



## Study on effect of fermentation to the quality parameter of cocoa bean in Indonesia

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### ABSTRACT

Most cocoa beans in Indonesia are traditionally produced by farmers using non fermented and sun drying method. The quality of cocoa beans produced by farmer in Yogyakarta may be improved by the fermentation method. However, it needs optimization for best fermentation process. The aims of the study was to improve quality of cocoa beans by fermentation of sun dried cocoa beans. The characteristic fermented cocoa beans was determined by measuring changes pH, acidity and fermentation indexes of cocoa beans during fermentation. This study used preconditioned cocoa beans to resemble obtain from the farmer. Preconditioning was done in order to get 15% moisture content of pulp at same level as moisture content of pulp from traditional process. Before fermentation, therefore sun dried cocoa beans was rehydrated to obtain a moisture content of pulp similar to fresh beans pulp, and then fermentation was conducted for 120 hours. Changes in acidity and fermentation indexes of cocoa beans during fermentation were measured. The fermentation process used 3 level treatment i.e. control (without inoculum), mixed culture of microbes added at the beginning fermentation. The result shows all cocoa beans acidity increase during fermentation from 4.48% to 6.45% for control, 4.64% to 6.39% for addition of inoculum at beginning of fermentation and from 4.45% to 6.59% for addition of inoculum at the beginning and middle of fermentation and fermentation indexes of cocoa beans increase for all level of inoculum addition i.e. 0.31 to 0.88 for control, 0.32 to 0.99 for addition of inoculum at the beginning fermentation and 0.33 to 1.03 for addition of inoculum at the beginning and middle of fermentation. The study indicated that addition of mixed culture microbes in fermentation improved the quality of cocoa beans that characterized by pH, acidity and fermentation indexes of cocoa beans.

**Key words :** Acidity, Cocoa beans, Fermentation, Fermentation indexes, Mixed culture microbes, pH.

### INTRODUCTION

Post-harvest handling of fresh cocoa beans is usually implemented in 2 methods. Firstly, the cocoa beans are fermented traditionally and dried by artificial drying. Second the cocoa beans are directly sun dried for several days without fermentation anymore. According to the Bureau of Statistics Data (Anonymous, 2013) that the dried cocoa production in 2013 reached 5.45 million tonnes and ± dry unfermented cocoa beans of ± 38.5000 tons. Dry unfermented cocoa beans consist of sun dried cocoa beans and dry half-fermented cocoa beans produced by cocoa farmer. Indonesian cocoa bean farmers produce ± as 93% of cocoa beans by simple method i.e. washing cocoa beans and it was directly dried by sun drying method without fermentation. While 7% of dry cocoa beans are produced by either the private company or national plantation company using fermentation method (Anonymous, 2013).

Fermented of cocoa beans is essentially a process to breakdown sugar compound in the pulp into organic acids such as citric acid and it was carried out by microbes (Camu, *et al.*, 2008, (Ardhana and Fleet, 2003). The produce organic acids during fermentation will induce enzymatic reactions

in the beans cotyledon, causing biochemical changes. Most of the are compound that give specific flavor, taste, and color of the cocoa beans (Biehl, *et al.*, 1985, Afoakwa, *et al.*, 2014). The fermentation process was carried out by using wood boxes for 5-7 days incubation and stirred reversally for every 2 days. The cocoa beans without fermentation will create bitter taste, astringency, and will not produce distinctive aroma of chocolate after roasting process (Schwan and Wheals, 2004).

Dry beans had lost most of the water content and several compound as substrate of microbes. The water content of cocoa beans during fermentation was utilized for enzymatic reactions in the beans cotyledon and also for microbial growth in cocoa beans pulp (Schwan and Wheals, 2004). Water will bring the enzyme to the substrate that is in the beans cotyledon, so that the hydrolysis and oxidation of the precursor of flavor, color, and aroma of the cocoa beans occurred. The water content of cocoa beans required in the fermentation of cocoa is more than 35%. Substrate in the fermentation of cocoa beans are sugar that is available in cocoa beans pulp and converted to organic acid such as citric acid. Organic acid will diffuse into the beans cotyledon

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and induces the enzymatic reactions to form precursor of flavor, aroma and color (Afoakwa *et al.*, 2014). Soaking of un-fermented cocoa beans was done to rehydration of cocoa beans before fermentation. Research on fermented dry cocoa beans using unfermented cocoa bean was done successfully in the engineering lab PAU, UGM., The aimed study to determine changes in the chemical properties of fermented dry cocoa beans drying.

## MATERIALS AND METHODS

**Preparation of cocoa beans :** Cocoa beans used were taken from ripe cocoa pods, which was a yellow or orange fruit that sounds hollow when tapped. Friuts without washing was cleaved to remove the beans cotyledon and dried in the cabinet dryer at 40 ° C, until the moisture content of the beans reached to  $\pm 15\%$ . Hundred (100) g of dry beans was soaked in 60 ml distilled water at room temperature for 5 days (120 hours). Soaked ccocoa beans were susequently fermented at room temperature for 5 days as well. Changes of total sugar, reducing sugar, pH, acidity, index of fermentation were determined and a succession of microbes during fermentation was also determined and it was compared to fermentated fresh cocoa beans.

**Preparation of the pulp samples:** The pulp was manually separated from the beans by rubbing the beans (with adhering pulp) between fingers and squeezing the pulp into clean sample bag.

**Preliminary analysis:** Preliminary analysis was conducted to determine the water content and reducing sugar of pulp and cocoa beans before and after drying. The water content of the cocoa beans is determined using thermogravimetric method, while reducing sugar content of the wet and dried pulp was analyzed by using Nelson-Somogy Method (Sudarmadji *et al.*, 1997).

**Fermented cocoa beans:** First fermentation, 100 g of fresh cocoa beans was fermented at room temperature for 5 days. Second fermentation, 100 g un-fermented cocoa beans was soaked in 60 ml of distilled water before fermentation and then they were fermented at room temperature for 5 days.

**Experimental design:** The experimental design used for the study was Randomized Complete Block Design (RCBD). The variable factors were cocoa treatments (fresh and sun dried cocoa beans) and fermentation time (0; 3 and 6 days). The pH, non-volatile (titratable) acidity, reducing sugars, fermentation index and total microbes (total plate count) were studied. All statistical analysis were performed using SPSS 13.00 statistical package program (2002). The data were analyzed by analysis of variance (ANOVA). If there is signicant different between treatmets, therefore it was followed by Duncan Multiple Range Test (DMRT) using 0.05% significancy.

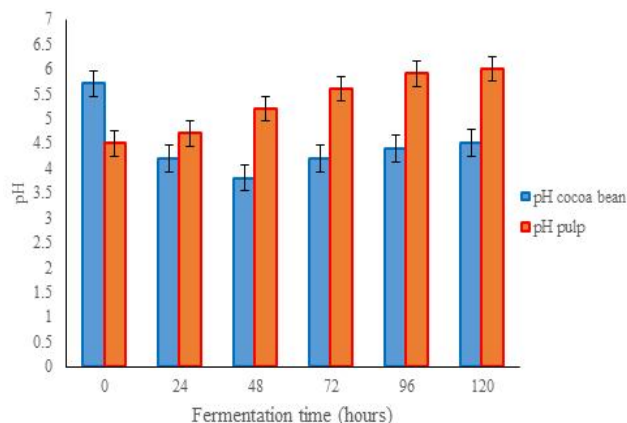
**pH and non-volatile (titratable) acidity:** pH and non-volatile (titratable) acidity of the pulp were determined using

the method as described by Nazaruddin *et al.* (2006) with slight modifications. Ten (10) g of the pulp was homogenized in 90 ml of hot distilled water, stirred manually for 30 s and filtered using Whatman filter paper No. 40 and cooled to 20–25°C. Twenty five (25) ml aliquot of the resulted filtrate was pipetted into a beaker glass and the pH was measured using a pH meter (Mettler Toledo MP 230), and calibrated with buffers at pH 4.01, 7.00 and 9.21. A further 10 ml aliquot was used to determine acidity by titration to an end point pH of 8.1 with 0.1 N NaOH solution and the values were reported as moles of sodium hydroxide (NaOH) per 100 g of sample.

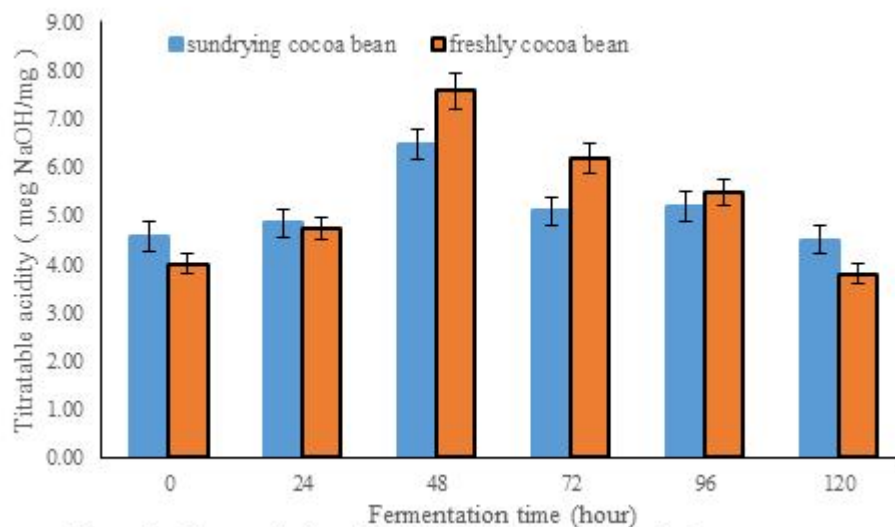
**Fermentation index determination:** Fermentation index of cocoa beans were determined using the method as described by Misnawi *et al.* (2002). Five hundred (500) mg of defatted cocoa powder were weighed into a 125 ml conical flask containing a mixture of 50 ml of methanol:hydrochloric acid (97:3), the mixture was cooled at  $8\pm 2^\circ\text{C}$  in a refrigerator for 16 –18 h. A clear extract was obtained by filtration through a Whatman filter paper No. 1. Fermentation index was calculated, based on the ratio of the absorbance at 460 nm to the absorbance at 530 nm.

## RESULT S AND DISCUSSION

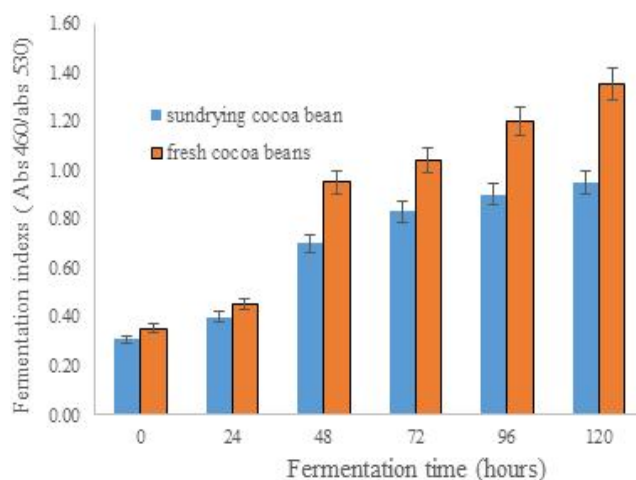
The pH of sun dried cocoa beans has been reported to range between 5 – 5.7 and pH of pulp between 6 – 6.2 (Figure 1). During the first two days of fermentation, the pH cocoa beans was recorded  $5.7\pm 0.1$ , and decreased to  $3.8\pm 0.1$  in 48 h fermentation, and at the end of fermentation was slight increased to  $4.5\pm 0.2$ . The beginning of fermentation, however, pH of pulp was  $4.5\pm 0.1$ , an slightly increased to  $6\pm 0.1$  during fermentation (Figure 1). Fermentation of cocoa beans caused significant ( $p \leq 0.05$ ) increasessed the acidity levels of cocoa beans, and reached at maximum level after 48 fermentation. After which the titratable acidity decreased considerably until the end of fermentation period, and this was noted for all treatments (Figure 2). The interaction between fermentation time and prepared of cocoa beans (with and without fermentation) gave significant effect ( $p \leq 0.05$ ) on acidity level of pulp. The fermentation index was close to 1 as



**Fig 1:** Changes in pH during cocoa beans fermentation.



**Fig 2:** Changes in titratable acidity of cocoa beans during fermentation



**Fig 3:** Change fermentation index cocoa bean during fermentation

indication for good fermentation. Comparison between dry cocoa beans obtained from treated cocoa beans and fermented fresh cocoa beans is showed in Figure 3. Fermentation index of sundried cocoa beans was  $0.35 \pm 0.1$ , and increased to  $1.35 \pm 0.1$ . While fermentation index of dry fresh cocoa beans was  $0.31 \pm 0.1$ , slightly increased to  $0.95 \pm 0.1$  after 120 h fermentation.

**Changes in pH profile of cocoa beans:** The acidity cocoa beans at the beginning of fermentation was moderate low (3 – 4). It was due to the content of citric acid, and it was preferable to growth of yeast. However, as fermentation on going, the dominant microbes was yeasts with good pectinolytic activity, and they degraded cocoa beans pulp to produce organic acids especially citric acid. Such citric acid will change fermentation condition that preferable to bacteria growth. This explained why the pH of pulp changed from 5.20.10 (after 48 h fermentation) to  $5.6 \pm 0.20$  (after 72 h fermentation). While pH of cocoa beans decreased from 5.7  $\pm 0.1$  to  $3.8 \pm 0.1$  (after 24h fermentation) and slightly

increased to  $4.5 \pm 0.10$  (after 72 h fermentation). Once the environment of fermentation became more aerobic, so that the lactic acid bacteria (LAB) would dominate fermentation process and converting the intermediate metabolites into lactic acids. At the latter stage of fermentation, acetic acid bacteria (AAB) oxidized the ethanol produced by yeasts and LAB into acetic acid and further more to carbon dioxide ( $\text{CO}_2$ ) and water. Nevertheless, the acetic acid was highly volatile and seldom accumulated under aerobic condition. Unlike, the pH, the titratable acidity were more appropriate indicator to measure the total acid level in any fermentation process and usually both parameters are negatively correlated. According to Beckett, (2009), the synthesis of lactic acid was very common among lactic acid fermentation, especially cocoa beans fermentation that carried out under anaerobic condition. The temperature rose steadily from 27 to 40°C (fresh cocoa beans) and from 26 to 38°C (sundried cocoa beans), respectively after the cocoa fermentation.

The steady increase on the temperature may be associated to the release of heat from cocoa biomass during the fermentation process. Initially, the yeasts were dominant species that utilize the available fermentable substrate (such as sugar) before converting them into ethanol and further to acetic acid via microbial succession. The conversion of fermentable substrate into desired metabolite by-products was performed exothermically, and hence it assisted to the increase of temperature (Schwan and Wheals, 2004).

**Changes in non-volatile (titratable) acidity of cocoa pulp:** During fermentation of cocoa beans, microorganisms breakdown the sugars in the pulp resulting in the production of alcohols and organic acids such as acetic acid -which then diffuse into the beans. Production of organic acids in the pulp is important in cocoa beans fermentation as these acids diffuse into the beans and subsequently induce the important biochemical reactions leading to well fermented

cocoa beans. The acidity level was highest at 48 h of fermentation as most of the pulp sugars were probably degraded into alcohols and then oxidized to acetic acid by acetic acid bacteria within 48 h of fermentation. Ardhana and Fleet (2003) reported that highest concentration of acetic acid (10 mg/g sample) in cocoa pulp occurred at 72 h (3 days) of fermentation. Acidity levels decreased after 3 days fermentation, it was due to most of the acid produced have diffused into the beans. Again, as the pulp volume reduced, there was improvement in aeration in the fermentation of biomass (pulp) leading to the evaporation of volatile acids such as acetic acid.

This might be due to the fact that reduced pulp volume per beans, caused reduction of pulp sugar content and thus, increased micro-aeration within the pulp. The decreasing of sugar content in pulp as result of yeasts activity during fermentation and consequently produced alcohol and acetic acid in the pulp (Biehl *et al.*, 1985). This suggested that cocoa beans preconditioning could be effectively employed to reduce acidity levels in cocoa beans during fermentation.

**Fermentation index:** The figures 3 shows that until the end of fermentation, however fermentation index of fermented sundried beans was not met, it was presumably because the temperature of fermentation was not reached. It was due to total population of microbes was not satisfactory for encouragement of fermentation process.

## CONCLUSION

The results of this study may be concluded that during drying of cocoa beans (covered by pulp) did not cause the microbe dead, so when cocoa beans were rehydrated the microbes were still survive. This evident may be seen in the succession of microbes that occurred with yeast (*S. cerevisiae*) population exchanged by LAB and followed by AAB during fermentation. The quality of sundried un-fermented cocoa beans compared with fresh fermented cocoa beans have in common. Fermentation index of sundried un-fermented cocoa beans was highest i.e. 0.95. From whole fermentation quality data it can be said that the quality of sundried cocoa beans may be improved through fermentation. The fermentation process of cocoa beans may be speed up by utilization of inoculum in order to improve the quality dry cocoa beans.

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