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The Effect of *Lactobacillus Plantarum* Hl-15 in Inhibiting the Growth of Mycotoxin-Producing Fungi during Fermentation of Cocoa Beans

Marwati T[1*], Djaafar TF[1*], Hatmi RU[1], Ni'Matuzzahra[2], Purwandari FA[2] and Rahayu ES[2*]

[1]*Postharvest Department, Assessment Institute for Agricultural Technology Yogyakarta, Indonesia*
[2]*Faculty of Agricultural Technology, Gadjah Mada University, Yogyakarta, Indonesia*

Corresponding author: Marwati T, Postharvest Department, Assessment Institute for Agricultural Technology Yogyakarta, Indonesia, Tel: +628128114249, E-mail: watipasca2@gmail.com

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Abstract

The existence of fungi in cocoa beans fermentation is undesired because it potentially produces mycotoxin. *Lactobacillus Plantarum* HL-15 is known to have the ability to inhibit fungi growth. The ability of lactic acid bacteria to inhibit fungal growth is likely to be influenced by cocoa bean varieties. Therefore, the objective of this study was to know the effect of *L. Plantarum* HL-15 inoculum additional to inhibit fungi growth in the fermentation of Criollo, Sulawesi, and Lonsum varieties and the quality of dried cocoa beans. The materials used in this research were Criollo, Sulawesi, and Lonsum varieties of cocoa beans from Gunungkidul Yogyakarta; and *L. Plantarum* HL-15 inoculum. The fermentation was conducted in a bamboo basket with a capacity of 2 kg of fresh cocoa beans, for 5 days at room temperature. The experimental design used was a completely randomized design with 6 treatment variables **(1)** Criollo variety without inoculum additional; **(2)** Criollo variety with inoculum additional; **(3)** Sulawesi variety without inoculum additional; **(4)** Sulawesi variety with inoculum additional; **(5)** Lonsum variety without inoculum additional; dan **(6)** Lonsum variety with inoculum additional. The result showed that the addition of *L. Plantarum* HL-15 inoculum in the fermentation process can inhibit fungi growth during fermentation of Criollo, Sulawesi and Lonsum cocoa beans with the decrease of total population of the fungi in Lonsum variety is greater than Sulawesi and Sulawesi variety is greater than Criollo. The addition of *L. Plantarum* HL-15 inoculum during fermentation can also reduce the total population of the fungi on the dried cocoa beans produced and did not affect the quality of cocoa beans according to SNI 2323: 2008.

Keywords: Cocoa Beans Fermentation; *Lactobacillus Plantarum* HL-15; Criollo Var; Sulawesi Var; Lonsum Var

Introduction

One of the main commodities in Indonesia is cocoa (*Theobroma cacao L.*). Indonesian cocoa plays an important role in the economy in Indonesia and international trade. Productivity and market demand for Indonesian cocoa commodities have increased from year to year. In 2017, Indonesian cocoa production amounted to 590,684 tons and in 2018 reached 593,833 tons [1].

Although the production of Indonesian cocoa commodities is high, the selling value of cocoa commodities, especially dry cocoa beans, tends to below. This is because the quality of cocoa in Indonesia is low. The low quality of cocoa is caused by a large number of dried cocoa beans contaminated with mycotoxins and mycotoxic fungal contaminants. The most common types of mycotoxins in cocoa beans are ochratoxins and aflatoxins produced from the types of *Aspergillus* and *Penicillium* [2]

Fungal contaminants in dried cocoa beans that are still commonly found. In smallholder plantations, the presence of fungi is caused by the lack of processing facilities and knowledge of farmers in the fermentation of cocoa beans. Fermentation of cocoa bean is influenced by many factors such as type cocoa, disease, climate and seasonal differences [3]. In addition, climate factors in Indonesia that is tropical and have high air humidity that are in accordance with the conditions of fungal growth can also be a factor that makes it easy for cocoa beans to be contaminated by fungi, especially mycotoxic fungi, during the processing process. This is in accordance with the study [4], that one type of mycotoxin (ochratoxin) was detected at all stages of postharvest activity, the highest level of contamination at the drying stage (0.569 ± 0.015 mg/kg). If the processing of cocoa beans is not done properly it can lead to the occurrence of mycotoxic fungal contamination. Therefore, needs to be controlled during the processing of cocoa beans to prevent the occurrence of biological contamination.

[*]**Main contributors**

Basically, cocoa bean fermentation is aimed at destroying pulp (external purpose) and seeking conditions so that chemical and biochemical reactions can occur in the beans which will affect the flavor and quality of the dried cocoa beans produced (internal purpose) [5]. Thus, the stages of fermentation in cocoa beans are an important step in obtaining good quality cocoa beans and determining the quality of the final product.

The purpose of the fermentation process can be achieved due to microbial activity in the process. Both Bacteria and fungi metabolized the pulp during fermentation [6,7]. The properties of pulp determine microbial development and metabolism. The pulp changes may affect the production of acids by lactic acid bacteria, yeasts and acetic acid bacteria [7]. The Microbes play an important role during the fermentation process. These three microbes can play a role in the formation of precursors forming aromas and flavors. In addition, several studies state that weak organic acids, one of which is produced by lactic acid bacteria, has an anti-microbial activity that plays a role in producing inhibitory effects on fungal growth. The presence of organic acids (lactic acid, acetic acid, and citric acid) can inhibit the growth of *Aspergillus* sp and suppress the mycotoxins produced [2]. Data have actually shown that many lactic acid bacteria (LAB) can inhibit mould growth and that some of them have the potential to interact with mycotoxins [8]. The selection of strains of Lactobacillus *Plantarum* HL-15 in this study was based on several reasons, among others, the type of strain was isolated from the fermentation process of cocoa at the same location where the research was conducted (Gunungkidul) [9]. The same location, in general will provide similar climatic conditions, so that the similarities of the types of mold that grow have greater opportunities [10]. The study also confirmed that this type of lactic acid bacteria was able to inhibit fungal growth and ochratoxin production so that in this study it was hoped that this strain would have a greater chance of inhibiting fungal growth.

Some mycotoxic fungi have been observed in the processing stages of cocoa in some country Côte d'Ivoire is the largest producer of cocoa beans in the world. In the research [11] with the cocoa samples from Côte d'Ivoire has found four (4) types of fungi in cacao pods, namely *aspergillus* (51.3%), *penicillium* (13.01%), alternaria (13.01%) and *fusarium* (9,23%), where the level of mycotoxin fungus contamination depends on the quality of the cocoa pod. On the other research, [12] said that cocoa from Ilheus-Bahia (Brazil) had the highest percentage of toxigenic fungi during the drying and storage stages of cocoa beans. Toxigenic fungi that were isolated were *A. flavus*, *Aspergillus parasiticus, Aspergillus nomius, Aspergillus niger* group, *Aspergillus carbonarius* and *Aspergillus ochraceus* group. The survey results in several stores in Ado, Ise, Emure and Ikere in Ekiti State of Nigeria showed that fungal mycotoxins were detected in cocoa beans during storage is *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* sp and *Fusarium* sp [13].

With the role of *L. Plantarum* HL-15 in the fermentation process, the fermentation process can be used as a step to control the biological contamination of dried cocoa beans. Because of the variety of cocoa varieties, research is needed to find out whether the anti-fungal activity produced by *L. Plantarum* HL-15 lactic acid bacteria can have the same effect on different cocoa varieties. Therefore, the objectives of this research are **(1)** knowing the effect of adding *L. Plantarum* HL-1

5 inoculum in inhibiting fungal growth in the fermentation of Criollo, Sulawesi, and Lonsum cacao beans **(2)** knowing the effect of adding *L. Plantarum* HL-15 inoculum on the fermentation of Criollo, Sulawesi, and Lonsum varieties of cocoa beans on the total fungal population in the dried cocoa beans produced and **(3)** knowing the effect of adding *L. Plantarum* HL-15 inoculum on the fermentation of Criollo, Sulawesi, and Lonsum varieties of cocoa beans on the quality of cocoa beans according to Indonesian National Standard (SNI) 2323: 2008.

Materials and Methods

Sample Collection

The materials used were Criollo, Sulawesi, and Lonsum varieties of cacao fruit originating from the Ngudi Raharjo II farmer group, Patuk District, Gunungkidul Regency, Yogyakarta, Indonesia. The lactic acid bacteria used are lactic acid bacteria strains of *L. Plantarum* HL-15.

Material and Equipment

The media used were Standard Plate Count Agar (APHA) (OXOID CM0463), Dichloran Rose Bengal Chloramphenicol Agar (DRBCA) (MERCK), de Man Ragosa Sharpe broth (MRSB) (MERCK), Bacteriological Pepton (LP0037 OXOID), D (+) Glucose (MERCK), Yeast Extract (LP0021 OXOID) and Malt Extract Agar (MEA) (MERCK), Bacteriological Agar (LPX11 OXOID), Calcium Carbonate ($CaCO_3$) (MERCK). The chemicals used include ethanol, aquades, chloramphenicol, NaCl, and Na-azide.

The equipment used in the fermentation process includes bamboo baskets with a diameter of 20cm and a height of 20cm (capacity of 2kg of fresh cocoa beans) for fermentation, banana leaves to coat the walls of bamboo baskets, burlap size 40x40 cm as a cover, base of bamboo baskets to accommodate fermented water, fermented shelves equipped with plastic hoods, stirrers, digital scales, knives, cocoa fruit beaters, and bases of wet cocoa beans (Figure 1). Equipment for analysis includes- analytic balance (METTLER PM4600), 1ml micropipette, 9cm petridish, 15ml conical tube, test tube rack, spatula, 250ml and 500ml Erlenmeyer, colony counter (QUEBEC), stomacher, autoclave, vortex mixer, 100 ml measuring cup, and pH meter.

Figure 1: Bamboo basket for fermentation process of cocoa beans

Method

The research has five steps, i.e. **(1)** Preparation of L. Plantarum HL-15 inoculum, **(2)** fermentation process of cocoa beans, **(3)** microbiology analysis during cocoa beans fermentation, **(4)** chemical analysis during cocoa beans fermentation, and **(5)** dry Cocoa beans Quality Test According to Indonesian National Standard (SNI) 2323: 2008. The each procedure of the step will be explained details below.

Preparation of L. *Plantarum* HL-15 inoculum: Preparation of liquid inoculum begins with the preparation of L. *Plantarum* HL-15 growth media in the form of MRS Broth (52g/L). Furthermore, inoculum of L. *Plantarum* HL-15 was taken from 0.2ml of culture stock in cryotube. Then, the inoculum was poured into a test tube containing 2 ml of sterile MRS Broth, followed by incubation at 37 °C for 24 hours. A total of 2ml of inoculum was then transferred to Erlenmeyer containing 20 ml of sterile MRS Broth, followed by incubation at 37 °C for 24 hours. The liquid inoculum is ready for use with a cell count of 109 cfu/ml.

Fermentation process of cocoa beans: The 2kg of fresh cocoa beans was weighed and put them in a bamboo basket. Furthermore, 20ml of liquid inoculum was poured into bamboo baskets for samples treated with an additional inoculum, while for samples not treated with inoculum no inoculum was added. Fermentation was carried out in bamboo baskets covered by banana leaves and given a cover of burlap cloth. Then put on a fermentation rack that was equipped with a plastic lid. Fermentation was carried out at room temperature for 5 days, accompanied by stirring cocoa beans every day and reversing on the 2nd day. This refers to the condition of fermentation in the Ngudi Raharjo II farmer group by combining reference based methods [14,10].

Microbiology analysis during cocoa beans fermentation: Microbiological analysis during fermentation involves calculating the total number of microbes by the pour plate method. Total population of lactic acid bacteria using media deMan Rogosa Sharpe Agar (MRSA) with the addition of $CaCO_3$ as an indicator of acid formation and Sodium Azida as a selective medium; the population of acetic acid bacteria uses Peptone Glucose Yeast Extract Agar (PGYEA) media with the addition of ethanol as a selection medium; the number of yeast population using Malt Extract Agar media with the addition of Chloramphenicol as an antibacterial; and total fungi using Dichloran Rose Bengal 134 Chloramphenicol Agar (DRBCA) media.

During the fermentation process from day 0 to day 5, 10 grams of samples were taken for microbiological analysis. 10 grams of the sample was inserted into the stomacher plastic then added 90 ml of 0.85% sterile NaCl solution, then the sample is destroyed with a stomacher until it breaks. The extracted sample was then diluted to 10-8 dilutions. Then the results of dilution were platted to the petri dish using the pour plate method aseptically. After the media was cured, the sample is incubated at 37 °C for 48 hours for bacteria and 25 °C for fungi and yeast. Enumeration of colonies is carried out using Quebec Colony Counter.

Chemical analysis during cocoa beans fermentation: Chemical analysis of pulp during fermentation was the strengthening of pH [12,15] and fermentation temperature. Temperature determination is done by inserting a thermometer into a pile of cocoa beans in a bamboo basket.

Dry cocoa beans quality test according to Indonesian National Standard (SNI) 2323: 2008: The test determines the level of seed quality based on the number of seeds per 100grams. The seeds were then sorted according to the type of flat seeds, skin, placenta, fused seeds (attached seeds), and whole cocoa beans of a certain quality. Sorting was done manually by hand based on observations. Aside from the shape of the seeds, it was also seen that the dirt on the skin. Then the whole seeds were treated with a split test to see the conditions in the seeds including salty seeds, moldy seeds, germinating seeds, and the presence of insects.

Each observation is calculated in number and compared with SNI for Cocoa Beans according to SNI 2323: 2008 to determine its quality.

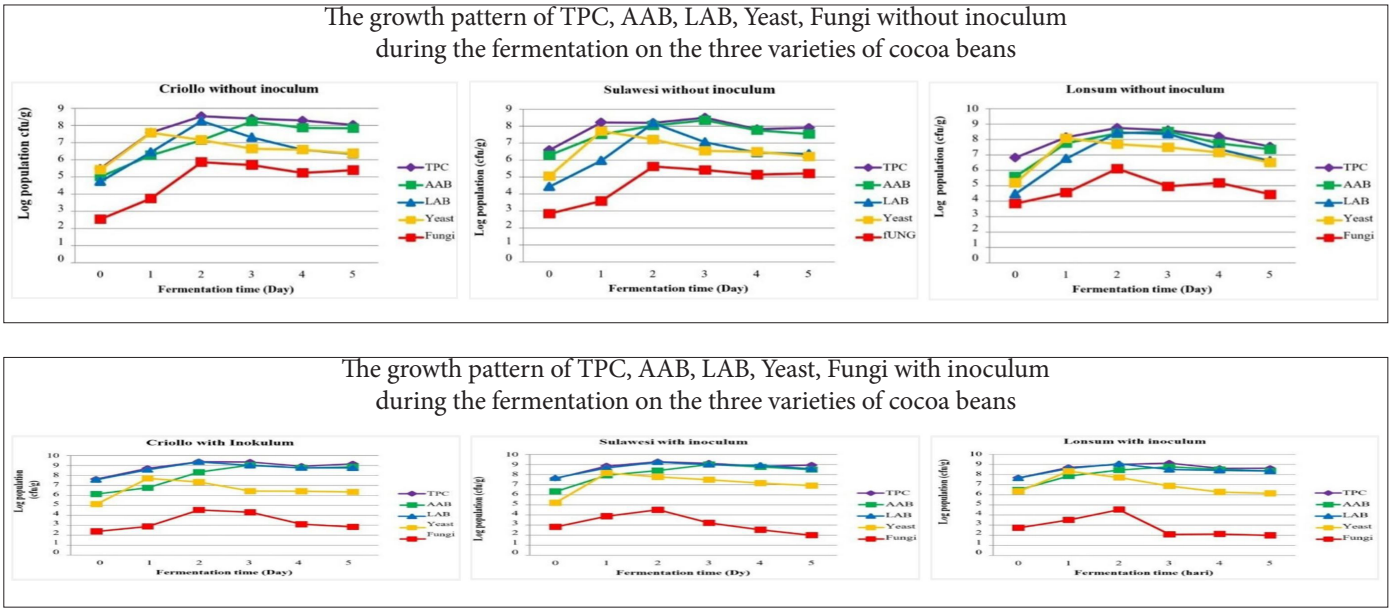
Results

Table 1 showed the volume of cocoa beans pulp. Result revealed that the difference of cocoa fruit variety (Criollo, Sulawesi, and Lonsum) cause the difference of volume of cocoa beans pulp. The Lonsum variety have the highest of pulp volume (2,46 ± 0,08 cm^3) compared to the others (Criollo dan Sulawesi).

	Criollo	Sulawesi	Lonsum
Pulp Volume (cm^3)	1,09 ± 0,04	2,04 ± 0,09	2,46 ± 0,08

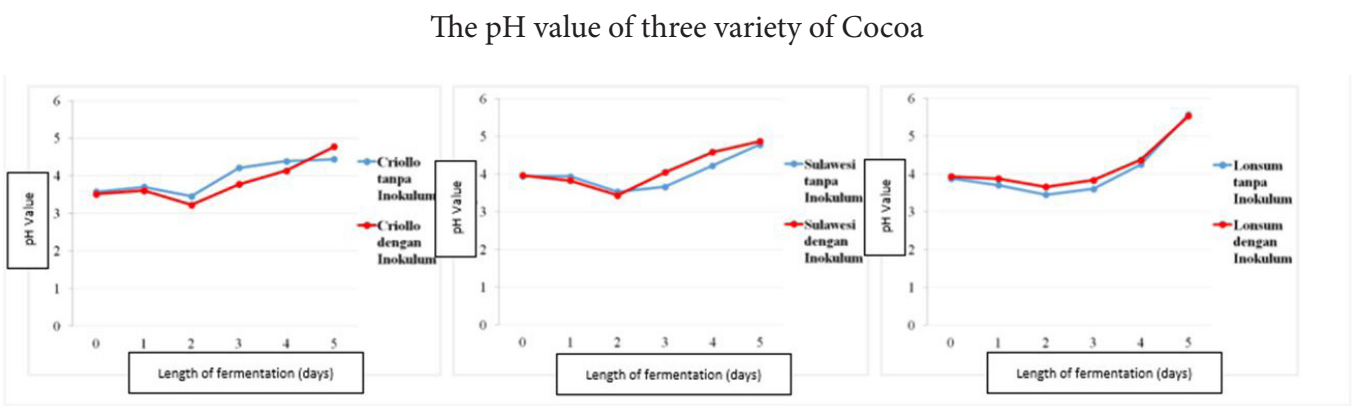
Table 1: Volume of Cocoa Beans Pulp

Figure 2 showed the growth of yeast, lactic acid bacteria, and acetic acid bacteria on Criollo var, Sulawesi var and Lonsum var cocoa beans during fermentation, without additions and with the addition of the inoculum L. *Plantarum* HL 15 have the same pattern. The fungi growth of three varieties of cocoa beans with the addition of inoculum on day 3 to day 5 can be inhibited. Its growth is lower than that which is not added by the inoculum. The yeasts dominate the first 24-36 hours of the fermentation process. This is in accordance with previous studies [3].



Keys: TPC: Total plate count; **AAB:** Acetic acid bacteria; **LAB:** Lactic acid bacteria; Yeast and Fungi
Figure 2: The growth pattern of lactic acid bacteria without and with inoculum on the three varieties of cocoa beans during the fermentation

Figure 3 showed pH values and temperature changes during fermentation. Result revealed that the chemical analysis of Criollo, Sulawesi and Lonsum varieties of cocoa beans for five days of fermentation in the form of pH values and temperature changes. The pH value of three varieties of cocoa beans with the addition of inoculum increased. The highest increase in pH values occurred in the Lonsum variety of cocoa beans. Both with addition inoculum and without addition inoculum, the temperature change during fermentation have the same pattern. The highest temperatures were achieved on 3rd day, i.e. 40 °C and then the temperature decrease.



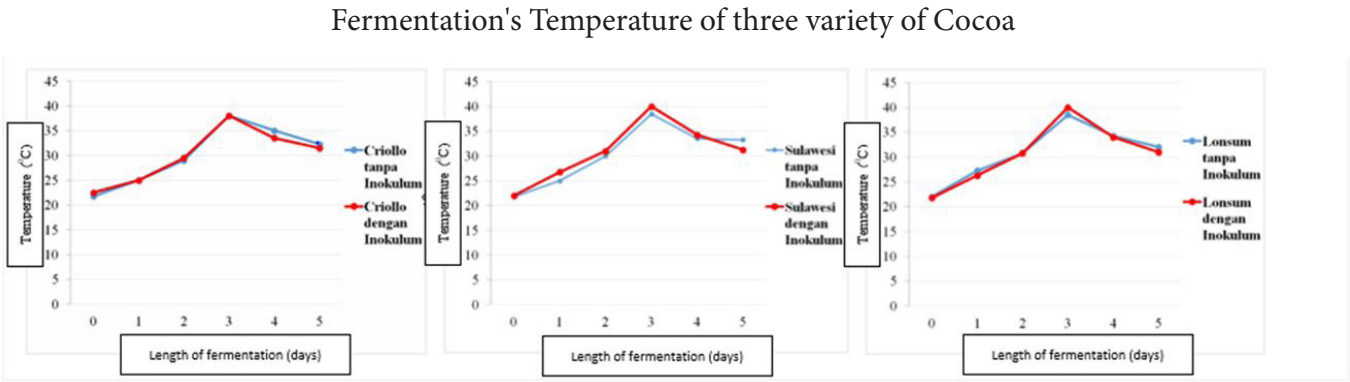


Figure 3: pH values and temperature changes during fermentation

Figure 4 showed lactic acid bacteria on dried cocoa beans. Result revealed that during the drying process, the total cell count of lactic acid bacteria in dry cocoa beans in all treatments was 4-5 log cycles. The dried cocoa beans of the three varieties with addition inoculum have higher lactic acid bacteria. Lonsum variety has highest of lactic acid bacteria, both with addition inoculum and without.

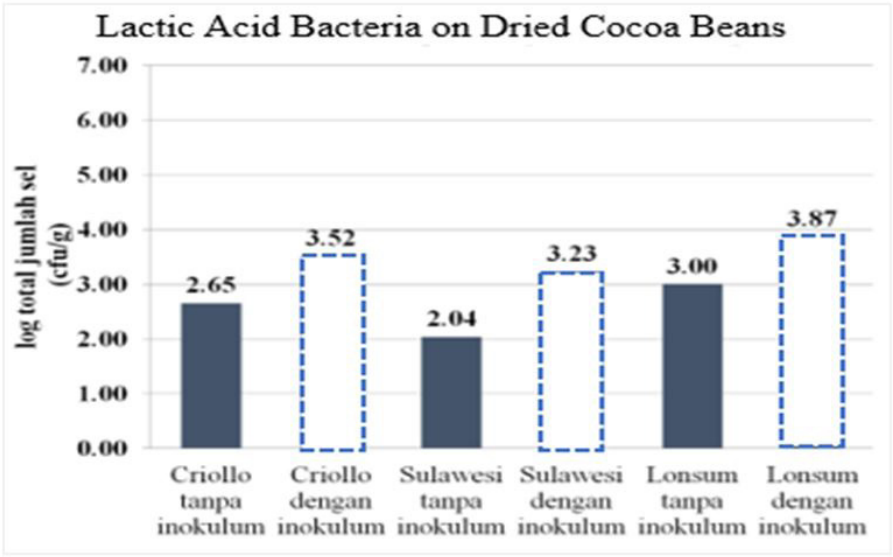


Figure 4: Lactic acid bacteria on dried cocoa beans

Figure 5 showed fungi on dried cocoa beans. Result revealed that the drying process is able to reduce the total number of fungal cells in all samples by 1-2 log cycles. Addition of inoculum to Sulawesi variety can inhibit the highest fungi.

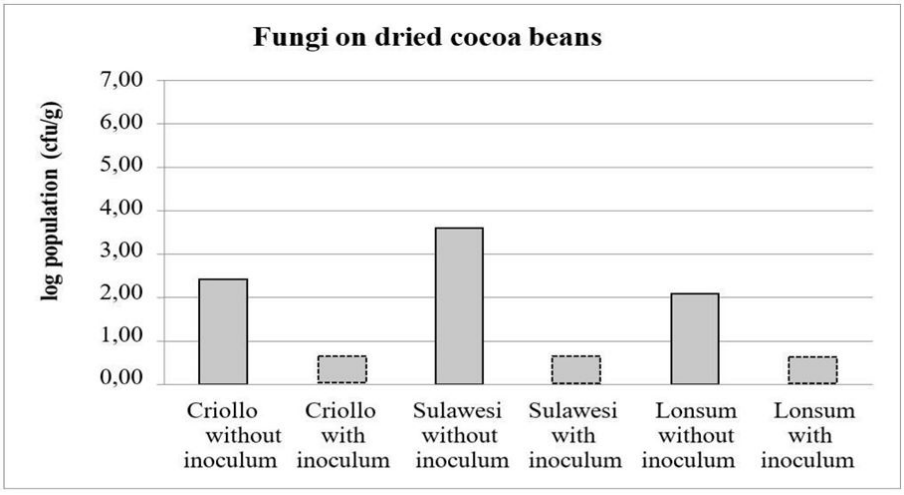


Figure 5: Fungi on dried cocoa beans

Table 2 showed analysis of Cocoa Beans Quality according to SNI. Result revealed there are no different in quality for cocoa beans with treatment and without treatment, the all of cocoa beans met SNI requirements. Addition of inoculum to Criollo and Lonsum variety, they have a level of quality of cocoa beans were better (II-F to I-F and II-B to I-B).

Varieties of Cocoa beans	Treatments	Total beans per 100 grams (bean)	Levels of moldy beans (%)	Levels of slatty beans (%)	Levels of beans contain insects (%)	Levels of dirty beans (%)	Levels of beans that germinates (%)	Bean's Quality
Criollo	Without inoculum	97	0	6,19	0	0	0	II-F
	With inoculum	88	0	2,27	0	0	0	I-F
Sulawesi	Without inoculum	85	0	2,35	0	0	0	I-B
	With inoculum	83	0	2,41	0	0	0	I-B
Lonsum	Without inoculum	96	0	3,12	0	0	0	II-B
	With inoculum	85	0	2,35	0	0	0	I-B

Keys: I-F: first quality of fine cocoa bean; **I-B:** first quality of bulk cocoa beans
Table 2: Analysis of Cocoa Beans Quality according to SNI

Discussion and Conclusions

Volume of Cocoa Beans Pulp

The fermentation process is influenced by several factors, such as the length of fermentation, uniformity of the speed of fermentation in the pile of cocoa beans, stirring, the influence of time between picking and storing cocoa fruit before fermentation, aeration, climate, fermentation method, and the effect of pulp volume [5]. In this study, the factors that become independent variables and can influence the fermentation process are the volume of the pulp layer in each of the different cocoa varieties. The pulp volume affects the fermentation process because it is related to microbes during fermentation and fermentation time. The difference in pulp volume of 3 varieties used in this study (Criollo, Sulawesi, and Lonsum) can be seen in Table 1. These factors are later thought to have an effect on microbiological tests during the fermentation process.

Microbiological analysis during fermentation

The total number of yeasts will increase on the first day (24-36 hours) of fermentation and begin to fall on the second day until the end of fermentation [3]. Yeast experienced an increase in population at the beginning of fermentation because the pulp is a substrate that contains a lot of sugar and pectin compounds so that it is in accordance with the growth conditions of the yeast. Similar data were obtained by [3], who reported that the yeasts quickly generate an alcoholic fermentation, and the sugars in the pulp are converted to alcohol and carbon dioxide. Yeast can secrete pectinase, so the pulp is destroyed (pulp viscosity decreases) and air cavities form which can increase aeration during fermentation [16,8]. An increase in aeration plus a reversal process on the second day can suppress yeast growth. So, that process becomes one of the factors in the total number of yeasts on the 2nd to 5th day decreasing. In addition, a decrease in yeast can also be caused due to increased concentrations of ethanol produced and acetic acid during fermentation due to increased activity of lactic acid bacteria and acetic acid bacteria, so that the condition suppresses the growth of yeast [3]. This condition indicates the end of the anaerobic phase and changed to aerobic phase.

On the aerobic phase, the growth pattern of lactic acid bacteria during the fermentation of the three varieties of cocoa beans is relatively the same, namely the total number of cells will increase until the second day and the peak activity and growth of bacteria occurs on the second day (over log 8 cfu/g). During fermentation, lactic acid bacteria will change glucose and produce lactic acid, alcohol, acetic acid, glycerol, manitol and CO_2 [17].

The highest total number of lactic acid bacteria for 5 days of fermentation was found on the second day with a cell count of log 8 - log 9 cfu/g. Furthermore, the total number of lactic acid bacteria decreased in the third day to the end of fermentation. There is a difference in the decrease in the total number of lactic acid bacteria in the sample treated with the addition of L. Plantarum HL-15 inoculum and samples not treated with the addition of *L. Plantarum* HL- 15 inoculum. The decrease in the total number of lactic acid bacteria in the sample treated with the addition of *L. Plantarum* HL-15 inoculums was not as large as the decrease in the total number of lactic acid bacteria in the sample which was not treated. This is because *L. Plantarum* belongs to lactic acid a bacterium which is resistant to changes in growth conditions, such as conditions with 10% ethanol content [15]

The further phases of fermentation, the microbes involved in the fermentation process are acetic acid bacteria. Acetic acid bacteria began to grow on the first and second days, with the total number of acetic acid bacteria log 6-7 cfu/g, up to the highest total number on day 3 of log 8-9 cfu/g. This is due to an increase in aeration due to the degradation activity of the pulp by the yeast plus a reversal process on the second day which causes the optimum aeration process. Then the total amount of acetic acid bacteria will decrease until the end of fermentation. Bacteria play a role in acidity formation during fermentation of cocoa beans, increased temperature in the fermentation substrate, and acid diffusion and protein hydrolysis in cotyledons [15].

Fungi were found in the analysis of the 0th day of fermentation in all samples with the total number ranged from log 2.39 - 3.84 cfu/g. The pattern of fungal growth in all samples is relatively the same, only the total number is different. The total number of fungi will increase until the second day and continue to decline until the last day. The increase in the total number of fungi can be caused due to air humidity during fermentation

Chemical Analysis during Fermentation

The pH value greatly modulates the production of the antifungal metabolite by lactic acid bacteria [Sathe, *et al.* 2007 in 8] [18]. During fermentation, there is a change in the pH of the cocoa beans. The previous research showed the variety of range of pH value for optimal production of inhibitory substances (5,5-7; 6,8; 6) [8]. The pH value measured is the pH of the pulp (surface pH of cocoa beans). The pH value decreased slightly in the first and second days and then increased on the third to fifth days. The pH value of all samples during the fermentation process increased at the end of fermentation with the highest pH of 5.57 in the sample of Lonsum varieties without the addition of *L. Plantarum* HL-15 inoculum. The increase in pH of the pulp is suspected because the acids produced during fermentation have diffused into cocoa beans [19,20]. The fermentation process of cocoa beans will cause biochemical changes in cocoa beans, such as the production of organic acids and heat, which support the process of diffusion of fermented products into cocoa beans. The diffusion of ethanol and organic acids resulting from fermentation into cocoa beans and an increase in temperature during fermentation is the main cause of seed mortality [19,20]. This situation is in line with the research conducted by Triyadi [10] and Kustyawati and Setyani [16].

During fermentation, microbial activity on the cocoa pulp generates heat [3], so the temperature of cocoa beans also changes. The essential factors that modulate lactic acid bacteria growth are temperature and fermentation periods. They influenced the amounts of antifungal metabolites produced [Batish, *et al.* in 8] [21]. The previous study reported that antifungal activity of lactobacillus *Plantarum* CUK501 was maximal temperature (1280 Au/ml) at 30 °C [Sathe, *et al*. 2007 in 3] [18]. In research, the initial temperature of fermentation in all treatments is the same, 22 °C with an ambient temperature of 23 °C. The pattern of temperature changes in all fermentation treatments is almost the same. On the first day of fermentation, temperature increases occurred in all treatments. This happens because of the yeast activity in converting sugar into alcohol. The reaction is exothermic, so in addition to alcohol and CO_2 heat release also occurs [5].

Microbiological Analysis of Dried Cocoa Beans

The total number of cells of lactic acid bacteria in dry cocoa beans varied in each sample. In the Criollo variety cocoa beans without the treatment of the addition of *L. Plantarum* HL-15 inoculums to log 2.65 cfu/g; Criollo variety cacao beans with the treatment of adding *L. Plantarum* HL-15 inoculum of 3.53 cfu/g; Sulawesi varieties of cocoa beans without the treatment of adding *L. Plantarum* HL-15 inoculums to log 2.04 cfu/g; Sulawesi varieties of cocoa beans with the treatment of adding *L. Plantarum* HL-15 inoculum to log 3.23 cfu/g; Lonsum cocoa beans without the treatment of addition of *L. Plantarum* HL-15 inoculum of log 3.00 cfu/g; and cocoa beans of Lonsum variety with the addition of *L. Plantarum* HL-15 inoculum of log 3.87 cfu/g.

It can be seen, that the sample treated with the addition of *L. Plantarum* HL-15 inoculum total number of cells of lactic acid bacteria was higher than the sample which was not treated with the addition of L. *Plantarum* inoculum. This is because the total number of cells of lactic acid bacteria in the sample treated with the addition of *L. Plantarum* HL-15 inoculum at the end of fermentation is still quite high, which is equal to log 8,35 - 8,76 cfu/g. The presence of *L. Plantarum* and *L. hilgardii* which can survive until the end of the fermentation process, and most likely remain alive during the process of storing dried cocoa beans [14].

The total number of fungal cells in the untreated sample added *L. Plantarum* HL-15 inoculum greater than the total number of fungal cells in the sample treated with the addition of *L. Plantarum* HL-15 inoculum. In Criollo varieties without the addition of *L. Plantarum* HL-15 inoculum, the total number of cells was log 2.41 cfu/g, while the Criollo variety cacao seeds were treated with the addition of *L. Plantarum* HL-15 inoculum total < log 1 cfu/g. In Sulawesi varieties of cocoa beans without the treatment of the addition of *L. Plantarum* HL- 15 inoculums the total number of cells was log 3.58cfu/g, while Sulawesi varieties of cocoa beans with the treatment of *L. Plantarum* HL-15 inoculums totaled < log1 cfu/g. And on the Lonsum cocoa beans without the treatment of the addition of *L. Plantarum* HL-15 inoculums, the total number of cells was log 2.08 cfu/g, while the Lonsum varieties were treated by adding *L. Plantarum* HL-15 inoculums totaling < log 1 cfu/g.

From the above data it can be seen that the addition of *L. Plantarum* HL-15 inoculum was able to inhibit fungal growth during fermentation and caused the total fungal population at the end of fermentation for samples treated with *L. Plantarum* HL-15 inoculum quite low, in the range < log2 cfu/g to log2 cfu/g. The addition of the inoculum had an effect on the number of initial microbial samples of cocoa beans on the 0th day of the drying process and caused the process of decreasing the fungi population faster and lower than the sample without the addition of the inoculum. Thus, the dried cocoa beans produced will also have a low total fungal population.

Quality Test of Dried Cocoa Beans According to SNI 2323:2008

Indonesian National Standard (SNI) 2323: 2008 is a regulation that regulates the quality of dry cocoa beans in Indonesia. That in all samples no fungi were found in cocoa beans.

However, based on the microbiological test of dried cocoa beans there are fungi with a total number of cells ranging from log 2 - 3 cfu/g for dried cocoa beans without the treatment of adding *L.Plantarum* HL-15 inoculum and < log 1 cfu/g for dry cocoa beans with treatment addition of *L.Plantarum* HL-15 inoculum. Therefore, the process of storing dried cocoa beans must be done well, so that internal fungal growth can be prevented and does not damage dry cocoa beans and reduce their quality. In this study, all parameters analyzed met the requirements specified in SNI 2323: 2008. Based on the data (Table 1), it can be said that there was no significant difference between the samples not treated with the addition of *L.Plantarum* HL-15 inoculum and those treated. Thus, the addition of *L.Plantarum* HL-15 inoculum has no effect on the quality of cocoa beans according to the parameters in SNI 2323: 2008.

Conclusion

Addition of *L. Plantarum* HL-15 inoculum in the fermentation of Criollo, Sulawesi, and Lonsum varieties of cocoa beans can inhibit fungal growth during fermentation of cacao beans, can reduce the total fungal population in the dried cocoa beans produced and did not affect the quality of cocoa beans according to SNI 2323: 2008.

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