadratic model, namely the addition of yeast 3.25%; added sugar 21.38%; and 124.73 hours of fermentation time resulted in a total phenol of 10.22 mg GAE/g.

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