The Usage of Dried Starter for Re-Fermentation of Unfermented Cocoa Beans

Hernani1*, T Hidayat1 and I Mulyawanti1
1Indonesian Center for Agricultural Postharvest Research and Development, Jl. TentaraPelajar 12 Bogor

*Email: umirizki57@gmail.com

Abstract. In order to improve the quality of cocoa beans, it has been made by re-fermentation of unfermented beans using dried starter. The methodology used in the fermentation process using a dry starter with the concentration of 1, 2 and 3% towards the quality of cocoa beans produced. The design of experiments was used completely randomized design (RAL) with two replications. Observations during the fermentation of cocoa beans were temperature, pH, alcohol and acid contents, whereas for the quality of dry beans were determined moisture content, ash, protein, fat, free fatty acid (FFA), fermentation index (IF). The flavor was also detected by GCMS. The results showed that during fermentation, the temperature was increased on the first day, then decreasing until day 6. Afterward, the pH tended to increase until day 7. Furthermore, alcohol and acid content (acetic and lactic acids) tends to decrease. The quality of dry beans gave moisture content, ash, protein, fat and FFA ranges from 8.12 to 9.21; 4.07 to 4.16; 21.28 to 22.06; 31.52 to 34.90 and 0.44 to 0.51, respectively. Index fermentation of 2% starter concentration gave the best value. Flavor compounds were detected on the re-fermented cocoa beans, namely the group of compounds pyrazines, ketones, aldehyde, alcohol, acid and pyron groups.

1. Introduction
Most of the cocoa in Indonesia produced without the fermentation process, and the quality has a low grade. Because of the low quality, the price is also low. The fermentation process is beneficial to increase the quality of cocoa beans because it will reduce the level of acidity, and maximize the flavor of chocolate [1,2]. Naturally, cocoa beans could fermentation spontaneously, because of pulp surrounding seeds have numerous microorganisms [3]. The microbial of cocoa fermentation involves various organisms, such as yeasts, bacteria (lactic acid and acetic acid bacteria, Bacillus species), and filamentous fungi. The major species involved in the control of fermentation are Hanseniaspora guilliermondii, Pichia kudriavzevii, Kluyveromyces marxianus(yeasts), Lactobacillus plantarum, L. fermentum (lactic acid) bacteria, and Acetobacter pasteurianus and Gluconobacter frateurii(acetic acid) bacteria [4].

In order to improve the quality of unfermented cocoa beans through the fermentation process, it should be used as the starter to help the fermentation. However, one of the constraints in re-handling to improve the quality of unfermented cocoa beans, the cocoa beans has lost most of the substrate and water content which is the absolute requirement of the fermentation process. Substrates for microbial growth in the fermentation of cocoa beans are the compounds of sugary such as glucose, fructose, sucrose, and pentose and also citric acid contained in the pulp [5]. Therefore, efforts to restore water content and dry cocoa beans substrate resemble the original condition through cocoa bean soaking and
the addition of external nutrients (as a substitute for pulp), namely: fructose: glucose: sucrose: citric acid (62: 41: 32 : 22.5)[6]. The other hand, the addition of dried starter to the fermentation process of cocoa beans aim to improve the quality of the fermentation process [5].

The starter is an important part of the cocoa fermentation process. Commonly, the starter contains microorganisms, i.e bacteria, mold, and yeast or a combination of these three types of those microbes that work through the fermentation process. The main requirements of the microbial starter are free of contamination, rapid growth process, produce distinctive flavor, resistant to bacterio phage and resistant to antibiotics. The development of starter for fermentation is used to control the fermentation process of cocoa beans to produce high-quality dry cocoa beans [7]. It has been reported that starter consisting of yeast (Saccharomyces chevalieri, a synonym of S. cerevisiae), LAB (L. lactis and L. plantarum), and AAB (A. aceti) succeeded in improving the quality of cocoa seed flavor. The usage of a starter consisting of Candida tropicalis, Lactobacillus sp., and Acetobacter sp. 1% reduced the fermentation time and quality of the resulting cocoa beans meet the SNI quality standard [8]. In addition, then [9] has been successfully utilized starter consisting of S. cerevisiae and A. aceti and sugar cane to improve the fermentation process of unfermented cocoa beans. The purpose of this research was to improve the quality (physico-chemical) of unfermented cocoa beans by a re-fermentation process using a dried starter of microbes.

2. Material and method
The experiment was used Completely Randomized Design (RAL) with two replications. Dried starter for fermentation was used from the mixture of S. cerevisiae: L. plantarum: A. aceti with the ratio 1: 2: 1 and viability of 6.7 x 10^10 cfu/g. The minimum viability of starter culture in fermentation is 10^6 Cf u / ml [10].

The raw material used unfermented cocoa beans with the amount of 5 kg, respectively. Before fermentation, unfermented cocoa beans were soaked in water for 1-2 hours. Then, preparing the medium was a mixture of fructose: glucose: sucrose: citric acid with a ratio of 62: 41: 32: 22.5. The medium was then stirred evenly with unfermented cocoa beans. Afterward, the dried starter was added to cocoa beans, stirred until mixed evenly, then put in fermentation box and covered with a gunny sack. The fermentation box was stored in a place protected from direct sunlight and after three days, the cocoa beans should be rotated. The fermentation process was carried out for 7 days. After the fermentation process was completed, the cocoa beans are straight to wash, drained and dried. The drying process used an oven with the temperature of 40°C. During the fermentation process, it was observed temperature, pH, alcohol content, and acid. The quality of dried beans was observed for moisture, ash, protein, and fat contents, FFA, fermentation index (IF), and flavor. The moisture content was analyzed by the gravimetric method and fat content by Soxhlet method [11]. Analysis of flavor compounds by GC-MS.

2.1. Fermentation index
Fermentation index of cocoa beans was determined using the method of [12]. Five hundred (500) mg of defatted cocoa powder were weighed into a 125 ml conical flask containing a mixture of 50 ml of methanol:hydrochloric acid (97:3), the mixture was cooled at 8±2°C in a refrigerator for 16 –18 h. A clear extract was obtained by filtration through a Whatman filter paper No. 1. Then, the extract is observed by a spectrophotometer at wavelength 460 nm and 530 nm. Fermentation index was calculated, based on the ratio of the absorbance at 460 nm to the absorbance at 530 nm.

3. Results and Discussion
During the fermentation process, the temperature was increased significantly on the first day of fermentation with relatively equal achievement from three dried starter concentrations, ranging from 40.6 to 42.03°C (Figure 1a). The rise of temperature at the beginning of fermentation is caused by an exothermic reaction occurring at the time of changing the external nutrients (a mixture of fructose: glucose: sucrose) into alcohol due to the yeast activity. Yeast is a dominant microbe at the beginning of
fermentation and quite significant as a consequence of the substrate conditions that contain lots of sugar [13]. In other hands, it has an important role in the fermentation of cocoa beans in producing alcohols in limited oxygen conditions and high sugar levels, which in turn is converted to acetic acid.

At 3% dried starter concentration, the fermentation temperature was relatively stable until day 3, while at 2% concentration the temperature decreased on the 2nd day then increased again until the 4th day. At the concentration of 1%, the temperature continued to decline after the first day of the fermentation process. This condition shows that the optimum temperature of cocoa fermentation, i.e. ± 40°C [13]. This can be achieved from 2-3% starter concentration.

At the concentrations of 2-3%, the growth of acetic acid bacteria is thought to increase after the availability of oxygen and alcohol resulted in the change of lactic acid bacteria and yeast. By acetic acid bacteria, the alcohol is oxidized to acetic acid, which is then oxidized to CO₂ and water. The reaction by acetic acid bacteria takes place exothermically which causes the fermentation temperature to increase.

![Figure 1](image1.png)

**Figure 1.** The temperature (a) and pH (b) during fermentation process

At the beginning of fermentation, cocoa beans have a pH 3.69 to 3.88. Therefore, during the fermentation process, the pH of cocoa beans was relatively constant until the third days of fermentation, however, after 3 days fermentation, the pH increased significantly until the seven days ranging from 6.45 to 6.61 (Figure 1b). When the pH higher than 5.1 gave the relatively better quality of beans [14]. It has been reported that pH in the fermentation of cocoa beans around 5.04 to 5.16 in 5 days without turning [15]. The utilized of a dried starter at various concentrations in the fermentation process exhibited similar pH patterns, particularly at concentrations of 1 and 3%. According to [16], low pH values are usually associated with high acidity levels in cocoa beans and have an indication of the low quality of cocoa beans.

Alcohol is one of the main products in the fermentation process. As for being seen in Figure 2 that alcohol content was tended to decrease during the fermentation process. For addition of starter 1% concentration showed decreased from the beginning of fermentation to seven days. However, it will give differences on 2% and 3% concentration. Subsequently, alcohol content tends to increase until day 7. These mean that increased alcohol levels will stop yeast growth [17].
The organic acids in the fermentation process produced acetic acid tended to decrease until fermentation day 5, however, at 3% concentration, the acetic acid was increased very sharply to the 7th day (Figure 3a). Then, the citric acid was also formed gradually for every day’s fermentation (Figure 3b). The fermentation process has usually reduced the levels of organic acids (acetic acid, lactic acid, and citric acid) because these compounds will cause an unpleasant aroma in cocoa beans [6]. The addition of starter would produce in the increasing amount of microbial fermentation, it will be more ethanol produced and degraded into acetic acid. This means that more acid in the dried cocoa beans showed the quality of the cocoa beans getting lower [18].

3.1. Index Fermentation
The index fermentation (IF) of cocoa beans from the various addition of concentration dried starter showed tended to increase with the length of fermentation (Figure 4). The cocoa beans have no good fermented when the IF less than 1.0, while perfectly fermentation when IF more than 1.0, that is 1.0 to 1.599 completely fermented and more than 1.600 as over-fermented [19]. From the treatment, it showed that the perfectly fermented cocoa beans obtained from a 3% starter addition because it had IF ≥ 1.0 while some of the other treatments only had IF close to 1.
3.2. Proximate analyses

Proximate analysis of cocoa beans can be seen in Table 1. The moisture content of cocoa beans showed significantly different values at dry starter concentrations of 1 and 2%, however, it had no difference between concentrations of 2 and 3%. Then, the cocoa bean ash content showed significantly different values, especially at 3% concentration, with values ranging from 4.07 to 4.16%. According to [20], the ash content would be decreased with the length of the fermentation time. This is because of the number of water-soluble minerals and fats that together will come out of the seeds during the fermentation process. The protein content of various dried starter concentrations was significantly different at 2% concentration (Table 1), ranging from 21.28 to 22.06%. It has been reported by [21] that protein content is ranged from 15.2 to 19.8%.

Table 1. Proximate analyses of cacao beans

| Starter concentration (%) | Moisture content (%) | Ash content (%) | Protein content (%) | Fat content (%) | FFA content (%) |
|---------------------------|----------------------|-----------------|---------------------|----------------|----------------|
| Control                   | 10.45<sup>c</sup>   | 4.94<sup>b</sup>| 22.56<sup>a</sup>  | 38.22<sup>b</sup>| 2.31<sup>b</sup>|
| 1                         | 8.12<sup>ab</sup>   | 4.08<sup>a</sup>| 21.55<sup>a</sup>  | 34.90<sup>ab</sup>| 0.51<sup>a</sup>|
| 2                         | 8.75<sup>b</sup>    | 4.07<sup>a</sup>| 22.06<sup>b</sup>  | 33.78<sup>ab</sup>| 0.44<sup>a</sup>|
| 3                         | 9.21<sup>b</sup>    | 4.16<sup>b</sup>| 21.28<sup>a</sup>  | 31.52<sup>b</sup>| 0.49<sup>a</sup>|

Fat content showed a significant difference between starter concentrations added, with range 31.52-34.90%. Fat content is an important quality index for cocoa beans. Generally, fat content ranges from 50.40 to 53.35% and 52.27-55.21% respectively for fermented and unfermented cocoa beans [21]. The free fatty acid content (FFA) ranged from 0.44-0.51%, not significantly different for all treated dry starter concentrations added. FFA levels for cocoa beans should not be more than 1% because cocoa beans containing FFA> 2.3% is considered to be already damaged. The Codex Standard establishes the maximum limit of free fatty acids in cocoa beans that is 1.75%.

Identification of flavor of unfermented cocoa beans by GC-MS was presented in Table 2 only for the best treatment, i.e., 3% concentration starter addition. Flavor compounds detected in cocoa beans resulted from re-treatment, namely compounds of pyrazine, ketone, aldehyde, alcohol, acid, and pyrone groups.
Table 2. The flavor compounds of re-fermented cocoa at 3% addition dried starter

| Groups of compounds       | Compounds                                                                 |
|---------------------------|---------------------------------------------------------------------------|
| Pyrazine                  | 3 Isobuthyl-2-metoxyopyrazine; 2,3, 5, 6 Tetra methyl pyrazine; 2Ethyl-3, 5 dimethyl pyrazine |
| Ketone and aldehyde       | 3 Methyl butanal; 2, 3Butadienone                                         |
| Alcohol                   | 2 Methyl propanol; Hexanol-2; Heptanol 2; 1, 3 Butadienol                |
| Acids                     | Cafeic acid; Acetic acid; Palmitic acid; Stearic acid; Oleic acid         |
| Pyrone                    | 3, 5 Dihydroxy-6-methyl-4-pyrone                                         |

The chemical compounds which is very important to contribute formation of seed cacao flavor, i.e., 3-methylbutanal, ethyl 2-methylbutanoate, hexanal, 2-isopropyl-3-methoxypyrazine, 2-ethyl-3,5-dimethyl pyrazine, and 2- and 3-methylbutanoate. Some of these compounds have been identified in re-treated cocoa beans with the addition of 3% dry starter, i.e. 3-methyl butanal, and 2-ethyl-3,5-dimethylpyrazine compounds.

4. Conclusion
The addition of dried starter to fermentation of unfermented cocoa beans would be improved the quality. The use of 3% dried starter gave the best result, which is indicated by the highest value of index fermentation and acetic acid content at the end of the fermentation process. This study suggested that dried starter can be developed for improving the quality of unfermented cocoa beans.

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