



Original Paper

The Effect of Fermentation Time and Yeast Application on the Physical and Chemical Properties of Dry Cocoa Beans

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Abstract— Cocoa is a product with high economic potential throughout the world. Fermentation of cocoa beans is the most important process in processing cocoa beans, because at this stage the distinctive taste and aroma of chocolate will be formed in the cocoa beans. Getting quality dry cocoa beans requires extensive post-harvest handling. Therefore, it is hoped that this research can speed up the fermentation process while maintaining the quality of cocoa beans by adding NKL yeast to the process. This research was conducted using a Completely Randomized Design (CRD) using 2 factors. The first factor was fermentation time which consists of 3 levels, namely 2 days, 4 days and 6 days. Meanwhile, the second factor was yeast concentration which consists of 3 levels, namely 0%, 1% and 2%. The parameters measured include the number of seeds per 100 grams, cut test, fat content, water content and temperature. The research results showed that interaction of the length of fermentation time with the concentration of yeast feeding had a significant effect on the variable number of seeds per 100 grams, and had a very significant effect on the variables of water content and fat content, while the effect was not significant on the cut test variable. The best combination of treatments was the combination of period of fermentation time (6 days) and yeast concentration of 2% (L₃R₃).

Keywords— Bulk cocoa, Fermentation, Yeast

I. INTRODUCTION

Cocoa is one of the commodities with high economic value globally [1]–[4]. Cocoa productivity in Indonesia reaches 728,046 tons, making it one of the country's leading export commodities. In addition, cocoa also ranks fourth in terms of plantation sub-sector area, after palm oil, coconut, and rubber [5]–[7]. Cocoa bean fermentation is a very important stage in processing, because in this process the distinctive taste and aroma of chocolate are formed. Cocoa bean fermentation usually lasts for 5-7 days [8]. During this process, the compound content in cocoa beans can increase or decrease, and gradual physical changes also occur [9].

Fermentation is an anabolic process that occurs in the first one to two days, with the microbial population dominated by yeast. The pectinolytic enzymes produced by yeast play a role in dissolving the mucilage around the cocoa beans, producing a fermented liquid that comes out through a hole in the bottom of

the fermentation box. This process increases oxygen levels, which supports the growth of lactic acid and acetic acid bacteria. The concentration of these two types of acids begins to increase in the mucilage after two days of fermentation [10].

In previous research, the addition of tape yeast to cocoa bean fermentation at a rate of 2.0% was tried by [11] with the best results occurring on 4 days of fermentation. However, what was tested in the study was more on the characteristics of cocoa bean pulp and its physical and chemical characteristics are not yet known. Based on these conditions, research is needed on the effect of adding tape yeast and fermentation duration on the physical and chemical characteristics of dry cocoa beans. The fermentation duration usually ranges from 2 to 6 days, depending on the type of cocoa and common practices in the local area [11], [12]. The fermentation duration used was 2 days, 4 days, and 6 days according to common practices carried out on plantations in Indonesia. This research is expected to accelerate the fermentation process while maintaining the quality of cocoa beans with the innovation of adding NKL brand tape yeast in the process. The acceleration of the fermentation period, in addition to accelerating the production process, can also maintain the quality of cocoa beans that will be further processed.

II. MATERIAL AND METHOD

A. Location and Time of the Research

This research was conducted at PTPN 1 Regional 5 Kendenglembu-Banyuwangi Plantation which was carried out from February 2024 to March 2024. Furthermore, the dry cocoa beans that had been obtained were characterized at the CDAST (Center for Development of Advanced Science and Technology) Laboratory, University of Jember.

B. Maintaining the Integrity of the Specifications

The materials used in this study were dry cocoa beans of the lindak/bulk cocoa type and NKL brand tape yeast, methanol, HCL, sterile aquades, petroleum benzene, N-Hexane, filter paper, alcohol, cotton, wool thread, and aluminum foil.

The tools used in this study include Soxhlet, oven, desiccator, pH meter, thermometer, analytical balance,

Analytical plus, mechanical dryer, glassware, water bath, vortex, incubator, spatula, magnetic stirrer, 40 mesh sieve, refrigerator, knife, plastic container, mortar and pestle, blue tip micro pipette, homogenizer.

C. Method

This study used a completely randomized design (CRD) with two factors: the first factor consisted of three levels, and the second factor consisted of three levels. So there were a total of 9 treatment combinations. There were 27 experimental units, with each treatment repeated three times. All factors will be treated as follows:

Factor I, namely fermentation time, consists of 3 levels, namely:

$L_1 = 2$ days

$L_2 = 4$ days

$L_3 = 6$ days

Factor II, namely the provision of yeast, consists of 3 levels, namely:

$R_1 = \text{Control (Without yeast addition)}$

$R_2 = 1\% (W/W)$

$R_3 = 2\% (W/W)$

D. Research Stages

The study was conducted by conducting a fermentation process that combines fermentation time with the provision of tape yeast. Fermentation was carried out using wet cocoa beans collected from several departments. Wet cocoa beans went through a sorting or picking test stage until wet cocoa beans weighing 500 grams were obtained in each treatment. The addition of 0%, 1%, and 2% tape yeast was used as a variation of the treatment. Furthermore, the cocoa beans were fermented for 2 days, 4 days, and 6 days. The tape yeast that will be

$$\% \text{ Unfermented Beans} = \sum \frac{\text{slaty colored seed halves}}{\text{total splitting of cocoa beans}} \times 100\% \dots\dots\dots (1)$$

$$\% \text{ Unfermented Beans} = \sum \frac{\text{purple colored seed halves}}{\text{total splitting of cocoa beans}} \times 100\% \dots\dots\dots (2)$$

$$\% \text{ Unfermented Beans} = \sum \frac{\text{brown colored seed halves}}{\text{total splitting of cocoa beans}} \times 100\% \dots\dots\dots (3)$$

added to the cocoa beans is first crushed by pounding, then sieved using a 40 mesh sieve to obtain fine tape yeast. After treatment, the time was calculated. After rinsing, the samples were dried in the sun for 48 hours. Furthermore, the cocoa beans were dried using a mechanical dryer at a temperature of 60–80 ° C for 12–20 hours. The dried cocoa beans obtained by cultivation were analyzed for their physical and chemical characteristics. Fig. 1 shows how cocoa bean samples were taken.

E. Physical Properties Testing of Dried Cocoa Beans

1) Number of seeds per 100 grams

Each treatment was weighed as much as 100 grams and then calculated how many seeds were in those 100 grams, as stated [13], which means that the test results are shown according to the number of seeds in 100 grams of test sample, namely as follows:

- I-AA = number of seeds no more than 85 seeds/100 grams
- I-A = number of seeds around 86-100 seeds/100 grams
- I-B = number of seeds around 101-110 seeds/100 grams
- I-C = number of seeds around 111-120 seeds/100 grams
- I-Sa = number of seeds around 121-140 seeds/100 grams
- I-Sb = number of seeds around 141-160 seeds/100 grams
- I-Sc = number of seeds exceeds 160 seeds/100 grams

2) Cut Test

In this study, the classification of beans was divided into three categories: the non-fermented class had a slaty color, the underfermented class had a purple color that was more dominant than the brown color, and the fermented class had the most dominant brown color [14]. The observation results are then calculated using the following formula:

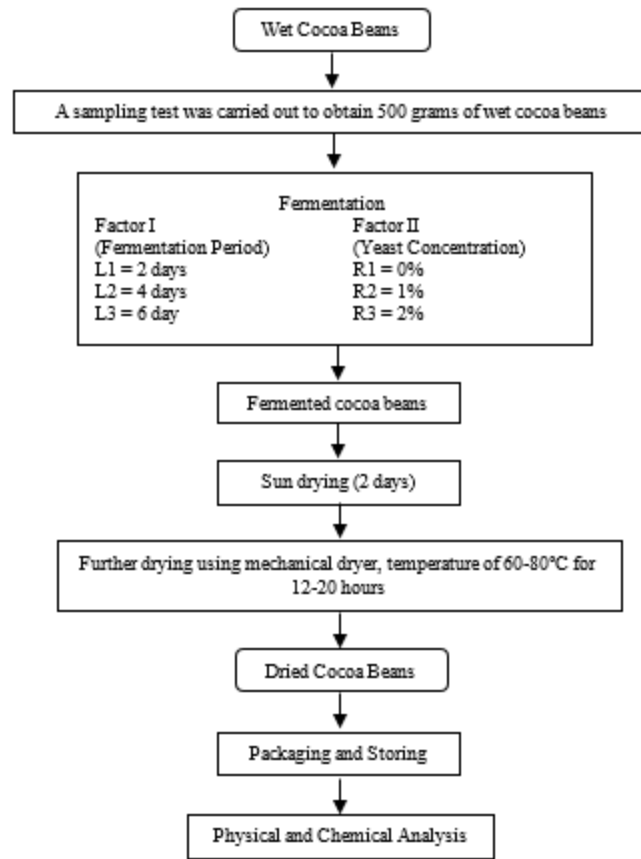


Fig. 1. Research Process Flowchart

F. Chemical Properties Testing of Dried Cocoa Beans

1) Fat Content

Fat content testing in this study used the Soxhlet method. The Soxhlet method is one of the fat analysis techniques that works based on the following principles. Soxhlet extraction and

extraction processes. After both processes are complete, the solution in the fat flask is heated in an oven at 105 ° C for one hour, and the flask is then cooled in a desiccator for 15 minutes before being weighed again [15]. The following formula (4) is used to calculate the weight of fat obtained:

$$\text{Fat Content (\%)} = \frac{(\text{Sample bottle} - \text{Empty bottle})}{\text{Weight of sample (g)}} \times 100 \quad (4)$$

2) Moisture content

Determination of water content is carried out using gravimetric (oven) method [13]. This process begins by weighing 10 grams of the broken test sample, then put into a closed cup that has been previously weighed for its empty weight (m_0). The cup and sample (m_1) are placed in an oven at a temperature of 103°C ± 2°C in an open condition for 16

hours without opening the oven during the drying process. After 16 hours, the cup is immediately closed, removed from the oven, and placed in a desiccator to cool. Furthermore, the closed cup and its contents are weighed again (m_2). The water content is calculated and expressed as a percentage of weight using the following formula.

$$\text{Moisture content} = \frac{(m_1 - m_2)}{(m_1 - m_0)} \times 100 \quad (5)$$

Note:

m_0 = weight of cup and lid (gr)

m_1 = weight of cup, lid and cocoa beans before drying (gr)

m_2 = weight of cup, lid and cocoa beans after drying (gr)

3) Temperature

Fermentation temperature measurements are carried out using a thermometer and measurements are carried out every 12 hours during the fermentation period.

G. Data Analysis

Analysis of variance (ANOVA) was used to analyze the research data. If there was a significant difference between treatments, the Duncan Multiple Range Test (DMRT) was used to continue the analysis with a 95% confidence level.

III. RESULTS AND DISCUSSION

The results of the analysis of variance carried out on all research variables are presented in Table I.

TABLE I. SUMMARY OF THE RESULTS OF THE ANALYSIS OF VARIANCE (F-COUNT) ON ALL RESEARCH VARIABLES

No	Variable	Value of F-Count		
		Period of fermentation (L)	Concentration of yeast (R)	Interaction (LR)
1	Number of Seeds per 100g	1,966 ^{ns}	4,042*	3,880*
2	Cut Test	32,860**	3,263 ^{ns}	2,256 ^{ns}
3	Moisture Content	64,733**	32,600**	19,322**
4	Fat Content	140,961**	20,770**	53,409**

The results of the analysis of variance in Table I. show that the interaction of the length of fermentation time with the concentration of yeast administration has a significant effect on the variable number of seeds per 100 grams, and has a very significant effect on the variables of water content and fat content, while having no significant effect on the cut test variable. The main effect of the length of fermentation time has a very significant effect on the variables of the cut test, water content, and fat content, but has no significant effect on the variable number of seeds per 100 grams. The main effect of the yeast concentration factor has a very significant effect on the variables of water content and fat content, and has a significant effect on the variable number of seeds per 100 grams, while having no significant effect on the cut test variable.

A. Amount of dry cocoa beans per 100 grams

TABLE II. DUNCAN MULTIPLE DISTANCE TEST RESULTS NUMBER OF DRY CACAO BEANS PER 100 GRAMS

Period of Fermentation (L)	Yeast Concentration (R)		
	R ₁ (0%)	R ₂ (1%)	R ₃ (2%)
L ₁ (2 days)	133.333 (b)	156.000 (b)	184.667 (a)
	C	B	A
L ₂ (4 days)	144.667 (b)	146.000 (b)	191.333 (a)
	B	B	A
L ₃ (6 days)	178.000 (a)	188.000 (a)	160.000 (b)
	AB	A	B

Table III shows the results of the classification of dry cocoa beans into several groups based on the weight of the beans.

TABLE III. NUMBER OF DRY COCOA BEANS PER 100 GRAMS

Fermentation	Amount of Beans	Criteria
L ₁ R ₁	133	I-Sa
L ₁ R ₂	156	I-Sb
L ₁ R ₃	185	I-Sc
L ₂ R ₁	145	I-Sb
L ₂ R ₂	146	I-Sb
L ₂ R ₃	191	I-Sc
L ₃ R ₁	178	I-Sc
L ₃ R ₂	188	I-Sc
L ₃ R ₃	160	I-Sb

The results of the test of the number of dry cocoa beans showed that the average number of beans ranged between 133 and 191 beans per 100 grams. This shows a fairly diverse variation of criteria, namely I-Sa, I-Sb, and I-Sc.

In Table III. It was found that the best result from the whole treatment was L₁R₁ with a number of seeds per 100 grams of 133 seeds with the I-Sa criteria. Overall, the number of seeds obtained in each treatment was still classified into the poor category, which was the good and quite good categories according to [13] are dry cocoa beans with categories I-AA to I-C.

This is because the texture of dry cocoa beans affects the weight of the beans which is related to the number of beans in a certain weight unit. Cocoa beans with a hollow texture have a lower density, so the number of beans in 100 grams tends to be more than beans with a compact or solid texture. Conversely, beans with a compact texture have a higher density, so the number of beans in the same weight unit becomes less. This shows that bean texture is an important factor that affects the measurement of weight and number of beans in post-harvest cocoa analysis. This is in line with research conducted by [16]. That the texture of cocoa beans can affect their weight. The number of beans per 100 grams will be more if the texture is more hollow. Conversely, if the texture is denser or more solid, the number of beans per 100 grams will be less.

B. Cut Test

TABLE IV. RESULTS OF MULTIPLE DISTANCE TEST OF CUT TEST VARIABLES.

Period of Fermentation (L)	Yeast Concentration (R)		
	R ₁ (0%)	R ₂ (1%)	R ₃ (2%)
L ₁ (2 days)	0 (c)	6,667 (b)	10,667 (b)
	B	A	A
L ₂ (4 days)	8 (b)	10 (b)	19,333 (a)
	B	B	A
L ₃ (6 days)	26,333 (a)	26,333 (a)	23 (a)
	AB	A	AB

Based on the cut test on dry cocoa beans, the results obtained can be seen in Fig. 2.

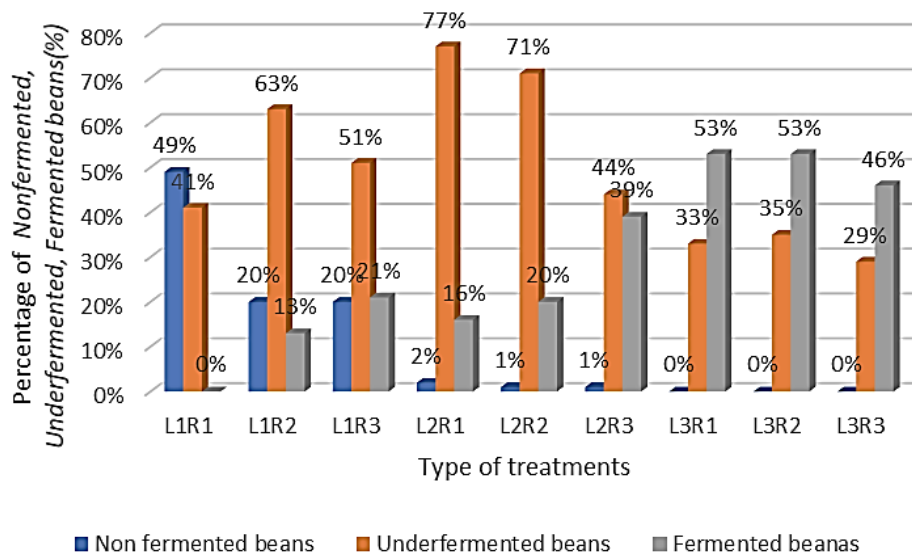


Fig. 2. Percentage of nonfermented, underfermented, and fermented dry cocoa beans

In this study, the results of the cut test with a brown color below 80% were obtained, but the best was found in samples L3R1 and L3R2 with the same value of 53% brown seeds (fermented).

Cocoa bean fermentation is considered successful if the beans produced undergo perfect fermentation, marked by an even brown color throughout the beans. Conversely, cocoa beans that are not fermented properly (slaty) will have a purple color [17]. The color change in cocoa beans is caused by the polyphenol compound content in them. During the fermentation process, polyphenols undergo oxidation triggered by the polyphenol oxidase enzyme with the help of air, producing tannin compounds that provide astringent taste and brown color. As fermentation progresses, polyphenol oxidation takes place, so that the color of the beans which were initially purple gradually changes to brown [18].

C. Moisture Content

Based on the data obtained, the best treatment recommendation to produce lower cocoa bean water content, although still above the SNI standard, is to apply fermentation for 2 days (L1) with the addition of 0% tape yeast (R1). This combination produces the lowest water content, which is 8.448%, which is closer to the maximum limit of the SNI 2323:2008 standard (7.5%) compared to other treatments.

TABLE V. MEASUREMENT OF MOISTURE CONTENT OF DRIED COCOA BEANS

Fermentation Period (L)	Yeast Concentration (R)		
	R ₁ (0%)	R ₂ (1%)	R ₃ (2%)
L ₁ (2 days)	8.448 (c)	9.392 (b)	9.868 (a)
	C	B	A
L ₂ (4 days)	9.535 (a)	10.984 (a)	9.631 (b)
	B	A	B
L ₃ (6 days)	8.870 (b)	9.084 (c)	8.597 (c)
	A	A	B

Based on the analysis of variance in Table V. shows that the effect of fermentation time and yeast concentration provides a very significant interaction on water content. In the

study conducted with a combination treatment of 2 days of fermentation time (L1) with the addition of yeast with a concentration of 0% (R1) had the lowest water content value. This is because during fermentation, microbes such as yeast and bacteria play a role in breaking down the pulp of cocoa beans. If the fermentation time is too short, microbial activity may not be enough to break down the pulp effectively. This causes less water to be bound in the bean structure, so that the water content tends to remain low [19].

According to [13] the best quality of cocoa beans for water content is not more than 7.5%. While the water content value obtained in this study was more than 7.5%, making this study not in accordance with the established standards. There are several things that affect the high water content in dry cocoa beans. Relative humidity and the desorption process are the main reasons for the high water content in dry cocoa beans. The water content of cocoa beans will reach equilibrium with the relative humidity (RH) around them. The higher the humidity, the more likely it is that cocoa beans will absorb water vapor from the environment. This causes the water content in cocoa beans to increase, even after the drying process. Furthermore, when cocoa beans are in an environment with lower humidity than the water content in them, the desorption process occurs. This is the process by which water molecules move from the beans to the air, reducing the water content in the beans. Conversely, if the humidity of the environment is high, desorption will decrease, and cocoa beans can absorb more water, increasing the water content in them [20].

D. Fat Content

TABLE VI. FAT CONTENT OF DRIED COCOA BEANS

Fermentation Period (L)	Yeast Concentration (R)		
	R ₁ (0%)	R ₂ (1%)	R ₃ (2%)
L ₁ (2 days)	36.600 (b)	38.452 (b)	34.830 (c)
	B	A	C
L ₂ (4 days)	38.871 (a)	39.154 (a)	40.246 (b)
	B	B	A
L ₃ (6 days)	38.721 (a)	39.389 (a)	43.835 (a)
	C	B	A

Based on the analysis of variance in Table VI. the interaction between fermentation time (L) and yeast addition (R) had a very significant effect on the fat content of dry cocoa beans. The simple effect of fermentation time (L) at the same yeast concentration (R) showed that at the R1 level (without yeast), 4-day fermentation (L2) produced a fat content of 38.871%, significantly compared to the 2-day fermentation time (L1) which only produced 36.600%. At the R2 level (1% yeast), 6-day fermentation time (L3) produced a fat content of 39.389%, which had no significant effect compared to the 4-day fermentation time (L2) which produced 39.154%. However, at the R3 level (2% yeast), 6-day fermentation time (L3) showed a very significant effect on the fat content with a value of 43.835%, compared to the 2-day fermentation time (L1) which produced a fat content of 34.830%.

The analysis of variance shown in Table VI shows that long fermentation time and yeast concentration have a very significant effect on fat content. This is in line with research [21] that cocoa bean pulp contains a lot of carbohydrates. During the fermentation process, microorganisms will break down sugar (carbohydrates) in cocoa beans into pyruvate. Carbohydrates undergo glycolysis which produces pyruvate, then pyruvate will be converted into acetyl CoA. Acetyl CoA is a raw material in the formation of fat through the process of lipogenesis. This process causes an increase in fat during fermentation. This study [22] also supports that longer fermentation time can improve biochemical processes, which ultimately increase the fat content of cocoa beans. Thus, the optimal combination to increase the fat content of cocoa beans is fermentation for 6 days (L3) with the addition of 2% yeast concentration (R3).

The high water content and fermentation process factors have an influence on the total fat content in dry cocoa beans. High water content in cocoa beans can cause a decrease in fat content. A study [16] shows that the higher the water content, the lower the fat content measured in cocoa beans. This is due to the fact that water acts as a diluent component that can reduce the fat concentration. If the beans are not dried properly after fermentation, the water content remains high, which results in low fat content.

Thus, not only the duration of fermentation and yeast concentration determine the increase in fat content, but high water content and optimal fermentation conditions are also important factors that need to be considered. Cocoa bean fermentation is an important stage in the processing process that plays a significant role in influencing the chemical composition of cocoa beans. During fermentation, the activity of microorganisms, especially bacteria and yeast, contributes to the decomposition of complex compounds found in beans,

including fat compounds. One of the processes that occurs is the activity of the lipase enzyme produced by these microbes. This enzyme functions to hydrolyze triglycerides into fatty acids and glycerol, which ultimately causes a decrease in the total fat content in cocoa beans. This process not only affects the chemical properties of the beans but also contributes to the formation of the final quality of cocoa beans, both in terms of chemistry and organoleptics [23].

E. Temperature

Based on the results of temperature measurements during the fermentation process, the results obtained will be presented in Fig. 3, Fig. 4, and Fig. 5.

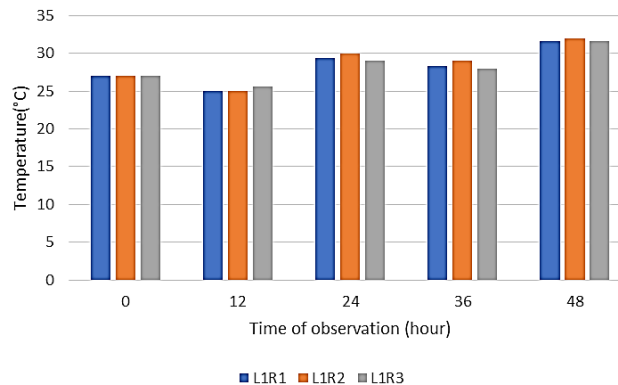


Fig. 3. Graph of Changes in Cocoa Bean Fermentation Temperature (L1)

Based on the graph of changes in cocoa bean fermentation temperature above, it shows that the temperature changes are increasing over time. Observations for 2 days in the L1 treatment obtained the highest temperature at 48 hours of fermentation, namely 32°C.

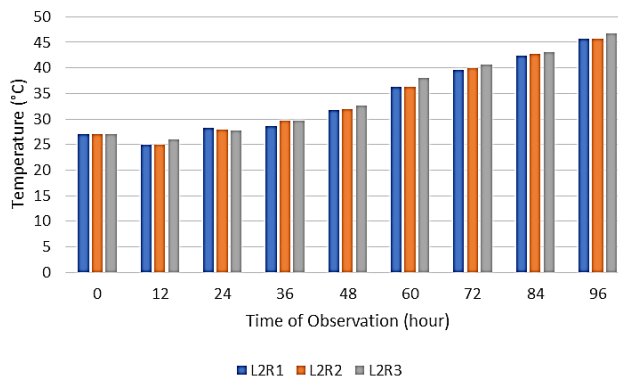


Fig. 4. Graph of Changes in Cocoa Bean Fermentation Temperature (L2)

Based on the graph of changes in cocoa bean fermentation temperature above, it shows that the temperature changes are increasing over time. Observations for 4 days in the L2 treatment obtained the highest temperature at a fermentation time of 96 hours, namely 46°C.

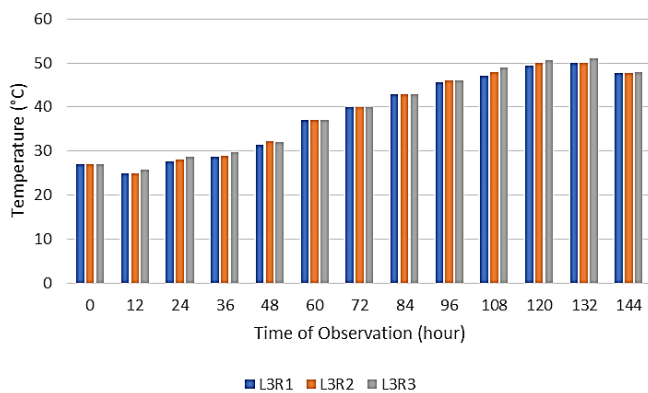


Fig. 5. Graph of Changes in Cocoa Bean Fermentation Temperature (L3)

Based on the graph of changes in the temperature of cocoa bean fermentation above, it shows that the temperature changes are increasing over time even though at some times the temperature decreases. Observations for 6 days in the L3 treatment obtained the highest temperature at a fermentation time of 132 hours, namely 51°C.

Because the fermentation temperature has reached 44–48°C in each treatment, the fermentation process is considered to be running well or successful. According to [24], fermentation is considered successful if the temperature of 44°C is achieved for at least 6 hours. Temperature changes during the fermentation process are influenced by the metabolic activity of microorganisms and the type of container used. In addition, other factors such as the weight of wet beans, the frequency of stirring or turning, aeration, and environmental temperature also affect the temperature changes. The influence of environmental temperature is quite influential in reducing the fermentation temperature. Occurring on the sixth day or measurement in 144 hours, rainfall on the 6th day was quite high causing a decrease in temperature, namely from 51°C down to 47°C. During fermentation, microbes produce heat as a result of metabolism. However, if the environmental temperature is too low, heat loss from the pile of cocoa beans will be higher than the heat production by microbes. This causes the fermentation temperature. The balance between heat production and loss is very important to keep the fermentation temperature within the optimal range [25].

CONCLUSION

Based on the research results, the following conclusions were drawn: The interaction of the length of fermentation time with the concentration of yeast given had a significant effect on the variable number of seeds per 100 grams, and had a very significant effect on the variables of water content and fat content, while having no significant effect on the cut test variable. The best treatment combination occurred in the interaction of the length of fermentation time of 6 days and the addition of 2% concentration (L3R3) to obtain water content and fat content.

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