Improvement of Cocoa Beans Fermentation by LAB Starter Addition

M. T. A. Penia Kresnowati, Lenny Suryani, and Mirra Affifah
Microbiology and Bioprocess Technology Laboratory, Department of Chemical Engineering
Bandung Institute of Technology, Bandung, Indonesia
Email: kresnowati@che.itb.ac.id

***Abstract*—Cocoa beans fermentation is an important step in the post-harvest processing of cocoa beans. This complex mix culture fermentation produces metabolic products that serve as the precursors for the flavor development process. Modification in the dynamics of microbial population during the fermentation may alter the overall microbial activity and thus may impact the fermentation process. Addition of microbial starter was thus suggested to improve the cocoa bean fermentation process. This paper discusses the effects of Lactic Acid Bacteria (LAB) starter addition to the cocoa bean fermentation. Dynamics in microbial population, i.e. yeast, lactic acid bacteria, and acetic acid bacteria were analyzed as well as the sugar components, metabolic products, and the fermentation index during the fermentation. The addition of LAB starter was observed to accelerate the growth of both lactic acid bacteria and acetic acid bacteria, leading to the increase in the ethanol, lactic acid, and acetic acid concentration. Overall it increases the fermentation index and potentially shorter the fermentation time.**

***Index Terms*—starter, fermentation, cocoa bean, LAB, metabolic products, dynamics.**

I. Introduction

Cocoa is one of the most important agricultural commodity products. The derivative products of cocoa range from food, beverage, and confectionery to pharmaceuticals and cosmetics. Every year the demand for cocoa increases by about 3% (World Cocoa Foundation, 2010). Cocoa beans are produced mostly in Ivory Coast, Ghana, Indonesia, and Ecuador.

Cocoa is harvested from the plantation as cocoa pods. The beans are then taken out from the pods, fermented and dried out before they are sold in the market. Various techniques of cocoa bean fermentations are applied, for example the box fermentation, the basket fermentation, the heap fermentation, or fermentation on a drying platform [1]. Further, in the cocoa processing industries they are first roasted to complete the curing or flavor development process.

During the cocoa beans fermentation, the mucilagous pulp (mucilage) surrounding the cocoa beans were removed by the actions of various microbial species indigenously present in the cocoa beans, such as yeast, lactic acid bacteria (LAB), and acetic acid bacteria (AAB) [2]. The species of microorganisms involved in the cocoa bean fermentation process vary with the geographical location of the plantation [3]-[5].

Sugary compounds, the main components of the mucilage, are fermented, releasing heat and yielding various metabolic products, among others are ethanol, lactic acid, and acetic acid. The combined effects are the death of the beans and the development of various precursors for the cocoa flavor formation. It also turns the color of the beans to brown-black, reduces the bitterness, improves the cocoa and nutty flavor, and hardens the cocoa bean shell. However, detailed mechanisms on this process have not been clearly understood.

Considering the role of microbial population during the fermentation, a change in the dynamics of microbial population may alter the overall activity and may impact the fermentation process. On the other hand, lactic acid bacteria is one of the indigenously present microorganisms in cocoa beans that is relatively easy to obtain. It was the aim of this research to map the effect of LAB starter addition to the cocoa bean fermentation, whether this could be used to improve the fermentation process. The dynamics in microbial population, sugar composition of the mucilage, formed metabolic products, as well as fermentation index were measured to analyze the effects.

II. Material and Methods

A. Cocoa Bean Fermentation

Cocoa beans var. Forastero were obtained from PTPN VII, Rajamandala, West Java, Indonesia. 16 gram of cocoa beans were used in each 50 mL Erlenmeyer fermentor. The fermentation process was performed to mimic the real cocoa bean fermentation in the plantation following Stoll [6], with some modification. For example the fermentation temperature was controlled as such to follow the increasing temperature profile of the cocoa bean fermentation [6].

Microbial strain used in the starter was *Lactobacillus plantarum* ITB CC 188. This strain was cultivated in MRS-A media for 2 days before it was inoculated in the beginning of the cocoa bean fermentation. 10^3 CFU of LAB per gram cocoa beans were used for each fermentation. For comparison, fermentations without

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starter addition (standard fermentation) were also performed.

Each fermentor provided for 1 time sample. The sample was prepared by crushing the fermented cocoa bean in 50 mL water. Each sample was used for the analysis of microbial composition, sugar compound and metabolic products, as well as fermentation index.

B. Analysis

The dynamics in microbial population was measured as the concentration of yeast, lactic acid bacteria, and acetic acid bacteria by Total Plate Count (TPC) method. Sample for yeast analysis were cultivated in Potato Destrose Agar (PDA) media, sample for lactic acid bacteria (LAB) analysis were cultivated in MRS-A media, whereas sample for acetic acid bacteria (AAB) analysis were cultivated in Acetobacter Agar. The petri was then incubated in 37 ℃ for 24 hours before the measurement of total colony formed.

The concentration of sugars: sucrose, glucose, and fructose, as well as citric acid and other metabolic products: ethanol, acetic acid, and lactic acid; were measured by HPLC using Aminex-HPX-87H Biorad column and Refractive Index Detector, using sulfuric acid as the eluent. The fermentation index was measured by spectrophotometry following Gourieva and Tserrevitinov [7].

III. Results

A. Dynamics in Microbial Composition

Yeasts, lactic acid bacteria, and acetic acid bacteria are the dominant microorganisms in cocoa beans fermentation. Yeast and lactic acid bacteria dominate the first 2-3 days of fermentation, followed by acetic acid bacteria which dominate until day 4-5. Later on fungi and *Bacillus* may appear and this indicates that the beans are over fermented [2].

Only yeast and lactic acid bacteria were observed significatly in the beginning of the fermentation, succedingly at concentration 2.7 x 10^5 and 1.4 x 10^5 CFU/gram (Fig. 1a). The yeast concentration increased and reached the maximum at day 3-4 of the fermentation. No significant difference was observed in the dynamics of yeast population between the standard and starter added fermentation (Fig. 1a). In another words, the addition of LAB starter did not significantly change the dynamics of yeast concentration throughout the fermentation.

As expected, the addition of LAB starter to the fermentation significantly changed the dynamics of LAB population. We observed the concentration of LAB abruptly increased and reached its maximum at day 2 in the fermentation with starter addition (Fig. 1b). On the other hand, the concentration of LAB increased slowly and reached the maximum concentration at day 3-4 at standard fermentation (Fig. 1b).

The addition of LAB starter accelerated the growth of AAB. We observed that the concentration of AAB reached 10^5 CFU/gram at day 2 of the fermentation with LAB starter addition, whereas at the same time no AAB was observed in the standard fermentation (Fig. 1c). The maximum concentration of AAB, however, were observed at day 3-4 for both standard fermentation and fermentation with starter addition (Fig. 1c).

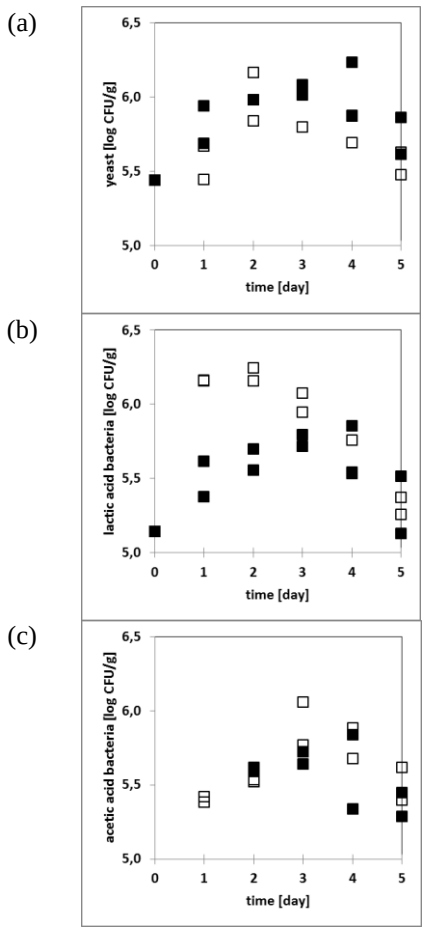

Figure 1. The effect of LAB starter addition to the dynamics in microbial population (colored symbol: standard fermentation, open symbol: fermentation with LAB starter addition).

B. Sugar Depletion in Cocoa Bean and Pulp

The mucilagous pulp surrounding the cocoa bean is rich in sugar which serves as the substrate for microbial fermentation [3]. We observed that sugary compounds of the pulp mainly composed of glucose, fructose, and sucrose; and their initial concentrations (before the fermentation) were consecutively 20.3 mg glucose/g, 22.2 mg fructose/g, and 6.1 mg sucrose /g (Fig. 2). Besides we also measured citric acid, that was reported to be an important component of mucilage [2].

The addition of LAB starter to the cocoa bean fermentation increased the sugar consumption rate such that faster decrease in glucose and fructose were observed in the fermentation with LAB starter addition (Fig. 2a-b). The final concentation of glucose and fructose in the fermentation with LAB starter addition were found to be successively 64% and 46% of those in the standard fermentations.

No significant difference was observed in the sucrose dynamics profile of the standard fermentation and the

fermentation with LAB starter addition (Fig. 2c). In both fermentation all sucrose was consumed and the final concentration was measured to be 3% of its initial concentration.

Compared to the sugars, the measured concentration of citric acid was relatively low. In the beginning of the fermentation, the concentration of citric acid was measured at 0.48 mg/g cocoa bean (Fig. 2d). The concentration of citric acid was observed to decrease along the fermentation. No significant difference was observed in the citric acid concentration dynamic profile between the standard fermentation and the fermentation with LAB starter addition.

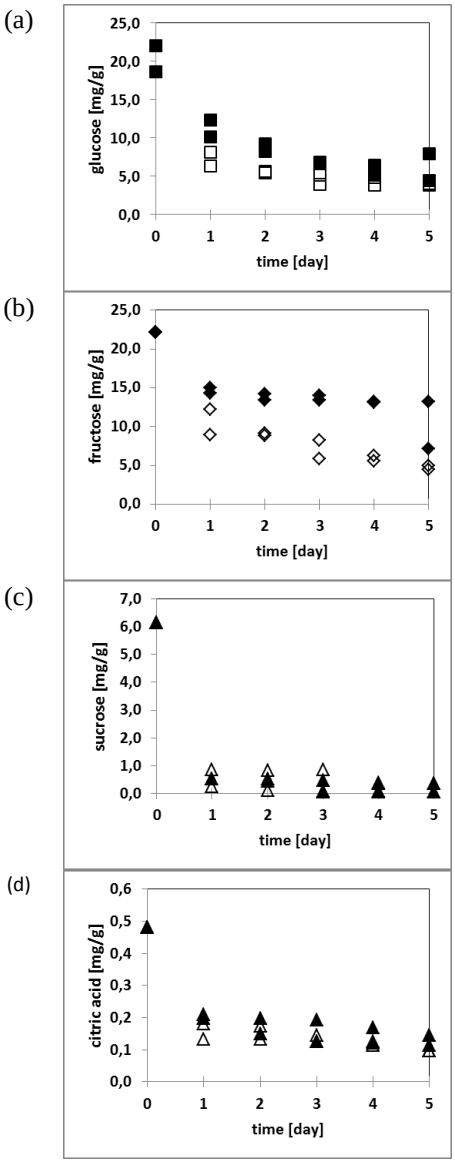

Figure 2. The effect of LAB starter addition to the dynamics in sugar composition (colored symbol: standard fermentation, open symbol: fermentation with LAB starter addition).

C. Metabolic Products Formation

Common metabolic products from the cocoa bean fermentation, that are ethanol, lactic acid, and acetic acid [2], were measured in both standard fermentation and the fermentation with LAB starter addition.

As expected, the addition of LAB starter increased the production of lactic acid. We observed the peak of lactic acid was shifted from day 3 in the standard fermentation to day 2 in the fermentation with LAB starter addition (Fig. 3a). The maximum concentration was also observed to be 5-6 times higher in the fermentation with LAB starter addition (Fig. 3a).

The addition of LAB starter to the cocoa fermentation increased the production of other metabolic products. The ethanol concentration increased up to 50 times in the fermentation with LAB starter addition (Fig. 3b), whereas the acetic acid concentration increased up to 4 times in the fermentation with LAB starter addition (Fig. 3c).

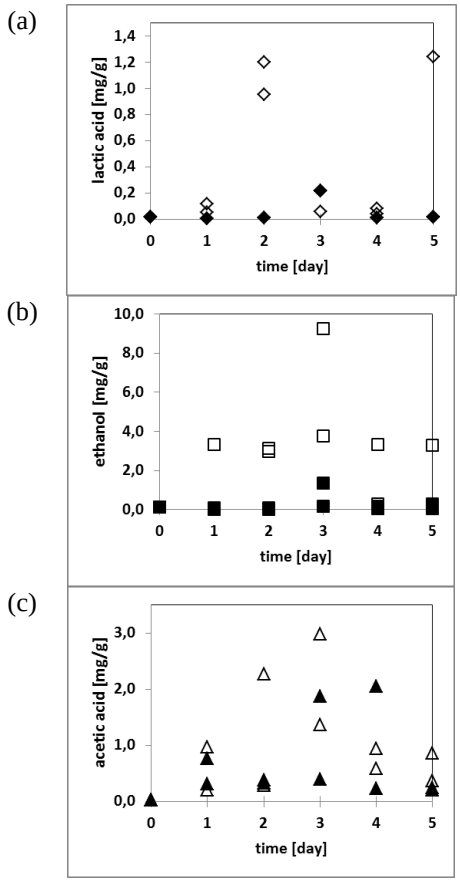

Figure 3. The effect of LAB starter addition to the dynamics in metabolic products (black symbol: standard fermentation, white symbol: fermentation with LAB starter addition).

D. Fermentation Index

TABLE I. EFFECTS OF LAB STARTER ADDITION TO COCOA BEAN FERMENTATION INDEX

Time [Day]	0	1	2	3	4	5
Standard Fermentation	0,38 ±0,00	0,44 ±0,03	0,55 ±0,01	0,67 ±0,06	0,75 ±0,06	0,87 ±0,03
Fermentation with LAB Starter Addition	0,38 ±0,00	0,45 ±0,00	0,56 ±0,08	0,69 ±0,02	0,82 ±0,08	0,96 ±0,05

The completeness of the cocoa bean fermentation is normally measurized by the fermentation index. We measured the fermentation index daily to study how the fermentation progressed. Both fermentations were observed to progress at the same pace initially, but later on the fermentation with LAB starter addition progressed faster. At day 5, the fermentation index of the fermentation with LAB starter was measured to be 0.95 compared to 0.86 of the standard fermentation (Table I).

IV. Discussion

The presented results showed that the addition of of LAB starter to the cocoa bean fermentation altered the dynamics in microbial population, sugar composition, as well as the metabolic products during the fermentation.

Higher growth of lactic acid bacteria and acetic acid bacteria were observed in the fermentation with LAB starter addition. Although we observed the acceleration in the growth of lactic acid bacteria and acetic acid bacteria, these did not depress the growth of other indigenous species as the yeast growth was observed to be normal. This might be explained by the abundantly available sugary compounds glucose, fructose, and sucrose that served as the substrate for the microbial fermentation. Even with the LAB starter addition, at fermentation day 5 there were still about 20% sugar left. As the comparison there were 30% glucose left and 46% fructose left at day 5 of standard fermentation. Although citric acid could be produced as metabolic products of microbial fermentation, its decreasing profile showed that it also served as the substrate in the cocoa bean fermentation. Overall, the mucilage of cocoa bean was depleted faster with the addition of LAB starter.

Interestingly, without increasing the yeast population throughout the fermentation, the addition of LAB starter also increased the level of ethanol in the fermentation. The increase level of ethanol provided the substrate for the acetic acid bacteria growth that in turn increased the level of acetic acid. The increase of ethanol concentration might be produced by some heterofermentative lactic acid bacteria strains, the growth of which might be triggered by the addition of LAB starter.

The increase level of metabolic products during the fermentation with LAB starter addition accelerated the production of cocoa flavour precursors as was observed in the increase of fermentation index. This measure indicated that the addition of LAB starter may fasten the cocoa bean fermentation and thus shortening the fermentation time. According to [8] the required fermentation time for cocoa beans were 2-3 days for var. Criollo and 4-6 days for var. Trinitario and var. Forastero. Another reference even indicated longer time for var. Forastero, that is 6-8 days [9]. Shortened fermentation time is a significant process improvement.

The research on the addition of yeast (*Saccharomyces cerevisiae* var. Chevalieri) to the cocoa bean fermentation suggested that the addition of yeast starter might also shorten the fermentation time [10]. The fermentation index of the fermentation with the addition of yeast starter at day 5 was 0.91 which was slightly lower than our results, 0.96. Considering that lactic acid bacteria starter would be easier to obtained compared to yeast starter, the addition of LAB starter offers a potential method to improve the cocoa bean fermentation in real life application.

V. Conclusion

The addition of LAB starter into cocoa bean fermentation modify the microbial composition during the cocoa bean fermentation and thus accelerate the depletion of sugary compound in the cocoa pulp and thereby intensify the metabolic products formation, in particular ethanol, lactic acid, and acetic acid. Overall this process was shown to improve the fermentation index that indicates that it can shorten the fermentation process.

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M. T. A. Penia Kresnowati is an assistant professor in Bioprocess Technology at Department of Chemical Engineering, Bandung Institute of Technology, Indonesia. In 2007 she obtained her PhD in Bioprocess Technology, Delft University of Technology (TU Delft), The Netherlands. Previously, in 2002 she obtained her Master degree in (Bio)Chemical Engineering from the same institution. Her bachelor degree

was obtained in Chemical Engineering, in 1999, from Bandung Institute of Technology, Indonesia.
She received Endeavour Fellowships and UNESCO-L'Oreal International For Women in Science Fellowships and she did a Post-doctoral research in BioEngineering Laboratory, Department of Chemical Engineering, Monash University, Australia. Her current research interests are on the topics of fermentation, biorefinery, and modeling in bioprocess.

Lenny Suryani graduated from Food Technology, Chemical Engineering Department, Bandung Institute of Technology, Indonesia. This article is the part of the results of her final research project.

Mirra Affifah graduated from Food Technology, Chemical Engineering Department, Bandung Institute of Technology, Indonesia. This article is the part of the results of her final research project