

Improvement of Small Scale Cocoa Fermentation Using *Lactobacillus fermentum* as Starter Culture

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ABSTRACT

Low harvest amount of cocoa smallholder has become a great constraint for farmer in order to carry out a proper postharvest practice. Low production of raw cocoa beans cause farmers are not able to ferment their cocoa beans that lead to low quality of cocoa beans produced. Addition of starter culture to improve the fermentation performance has been previously reported by some researchers. In this study, *Lactobacillus fermentum* (LF) inoculum was used as stater culture for small scale cocoa fermentation (15 kg). The LF culture (10^7 CFU/gr) was added in several concentration (1, 2.5, and 5% w/w) prior cocoa fermentation. The fermentation was carried out in 4 days (96 h) with once turning in 48 h. The result showed that the addition of LF in small batch of cocoa fermentation could improve the performance of fermentation and resulted in higher amount of fermented cocoa beans (70,34%, 5% LF) compared to natural fermentation and fair average quality (FAQ) beans (45% and 41%, respectively). This research result is significantly important solving the issues of fermentation concerning with minimum quantity of cocoa needed. With this approach, small batch of cocoa fermentation even could result in comparable quality to full-batch fermentation.

Keywords: Starter culture, small batch fermentation, lactic acid bacteria, cocoa, *Lactobacillus fermentum*

INTRODUCTION

Cocoa pulp which consists of 80% water, 15% glucose and fructose provides substrate for various microorganisms essential in cocoa beans fermentation (Figueira *et al.*, 1993; Minnifie, 1999). Schwan *et al.* (1995) mentioned that there were successions of microorganism including yeast, lactic acid bacteria, and acetic acid bacteria occurred during the fermentation in which over 100

aerobic spore-forming bacteria aside from yeast were isolated from cocoa fermentation in Bahia. It has also been reported that various endogenous enzymes such as protease, glycosidases, and polyphenol oxidases played crucial roles in the production of flavor precursors and pigment degradation during the fermentation process (Hansen *et al.*, 1998). According to Puziah *et al.* (1998), fermentation is needed to induce biochemical changes and development of flavor precursors

in cocoa beans, and is a prerequisite to obtain cocoa-specific aroma upon roasting. During the fermentation process, free amino acids, peptides and reducing sugar will be developed and act as precursors of cocoa flavor during Maillard non-enzymatic browning to produce cocoa flavor during roasting (Mohr *et al.*, 1976). In term of desirable cocoa flavor development, fermentation is crucial step in cocoa beans processing in which the unique cocoa flavor will only be generated during the cocoa beans fermentation and subsequently during roasting process (Lopez, 1986; Lopez *et al.*, 2007).

Nowadays, majority of the world cocoa fermentation is carried out using heaps covered with banana leaves or in a basket; whereas the other half cocoa beans were fermented using some types of box (Rohan, 1963; Thompson *et al.*, 2001). However, the application and manner of cocoa fermentation varied from country to country and mostly depend on the scale of harvest. Small scale production due to low amount of harvest usually utilize traditional method of cocoa fermentation that can be carried out using banana leaf-lined holes in the ground, derelict canoes, or in make shift banana and bamboo frames. In other cases, farmer utilizes fruit boxes, baskets, plastic buckets, fertilizer bags and any convenient facilities, or cocoa beans are simply piled on a sheet and covered with any handy material (Thompson *et al.*, 2001). However, permanent facilities for fermentation made from batteries of wood or fiber-glass boxes usually used by large plantation which can produce adequate amount of fresh cocoa beans.

Previous result reported by Jespersen *et al.* (2005) showed that the amounts of cocoa beans processed in fermentation significantly affect the succession of microorganisms which then combined by such factors as season and handling method of cocoa beans

resulted in various performance of cocoa fermentation. Correct succession of microorganisms during cocoa fermentation will lead into the production of alcohol, acetic acid, lactic acid and generate heat that causes bean death and triggering the biochemical process in the beans (Hashim *et al.*, 1998). This biochemical process which mainly occurs due to the enzyme activity such as proteases, glycosidases and polyphenol oxidase will thus affecting the flavour precursors development in cocoa beans (Lopez & Dimmick, 1991). Successful fermentation will produce high quality cocoa beans with high intensity of chocolate flavour. Unpredictable result due to the variability of processing method and the under-performed fermentation caused by the constraint in amount of cocoa beans used in fermentation require wholesome solution which can produce cocoa with predictable quality. Schwan (1998) previously reported that the used of defined microbial cocktail inoculum can be a solution to overcome the unpredictable natural fermentation of cocoa. On the same approach, the use of defined inoculum should also able to improve the performance of low amount cocoa fermentation by regulating the succession of microorganisms during cocoa fermentation in which will be the focus of this study.

MATERIALS AND METHODS

Cocoa pods were obtained from small-holder plantations in Banyuwangi which was acquired in intact pod form. The fresh cocoa beans was obtained by breaking the pods prior fermentation treatment. The *Lactobacillus fermentum* (LF) inoculum was obtained from Indonesian National Culture Collection (LIPI, Bogor). The inoculum of LF utilized in fermentation was derived from serial culturization of LF in MRS media and skim

milk media. The liquid culture of LF was then powdered using the wheat flour to obtain the final concentration of LF 10^7 CFU/gr.

Cocoa beans fermentation was carried out immediately after breaking the cocoa pods. Approximately around 15 kg of cocoa beans were transferred into styrofoam box which were previously lined with banana leaves. The styrofoam box itself had been designed to be as similar as wooden box usually used for cocoa fermentation equipped with holes on the bottom of the box to allow drainage process. After the box was filled with fresh cocoa beans, LF inoculum was added with defined concentration (1%, 2.5% and 5% of total weight) and three replications including control was carried out. On the other hand, directly sun-dried cocoa beans was also processed to obtain fair average quality (FAQ) cocoa beans which would be used for comparison. The fermentation process was carried out for 4 days (96 hours). After 48 hours of fermentation, the cocoa beans were turned. After the fermentation, the beans were then sun dried until enough moisture content was achieved (around 7.5%). Dried cocoa beans were then stored in cool and dry place until analysed.

The cut-test criteria is based on Indonesian National Standard for cocoa beans (SNI 2323-2008) (BSN, 2017) except the partly and full purple beans will be scored separately from slaty beans. The brown colored cocoa nibs represent the fermented cocoa beans condition, whereas slaty represent unfermented cocoa beans and full or partly purple represent partially fermented cocoa beans. Fermentation index was determined using the method of Gourieva & Tserevitinov (1979), based on the ratio of oxidized and polymerized anthocyanidins (460 nm) and its monomer (530 nm). A 0.5 g of cocoa powder sample was weighed in a 125 mL conical flask and 50 mL of methanol : hydrochloric

acid (97:3) mixture was then added. The mixture was cooled to temperature of 8p C in a refrigerator for 16–18 hours. A clear extract was obtained by filtration through a Whatman no. 1 filter paper; then its absorbance was measured at 460 nm and 530 nm.

Data analysis was performed using general liner model (GLM) and post-hoc analysis was done using Tukey HSD (Honestly Significant Difference) in statistical package for social science (SPSS) software version 17.0 (IBM Corporation, Armonk, New York, USA). The statistical analyses were performed at 5% significance level.

RESULTS AND DISCUSSION

Cut test analysis result showed that treatment using LF as starter in 15 kg fermentation capacity resulted in significantly better performance of fermentation compared to natural and FAQ, represented with slaty free result and lower percentage of full purple beans compared to that of natural fermentation and FAQ. The full purple beans percentage of the treatment ranged from 7.22% to 15.33%, in which the result of treatments 1% and 2% were not significantly different with that of natural fermentation (Figure 1), however all the treatment results were significantly lower than FAQ. Schwan (1998) previously mentioned that the use of microbial inoculum including lactic acid bacteria added at time zero could produce similar result with normal fermentation, in which study, the fermentation was carried out in 200 kg of raw cocoa beans capacity.

The content of full purple beans in our natural fermentation trial was not significantly different with that of FAQ, moreover it also possessed higher amount of partly purple beans but free from slaty beans. Said & Samarakhody (1986) previously mentioned that the ratio of surface area to cocoa mass

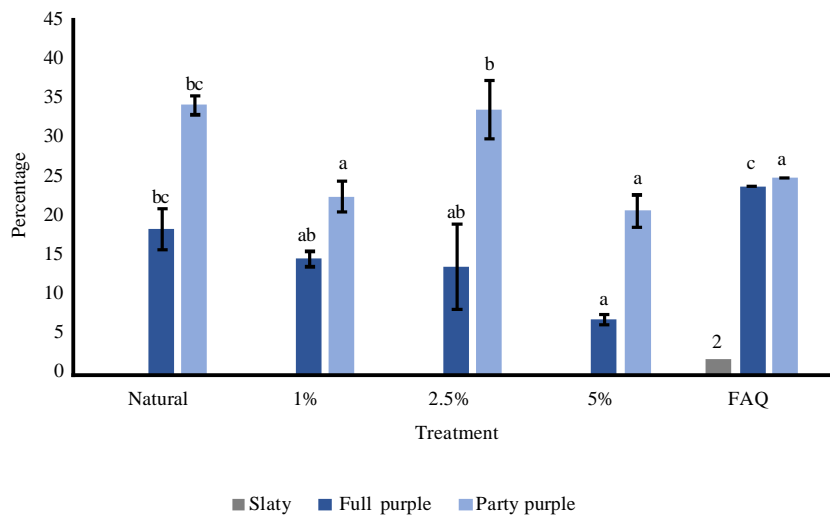


Figure 1. Percentage of slaty, full purple and partly purple beans as the result of different treatment of fermentation and FAQ (Mean (n=3) value with different letters in same category were significantly different (Tukey HSD, $p < 0.05$). Bars represent standard deviation)

significantly affected the performance of hydrolysis. The larger ratio of surface area to cocoa mass resulted in lower temperature rise and larger loss in heat during fermentation. The temperature loss of the system to contain heat during fermentation is a crucial factor in fermentation since as mentioned by Lopez & Dimmick (1995) that the purpose of the fermentation is to provide heat and acetic acid for killing the beans which then subsequently induce biochemical changes in cocoa beans. Normal fermentation of cocoa is usually done in minimum of 40 kg, and in larger plantations, the capacity could reach from 600 kg to 2 tonnes of cocoa bean per batch mainly using depth wooden box to provide smaller ratio of surface area to cocoa mass. The batch used in this trial was 15 kg, thus contained larger ratio compared to that of 40 kg, of fermentation. The larger ratio would not provide good temperature rise and ability to contain the heat thus it was reasonable that the fermentation was under performed.

It was convinced since the fermented beans resulted from those treatment were not significantly different (Figure 2). The FAQ beans which was supposed not producing fermented beans, actually produce fully fermented beans even though it was not fermented in styrofoam box and directly sundried.

The occurrence of fermented beans in FAQ practices can be related to the report of Lopez & Dimmick (1995) based on previous practices in the processing of criollo beans. It was mentioned that the fermentation of criollo beans in Ecuador is done in drying platforms and fermentation occurred during drying (aerobic) and when the cocoa is piled up at night (anaerobic) and sufficient to produce fully fermented criollo beans. However, this practice of fermentation is not sufficient for forastero or trinitario varieties that requires longer fermentation time, but it is not impossible that such a practice could also result in fully fermented beans as in agreement with our FAQ result.

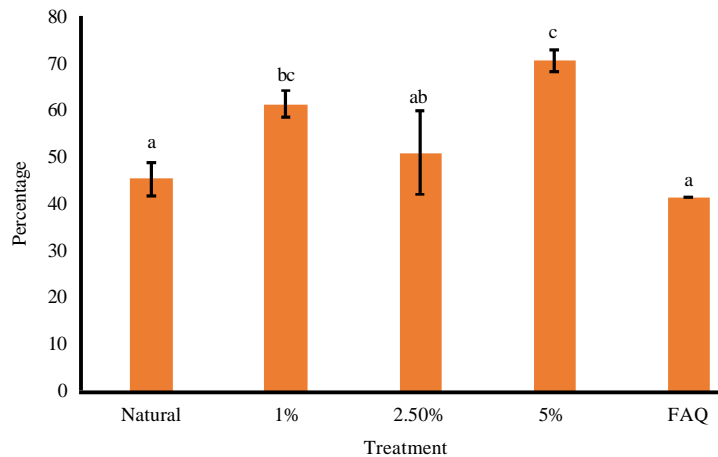


Figure 2. Percentage of fermented beans as the result of different treatments of fermentation and FAQ (Mean (n=3) value with different letters are significantly different (Tukey HSD, $p < 0.05$). Bars represent standard deviation)

Figure 2 also shows that the addition of LF inoculum for cocoa fermentation at 1% and 5% concentration resulted in higher number of fully fermented cocoa beans. On the other hand, the result of the treatment using 2.5% was not significantly different from natural and FAQ mainly due to high variation of the result obtained. In its highest performance, the addition of 2.5% LF treatment could produce comparable result of fermented cocoa beans to that of 1% and 5%. Addition of lactic acid bacteria in the start of fermentation might boost the cocoa beans fermentation performance in term of providing lactic acid in early stages of fermentation to accelerate the bean death process. The result was supported by the report of Schwan (1998) which found that in the fermentation added with cocktail inoculum involving lactic acid bacteria at early stage of fermentation could resulted in the same performance of well carried out fermentation which indicated by high production of lactic acid during the first 48 hours of fermentation. The production of acid is crucial in

cocoa fermentation, since when it is existed together with heat and ethanol it will lead into bean death and induce the biochemical changes in cocoa beans (Hashim *et al.*, 1998).

Our analysis of fermentation index on the samples also showed that the treatment using LF as inoculum produced not significant different result to that of natural fermentation, whereas the FAQ beans showed lowest fermentation index (0.35) means that the beans were clearly under-fermented. However, the result might be contradictive to that of mentioned in Figure 2 which stated that the fermented bean count of natural fermentation was not different from FAQ. The main determinant factor could be the high occurrence of slaty and full purple beans in FAQ, since fermentation index measures brown-yellow color intensity read at absorbance of 460 nm and compared to purple color intensity read at absorbance of 530 nm (Gourieva & Tserevinov, 1979) the occurrence of brownless cocoa beans could resulted in lower fermentation index obtained.

On the other hand, our cut test result on the mouldy, insect infested and germinated beans was shown in Figure 3. All the treatments were free of mouldy beans, and some of the treatment possessed germinated beans (1%, 5% and FAQ). The occurrence of mouldy beans as resulted by fermentation process mostly indicate that the fermentation was excessive whereas the occurrence of germinated beans means that the acid and heat induced bean death was not achieved in some parts of cocoa beans during fermentation. Basically, all the fermentation treatments in this study were generally under-performance compared to medium to large scale of fermentation. Schwan (1998) previously mentioned that fermentation of cocoa beans involved around 200 kg per batch in wooden box could resulted more than 90% of fully fermented beans within 7 days of fermentation. On smaller scale and shorter duration of fermenta-

tion, Bariah (2014) showed that the fermentation of 120 kg of cocoa beans in shallow wooden box could produce more than 82% of fully fermented beans after 5 days of fermentation. However, different condition and practices resulted in different quality obtained. As mentioned by Leal Jr. *et al.* (2008) which obtained only 31 to 36% of fully fermented cocoa beans inspite of using 45 kg of raw cocoa beans per batch (plastic basket fermentation, 144 h). Thus, the achievement obtained in this research is significantly important to solve the issue of fermentation concerning with minimum quantity of cocoa beans needed. In Indonesia, low harvests inhibit the smallholder to do the proper fermentation practices. With this approach, even small batch of cocoa fermentation could result in comparable quality to that of full-batch fermentation.

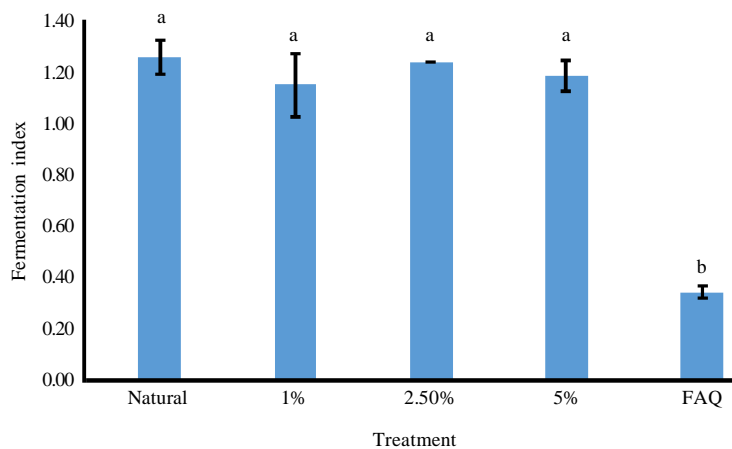


Figure 3. Fermentation index of dried bean obtained from different treatments (Mean (n=3) value with different letters were significantly different (Tukey HSD, $p < 0.05$). Bars represent standard deviations)

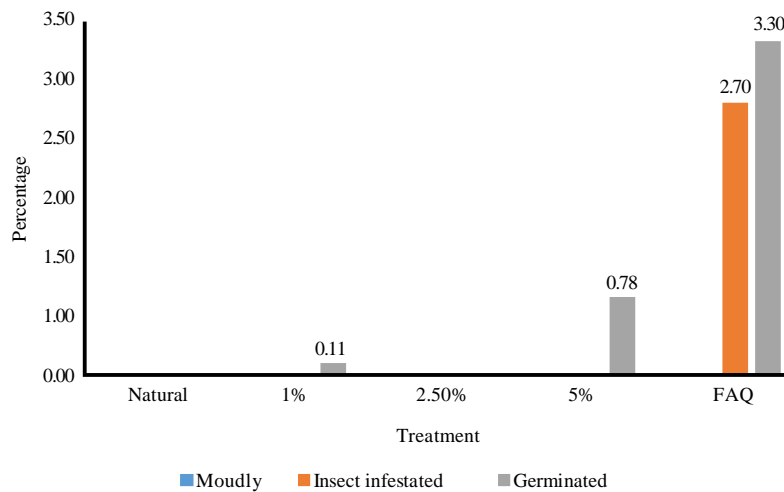


Figure 4. Percentage of mouldy, insect infested and germinated beans as the result of different treatments of fermentation and FAQ (Mean (n=3) value with different letters in the same category were significantly different (Tukey HSD, p<0.05). Bars represent standard deviation)

CONCLUSION

The fermentation of cocoa beans using the *Lactobacillus fermentum* inoculum as starter has been successfully carried out in this study. The addition of LF prior to cocoa fermentation in small scale batch (15 kg) resulted in better quality compared to that of spontaneous fermentation and FAQ and could produce around 70% of well fermented cocoa beans on its optimum concentration (5%). This finding could be a solution in order to solve the quantity constrain occurred in smallholder which cannot process their cocoa beans due to the lack of sufficient quantity. However, further study is needed to gain more information regarding the flavor potential of LF fermented cocoa beans and also its biochemical changes.

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