The Effect of Pod Storage on Chemical and Microbiological Characteristics of Organic and Non–organic Balinese Cacao Pulps

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Abstract. The purpose of this study was to determine the effect of Organic and Non-Organic Balinese Cacao (*Theobroma cacao* L.) storage duration on chemical and microbiological levels in order to know the exact storage period to get a good substrate for fermentation. This research is the first step in preparing Specialty Cocoa. Chemical parameters such as reducing sugar, total sugar, protein content, moisture content, and pH along the pod storage were determined in this study. The ideal substrate condition for cocoa fermentation was suggested on the 4th d, in which the pulp contained total sugar of 12.5 % and moisture content of 86 %. Meanwhile, the statistical analysis was done to test whether the duration of pod storage has a significant effect on the presence of bacteria related to fermentation. The test showed that the duration of pod storage did not have a significant effect on either lactic acid bacteria or acetic acid bacteria content (P value > 0.05). Nevertheless, the research found that lactic acid bacteria content was up to 1.9 log CFU mL^{-1} and acetic acid bacteria content was up to 2.72 log CFU mL^{-1} during the 5 d of pod storage.

Keywords: Fermentation, moisture, storage, sugar, *Theobroma cacao* L.

1 Introduction

Currently, the market of chocolate industry in developed countries continues to grow, especially in the market segment known as specialty cocoa or Fine and Flavor Cocoa (FFC) [1]. In order to produce FFC, it requires good cocoa handling at the farm level because its precursor compounds are formed especially during fermentation which is usually carried out at the farm level in the country of origin. Although some studies have been carried out on dry seeds such as activation of flavor precursors [2, 3] and starter culture formulations [4–6], it can still be seen that many efforts need to be made and are still constrained. Even the flavor modification of dried cocoa beans is not necessary if the natural fermentation process is carried out properly.

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Indonesia is the third-largest cocoa producer in the world. The seven largest cocoa-producing countries in the world today are Ivory Coast, Ghana, Indonesia, Nigeria, Brazil, Ecuador, and Malaysia. Indonesia contributes around 8 % to 17 % of world production [7]. Therefore, research support needs to be carried out from various parties to maintain the continuity of cacao (*Theobroma cacao* L.) cultivation in Indonesia and the sustainability of cocoa raw materials from Indonesia.

The focus of the attention to the quality of cocoa in the country of origin should be the fermentation stage, and the fermentation process needs a proper preparation or a pre-conditioning stage. This preconditioning includes storage of cocoa pods, pre-drying, and reduction of cocoa pulp [8–10]. This research was initial research and focused on the storage stage of cocoa pods and the use of pectinase and Ca^{2+} enzymes during fermentation. It was hoped that after this research was carried out, there would be one standard method to prepare the fermentation process, especially the desired composition of cocoa pulp including what parameters must be controlled and a solution to be found so that the fermentation takes place properly and it can produce quality dry cocoa beans.

Several parameters of cocoa quality based on SNI 2323.2008 concerning cocoa beans are only related to the number of beans, moisture content, fat content, and total microbial pollutants. Several organoleptic parameters such as cocoa beans that are not acidic, less bitter, not as spicy, and have a distinctive taste are usually required in the specialty cocoa market. The research that has been conducted by [11–14] stated that increasing the time of cocoa pods storage days, can reduce bitter and sour taste although the polyphenol compounds are also reduced. However, [1] in his review stated that detailed research is needed in terms of reducing the protein and sugar content of cocoa pulp to reduce the sour and bitter taste. This study aimed to determine the effect of cocoa pod storage time on the total sugar content of cocoa pulp before fermentation to determine the effect of cocoa pod storage time on protein content in cocoa pulp before fermentation. The urgencies of this study were the increasing trend of the chocolate product market on demand for the best and the distinctive taste of cocoa as its raw material has prompted research support from various groups to get the best cocoa by performing good fermentation. However, in order to carry out good fermentation, it was necessary to prepare an ideal substrate beforehand. The conditioning of cocoa pulp as a substrate and raw material for fermentation was obtained from preconditioning fermentation of cocoa, one of which was determining the length of time for the fermentation. This research was the beginning of further research in an effort to produce the best-fermented cocoa from various sources in Indonesia that have different conditions for cocoa pods.

2 Methods

The research was conducted in two stages, namely testing the time of cocoa pods storage and testing the addition of pectinase and Ca^{2+} during fermentation of cocoa beans. This article reported the first step which is an analysis of cocoa pod storage time in a farm. The organic and non–organic cocoa pods were obtained from cocoa plantations in Bali. Cocoa pods were opened according to the day of observation, every day from 0 d to 5 d. The pulp was manually separated from the beans by rubbing the beans (with adhering pulp) between fingers and squeezing the pulp into a clean sample bag. The pulp was then stored at –200 °C prior to analyses.

Determination of reducing sugar content in a sample can be determined using a DNS reagent or dinitro salicylic acid / 3,5–dinitrosalicylic acid. DNS acts as an oxidizer and in alkaline conditions, it will react by reducing sugars to form 3–amino–5–nitrosalicylic acid [15]. DNS standard solutions are made using glucose dissolved in distilled water with a

concentration of 1 000 mg kg^{-1} and diluted to 200 mg kg^{-1}, 400 mg kg^{-1}, 600 mg kg^{-1}, and 800 mg kg^{-1} [15].

Total sugar standard solution is made using sucrose which is dissolved in distilled water with a concentration of 100 mg kg^{-1} and diluted to 20 mg kg^{-1}, 40 mg kg^{-1}, 60 mg kg^{-1}, and 80 mg kg^{-1}. 1 mL of the sample was put in a test tube and 1 mL of phenol acid reagent was added and shaken until homogeneous. After that, 5 mL of concentrated sulfuric acid was added to the mixture. The mixture was then shaken until homogeneous and left for 10 min. After leaving, the mixture was placed in a water bath at a temperature of 25 °C to 30 °C for 15 min. The absorbance of the sample was measured at a wavelength of 488 nm [16].

The next step was testing the water content [17], measuring the weight of a sample of fresh cocoa pulp (W1), drying them in the oven with a temperature of 102 °C until it reached the weight constant (W2). Then the percentage of water content was calculated [18, 19].

The measurement of protein content used Kjeldahl Method (SNI–01–2782–1990). The stages of protein analysis using the Kjeldahl method included digestion, distillation, and titration [18, 19]. The destruction aimed to release the element N from the protein which was converted into ammonium sulfate. In the distillation stage, the ammonium sulfate was converted into ammonia which was captured by the standard acid solution excess. The remaining acid which did not react with ammonia was titrated so that the amount of ammonia from the N protein sample could be determined..

Microbiological Testing. The ability of the cocoa pulp medium to support microorganisms is very important for the fermentation process to run well. To see the ability of the medium to grow yeast, a PDA with the addition of chloramphenicol, Lactic Acid Bacteria (LAB) with MRSA media, Acetic Acid Bacteria (BAA) with NA media can be used [20].

Data Processing Techniques. After obtaining the results of the analysis of each test material, an analysis of the effect of time cacao $pods^{-1}$ was analyzed on the total sugar content, protein content, and amount of yeast, LAB, and BAA using statistical analysis of variance (one way ANOVA) followed by Tukey's comparison to test if H1 was accepted. The independent variables for the ANOVA test were storage time of cocoa $pods^{-1}$ (P0, P1, P2, P3, P4, P5). The dependent variables for the ANOVA test were the total sugar content, protein content, and amount of yeast.

3 Result and discussions

3.1 Reducing sugar

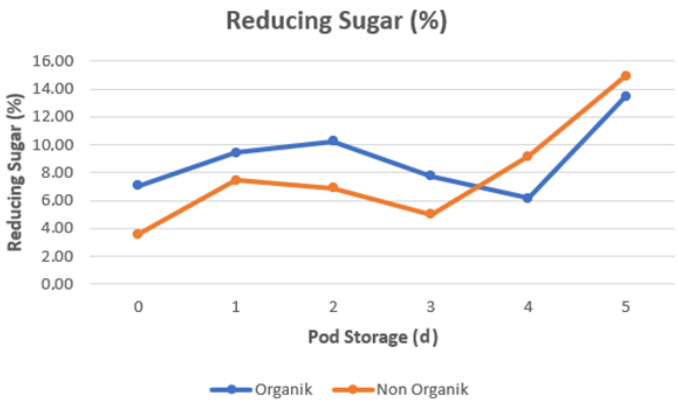

Fig. 1. Effect of pod storage (d) on reducing sugar content

The statistical test results of reducing sugar data showed that the pod storage time had a significant effect on reducing sugar levels (*P* value < 0.05), which means the longer the pod storage time, the higher the tendency of reducing sugar levels. The results of statistical tests showed that the variation of pod storage time had an interaction with the reducing sugar content of cocoa pulp (*P* value > 0.05).

In [14], the reduced sugar content of cocoa that has been stored for 10 d decreased from 7.5 % to 4.8 %, slightly different from the results of the observations in this study that only decreased the reducing sugar levels after 1 d and 2 d of storage. This reduction in reducing sugar levels is caused by the existing reducing sugars being converted into energy for physiological processes and metabolic activities that are present in the cocoa pods. Sampling for the next fermentation test was taken from the 4th d storage sample because it had the lowest value, namely 6.14 %. The adequate sugar content can reduce the level of a sour taste in dry cocoa beans, and speed up the fermentation time [1].

3.2 Total sugar

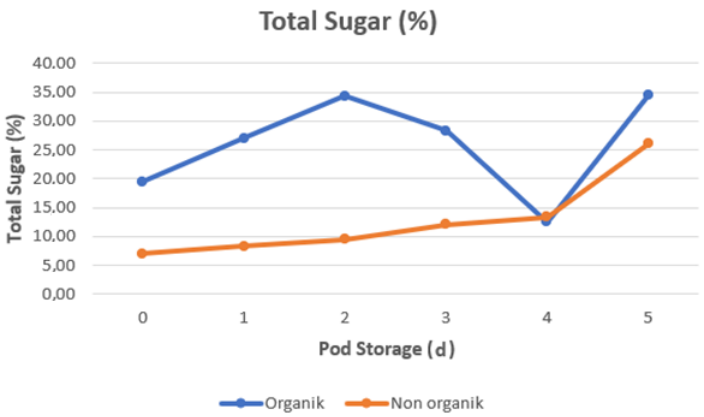

Fig. 2. Effect of pod storage (d) on total sugar

The statistical test results from the total sugar data showed that the cacao did not have a significant effect on the total sugar content (*P* value > 0.05).

Total sugar data shows that the total sugar content was quite high in the organic cocoa pulp so the highest value was 34.4 % after the 2nd d of storage and reached the lowest level after the 4th d of storage to reach 12.5 %. This is influenced by the physiological activity of the pulp breaking down into simpler compounds. The published result stated that cocoa pulp contains 80 % to 90 % water, 10 % to 13 % sugar, 1 % pectin, so sampling to carry out the next fermentation process can be carried out on organic cocoa pulp after the 4th d of storage, based on this data (total sugar 12.5 %).

3.3 Water content

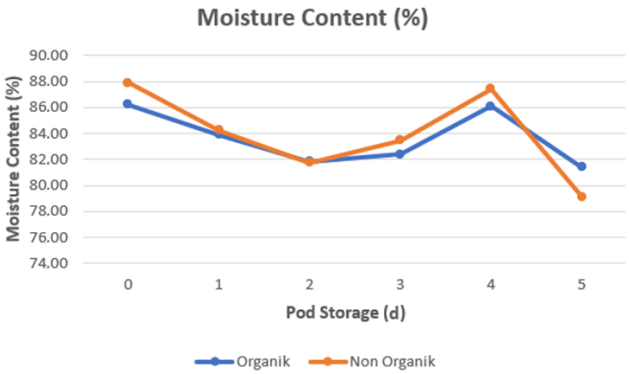

Fig. 3. Effect of pod storage on moisture content

The published result stated that cocoa pulp contains 80 % to 90 % water and this research shows that it contains 79 % to 86 % it seems that the post-harvest respiration and transpiration processes at this point produce the highest moisture content.

Taking samples for the next fermentation process is better done at 4 d of storage because after the 4th d of storage, the water content tends to decrease. Low water content provides unfavorable conditions for microbial activity during fermentation.

3.4 pH

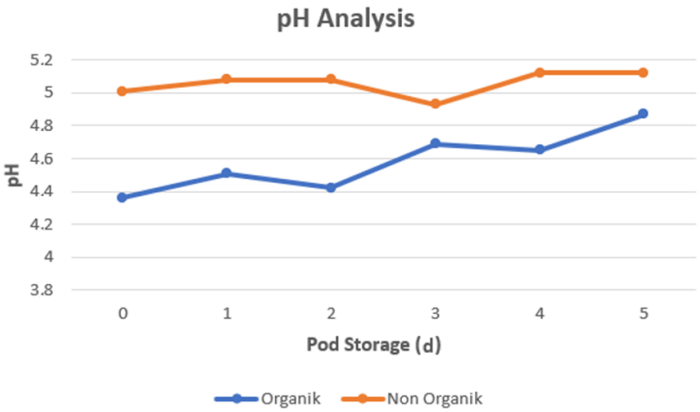

Fig. 4. Effect of pod storage (d) on pH

The results of statistical tests from pH data show that the storage time has a significant effect on the pH value (P value < 0.05). From the pH observation data, it can be seen that organic cocoa pulp is lower than non–organic cocoa pulp and increases with increasing pod storage days. The pH of several other cocoa varieties outside Indonesia ranges from pH 3 to pH 3.5 so the pH of Balinese cocoa is relatively higher than cocoa in other countries. The pH conditions play a role in good fermentation conditions, so pod storage until the 4 d can still be used because after 4 d of storage the organic cocoa pulp has increased.

3.5 Protein

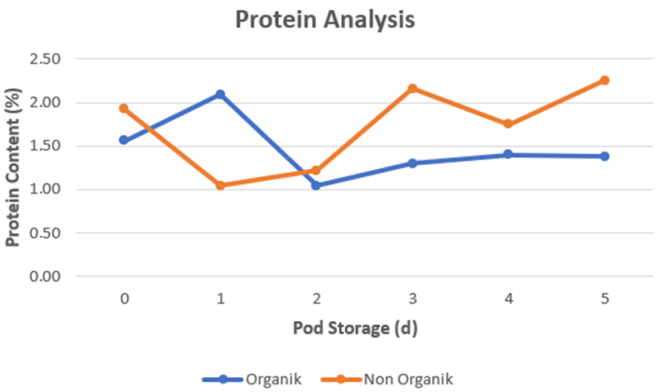

Fig. 5. Effect of pod storage on protein content

From the protein observation data, it can be seen that the protein content ranges from 1.04 % to 2.25 %, which is relatively small compared to some data on the protein content of other cocoa varieties, which is around 10 % to 24 %. Data on protein content in organic and inorganic cocoa pulp were statistically significant ($P < 0.05$).

3.6 Microbiology test

Production of acids in the pulp is important in cacao fermentation as these acids are diffused into the beans and subsequently induce the important reactions leading to well-fermented cocoa beans. Acid production in the fermentation stage is influenced by the existence of lactic acid bacteria and acetic acid bacteria in the fermentation stage. However, the aim of this research was to get a proper preparation (pre-conditioning stage) before fermentation, not yet focused on the number of those bacteria. Thus, the statistical analysis was done to test whether the duration of pod storage has a significant effect on the presence or absence of those bacteria. The statistical test showed that the duration of pod storage had a significant effect on neither lactic acid bacteria nor acetic acid bacteria content (P value > 0.05). Nevertheless, the research found that lactic acid bacteria content was up to 1.9 log CFU mL^{-1} and acetic acid bacteria content was up to 2.72 log CFU mL^{-1}.

Table 1. Microbiological result

Pod Storage (d)	Lactic Acid Bacteria (log CFU mL^{-1})		Acetic Acid Bacteria (log CFU mL^{-1})	
	Organic	Non–organic	Organic	Non–organic
0	0.00	0.00	1.29	1.30
1	0.00	1.90	2.72	2.50
2	0.00	1.30	0.00	2.34
3	0.00	1.45	1.00	1.00
4	1.30	1.45	2.07	1.75

4 Conclusions

The next step of the research will be using organic cocoa pods from Bali with a pod storage time of 4th d. Observations will be conducted on the fermentation process with the addition of pectinase and Ca^{2+}. The final result of the fermentation process will not only be tested

according to the provisions of SNI 2323–2008 but also be tested with international standard flavor and taste by trained panelists from Puslitkoka Jember, East Java, Indonesia.

This research was funding by Hibah Internal of Surabaya University, East Java, Indonesia with agreement letter number 050/SP/Lit/LPPM-01/FTB/XII/2019. The authors thank the Surabaya University, East Java, Indonesia, and declare no conflict of interest.

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