

## Analysis of pH and Color of Fermented Cocoa (*Theobroma cacao* L) Beans using Response Surface Methodology

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### ABSTRACT

Cocoa (*Theobroma cacao* L) is a tropical plant that is widely used as a basic ingredient in chocolate production. The quality and taste of chocolate are greatly influenced by the stages and processes of post-harvest handling. Some post-harvest processes that are recognized for improving cocoa quality are pod storage, fermentation, and drying. The quality of cocoa beans can be known based on several physical and chemical parameters, including the color and pH of cocoa beans. This study aimed to analyze the effect of pod storage before fermentation ( $X_1$ ), aeration rate ( $X_2$ ) and aeration mode ( $X_3$ ) during the fermentation process on pH ( $Y_1$ ), chroma ( $Y_2$ ), and hue ( $Y_3$ ) of cocoa beans after fermentation and drying. Response surface methodology (RSM) was used to observe the relationships among the  $X_1$ ,  $X_2$ ,  $X_3$  variables and the  $Y_1$ ,  $Y_2$ , and  $Y_3$  of dried cocoa beans, with the following treatments:  $X_1 = 1; 5; 9$  days,  $X_2 = 0.2; 0.3; 0.4$  liters/minute,  $X_3 =$  intermittent 1; intermittent 2; and continue. The pH, chroma and hue of dried cocoa beans were: 4.873-6.517, 9.99-32.20, and 28.85-71.53, respectively. Furthermore, analysis of variance showed that pod storage before fermentation process significantly affected the hue but did not significantly affect

the pH and chroma. The aeration rate had a significant effect on the pH and hue but did not have a significant effect on the chroma, while the aeration mode had no significant effect on the pH, chroma, and hue of dried cocoa beans.

**Keywords:** Aeration, cocoa bean fermentation, color  $L^* a^* b^*$ , pH, pod storage

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## INTRODUCTION

The post-harvest handling processes of cocoa beans which have large impacts on the quality of dried cocoa beans are pod storage before fermentation (cacao pod ripening), fermentation, and drying of cocoa beans (Kongor et al., 2016; Krähmer et al., 2015). The quality of cocoa beans is also greatly influenced by complex chemical and biochemical changes in cocoa beans during fermentation and drying processes (Afoakwa et al., 2012). Even the fermentation and drying process is noticeably able to increase the flavor precursors of cocoa beans (Misnawi, 2008), and to determine the characteristics of quality cocoa in chocolate industry (Barrientos et al., 2019).

In addition to changes in air temperature, changes in pH, color, aroma, taste, and other physical appearances of cocoa pulp and nib also indicate that a fermentation process has occurred. A change in the color of fresh cocoa beans from purple to brown after fermentation and drying processes is an indicator that should be considered in determining the quality of cocoa beans (Hartuti et al., 2018). In general, color assessment used is color model  $L^* a^* b^*$  (Mendoza, et al., 2007; Afoakwa et al., 2012; Akhiriani et al., 2012). Hartuti et al. (2019) stated that pod storage time before fermentation, aeration rate, and fermentation temperature affected the color values of  $L^*$ ,  $a^*$ ,  $b^*$ , chroma, hue, and RGB of dried cocoa beans. Fermented cocoa beans have higher reddish and yellowish colors than unfermented cocoa beans. Afoakwa et al. (2012) also stated that an increase in pod storage could make the colors of cocoa beans darker, more yellowish and less reddish.

In addition to the color, the quality of fermented cocoa beans can also be determined by the acidity (pH) of cocoa beans (Apriyanto et al., 2016). Kongor et al. (2016) mentioned that the color and acidity of cocoa beans were indicators that could be used to observe the quality of cocoa beans. The characteristics of fermented cocoa beans are also determined by measuring any changes in the pH, acidity and fermentation index of cocoa beans during fermentation process (Apriyanto et al. 2016). Increased pod storage increases the pH of the cocoa beans at the end of fermentation. The pH of cocoa beans increased from 4.80 for non-stored pods to 7.01 for 10-days-stored pods, after 6 days of fermentation (Afoakwa et al., 2013). Meanwhile, Muzaiifa et al. (2017) stated that cocoa beans fermented for 6 days with 2 different aeration intervals (stirring every 24 and 48 hours) had pH of 5.88 and 6.51 at the end of fermentation, respectively.

Response surface methodology (RSM) is a method that can be used to determine the relationship and interaction between independent variables and response variables. RSM analysis for food processing, such as pH, color and others, has been used by other researchers, e.g. to observe: the effect of flour replacement and particle size on the color of wheat bread (Marcin et al., 2016), the effect of a change in the time and temperature of frying pan on the change of the color of papaya chips (Wexler et al., 2016), the optimization

of an ultrasound-assisted extraction condition for flavonoid compounds from cocoa pods using RSM (Yusof et al., 2019), the effect of pod storage, aeration rate and temperature on the color of dried cocoa beans using RSM (Hartuti et al, 2019), the effect of polyphenol and pH on cocoa flavor (Noor-Sofalina et al., 2009), the changes in nib acidification and biochemical composition during fermentation of cocoa (Afoakwa et al., 2013).

The purpose of this study was to analyze and evaluate the combined effects of pod storage before fermentation, as well as the effects of aeration control and aeration mode during fermentation, on the pH and color of dried cocoa beans using the RSM.

## **MATERIALS AND METHODS**

### **Sample Preparation**

The samples of cocoa pods used in this study were obtained from a farmer's plantation in Hargobinangun Village, Pakem Subdistrict, Sleman Regency, Yogyakarta, Indonesia. The cacao pods (a variety of Trinitario, which is a hybrid of Criollo and Forastero) were chosen based on uniform maturity. They were marked by -green, full yellow, and slightly orange-yellow of the pods skins with average harvest ages of 5-6 months since flowering. Harvested cocoa pods were stored in a room with ambient temperatures of 28 - 31 °C and relative humidity of 80 - 90% for 1, 5, and 9 days before fermentation. After the pod storage time was over, the cocoa pods were opened and separated from their skins and placentas, without any treatment before fermentation, except the pod storage process. One (1) kg of cocoa beans was prepared and put into a tightly closed reactor and fermented for 5 days for each treatment.

### **Fermentation Process**

The cocoa fermentation process was carried out using a packed-bed fermenter. The fermentation equipment used in this study referred to the fermentation equipment used by Saludes et al. (2007), but with some modifications. The modifications were made to the type and amount of material used as fermenter heat sources. A heater in the equipment used by Saludes et al. (2007) was replaced with 4 incandescent lamps and 4 fans as shown in Figure 1. The reactor (cocoa bean container) was placed in a fermentation chamber equipped with an air temperature control system, aeration rate, and aeration mode. Fermentation temperature was measured and controlled using temperature sensors inside and outside the reactor, which were connected to a data logger. The temperature of the fermenter was controlled to minimize heat loss from the inside of the reactor into the surrounding environment. The aeration rate was measured using an airflow meter and flowed into the reactor inlet.

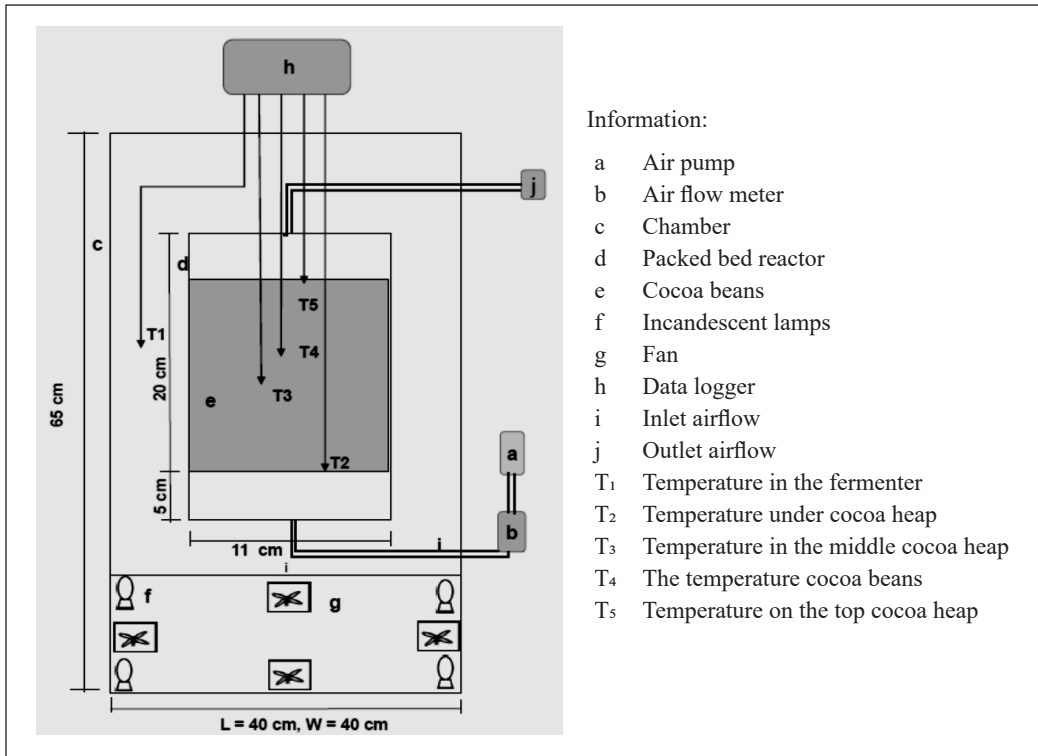


Figure 1. Schematic diagram of experimental cocoa fermentation system

The fermentation process was carried out for 5 days. Initially, 1 kg of cocoa beans were put into the reactor tube, then closed and left for 1 day. Air aeration started to be performed on the second day of the fermentation or after 24 hours fermentation process. Aeration was given with 3 variations of aeration rate (0.2; 0.3; 0.4 liters/minute) and aeration mode (intermittent 1; intermittent 2; and continuous). Intermittent aeration mode was given during the fermentation of cocoa beans at certain time intervals. Intermittent 1, air aeration was given at intervals of 1-hour aeration (1-hour aeration on) and 2 hours without aeration (2 hours aeration off). Intermittent 2, air aeration was given at intervals of 2 hours aeration (2 hours aeration on) and 1 hour without aeration (1-hour aeration off). Furthermore, for the continuous aeration mode, air aeration was given continuously until the end of the fermentation process. After the cocoa bean fermentation process was finished, cocoa beans were dried using a drying cabinet (Shumisu, model PSN-150 with a 12 kW heater and a maximum temperature of 200 °C) with a temperature of 50 °C for 22-25 hours (until the moisture content of dried cocoa beans reached 7 – 7.5%), and then the color and pH analysis of the dried cocoa beans was carried out.

**pH Determination**

The measurement of the pH of the dried fermented cocoa beans was done in several steps. Firstly, a pH meter and a standard buffer solution of pH 4.0 and pH 7.0 were prepared. Then the samples consisting of about 12-20 seeds were taken and tested. Then, the outer skin and ground cocoa beans were separated using a blender and weighed about 10 g to be put into a beaker. 90 ml of hot (between 70°C to 80°C) distilled water was added and stirred slowly until a “no lump” suspension was formed. Then, the filtrate was filtered and cooled to a temperature of  $27 \pm 2^\circ\text{C}$ , then the pH was determined as quickly as possible at the temperature (BSN, 2008).

**Color Determination**

The color of each sample was measured with a color meter TES 135A. The measurement was based on the  $L^*a^*b^*$  color system. The  $L^*$ ,  $a^*$  and  $b^*$  colour space parameters of the samples were calculated as:  $L^*$  ( $L_s - L_0$ ) for lightness;  $a^*$  ( $a_s - a_0$ ) for extent of green color;  $b^*$  ( $b_s - b_0$ ) for blue color (Mendoza et al., 2007; Afoakwa et al., 2012; Marcin et al., 2016; Saunshia et al., 2018). Moreover, based on the obtained color values of  $L^*$ ,  $a^*$ , and  $b^*$ , the chroma and hue were calculated according to Equations 1 and 2 (Wexler et al., 2016; Afoakwa, 2016; Hartuti et al., 2019).

$$C^* = \sqrt{a^{*2} + b^{*2}} \tag{1}$$

$$h = \arctan\left(\frac{b^*}{a^*}\right) \text{ or } h = \tan^{-1}\left(\frac{b^*}{a^*}\right) \tag{2}$$

Where,  $C^*$  is the chroma coordinates to indicate brightness; and  $h$  is the hue angle value.

**Data Analysis**

The experimental design in this study used a central composite design (CCD) of three factors (pod storage, aeration rate, and aeration mode) on the pH and color of dried cocoa beans. The experimental design, limit, and level of the variables in this study are as shown in Table 1.

Table 1  
*Limits and levels of independent variables on the fermentation of cocoa beans*

Variable (X)	Limits and Levels		
	-1	0	+1
Pods storage, $X_1$ (days),	1	5	9
Aeration rate, $X_2$ (L/min)	0.2	0.3	0.4
Aeration mode, $X_3$ (hours/hours)	Intermittent 1	Intermittent 2	Continue

The data were analyzed using the RSM computed with Minitab 14 software package to determine the effects of pod storage before fermentation ( $X_1$ ), aeration rate ( $X_2$ ), and aeration mode ( $X_3$ ) variables on the pH, chroma and hue of dried cocoa beans, which consisted of 20 treatments. The data analysis using the RSM will deliver a mathematical Equation 3 (Bezerra et al., 2008; Hartuti et al., 2019) as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad [3]$$

Where, Y is response to pH, chroma, and hue of dried cocoa beans;  $\beta_0$  is intercepting/constant;  $\beta_1, \beta_2, \beta_3$  are linear coefficients;  $\beta_{11}, \beta_{22}, \beta_{33}$  are quadratic coefficients;  $\beta_{12}, \beta_{13}, \beta_{23}$  are interaction coefficients.

### RESULTS AND DISCUSSIONS

The results showed that the storage of cocoa before fermentation, aeration rate, and aeration mode affected the pH and color of the dried cocoa beans. The results of the calculations of the average pH of the cocoa beans after fermentation and drying are as shown in Figure 2, while those of the average color of  $L^*, a^*, b^*$ , chroma, and hue are as shown in Table 2. The pH of the dried cocoa beans in the study was 4.873-6.517. Some samples of the cocoa beans had pH values outside the range of 5.0-5.5 as the expected pH values in order to obtain good flavor (Biehl et al., 1989). Meanwhile, fermented cocoa beans with pH values of 4.0-4.5 have a lower flavor potential (Biehl & Voigt, 1994 in Araujo et al., 2018). In general, the pH values resulted in the study were quite good because cocoa beans that have a higher pH can produce less sour chocolate products, which is one of the characteristics of a good chocolate product (Araujo et al., 2018).

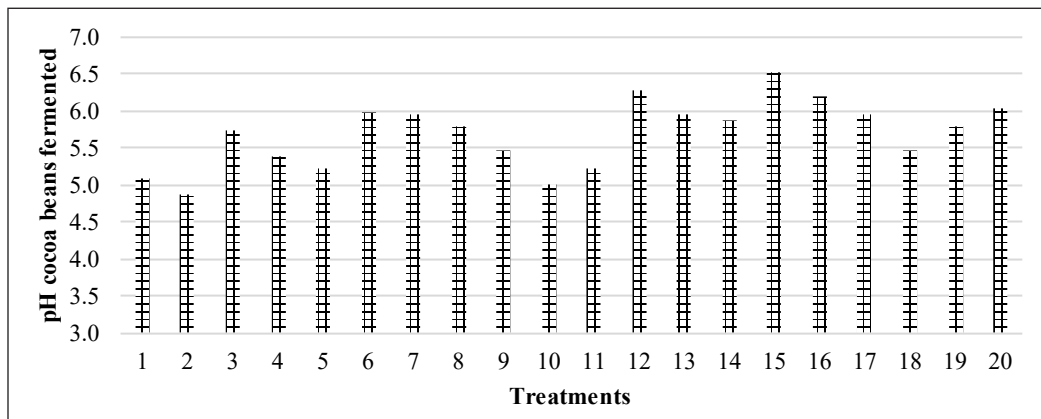


Figure 2. pH cocoa beans

Table 2  
*L\**, *a\**, *b\** color, chroma, and hue of dried fermented cocoa beans

No.	Treatments			Average color measurement			Color of dried fermented cocoa beans	
	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	L*	a*	b*	Chroma ©	Hue (H)
1	-1	-1	-1	25.70	5.93	8.03	9.99	53.55
2	+1	-1	-1	19.57	8.37	23.43	24.88	70.35
3	-1	+1	-1	19.13	6.57	9.70	11.71	55.90
4	+1	+1	-1	17.07	6.03	8.67	10.56	55.16
5	-1	-1	+1	26.47	12.00	15.12	19.30	51.58
6	+1	-1	+1	24.23	7.98	13.50	15.68	59.40
7	-1	+1	+1	35.69	15.38	8.47	17.56	28.85
8	+1	+1	+1	26.00	14.53	10.59	17.98	36.09
9	-1	0	0	27.20	11.42	6.45	13.12	29.46
10	+1	0	0	33.02	12.64	9.97	16.10	38.26
11	0	-1	0	12.20	4.93	14.77	15.57	71.53
12	0	+1	0	14.97	8.00	7.87	11.22	44.52
13	0	0	-1	20.30	14.42	15.33	21.05	46.75
14	0	0	+1	25.27	18.53	26.33	32.20	54.87
15	0	0	0	20.70	11.53	13.80	17.98	50.11
16	0	0	0	17.79	10.63	12.70	16.56	50.07
17	0	0	0	23.60	9.23	10.40	13.91	48.40
18	0	0	0	17.87	6.50	9.67	11.65	56.08
19	0	0	0	26.17	15.83	25.67	30.15	58.34
20	0	0	0	14.90	4.77	9.77	10.87	63.99

The chroma and hue analysis was used to measure the color levels. The findings showed that the color indicators, both the chroma and the hue scores were different in each treatment. The highest chroma from the dried cocoa beans was obtained in treatment 14 (32.20), while the lowest chroma was obtained in treatment 1 (9.99). Meanwhile, the highest hue from the dried cocoa beans was obtained in treatment 11 (71.53), while the lowest hue was obtained in treatment 7 (28.85) (Table 2).

### Analysis of pH of Fermented Cocoa Beans using RSM

The analysis results of the response surface methodology showed that pod storage, aeration rate, and aeration mode had significant effects on the pH of cocoa beans, as presented in Table 3. The pH values of the dried cacao beans are presented in Figure 3-5. Figure 3 shows that the treatment variations of pods storage (X<sub>1</sub>) and aeration rate (X<sub>2</sub>); at aeration mode of (X<sub>3</sub>) = intermittent 2, resulted in pH values of 5.0 - 6.0, where a higher aeration rate will cause a higher pH and vice versa. Figure 2 also shows that aeration rates of < 0.3 liters/minute with 1-9 days pod storage resulted in lower pH values than the aeration rates of (X<sub>2</sub>) > 0.3 liters/minute with 1-9 days pod storage.

Table 3  
Regression coefficients and R<sup>2</sup> values of the RSM for each response variable

Variables	pH (Y <sub>1</sub> )	Chroma (Y <sub>2</sub> )	Hue (Y <sub>3</sub> )
Constant	5.8479*	17.576*	51.9294*
X <sub>1</sub>	-0.0462	1.352	3.9920*
X <sub>2</sub>	0.2729*	-1.639	-8.5890*
X <sub>3</sub>	0.1739	2.453	-5.0920
X <sub>1</sub> *X <sub>1</sub>	-0.5125*	-4.049	-14.2159*
X <sub>2</sub> *X <sub>2</sub>	-0.0210	-5.264	9.9491
X <sub>3</sub> *X <sub>3</sub>	0.1610	7.966*	2.7341
X <sub>1</sub> *X <sub>2</sub>	-0.1328	-1.500	-2.2650
X <sub>1</sub> *X <sub>3</sub>	0.1497	-2.118	-0.1250
X <sub>2</sub> *X <sub>3</sub>	-0.0760	1.645	-4.1500
R-Sq	74.3%	54.4%	75.8%

Notes: \* significant at p < 0.05; X<sub>1</sub> = pods storage; X<sub>2</sub> = aeration rate; X<sub>3</sub> = aeration mode

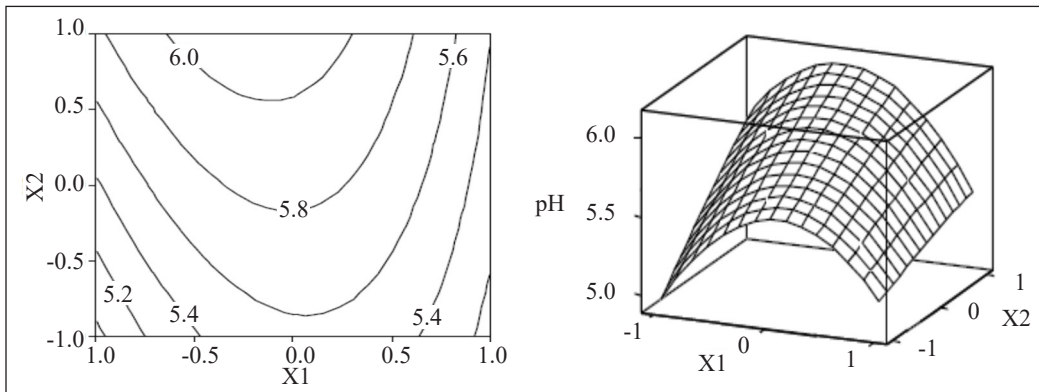


Figure 3. The plot contour and surface of pH of dried fermented cocoa beans (Y<sub>1</sub>) in relationships with X<sub>1</sub> and X<sub>2</sub> at X<sub>3</sub> = Intermittent 2

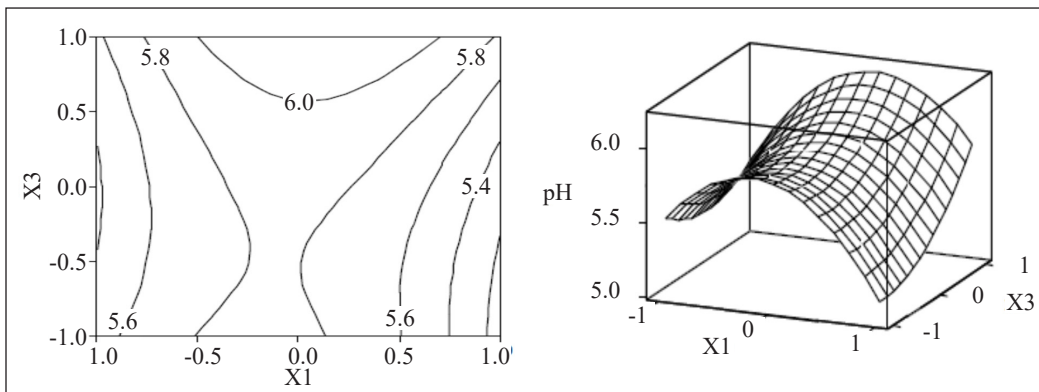


Figure 4. Plot contour and surface of pH cocoa beans fermented (Y<sub>1</sub>) relationships with X<sub>1</sub> and X<sub>3</sub> at X<sub>2</sub> = 0.3 liters/minute



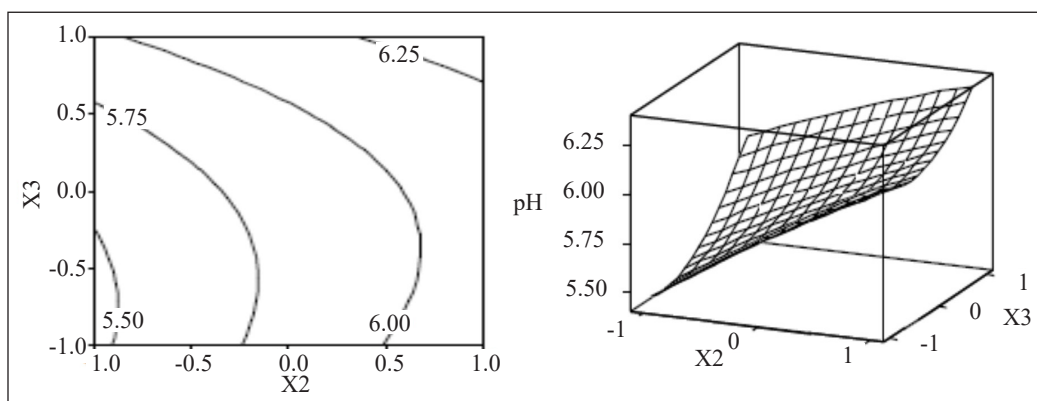


Figure 5. Plot contour and *surface* of pH cocoa beans fermented ( $Y_1$ ) relationships with  $X_2$  and  $X_3$  at  $X_1 = 5$  days

The changes in the pH values of the dried fermented cocoa beans as shown in Fig. 4 were 5.1-6.1, with the variations of pod storage ( $X_1$ ) and aeration mode ( $X_3$ ), at aeration rate ( $X_2$ ) of 0.3 liters/ minute. The highest pH of the dried cocoa beans was 6.1, which was obtained in treatments  $X_1 = 5$  days,  $X_3 =$  continuous mode, and  $X_2 = 0.3$  liters/minute. Meanwhile the lowest pH of the dried cocoa beans (5.1) was obtained in treatments  $X_1 = 9$  days,  $X_3 =$  intermittent 1, and  $X_2 = 0.3$  liters/minute. Meanwhile, at all variation levels of  $X_2$  and  $X_3$ , and when  $X_1 = 5$  days, the pH values of the dried fermented cocoa beans were 5.4-6.3, as shown in Fig. 5. The highest pH was obtained at continuous aeration mode and aeration rate of 0.4 liters/minute, while the lowest pH was obtained at an aeration rate of 0.2 liters/ minute and aeration mode of intermittent 1.

RSM analysis (based on ANOVA analysis, surface and contour plot) showed that aeration rate ( $X_2$ ) significantly affected the pH of the dried cocoa beans. It is known that an increase in aeration rates will also increase the pH values of cocoa beans because an increase in aeration rate causes more water vapor containing acidic properties in cocoa beans to be pushed out of the reactor, thus increasing pH value. Nevertheless,  $X_1$  and  $X_3$  variables had smaller effects on the pH values. Table 3 shows that  $X_1$  and  $X_3$  variables had no significant effect on the pH values of the dried fermented cocoa beans.

### Analysis of Color of Fermented Cocoa Beans using RSM

Based on the analysis of the variants of the chroma and hue of the dried cocoa beans as listed in Table 3,  $R^2$  values were 54.4% and 75.8%, respectively. It means that pod storage, aeration rate, and aeration mode had effects of 54.4% on the chroma and 75.8% on the hue of the cocoa beans. Therefore, it is assumed that other factors may affect the changes of the chroma and hue of the dried cocoa beans.

Table 3 shows that pod storage, aeration rate, and aeration mode had no significant effects on the chroma. Nonetheless, pod storage and aeration rate had significant effects on the hue, while aeration mode did not. The changes of the chroma and hue of dried cocoa beans can also be known based on the contour and surface plots produced by RSM analysis. Figure 6 shows that the highest chroma was 17.8, which was obtained at  $X_1 = 5-6$  days,  $X_2 = 0.3$  liters/minute, and  $X_3 =$  intermittent 2, while the lowest chroma was 7.20, which was obtained at  $X_1 = 1$  day,  $X_2 = 0.4$  liters/minute, and  $X_3 =$  intermittent 2. The surface and contour plots as shown in Figure 7 show that the highest chroma was 28.01, which was obtained at  $X_1 = 5$  days,  $X_3 =$  continuous, and  $X_2 = 0.3$  liters/minute, while the lowest chroma was 11.58, which was obtained at  $X_1 = 1$  day,  $X_3 =$  intermittent 2 and  $X_2 = 0.3$  liters/minute. Furthermore Figure 8 shows that the highest chroma was 27.99, which was obtained at  $X_1 = 5$  days,  $X_2 = 0.3$  liters/minute and  $X_3 =$  continuous, and the lowest chroma of the cocoa beans was 10.17, which was obtained at  $X_1 = 5$  days,  $X_2 = 0.4$  liters/minute and  $X_3 =$  intermittent 2.

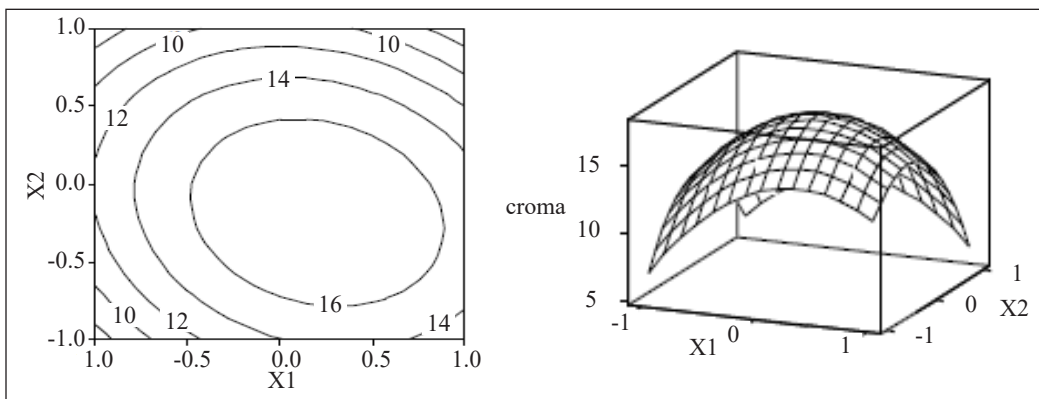


Figure 6. Plot contour and surface of chroma cocoa beans fermented ( $Y_2$ ) relationships with  $X_1$  and  $X_2$ , at  $X_3 =$  intermittent 2

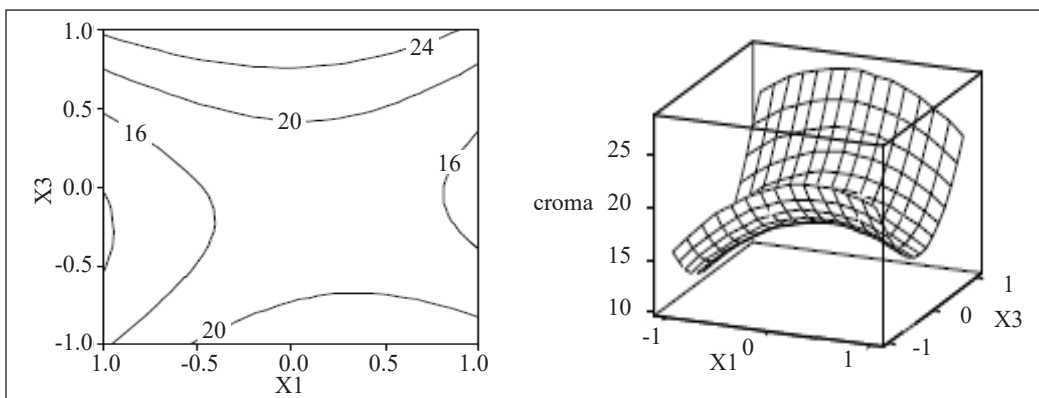


Figure 7. Plot contour and surface of chroma cocoa beans fermented ( $Y_2$ ) relationships with  $X_1$  and  $X_3$ , at  $X_2 = 0.3$  liter/minute

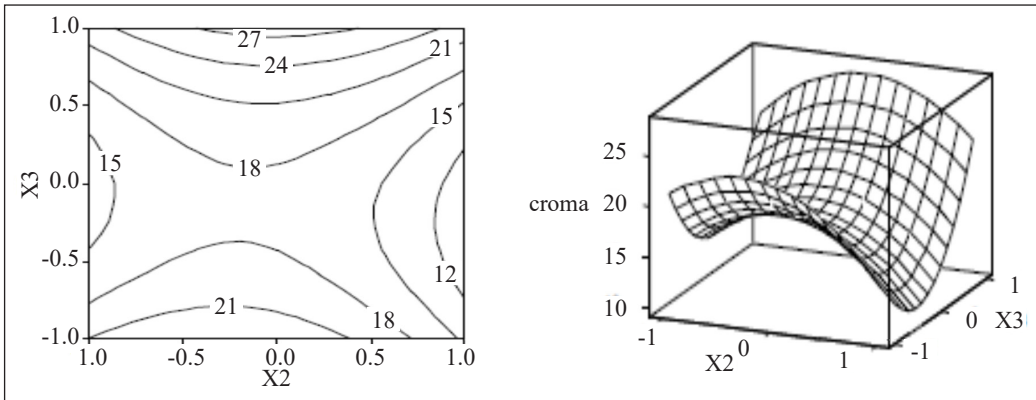


Figure 8. Plot contour and surface of chroma cocoa beans fermented ( $Y_2$ ) relationships with  $X_2$  and  $X_3$  at  $X_1 = 5$  days

Figure 9 shows that the highest hue of the cocoa beans was 70.86, which was obtained at  $X_1 = 5$  days,  $X_2 = 0.2$  liters/minute, and  $X_3 =$  intermittent 2, while the lowest hue was 33.21, which was obtained at  $X_1 = 1$  day,  $X_2 = 0.35$  liter/minute, and  $X_3 =$  intermittent 2. Moreover, the surface and contour plots as shown in Figure 10, where at  $X_2 = 0.3$  liters/minute, the highest hue of the cocoa beans was 60.03, which was obtained at  $X_1 = 5$  days and  $X_3 =$  intermittent 1. Meanwhile, the lowest hue was 31.71, which was obtained at  $X_1 = 1$  day,  $X_3 =$  continuous. Furthermore, Figure 11 shows that the highest hue of the cocoa beans was 73.82, which was obtained at  $X_2 = 0.2$  liters/minute and  $X_3 =$  intermittent 1, and the lowest hue was 45.51, which was obtained at  $X_2 = 0.36$  liters/minute and  $X_3 =$  continuous, and  $X_1$  set at 5 days.

The findings showed that hue is significantly affected by pod storage and aeration rate. Sulaiman et al. (2017) also stated that the color of cocoa beans was significantly affected by the duration of fermentation and pod storage. However, aeration mode does not significantly

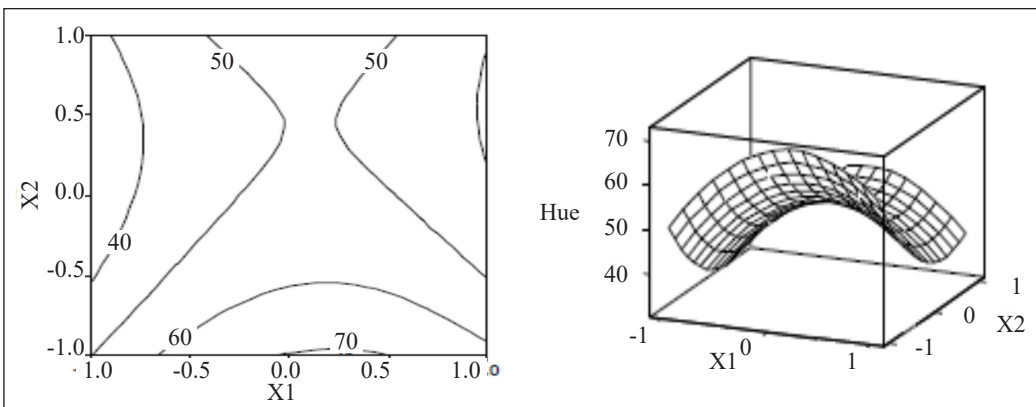


Figure 9. Plot contour and surface of hue cocoa beans fermented ( $Y_3$ ) relationships with  $X_1$  and  $X_2$ , at  $X_3 =$  Intermittent 2

affect the hue because the aeration given was very small, so the air contained in the reactor tube could not penetrate the pulps and cocoa beans properly. As a result, aeration mode did not affect the changes in the colors of the cocoa beans. In fact, the color characteristics of cocoa beans are not the only indicators used to measure the quality of cocoa beans.

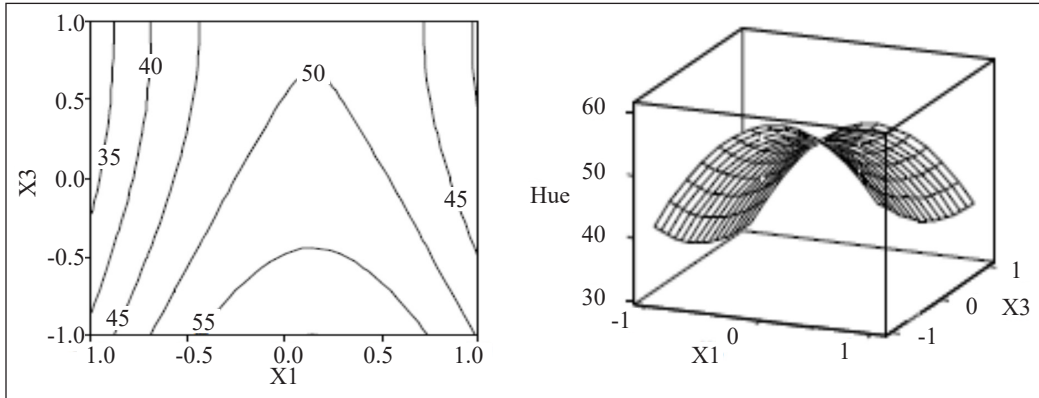


Figure 10. Plot contour and surface of hue cocoa beans fermented ( $Y_3$ ) relationships with  $X_1$  and  $X_3$  at  $X_2 = 0.3$  liter/minute

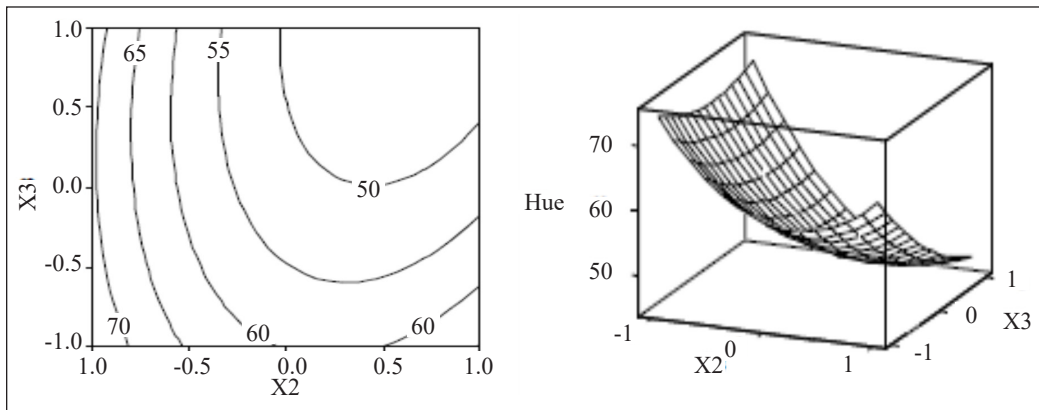


Figure 11. Plot contour and surface of hue cocoa beans fermented ( $Y_3$ ) relationships with  $X_2$  and  $X_3$  at  $X_1 = 5$  days

## CONCLUSIONS

Pod storage before fermentation, aeration rate, and aeration mode have different effects on pH, color  $L^*$ ,  $a^*$ ,  $b^*$ , chroma, and hue of dried cocoa beans. The pH, chroma, and hue values of the dried cocoa beans are 4.873-6.517, 9.99-32.20, and 28.85-71.53, respectively. The results of the ANOVA analysis showed that pod storage affects hue significantly, but it does not affect the pH and chroma significantly. The aeration rate has a significant effect on pH and hue color, but it does not have a significant effect on chroma. Additionally, aeration mode does not have a significant effect on pH, chroma, and hue of dried cocoa beans.

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