Packaging and storage of cocoa beans fermented with *Lactobacillus plantarum* HL-15 in Agricultural Technology Park Nglanggeran, Yogyakarta

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**Abstract.** In Gunungkidul, there is Agricultural Techno Park (ATP) Nglanggeran which processes fermented cocoa beans from local farmers for making chocolate products. This research aimed to obtain a suitable packaging method for fermented cocoa beans during storage in ATP Nglanggeran. This research was carried out using a completely randomized factorial design. The first factor was the addition of starter (B), which consisted of B1 (with *Lactobacillus plantarum* HL 15) and B2 (without *Lactobacillus plantarum* HL 15). The second factor was the packaging methods (K), which consisted of K1 (vacuumed PP plastic 0.8 mm in thickness), K2 (non-vacuumed PP plastic 0.8 mm in thickness), K3 (plastic container), K4 (vacuumed PP plastic 0.8 mm in thickness stored within the plastic container), and K5 (nylon sack). The third factor was the storage time (P), which were P0 (0 month), P1 (1 month), and P2 (2 months). The result showed that the samples with the addition of the starter had a lower number of fungal colonies than those without the addition. Besides, the packaging using vacuumed PP plastic 0.8 mm in thickness stored within a plastic container gave the best result on minimizing the total fungal growth contamination and the peroxide value.

1. Introduction

Indonesia is the third-largest cocoa producer in the world after Ghana and Ivory Coast. The top five importing countries for Indonesian cocoa are Malaysia, America, China, India, and the Netherlands. In addition to the increasing open export opportunities, the domestic cocoa bean market is still quite large, including for the cocoa processing industry in Java [1]. The production in Yogyakarta in 2018 was 1,362 tons, and it was predicted to have an increase of 10% in 2019 and 2020 up to 1,498 tons, and 1,648 tons, respectively [2]. One of the cocoa productions centers in the Special Region of Yogyakarta is in the...
Patuk District of Gunungkidul Regency. In Nglanggeran Village, Patuk Subdistrict, Gunungkidul, there is an Agricultural Techno Park (ATP) that processes fermented cocoa beans from local farmers for making chocolate products. ATP Nglanggeran needs to be supported by some studies on fermentation technology, packaging, and storage of cocoa beans to be able to produce quality and safely processed chocolate.

Several critical stages of the contamination of mycotoxin-producing fungi in handling cocoa beans are fermentation, drying, and storage [3, 4]. During the fermentation process, several types of microbial growth occur, such as lactic acid bacteria (BAL), acetic acid bacteria (BAA), and yeast. These microorganisms have their respective roles in the fermentation process of cocoa beans [5]. In the fermentation process, the fermentation box also plays an important role. The use of fermentation box (single box with a hand crank design) requires more efficient material and space, needs to have better aeration and optimal temperature (>40 °C), and needs to be able to produce the lowest fungi level (1.33%) in the five-day fermentation [6]. If the fermentation, drying, and storage conditions are not controlled, fungus contamination will occur then cause damage and even produce toxins.

Aspergillus sp., Fusarium sp., Trichoderma sp., and Penicillium sp. are able to produce mycotoxins and cause uncontrolled fermentation processes [7]. Mycotoxin-producing fungi can be inhibited by the addition of Lactobacillus plantarum HL-15 culture, which is isolated from cocoa beans during fermentation [8]. To extend the shelf life of the culture and for an easier application, the starter culture is produced in a dry form. The method of drying the L. plantarum HL-15 culture can be performed with a spray dryer with a rice flour matrix [9], a rack-type dryer with a rice flour matrix [10], or an oven dryer with a tapioca matrix [11]. The use of dry starter culture of L. plantarum HL-15 in the fermentation process can provide increased value added to dried cocoa beans [12].

Lactic acid bacteria that have been proven to have the power to inhibit fungi from organic acid production are from the species Lactobacillus fermentum and Lactobacillus plantarum [13]. The fermentation process with the addition of lactic acid bacteria shows an increase in the fermentation index which indicates that the fermentation process can be accelerated [14]. Improvement of the fermentation process of dried cocoa beans can be done by adding cane drops, yeast, and lactic acid bacteria [15]. The aroma of unfermented cocoa beans can be enhanced by the fermentation process with the addition of S. cerevisiae, L. plantarum, and A. aceti cultures [16]. The application of Lactobacillus fermentum in the fermentation process can produce good quality cocoa which is free of mycotoxins [17]. Controlling mycotoxin-producing fungal contaminants to improve the quality of cocoa beans can use lactic acid bacteria [18,19]. Besides, L. plantarum HL-15 can inhibit fungal growth and synthesis of oxytocin A in cocoa beans during fermentation and drying [20]. The addition of L. plantarum HL 15 as a culture starter in the old and new fermentation boxes has a smaller level of fungus contamination compared to the treatment without L. plantarum HL 15 as the culture starter [21].

In the cocoa beans, Eurotium sp was still detected to be carried by the cocoa powder being produced. The presence of Eurotium in cocoa beans will be followed by the growth of Aspergillus and Penicillium [21]. In the storage of cocoa beans, there were also found some fungi (Aspergillus, Paecilomyces, Talaromyces, Penicillium, Pseudophitomyces, Cimplicillium, and Aspergillus carbonarius) [3]. Besides, there were found fungus A. flavus, A. niger, A. fumigatus, Penicillium sp, Fusarium sp, Trichoderma sp., Rhizopus sp., Mucor sp., and Verticillium sp. in the dried cocoa beans at the farmer level. Meanwhile, at the trader level, A. flavus, A. niger, Penicillium sp., Fusarium sp., Trichoderma sp., and Mucor sp. were found, and at the exporter level, there were found fungi A. flavus, A. niger, Penicillium sp, Trichoderma viridae, and Geotrichum sp. The total population of mycotoxigenic fungi at the levels of farmers, traders and exporters were respectively 1.4 x 109 cfu/ml, 6.5 x 107 cfu/ml, and 6.0 x 105 cfu/ml [22]. The Penicillium species which was found mostly in the cocoa belongs to P. citrinum which
is likely to have a capability in the production of OTA [23]. OTA was detected in the beans at all stages of post-harvesting operations, such as pod-opening, fermentation, drying, and storage [24]. Black Aspergillus was the dominant species of Aspergillus in cocoa beans, and A. carbonarius was the OTA producer among the black Aspergillus [25].

Packaging plays an important role in maintaining the water content of fermented cocoa beans to keep the seeds from being attacked by the fungi during storage. Proper packaging and storage of cocoa beans are as important as proper fermentation and drying processes. According to Owusu [26], cocoa beans must be packaged in clean bags that are strong enough and properly sealed. The bags used must be made of non-toxic food-grade material. After the drying and sorting process, cocoa beans must be put in an appropriate bag and stored. Furthermore, packaged cocoa beans must be placed in a weather-resistant storage warehouse, free from pests, away from smoke, and other odors. Packaged cocoa beans also need to be placed on the surface of the soil and away from the wall.

The quality of dried cocoa beans in the international trade is assessed by the level of the total percentage of fungi, unfermented beans, purple seeds, seeds that are attacked by insects, and germinated seeds. In the recent cocoa trade, from the scientific point of view, much emphasis is put on free fatty acid content (FFA) which is influenced by many factors, such as humidity, oxygen, and insect infestation [27]. The facts show that the high water content of cocoa beans causes fungal infections, which are related to FFA values in cocoa beans. The increase in FFA during storage can be related to the activity of the lipase enzyme which is naturally present in cocoa beans [28]. The enzyme becomes active due to changes in the moisture content of the seeds and high-temperature storage environment [29]. Therefore, storage management plays an important role to maintain the quality of fermented cocoa beans.

This study aims to determine the effect of adding Lactobacillus plantarum HL-15 culture to the fermentation process and packaging method on the quality of cocoa beans during storage in ATP Nglanggeran. This starter of Lactobacillus plantarum HL-15 was added at the beginning of the fermentation process to minimize the total fungal growth in the cocoa beans. The packaging methods were also studied to select which one had the best effect on maintaining the quality of cocoa beans.

2. Materials and methods

2.1. Materials

The fermentation and packaging of cocoa beans were carried out at UPH Ngudi Raharjo II Gunungkidul Yogyakarta, while the storage was carried out at ATP Nglanggeran. The cocoa beans were obtained from Gunungkidul. The starter culture of Lactobacillus plantarum HL-15 was obtained from FNCC Gadjah Mada University, Yogyakarta [6]. The packaging materials were PP 0.8 mm plastic, nylon sacks, raffia, and plastic containers. Chemicals for conducting the chemical and microbiological analyses included solid KI (MERCK), KIO3 1% (MERCK), HCl 2N (MERCK), sodium thiosulfate (Na2S2O3) MERCK brand, 1% starch, chloroform (CH3Cl), methanol, Na2SO4 anhydrous (MERCK), glacial acetic acid, DG-18 (OXOID) plating media, chloramphenicol, glycerol, sodium chloride (NaCl) MERCK brand, and 0.05% Bayclin brand disinfectant (purchased in a local supermarket in Yogyakarta).

2.2. Methods

Fresh cocoa fruits, after harvesting, were carefully broken down using a wooden beater, or by hitting one fruit with another fruit. Then the cocoa beans were removed from the fruit and separated from the pith attached to the seeds. Afterward, the sorting of cocoa beans was done by separating the fully ripe cocoa beans from young seeds and deformed seeds due to pests, fungi, or sprouting.
The study of this fermentation process was carried out using a field scale with a 40 kg capacity of fermentation box. The fermentation process of cocoa, using starter culture, is a modification of the fermentation method performed by [14,15,16,17,18,19,20]. The fermentation was carried out for five days. On the third day, the cocoa seeds were turned upside down so that they were evenly fermented. On day 5, the cocoa beans were removed from the fermentation box, soaked, washed, and ready to be dried. Next, the drying was done by a combination of manual drying and rack type dryer at the temperature between 55-66°C to reach 6-7% water content. After that, the dried cocoa beans were packaged using the packaging materials and then stored at room temperature in ATP Nglanggeran.

This research was carried out using a completely randomized factorial design. The first factor was the addition of the starter (B), which were B1 (with \textit{Lactobacillus plantarum} HL-15) and B2 (without \textit{Lactobacillus plantarum} HL-15). The second factor was the packaging methods (K), which were K1 (vacuumed PP plastic 0.8 mm in thickness), K2 (non-vacuumed PP plastic 0.8 mm in thickness), K3 (plastic container), K4 (vacuumed PP plastic 0.8 mm in thickness stored within a plastic container), and K5 (nylon sack). The third factor was the storage duration (P), which were P0 (0 month), P1 (1 month), and P2 (2 months) as seen in table 1.

| Packaging methods (K) | Addition of the starter (B) | With \textit{L. plantarum} HL-15 (B1) | Without \textit{L. plantarum} HL-15 (B2) |
|----------------------|-----------------------------|---------------------------------|---------------------------------|
| Vacuumed PP plastic 0.8 mm in thickness (K1) | v | v |
| Non-vacuumed PP plastic 0.8 mm in thickness (K2) | v | v |
| Plastic container (K3) | v | v |
| Vacuumed PP plastic 0.8 mm in thickness stored within a plastic container (K4) | v | v |
| Nylon sack (K5) | v | v |

2.3. Analyses of dried cocoa beans
A number of analyses conducted on the dried cocoa beans before and during the storage: (1) the quality assessment based on SNI 2003: 2828; (2) moisture content; (3) seed water activity; (4) peroxide value; and (5) total mushroom contamination. The data were analyzed using SPSS software version 22.0. One-way analysis of variance (ANOVA) with a significance level of 5% was used.

3. Results and discussion

3.1. Temperature and relative humidity of storage room
The storage of fermented cocoa beans was carried out at ATP Nglanggeran, Gunungkidul. The optimal room temperature for storing cocoa beans is below 30°C with a maximum relative humidity (RH) of 75% [30]. The temperature and RH of the room during the storage were quite fluctuating, with the lowest temperature of 26.6 °C and the highest temperature of 28.8 °C, and with the lowest relative humidity of 60% and the highest of 69%. The range of temperatures and RH values did not exceed the upper limit of temperature and relative humidity for optimal cocoa bean storage [30]. This indicated that the temperature and relative humidity of the room in ATP Nglanggeran for two months of storage of cocoa beans was always in the optimal range.
3.2. The quality of dried cocoa beans based on the Indonesian National Standards (SNI)

The analysis results of the quality of cocoa beans which include moldy seeds, levels of unfermented beans (slaty), levels of germinated seeds, levels of insect-containing seeds, broken seeds, and flat beans can be seen in table 2. In the first month of storage, the cocoa beans without or with the addition of starter *L. plantarum* HL-15, in all packaging methods, did not show fungal growth. This is in line with research by Yanti [31] which showed that the addition of local microbes in the form of lactic acid bacteria did not exhibit fungal growth in cocoa beans. In the cocoa bean sample, after two months of storage, it was found that the growth of this external fungus was characterized by grayish-white spots and grayish-white fine fibers on the surface of the dried cocoa beans as the result of the fermentation using culture or without the addition of culture.

In all samples of the cocoa beans, the number of seeds germinated and contaminated by insects show a 0% level. This means that no sample had been germinated or contaminated by insects. Seed germination is undesirable in dried cocoa bean products because germination shows that the seeds are still alive, the fermentation is not going well, and the flavor-forming precursor compounds cannot appear. The seeds that are contaminated by insects are also undesirable because they will reduce the quality of the cocoa beans, especially physically. Some examples of insects that commonly attack cocoa beans include fig moth (*Cadra cautella*), gray moth (*Cadra elutella*), *Araecerus fascicularis*, *Lasioderma serricorne*, red flour beetle (*Tribolium castaneum*), and khapra beetle (*Trogoderma granarium*). Damage caused by the insects varies from the cavity on the surface of the seeds, dirt in the form of larvae, cocoon shells, to silk spun threads [32].

From all the parameters being analyzed, the values obtained were compared with the Indonesian National Standards for cocoa beans. From this comparison, it can be seen that the quality of the samples of cocoa beans was homogeneous. The samples of 0 month, the first month, and the second month were all included in the I-B class. Meanwhile, the maximum percentages for the moldy seeds, the slaty seeds, the insect-containing seeds, the impurities, and the germinating seeds were 2%, 3%, 1%, was 1.5%, and 2%, respectively.

3.3. The moisture content of cocoa beans

The moisture content of the cocoa beans during storage is shown in table 3. From table 3, it can be seen that statistically, the factor of addition of *L. plantarum* HL-15 starter and the packaging methods did not significantly influence the water content. After two months of the storage, there was a significant increase in water content, but it still met the export quality standards. The standard moisture content of export quality cocoa beans is a maximum of 7.5%. If it is higher than this value, cocoa beans will not be safe to be stored for a long time, but if the moisture content is too low, cocoa beans tend to be fragile [33]. Besides, if the moisture content is too low (<5%), the cocoa beans will become brittle and break easily during transportation [34].

3.4. The cocoa beans water activity

The moisture content of cocoa beans during storage is shown in table 4. Aw value is closely related to the potential for growing microorganisms including fungi that can damage dried cocoa beans during storage. Statistically, the factors that has a significant effect on Aw sample are packaging and the duration of storage. Because the p values of both are less than 0.05, while the starter factor had a p-value of 0.707, it can be concluded that it does not have a significant effect. However, the interaction between the starter and packaging factors significantly influences the Aw values of the samples. In conclusion, the addition of a starter will have a significant effect on Aw samples if followed by packaging variations.
Table 2. The quality of dried cocoa beans based on the Indonesian National Standard including moldy seeds, levels of unfermented (slaty) beans, levels of germinated seeds, levels of insect containing seeds, broken seeds, and flat beans.

| Month | Cocoa beans Fermentation | Packaging | Number of seeds / 100 grams | Moldy seeds (%) | Unfermented (slaty) beans (%) | Germinated seeds (%) | Insect containing seeds (%) | Broken seeds (%) | Flat beans (%) | Quality | Size group |
|-------|--------------------------|-----------|-----------------------------|----------------|-----------------------------|-------------------|-----------------------------|----------------|--------------|---------|------------|
| 0     | With \( L.\) *plantarum* HL-15 | Vacuumed PP plastic 0.8 mm in thickness | 102 ± 1.41 | 0 | 0 | 0 | 0.5 | 0 | I-B | B |
|       | Without \( L.\) *plantarum* HL-15 | Vacuumed PP plastic 0.8 mm in thickness | 106 ± 0 | 0 | 0 | 0 | 0 | 0.47 | 0 | I-B | B |
| 1     | With \( L.\) *plantarum* HL-15 | Non-vacuumed PP plastic 0.8 mm in thickness | 105 ± 2.83 | 0 | 0 | 0 | 0 | 0.47 | 0 | I-B | B |
|       | Plastic container | Vacuumed PP plastic 0.8 mm in thickness stored within a plastic container | 101 ± 5.66 | 0 | 0 | 0 | 0 | 0 | 0 | I-B | B |
|       | Nylon sack | Vacuumed PP plastic 0.8 mm in thickness | 111 ± 2.83 | 0 | 0 | 0 | 0 | 0 | 0 | I-B | C |
|       | Without \( L.\) *plantarum* HL-15 | Vacuumed PP plastic 0.8 mm in thickness | 109 ± 14.14 | 0 | 0 | 0 | 0 | 0 | 0 | I-B | B |
|                | With L. plantarum HL-15 | Without L. plantarum HL-15 |
|----------------|-------------------------|-----------------------------|
| **Non-vacuumed PP plastic 0.8 mm in thickness** |                         |                             |
| Plastic container | 121 ± 5.66 0 0 0 0 0 0 | 109 ± 2.12 0.92 0 0 0 0.47 1.37 I-B B |
| Vacuumed PP plastic 0.8 mm in thickness stored within a plastic container | 108 ± 1.41 0 0 0 0 0 0 | 118 ± 3.54 0 0.47 0 0 0.95 I-B B |
| Nylon sack | 118 ± 2.12 0 0 0 0 0 0.42 0 | 112 ± 1.41 0.89 0.44 0 0 0.44 0.44 I-B C |
| Vacuumed PP plastic 0.8 mm in thickness | 110 ± 1.41 0.9 0.46 0 0 0 | 110 ± 1.41 0.9 0.46 0 0 0.92 I-B B |
| Non-vacuumed PP plastic 0.8 mm in thickness | 108 ± 2.83 0.46 0 0 0 0 | 108 ± 2.83 0.46 0 0 0 0.46 I-B B |
| Plastic container | 118 ± 3.54 1.72 0.42 0 0 0.44 1.74 I-B C | 102 ± 6.36 0 0.47 0 0 0 | 102 ± 6.36 0 0.47 0 0 0.95 I-B B |
| Nylon sack | 112 ± 1.41 0.89 0.44 0 0 0.44 0.44 I-B C | 109 ± 2.12 0.92 0 0 0 0.47 1.37 I-B B |
|                | Non-vacuumed PP plastic 0.8 mm in thickness | Plastic container | Vacuumed PP plastic 0.8 mm in thickness stored within a plastic container | Nylon sack |
|----------------|---------------------------------------------|-------------------|--------------------------------------------------------------------------|------------|
| L. plantarum   | 121 ± 2.83 1.67 0.41 0 0 0.42 0.42 I-B S    | 112 ± 0.71 0 0 0 0 0 0 I-B C                                   | 112 ± 1.41 0.9 0 0 0 0 0.45 I-B C                                    | 103 ± 4.24 0 0.5 0 0 1 0 I-B B |
| HL-15          |                                             |                   |                                                                          |            |
Table 3. The moisture content of cocoa beans during storage.

| Cocoa beans                                                                 | Moisture content (%) during storage |
|----------------------------------------------------------------------------|-------------------------------------|
|                                                                            | 0 Month     | 1 Month     | 2 Months    |
| With L. plantarum HL-15                                                   |            |            |             |
| Vacuumed PP plastic 0.8 mm in thickness                                     | 6.24<sup>aA</sup> | 6.18<sup>aA</sup> | 7.05<sup>bB</sup> |
| Non-vacuumed PP plastic 0.8 mm in thickness                                | 6.24<sup>aA</sup> | 6.18<sup>aA</sup> | 6.73<sup>bB</sup> |
| Plastic container                                                          | 6.24<sup>aA</sup> | 6.56<sup>aA</sup> | 7.39<sup>bB</sup> |
| Vacuumed PP plastic 0.8 mm in thickness stored within a plastic container  | 6.24<sup>aA</sup> | 6.02<sup>aA</sup> | 6.39<sup>bB</sup> |
| Nylon sack                                                                | 6.24<sup>aA</sup> | 6.30<sup>aA</sup> | 7.20<sup>bB</sup> |
| Without L. plantarum HL-15                                                 |            |            |             |
| Vacuumed PP plastic 0.8mm in thickness                                     | 7.02<sup>aA</sup> | 6.36<sup>aA</sup> | 6.91<sup>bB</sup> |
| Non-vacuumed PP plastic 0.8mm in thickness                                 | 7.03<sup>aA</sup> | 7.02<sup>aA</sup> | 7.79<sup>bB</sup> |
| Plastic container                                                          | 7.03<sup>aA</sup> | 6.28<sup>aA</sup> | 7.23<sup>bB</sup> |
| Vacuumed PP plastic 0.8mm in thickness stored within a plastic container   | 7.03<sup>aA</sup> | 7.55<sup>aA</sup> | 7.07<sup>bB</sup> |
| Nylon sack                                                                | 7.03<sup>aA</sup> | 6.28<sup>aA</sup> | 7.06<sup>bB</sup> |

Remark: Figures followed by different lowercase letters (a, b, c) in the same column and figures followed by different capital letters (A, B) in different columns show significant difference.

The highest average Aw values are found in the samples with K5 packaging variations, followed by K1, K3, K4, and K2, respectively. The K5 package is a nylon sack packaging as used by cocoa farmers in the Gunungkidul area to store their dried cocoa beans. In terms of air permeability, this type of packaging is easier to penetrate than other types of packaging because nylon sacks have gaps on wider surfaces. Thus, the dried cocoa beans inside will be more susceptible to contact with the surrounding air. The statistical analysis shows that the packaging treatment using 0.8 mm un-vacuum PP (K2) plastic is actually able to maintain the Aw value better than the 0.8 mm PP plastic package that is vacated (K1). This can be seen from the average Aw value of each treatment each month, where for K2, the mean value is lower than K1, as well as the change in Aw that occurs, which also has a smaller minimum value for K2 when compared to K1. Through Duncan's post hoc test, it is found that the variation of K2 packaging is significantly different from K1.

3.5. The peroxide value in cocoa beans

The peroxide value in the cocoa beans during storage is shown in table 5. From table 5, it can be seen that the type of packaging and the storage duration significantly affect the peroxide value while the addition of a starter has no effect on it. The longer the seeds are stored, the larger would be the average peroxide value, which indicates an increase in fat damage.
Table 4. The cocoa bean water activity during storage.

| Cocoa beans                                                                 | Water activity during storage |
|----------------------------------------------------------------------------|-------------------------------|
|                                                                             | 0 Month          | 1 Month         | 2 Months        |
| **With L. plantarum HL-15**                                                |                 |                 |                 |
| Vacuumed PP plastic 0.8 mm in thickness                                    | 0.73<sup>bcA</sup> | 0.74<sup>bcaB</sup> | 0.78<sup>bcB</sup> |
| Non-vacuumed PP plastic 0.8 mm in thickness                                | 0.71<sup>aA</sup>  | 0.72<sup>aAB</sup> | 0.77<sup>abB</sup> |
| Plastic container                                                          | 0.75<sup>bcA</sup> | 0.75<sup>bcaB</sup> | 0.76<sup>bcB</sup> |
| Vacuumed PP plastic 0.8 mm in thickness stored within a plastic container  | 0.71<sup>abA</sup> | 0.72<sup>abAB</sup> | 0.74<sup>abB</sup> |
| Nylon sack                                                                 | 0.78<sup>cA</sup>  | 0.79<sup>cAB</sup> | 0.76<sup>cB</sup>  |
| **Without L. plantarum HL-15**                                             |                 |                 |                 |
| Vacuumed PP plastic 0.8 mm in thickness                                    | 0.74<sup>bcA</sup> | 0.75<sup>bcaB</sup> | 0.77<sup>bcB</sup> |
| Non-vacuumed PP plastic 0.8 mm in thickness                                | 0.72<sup>aA</sup>  | 0.72<sup>aAB</sup> | 0.75<sup>abB</sup> |
| Plastic container                                                          | 0.74<sup>bcA</sup> | 0.75<sup>bcaB</sup> | 0.76<sup>bcB</sup> |
| Vacuumed PP plastic 0.8 mm in thickness stored within a plastic container  | 0.76<sup>abA</sup> | 0.76<sup>abAB</sup> | 0.74<sup>abB</sup> |
| Nylon sack                                                                 | 0.75<sup>cA</sup>  | 0.75<sup>cAB</sup> | 0.71<sup>cB</sup>  |

Remark: Figures followed by different lowercase letters (a, b, c) in the same column and figures followed by different capital letters (A, B) in different columns show significant difference.

The lowest peroxide value is found in the cocoa beans with K4 packaging, while the highest is in the cocoa beans with K5. Indeed, K4 packaging has a barrier to environmental conditions. It is oilier than other variations of packaging so that it has the ability to inhibit the greatest oxidation of fat as well. For example, it can inhibit direct contact with light that can cause photooxidation and inhibit direct contact with air that can trigger autoxidation. This is in contrast to the variation of K5 packaging, where nylon sack packaging is the least airtight packaging so that air can easily enter and make contact with the seeds.

The peroxide limit in dried cocoa beans is 5 meqiv O2/Kg sample [35]. In fact, the peroxide values obtained from the cocoa seeds for all the treatments are below the threshold.

3.6. The total fungal contamination of cocoa beans
The total fungal contamination of cocoa bean during storage is shown in table 6. Table 6 records that the type of packaging has not shown an effect on the total fungal colonies, both on fermented cocoa beans by adding L. plantarum HL-15 or without adding L. plantarum HL-15 for the two-month storage. Meanwhile, the storage time has a significant effect on the growth of fungal colonies, both on fermented cocoa beans with the addition of L. plantarum starter and without the addition of plantarum HL-15.
starter. It is obvious that the number of fungal colonies increases significantly after the two-month storage.

High air humidity can trigger mold growth [30]. Hence, besides using vacuum packaging, other treatments should be taken during storage to maintain the quality of cocoa beans. In visual observation based on SNI, there was no fungus, but based on the results of microbial observations in the laboratory analysis with pour plate method, there were still internal mushroom populations. Therefore, a good handling is necessary during the process of storing dried cocoa beans so that the growth of internal mold can be prevented and does not damage the dried cocoa beans and reduce its quality.

Table 5. The peroxide value in cocoa beans during storage.

| Cocoa beans | The peroxide value (5 meqiv O₂/Kg sample) during storage |
|-------------|---------------------------------------------------------|
|             | 0 Month | 1 Month | 2 Months |
| **With** L. plantarum HL-15 | |
| Vacuumed PP plastic 0.8 mm in thickness | 0<sub>abA</sub> | 0.46<sub>abB</sub> | 0.59<sub>abC</sub> |
| Non-vacuumed PP plastic 0.8 mm in thickness | 0<sub>bA</sub> | 0.55<sub>bB</sub> | 0.65<sub>bC</sub> |
| Plastic container | 0<sub>abA</sub> | 0.47<sub>abB</sub> | 0.58<sub>bC</sub> |
| Vacuumed PP plastic 0.8 mm in thickness stored within a plastic container | 0<sub>aA</sub> | 0.38<sub>aB</sub> | 0.49<sub>aC</sub> |
| Nylon sack | 0<sub>cA</sub> | 0.80<sub>cB</sub> | 1.07<sub>cC</sub> |

| Without L. plantarum HL-15 | |
| Vacuumed PP plastic 0.8 mm in thickness | 0<sub>abA</sub> | 0.48<sub>abB</sub> | 0.52<sub>abC</sub> |
| Non-vacuumed PP plastic 0.8 mm in thickness | 0<sub>bA</sub> | 0.66<sub>bB</sub> | 0.61<sub>bC</sub> |
| Plastic container | 0<sub>abA</sub> | 0.47<sub>abB</sub> | 0.60<sub>abC</sub> |
| Vacuumed PP plastic 0.8 mm in thickness stored within a plastic container | 0<sub>aA</sub> | 0.39<sub>aB</sub> | 0.41<sub>aC</sub> |
| Nylon sack | 0<sub>cA</sub> | 0.63<sub>cB</sub> | 0.69<sub>cC</sub> |

Remark: Figures followed by different lowercase letters (a, b, c) in the same column and figures followed by different capital letters (A, B) in different columns show significant difference.
### Table 6. The total fungal contamination of cocoa beans during storage.

| Cocoa beans | Total fungal contamination (log CFU/g) during storage |
|-------------|-----------------------------------------------------|
|             | 0 Month | 1 Month | 2 Months |
| With L. plantarum HL-15 |          |          |          |
| Vacuumed PP plastic 0.8 mm in thickness | 2.0<sup>A</sup> | 2.16<sup>aA</sup> | 3.14<sup>abB</sup> |
| Non-vacuumed PP plastic 0.8 mm in thickness | 2.18<sup>aA</sup> | 2.38<sup>aA</sup> | 3.51<sup>abB</sup> |
| Plastic container | 2.0<sup>aA</sup> | 2.32<sup>aA</sup> | 3.27<sup>abB</sup> |
| Vacuumed PP plastic 0.8 mm in thickness stored within a plastic container | ND | ND | 3.25<sup>abB</sup> |
| Nylon sack | 2.22<sup>aA</sup> | 2.54<sup>aA</sup> | 3.26<sup>abB</sup> |
| Without L. plantarum HL-15 |          |          |          |
| Vacuumed PP plastic 0.8 mm in thickness | 2.53<sup>aA</sup> | 3.16<sup>aA</sup> | 3.41<sup>abB</sup> |
| Non-vacuumed PP plastic 0.8 mm in thickness | 2.0<sup>aA</sup> | 2.52<sup>aA</sup> | 3.21<sup>abB</sup> |
| Plastic container | 2.0<sup>aA</sup> | 2.05<sup>aA</sup> | 3.35<sup>abB</sup> |
| Vacuumed PP plastic 0.8 mm in thickness stored within a plastic container | 2.0<sup>aA</sup> | 2.18<sup>aA</sup> | 3.0<sup>abB</sup> |
| Nylon sack | 2.26<sup>aA</sup> | 2.34<sup>aA</sup> | 3.42<sup>abB</sup> |

Remark: Figures followed by different lowercase letters (a, b, c) in the same column and figures followed by different capital letters (A, B) in different columns show significant difference.

The primary role of vacuum packaging is to remove oxygen to help prevent food spoilage and to minimize the absorption of water vapor by the packaged product. This is related to the amount of free water (aW) in food products that can be used by microorganisms such as fungi and bacteria to grow [36].

### 4. Conclusion

This research concluded that the addition of *Lactobacillus plantarum* HL-15 starter during the fermentation of cocoa beans did not have a statistically significant effect on the growth of fungi that contaminated the dried cocoa beans during the two months of storage. Packaging with 0.8 mm PP plastic that was vacuumed and put into a plastic container had a significant effect on the value of peroxide but did not have a significant effect on the total fungus contamination during the 2 months of storage.
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