Small-Scale Fermentation of Cocoa Beans and the Parameter for on-Process Monitoring

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Abstract

Cocoa bean fermentation is a critical step in the formation of chocolate aroma precursors. Small-scale fermentation is needed to accommodate fermentation practice during low season and fruit scarcity. The study was conducted to address the minimum amount of wet cocoa beans required for a successful fermentation. In the trials, wet cocoa beans at different quantities, i.e. 1, 2.5, 5, 7.5, 10, 15, and 40 kg, were incubated in separate wooden boxes for 4 days (96 hours) and turned once after 48 hours. Temperature and pH of the pulp and beans were measured every 6 hours. Dry beans were visually inspected to determine the number of grayish and purple cotyledons indicating unfermented beans. In general, smaller scale fermentations, i.e. 1 to 15 kg, resulted in similar profiles with that of standard mini-box fermentation (40 kg) in terms of temperature, pulp pH and bean pH. The 40 kg fermentation resulted in the lowest percentage of purple beans with no grayish beans, meanwhile smaller scale fermentations showed higher number of unfermented beans. The logarithmic regression indicates that as much as 1.4 kg beans my producing unfermented beans of less than 20% which meets the criteria of grade 3 based on the Indonesian National Standard for cocoa bean (SNI 2323:2008/Amd1:2010). Data analysis shows that temperature below 35°C for 42 hours was associated with higher percentage of grayish and purple beans. It is concluded that cocoa bean fermentation can be carried out at least at 5 kg scale, and temperature at 42 hours could be a parameter for process monitoring.

Keywords: cocoa, fermentation, quality, small-scale, smallholder

INTRODUCTION

Cocoa, as the main ingredient of chocolate, has continuously been growing in the context of global demand. World consumption is estimated to increase of up to 30% by 2020 (Beg *et al.,* 2017). Indonesia, as one of major cocoa producing countries, is notorious for low quality beans due to high number of unfermented beans and waste, particularly those from smallholder farms (Neilson, 2007). There are several reasons underlying the non-fermentation practice of smallholder farmers in Indonesia. A survey conducted by Raharto (2019) revealed that the main factor is the unappealing price difference between unfermented and the fermented beans. Another factor is farmers' perception on complicated process of fermentation and uncertain quality of the products.

The quantity of wet beans is important for a successful fermentation. The typical quantity for fermentation is around 200–1000 kg of wet beans (Ardhana & Fleet, 2003; Camu *et al.*, 2008; Fahrurrozi *et al.,* 2015). A shallow box fermentation had also been introduced, involving 150 kg of wet beans (Sulaiman & Yang, 2015). Furthermore, protocols for smaller scale fermentation, i.e. 40 kg (Pereira

et al., 2013) and 20 kg (Lefeber *et al.*, 2011) had been introduced.

However, the adequate quantity for a proper cocoa bean fermentation in Indonesia has been challenging. Indonesian cocoa beans are mostly produced by smallholder farmers with average farm size that is less than two hectares with dry bean production is merely 350 kg.ha^{-1}.year^{-1} (Tothmihaly *et al.,* 2017). In certain months, the production of wet cocoa beans in a farm will plummet into less than 10 kg per harvesting period (Santosa *et al.,* 2018). High variation in the quantity wet beans a protocol for handling for lower amount cocoa beans. In addition, to determine a successful fermentation, an on-process monitoring indicator should be established.

In this study, cocoa fermentations were conducted using several different quantities of wet beans. In the standard protocol of fermentation, minimum quantity of wet beans at 40 kg with 40 cm of depth is recommended (Yusianto, 1990). The objective of this study was to assign the lowest possible quantity that allows minimum quality of cocoa beans based on Indonesian National Standard, SNI 2323:2008/Amd1:2010 (BSN, 2010). The on-process monitoring indicator was established by means of correlational analysis between fermentation temperature and the percentage of grayish and purple beans.

MATERIALS AND METHODS

Cocoa Bean Preparation and Fermentation

The trial applied completely randomized design, with a single factor, i.e. seven level of box capacities applying three replications, resulting 21 experimental units. Cocoa pods were obtained from Kaliwining Experimental Station of ICCRI located in Jember. Pods were manually opened and beans were collected. Beans were stirred for homogenization before being distributed to separate wooden boxes at quantities 1, 2.5, 5, 7.5, 10, 15, and 40 kg. Fermentation box has a fixed width, and the length was adjusted with partitioning board from the same wood material, to maintain the depth of the beans not less than 40 cm (Yusianto, 1990). Beans were covered with jute sheets and being fermented for 96 hours with turning once after 48 hours. In the end of fermentation, beans were sun-dried on a tarp sheet and the moisture content was measured every day using a digital moisture tester. The drying was stopped when moisture content reached 7%, and the beans were stored at room temperature.

Observation and Analysis

Throughout fermentation, temperature and pH of the pulp were measured every six hours by probing thermometer and pH-meter into the fermenting mass, at depth ± 15 cm from the surface to reach center point inside the mass. At every time point, five beans were taken out, cleaned from the testa, pounded and added with 50 mL of distilled water to measure the pH of cotyledon. Dried beans after fermentation were cut length wise to assess the physical quality including the percentage of grayish, fully purple, and partly purple beans. The result was then evaluated according to Indonesian National Standard for cocoa beans, SNI 2323:2008/Amd1:2010 (BSN, 2010).

Statistical Analysis

Data from observation and physical quality analysis were collected. The mean and standard deviation values were calculated from three replications. The data were tested for Shapiro-Wilk's normality and Levene's homogeneity. Data which passed the tests were proceeded to one-way Analysis of Variance and Tukey's HSD post-hoc test.

Data that did not pass the tests were proceeded to a non-parametric Kruskal-Wallis test. Correlational analysis was done to evaluate the relationship between observational data (temperature and pH during fermentation) and physical quality (slaty and grayish bean numbers). A regression was performed on the bean weight against the number of unfermented beans to determine the lowest possible fermentation scale that allows inclusion in the criteria of SNI 2323:2008/Amd1:2010 (BSN, 2010).

RESULTS AND DISCUSSION

Fermentation is a simple yet crucial step in cocoa bean processing. This step enables the development of aroma precursors and at the same time, eliminating of bitterness and astringency. The fermentation consists of two phases, i.e. anaerobic and aerobic phases. Anaerobic phase is mostly occupied by yeasts and lactic acid bacteria. The yeast breaks down carbohydrate in the pulp resulting in the glucose, fructose and ethanol. Lactic acid bacteria then utilize yeast degradation products to yield lactic acid, acetic acid, ethanol and mannitol. Aerobic phase is commenced by turning during which oxygen is introduced. In the aerobic condition, acetic acid bacteria convert ethanol into acetic acid (de Vuyst & Leroy, 2020). The lactic acid produced in the anaerobic phase is utilized by acetic acid bacteria for growth (Pelicaen *et al.,* 2019).

Temperature and pH

The temperature profile of cocoa bean fermentation typically increases over the course of fermentation, as a consequence of growth and metabolism of the microorganism in the pulp (Lefeber *et al.,* 2011; Pereira *et al.,* 2013; Fahrurrozi, 2015). Ascending temperature profile was demonstrated in the fermentations at 7.5, 10, 15 and 40 kg, whose temperature increased at 48 and 96 hours. On the other hand, temperature in the fermentation at 1-5 kg rose only in the first 48 hours, then declined after turning to final temperature higher than the initial state (Figure 1). This suggests slower metabolism of acetic acid bacteria due to insufficient substrates given low amount of pulp in the fermenting mass (Pelicaen *et al.,* 2019; de Vuyst & Leroy, 2020).

There was rapid increase at 24 hours temperature in the 40 kg-fermentation, potentially triggered by aggressive yeast metabolism towards abundant pulp. Similarly, this is also occurred in the 5 kg fermentation. Yeast metabolism might have been accommodated at 5 kg scale, considering the availability of pulp compared with 1 and 2.5 kg fermentations. However, a rapid 24 hours temperature increase was not observed in the 7.5, 10, and 15 kg fermentations. In the anaerobic phase, the fermenting mass becomes less dense as the pulp leaches. In the 5 kg
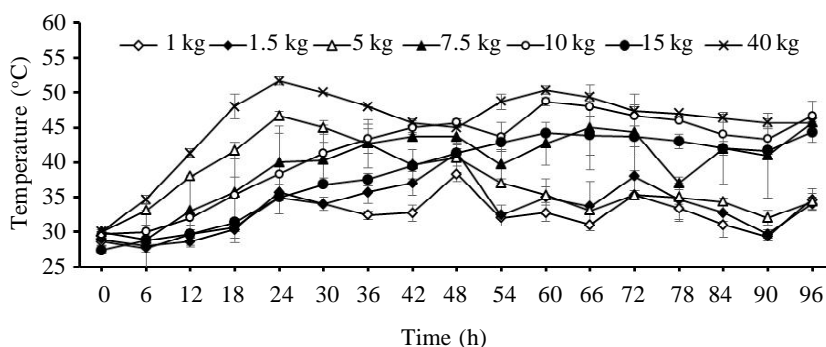

Figure 1.   Temperature profile of cocoa fermenting mass at several different scales; 1 kg, 2.5 kg, 5 kg, 7.5 kg, 10 kg, 15 kg, and 40 kg as the control treatment. Data are means of three replications

fermentation, the density might have been lower than fermentation at higher scales. This allows some oxygen to enter the fermenting mass and enhance the metabolism of some type of yeasts. *Saccharomyces cerevisiae* had been reported to have better metabolism in slightly aerated condition (Deesuth *et al.,* 2016).

Pulp pH gradually increased throughout fermentation (Figure 2). The source of acidity in the initial stage of fermentation is citric acid in the pulp. As pulp is degraded by yeast, it leaches and loses the citric acid. Additionally, organic acids produced by lactic acid and acetic acid bacteria diffuse into the interior part of the beans, inducing lower pH in the cotyledon and leaving pulp at higher pH (Ardhana & Fleet, 2003; Camu *et al.,* 2008). All treatments showed rising pH of the pulp indicating the successful pulp degradation and acid loss.

In the other hand, cotyledon pH decreased as fermentation progressed (Figure 3), confirming the acid transfer from pulp to cotyledon. In all treatments, rapid decreases occurred in the first 42 hours and slight increases were observed afterwards. The final pH was averagely 0.97–2 points lower than initial pH. The acidity of bean is critical for development of amino acids as precursors for chocolate aroma. Inside the beans, lower pH is important for activity of aspartic endoprotease that breaks down protein into peptides, then a slightly higher pH is needed for carboxypep-
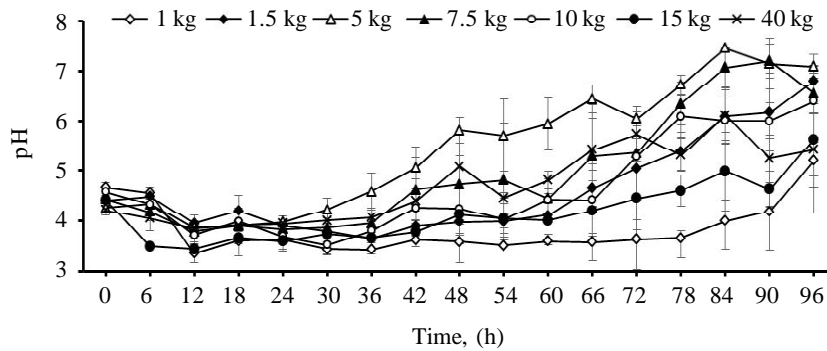

Figure 2.   Acidity (pH) profile of cocoa pulp during fermentation at several different scales; 1 kg, 2.5 kg, 5 kg, 7.5 kg, 10 kg, 15 kg, and 40 kg as the control treatment; Data are means of three replications
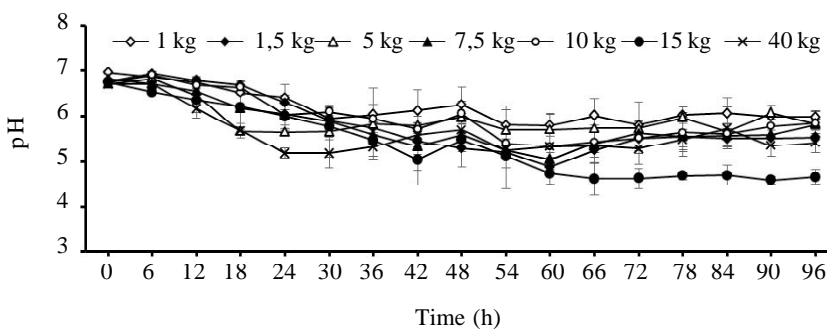

Figure 3.   Acidity (pH) profile of cocoa cotyledons during fermentation at several different scales; 1 kg, 2.5 kg, 5 kg, 7.5 kg, 10 kg, 15 kg, and 40 kg as the control treatment; Data are means of three replications

tidase activity to break peptides into hydrophobic amino acids. The hydrophobic amino acids are precursors of chocolate aroma (Misnawi *et al.*, 2002).

Interestingly, pulp pH level of the treatment at 5 kg was significantly higher at 48-66 hours than that of 1 kg treatment (p<0.05). It was possible that the mass structure at 5 kg fermentation allowed a better aeration for the growth of acetic acid bacteria which rapidly utilize lactic acids. On the other hand, the pulp pH level in 1 kg fermentation remained mostly low, suggesting accumulation of organic acids in the pulp instead the cotyledon. This explanation is supported by the elevated pH level in the cotyledon.

Cotyledon Colors

There are various approaches to evaluate a successful fermentation. The easiest technique might be observing the color development of cotyledon. Fresh cotyledon from Forastero type has a purple color which is brought by anthocyanin, a member of polyphenol group. Well fermented beans are attributed to brown color, while unfermented beans exhibit grayish and purple color (Camu

et al., 2008; Sulaiman & Yang, 2015). The number of grayish and full purple beans are regulated in the Indonesian National Standard, SNI 2323:2008/Amd1:2010 (BSN, 2010). The grayish and fully purple (on more than 50% of interior surface) beans are considered unfermented, while brown and partly purple (on less than 50% of interior surface) are classified as fermented. In the standard, the lowest grade 3 permits 20% of unfermented beans.

Treatments above 1 kg scale had successfully produced unfermented beans with proportion meets the standard for grade 3. Neither grayish nor fully purple beans were present in the 40 kg fermentation, which meets the criteria of grade 1. Lower scale fermentation at 5-15 kg resulted in the grayish and full purple beans that can be categorized into grade 2. Fermentation at 2.5 kg produced beans at grade 3, while fermentation at 1 kg produced beans that was out of the standard due to unfermented bean content exceeding 20% (Figure 4). In addition, fermentation at 1 and 2.5 kg had higher variance than that of higher scales. Non-fermentation beans obtained by drying beans right after extraction from the pods were attributed with very high per-
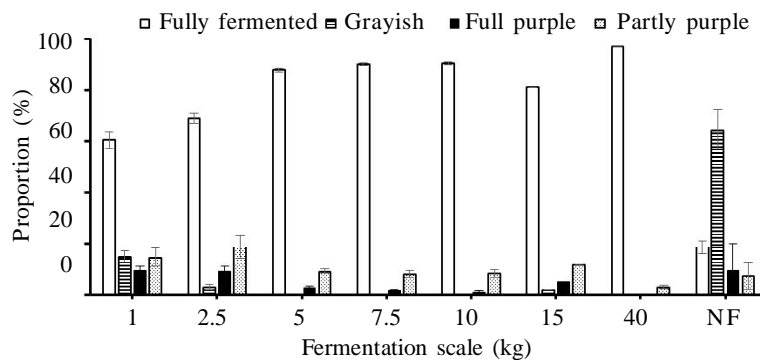

Figure 4. Analysis result of dried cocoa beans from fermentations at several different fermentation scales; 1, 2.5, 5, 7.5, 10, 15, 40 kg and non-fermentation (NF) beans. Parameter includes percentages of fully fermented, grayish, fully purple, and partly purple beans. Data are means of three replications.

centage of grayish beans (>60%), besides some amount of purple beans.

During fermentation, cocoa beans lose viability due to exposure towards heat, ethanol and acetic acid that diffuse through testa. Ever since bean dies, the compartments inside the cell become disorganized, enabling anthocyanin to interact with respective enzyme, polyphenol oxidase. The enzyme oxidizes anthocyanin resulting insoluble material that has brown color (De Vuyst & Leroy, 2020). It is hypothesized that in the fermentation lower than 40 kg, the ethanol and acetic acid concentrations were limited. This has led to hindered disorganization event inside cotyledon cells, and consequently anthocyanin oxidation.

The quantity of wet beans moderately correlated with the percentage of grayish and purple beans. If the weight was plotted against percentage of grayish and purple beans, a logarithmic regression may apply ($R^2 = 0.9557$). The resulted equation is $y = -10.68\ln(x)+23.157$, whereas y is the percentage of unfermented (sum of grayish and full purple) beans, and x is the weight of wet beans (kg) (Figure 5). Using this equation, as low as 1.4 kg wet cocoa beans is required to result unfermented beans 19.6% which is permitted in the SNI standard grade 3. However, due to large variations occur in fermentations at 1 and 2.5 kg, it is suggested to ferment not less than 5 kg of wet beans.

Monitoring Fermentation

Correlational analysis revealed that higher temperature at 42 hours was associated with lower amount of unfermented beans ($r > -0.8$) (Table 1). Fermentations whose temperature at 42 hours were below 35°C had produced unfermented beans higher than 20%. Temperature at 42 hours was also strongly correlated with the preceding temperature levels. Therefore, the temperature rise during anaerobic phase (6-42 hours) effectively predicts the development of unfermented beans. Initial temperature (0 hours) is not strongly correlated with temperature in the subsequent time points.
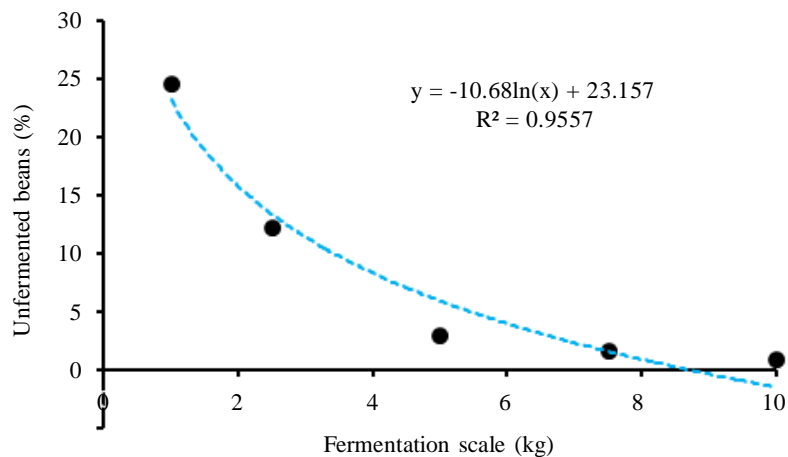

Figure 5. Relationship between percentage of unfermented beans and fermentation scales

Table 1. Pearson's coefficient of correlation (r) between bean color and temperature at several time points

Time point (h)	Grayish	Partly purple	Full purple	Unfermented (grayish + full purple)
0	-0.48	-0.49	-0.58	-0.56
6	-0.45	-0.58	-0.57	-0.54
12	-0.46	-0.72	-0.65	-0.58
16	-0.49	-0.73	-0.67	-0.60
24	-0.48	-0.67	-0.62	-0.58
30	-0.6	-0.84	-0.81	-0.74
36	-0.77	-0.82	-0.88	-0.87
42	-0.82	-0.74	-0.87	-0.90
48	-0.71	-0.6	-0.72	-0.76
54	-0.62	-0.81	-0.84	-0.76
60	-0.6	-0.75	-0.82	-0.74
66	-0.59	-0.63	-0.76	-0.71
72	-0.52	-0.63	-0.69	-0.64
78	-0.5	-0.68	-0.72	-0.63
84	-0.61	-0.73	-0.81	-0.74
90	-0.58	-0.74	-0.81	-0.72
96	-0.51	-0.66	-0.75	-0.65

CONCLUSIONS

This study concludes that cocoa bean fermentation is accommodated by small amount of wet beans of less than 40 kg. Fermentation at 5-15 kg and 2.5 kg produced beans that met criteria of SNI 2323:2008/ Amd1: 2010 grade 2 and 3, respectively. Only treatment of 40 kg scale is able to produce beans meeting the standard grade 1. Fermentation monitoring can be carried out by observing the temperature of fermenting mass at the 42 hours, whereas temperature below 35°C is associated with higher percentage of unfermented beans.

AUTHOR CONTRIBUTION

Ariza Budi Tunjung Sari is the main author that was responsible for planning and conducting experiment, data analysis and manuscript preparation. Hendy Firmanto is the co-author contributing in the data analysis and interpretation. Teguh Wahyudi is co-author contributing in data interpretation and proofreading.

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