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The effect of techniques and fermentation time on cocoa beans quality (*Theobroma cacao* L.)

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Abstract. This research was aimed to know the effect of techniques and fermentation time on of cocoa beans quality. Experiment was conducted using two variables and three replications. Variable fermentation techniques was with two factors; spontaneous and controlled. Variable fermentation time was with three factors namely 72, 120 and 168 hours. Cocoa quality was determined by physical quality test consists of temperature during fermentation. Required quality for common and special beans is in accordance to SNI 2323: 2008. Chemical test for cocoa qualities were fat, moisture content, ash, protein, carbohydrates and pH. Data were analyzed using ANOVA followed with Duncan Test. Results showed fermentation techniques and time were significantly affect to temperature, fat contents, ash, pH. Especially the fermentation time, it's also affect significantly to the moisture and starch. Best result was shown in cocoa beans with controlled fermentation techniques at 72 hours as shown by temperature at 44,67°C, 31,46% fat content, 2,97% moisture, 1,99% ash, 39,37% starch and 5,69 pH. Following this research, better quality of cocoa beans could obtain in the next study using different microorganism (heterofermentative).

1. Introduction

Cocoa (*Theobroma cacao* L.) is one of the leading commodities in the plantation sub-sector. The cocoa commodity has consistently played a role as a source of foreign exchange that makes an important contribution to the structure of the Indonesian economy [1]. The cocoa commodity is also an employment provider since it is able to absorb a large workforce. In addition, cocoa also plays a role in encouraging the regional and agro-industrial development [2]. Cocoa is an agricultural product that has an important and reliable role in realizing agricultural development. In the last two decades, the area of cocoa plantation was grow and increase the total of national cocoa production. Cocoa is the third largest foreign exchange commodity in the plantation sub-sector after oil palm and rubber [3].

Indonesia is the second country which has largest cocoa production in the world in 2012 after Ivory Coast, with production at 2010, 2011, 2012 of 837,918, 712,231 and 740,513 tons respectively [4]. The processing of cocoa beans, especially the fermentation stage, determines the quality of final product from cocoa because in the fermentation process there is a formation of a candidate taste (precursor) that is unique to cocoa and it's also reduction of unwanted harsh and bitter tastes [5].

Saccharomyces cerevisiae is the dominant yeast during cocoa fermentation because of its high survival rate [6]. At the beginning of 24 hours fermentation, yeast dominates the fermentation, then it decreases and replaced by the growth of lactic acid bacteria. Initially, the role of lactic acid bacteria in



cocoa fermentation was not well known, until the discovery of *Lactobacillus plantarum* which was consistent during fermentation [7]. Based on this, the controlled fermentation technique uses the addition of *Saccharomyces cerevisiae* and *Lactobacillus plantarum*. The natural fermentation process takes relatively longer, around 5–7 days (120-168 hours) which causes cocoa farmers to be reluctant to ferment beans due to technical and economic problems [8]. However, chocolate with criollo varieties tended to ferment faster (72 hours) and chocolate with forastero varieties for 120 hours. Based on this, the fermentation time used in this study were 72, 120 and 168 hours.

Cocoa beans that are dried without fermentation, will have low quality because do not have chocolate flavor candidate. For this reason, fermentation process is very important in cocoa processing process aimed good cocoa quality resulted. The purpose of this study was to determine the effect of the technique and time of fermentation on the quality of cocoa beans.

2. Materials and methods

2.1. Materials

The research materials were cocoa pods, bacterial starter namely *Saccharomyces cerevisiae* and *Lactobacillus plantarum*. Materials for proximate analysis, physical and chemical quality of cocoa beans were selenium, concentrated H₂SO₄, NaOH, boric acid (H₃BO₃), indicators of BCG-MM, and HCl. The tools used for cocoa fermentation process were box made of wood for accommodating ± 5 kg of cocoa beans, a wooden bat, banana leaves for base and cover of the box, bucket, scale, rubber gloves, thermometer, knife, spatula and oven. The tools used for proximate analysis consist of weighing paper, porcelain dishes, ovens, kilns, desiccators, analytical scales, filter paper, Soxhlet extraction, Kjeldahl tubes, Erlenmeyer, measuring cups, and pipettes.

2.2. Methods

2.2.1. Preparation of cocoa pod. The cocoa sample in this study was cacao with yellow color. Pods was cracked using wood stick or knife, but the cocoa beans were avoided to touch using metal. Cocoa beans are removed by hand or wooden spoon. Cocoa beans are taken either from the pods that are still clustered in the placenta / heart / liver of the cocoa pods. The cocoa seed that not removed from the placenta will affect the appearance of dry cocoa beans produced.

2.2.2. Sortation and weighing. The cocoa that has been collected from the shell is sorted and only the white one was used. The sorted cocoa beans are weighed as much as 5 kg and then put into the fermentation box.

2.2.3. Fermentation. Cocoa fermentation technique was divided into 2 levels; spontaneous fermentation (without any addition) and controlled fermentation (with the addition of *Saccharomyces cerevisiae* in the form of yeast as much as 10 g / kg of cocoa beans and *Lactobacillus plantarum* as much as 107 CFU). The fermentation time is divided into 3 levels, namely for 72, 120 and 168 hours (stirring was performed once every 48 hours). During the final fermentation process, temperature and pH were measured as dependent variables in the fermentation process. The method of spontaneous cocoa fermentation is that the cocoa beans are directly put into the fermentation box. For controlled cocoa fermentation, put cocoa beans into the fermentation box (5 kg), add *Saccharomyces cerevisiae* 10 g / kg cocoa beans and stir until evenly distributed. Cover the cocoa beans in the box with banana leaves (Figure 1). After 24 hours, add *Lactobacillus plantarum* 1 ml / kg cocoa beans to the fermentation box, stir until evenly and cover the fermentation box with banana leaves.



Figure 1. Fermentation process using box

9. Results and discussion

3.1. Fermentation temperature

The ANOVA showed that there was an interaction between fermentation time and temperature on the quality of cocoa beans. Fermentation time affects the temperature during fermentation. The highest temperature of cocoa beans was found at fermentation for 72 hours (Table 1).

Table 1. Fermentation temperature

Fermentation Technique	Time of fermentation (0°C)			
	0 jam	72 jam	120 jam	168 jam
Spontaneous	28.67 ± 0.58 ^c	40.67 ± 1.15 ^b	41.67 ± 0.58 ^b	41.33 ± 0.58 ^b
Controlled	29.33 ± 0.58 ^c	44.67 ± 1.53 ^a	40.33 ± 1.15 ^b	37.33 ± 0.58 ^c

Note: remarks with different number show significant at $\alpha=0.05$

At 120 and 168 hours fermentation time, the temperature of cocoa beans was not significantly different. Meanwhile, the lowest temperature was at the fermentation time of 0 hour. Temperature increase during fermentation is caused by an exothermic reaction that occurs when the pulp sugar changes to ethanol by yeast activity. As a result, the pulp melts, moisture and oxygen droplets will flow into the pile of seeds. This aeration causes a sharp rise in temperature and results in seed death. When the seed dies, the chemical reactions began in cotyledons. The temperature decrease occurs because the death process of the beans in cocoa is over and the exothermic reaction has been completed, the sugar in the cocoa pulp has completely broken down into ethanol by the activity of microorganisms [9].

The temperature increase occurs due to the metabolic processes of microorganisms during the fermentation process. The temperature decrease occurs because the metabolic process has stopped so that the temperature decreases again. The best fermentation temperature is between 40 to 50°C [10]. In spontaneous fermentation temperature ranged from 40-50°C was in fermentation for 72, 120 and 168 hours. However, highest temperature for spontaneous fermentation was during fermentation for 168 hours. In controlled fermentation, temperatures in the range of 40-50°C were in fermentation for 72 and 120 hours. However, the highest temperature in controlled fermentation was at 72 hours fermentation.

At fermentation temperature, it can be concluded that controlled fermentation saves more time than spontaneous fermentation. This can help farmers to get fermented cocoa in less time. This study supports the research that fermentation with the addition of microorganisms produces different temperatures and increases faster than spontaneous fermentation [9].

3.2. pH during fermentation

The reaction by microorganisms takes place exothermally, causing the temperature in the pulp to increase and play a role in the formation of acidity levels in cocoa beans [9]. The pH of cocoa beans is measured at 0 hours (just put into the fermentation box) and measured again when the cocoa beans to be harvested, namely at 72, 120 and 168 hours. The data on the pH of cocoa beans in the fermentation box can be seen in Table 2.

Table 2. pH

Fermentation Technique	Time of fermentation (0°C)			
	0 jam	72 jam	120 jam	168 jam
Spontaneous	5.37 ± 0.13	4.43 ± 0.13	4.97 ± 0.04	6.54 ± 0.21
Controlled	5.69 ± 0.32	4.61 ± 0.32	5.52 ± 0.34	6.62 ± 0.35

Note: remarks with different number show significant at $\alpha=0.05$

In spontaneous fermentation, the pH of cocoa beans tends to decrease, seen from the fermentation time of 0 and 72 hours, from 5.37 to 4.43 then it tends to increase when the fermentation time is 120 hours and 168 hours from 4.97 and 6.54. In controlled fermentation, the pH of cocoa beans tended to decrease seen from the fermentation time of 0 and 72 hours from 5.69 to 4.61 then tends to increase at the fermentation time of 120 and 168 hours at 5.52 and 6.62.

In this study, it was found that the fermentation rate showed significant difference in pH of dry cocoa beans, longer fermentation time the pH value fluctuated and decreased again. This occurs due to the formation of organic acids such as lactic acid and acetic acid [6]. However, pH of the cocoa beans must not be too acid, but must be above 5.0 to have a good taste. The acidity of the seeds is very important aspect of chocolate flavor because it is not only related to sour taste, but also determines the course of the reaction in which flavor compounds are formed. The formation of flavor is more potential to occur in seeds pH 5.0-5.5 than pH 4.0-4.5 [10].

At the pH of fermentation, it can be concluded that the best fermentation is controlled fermentation with fermentation time of 120 hours and it is suspected that the best spontaneous fermentation is fermentation with a time of 144 hours, because fermentation for 120 hours has not shown a potential pH and fermentation for 168 hours exceeds the potential pH of the fermentation process of cocoa beans. This result show that fermentation with the addition of microorganisms produces different temperatures and increases faster than spontaneous fermentation [9,10].

3.3. Fat content

In spontaneous fermentation, the average fat content tends to increase seen (Table 3) from the fermentation time of 72 hours, 120 hours to 168 hours, namely from 26.77, 27.04, to 31.72%. In controlled fermentation, the average fat content tends to increase seen from the fermentation time of 72 hours, 120 hours to 168 hours, namely 31.46, 22.05, to 32.41%.

Table 3. Fat content

Fermentation Technique	Time of fermentation (0°C)		
	72 jam	120 jam	168 jam
Spontaneous	26.77 ± 0.52c	27.04 ± 1.12c	31.72 ± 0.56b
Controlled	31.46 ± 0.61b	32.41 ± 0.75b	37.20 ± 1.39a

Note: remarks with different number show significant at $\alpha=0.05$

The fermentation time of cocoa beans affects the fat content of cocoa beans. In the anaerobic fermentation (without oxygen), the microorganisms that grow increase fat content by converting compounds such as polyphenols, proteins and sugars. This is what causes longer fermentation time

affect to the fat content of cocoa beans. The longer fermentation process, the fat content will increase due to the increasing number of compounds being changed [11].

3.4. Moisture content

The highest moisture content in cocoa beans (Table 4) was during the fermentation time for 120 hours, while the lowest moisture content in cocoa beans was at the 72-hour fermentation time. Fermentation time affects the moisture content of cocoa beans. At the time of fermentation, longer fermentation process will decrease the moisture content. This is due to the activity of microbes and enzymes in degrading complex tissues (pulp) into simple organic compounds which more active so that the pulp is destroyed, as a result the pores are opened which makes it easier to remove moisture during drying [5].

Table 4. Moisture content

Fermentation Technique	Time of fermentation (0 ^o C)		
	72 jam	120 jam	168 jam
Spontaneous	4.27 ± 0.22 ^b	4.48 ± 0.30 ^b	4.58 ± 0.51 ^b
Controlled	2.97 ± 0.07 ^c	4.35 ± 0.13 ^b	5.29 ± 0.31 ^a

Note: remarks with different number show significant at $\alpha=0.05$

More time of fermentation causing the increase in moisture content [10]. Acid that arises in the fermentation as result of microorganisms' activity, breakdown cells in the pulp into liquid. This is the causing factor that increase in moisture content during the fermentation process. The best moisture content in perfectly fermented cocoa beans is a moisture content ranging from 5 - 7.5% with the lowest moisture content being the best fermentation time. In this study, the spontaneous fermentation which had the best moisture content was in the fermentation time of 72 hours, with the yield of moisture content of 4.27%, in controlled fermentation, the best moisture content was 72 hours with a moisture content of 2.97%.

3.5. Ash content

In spontaneous fermentation, the average ash content (Table 5) tended to decrease, seen from the fermentation time of 72 hours, 120 hours to 168 hours from 1.97% to 1.95%, then to 1.95%. In controlled fermentation, the average ash content tends to decrease seen from the fermentation time of 72 hours, 120 hours to 168 hours from 1.99% to 1.99%, then to 1.93%.

Table 5. Ash content

Fermentation Technique	Time of fermentation (0 ^o C)		
	72 jam	120 jam	168 jam
Spontaneous	1.97 ± 0.04 ^b	1.95 ± 0.01 ^b	1.95 ± 0.04 ^b
Controlled	1.99 ± 0.04 ^a	1.99 ± 0.03 ^a	1.93 ± 0.02 ^b

Note: remarks with different number show significant at $\alpha=0.05$

In this study, the ash content decreased in both spontaneous and controlled fermentation. Total ash produced has decreased with increasing fermentation time because the fermentation process triggers the release of compounds and moisture from the cocoa beans [12].

3.6. Protein content

In spontaneous fermentation, the average protein content (Table 6) tended to increase seen from the fermentation time of 72 hours, 120 hours to 168 hours, from 10.77% to 12.11% then to 12.30%. In controlled fermentation, the average protein content tended to decrease seen from the fermentation time of 72 hours, 120 hours to 168 hours, namely from 11.64% to 13.47% then to 13.94% (Table 5).

Table 6. Protein content

Fermentation Technique	Time of fermentation (0 ⁰ C)		
	72 jam	120 jam	168 jam
Spontaneous	10.77 ± 0.03 ^a	12.11 ± 0.39 ^a	12.11 ± 0.39 ^a
Controlled	11.64 ± 0.65 ^a	13.47 ± 0.28 ^a	13.47 ± 0.28 ^a

Note: remarks with different number show significant at $\alpha=0.05$

Protein content increases with the increasing fermentation time and drying temperature. During drying process, the product will lose its moisture content so that the amount of protein that is dried is higher or more concentrated compared to the protein of cocoa beans without fermentation [12].

3.7. Carbohydrate content

In spontaneous fermentation, the average of carbohydrate content tends to decrease, seen from the fermentation time of 72 hours, 120 hours to 168 hours, from 53.03% to 50.47%, then it tends to decrease to 36.89%. In controlled fermentation the average protein content tended to increase seen from the fermentation time of 72 hours to 120 hours from 48.08%. to 55.30%, then it tended to decrease at the fermentation time from 120 to 168 hours to 39.37% (Table 6).

Table 7. Carbohydrate content

Fermentation Technique	Time of fermentation (0 ⁰ C)		
	72 jam	120 jam	168 jam
Spontaneous	36.89 ± 1.39 ^c	50.47 ± 1.94 ^b	53.03 ± 1.30 ^a
Controlled	39.37 ± 0.76 ^c	48.08 ± 0.95 ^b	55.30 ± 0.28 ^a

Note: remarks with different number show significant at $\alpha=0.05$

Further test showed that the fermentation time of cocoa beans affected the carbohydrate content of cocoa beans. The highest carbohydrate content in cocoa beans was during fermentation for 120 hours, while the lowest carbohydrate was during fermentation for 168 hours. The Maillard reaction causes a reduction in the content of reducing sugars, amino acids and peptides in cocoa beans, so that the relative carbohydrate content will increase [13]. Some of the flavor compounds formed are volatile compounds. This reaction is increasing in roasting perfectly fermented cocoa beans, so that the carbohydrate content also increases. Perfect fermentation is fermentation that produces large carbohydrate content [14]. In this study, both spontaneous and controlled fermentation showed highest carbohydrate content in fermentation for 120 hours.

4. Conclusion

From the study concluded that the fermentation technique gave significant difference in the temperature, fat content, ash content and pH of cocoa beans during fermentation. Meanwhile, the fermentation time had a significant effect on temperature during fermentation, fat content, moisture content, ash content and pH of cocoa beans during fermentation. There is a significant interaction between fermentation technique and time at temperature and pH of cocoa beans during fermentation, fat content, moisture content, and ash content. The best treatment was obtained from a controlled fermentation technique with a fermentation time of 72 hours. The results of this best treatment test were temperature at 44.67°C, fat content of 31.46%, moisture content of 2.97%, ash content of 1.99%, protein content of 11.64%, carbohydrate content of 39.37%, and a pH value of 5.69. The fat content in fermented cocoa beans is higher than the unfermented fat content, where the fat content will give a better aroma after roasting the cocoa beans.

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