A Study of Bread Yeast and Sucrose to the Successful Process of Cocoa Beans Fermentation

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Abstract. This research aims to know the effect of the concentration of sucrose and bread yeast to the successful process of cocoa beans fermentation. The research was conducted in the form of Completely Random Design of Factorial Pattern, two Factors. The first factor is sucrose consisted of 0% (control), 1%, 2%, and 3% sucrose. The second factor was the bread yeast consists of two (2) concentrations: 0.5% and 1%, and each repeated twice to obtain 16 experimental units. The observed parameters were fermentation index, salty beans and the pH of cocoa beans. The result of the research showed that there was an interaction between sucrose and bread yeast in the successful process of cocoa beans fermentation in small scale. The treatment without sucrose and adding 1% of bread yeast had a better effect to the fermentations index value of 1.72; salty beans were 1.56 and pH beans was 5.30.

1. Introduction

Cocoa is an industrial crop that has potential as a producer of foreign exchange in non-oil and gas sector. The economic value of cocoa beans is significant in its contribution to the life of society and the source of foreign exchange. Therefore, the development of cocoa is continuing both in cultivation and post-harvest aspects. Indonesia is the third largest producer and exporter cacao in the world, after Ivory Coast and Ghana, with a total area of 1.65 million hectares of plantations and the total production of 440,000 tons in 2010/2011; 87 percent is produced in local farms [1]. The fundamental disadvantage in the production of local cocoa is low quality and failure to achieve the properties required in international standards of trades [2].

The farmers mostly produce the production of cocoa seeds in Indonesia. Around 965,000 farmers are involved in cocoa farming. In 2005, there was 887,735 ha (89,45%) cocoa plantation in Indonesia as smallholdings of cocoa farmers. Meanwhile private extensive plantation covering 54,737 ha (5,51%) and state extensive plantation only 49,976 ha (5,04%). Thus, the cocoa farmers contribute about 90% of national production. However, from the existing cocoa plantation in Indonesia, the value of its productivity is still low: 897 kg/ha/year, whereas the potential productivity usually reaches more than 2,000 kg/ha/year [3].

Even though it has a good marketing prospect, the commodities of Indonesian cocoa are still facing a problem on the image of poor quality that could affect to the competitiveness of product in the international market. It is because the cocoa seed still dominates the production of Indonesian cocoa has not been fermented. It still appeared in dirt seeds and contaminated with insect and fungi. Processing cocoa, in essence, is an attempt to process cocoa into dried cocoa seeds that fulfill the standard of quality and can elicit the distinctive characteristic of cocoa, especially the taste. Stage of processing that considered as the most influence to the quality of dried cocoa seed is fermentation. Fermentation of cocoa seeds aims to destroy the pulp and cultivate the condition for biochemical reactions in the seeds, which contribute to the formation of the taste precursors and the color of
chocolate. The crushed pulp will be smooth to escape from the seed, forming pulp fluid dripping out from the seeds [4].

Lopez and Dimic stated the fermentation of cocoa beans is the process of transforming the sucrose into alcohol and then into organic acids performed by a consortium of fermenting microbes [3]. Those organic acids will induce the reaction of enzymatic in the seeds; it results in biochemical change that will form a compound that gives a smell, taste, and color to the cocoa beans [5].

The non-fermented dry cocoa beans have the different characters to the wet cocoa beans when the fermentation is used. The dried cocoa beans have lost some content of water and substrate which is the requirement in the fermentation of cocoa. The water will combine the enzyme and substrate in the seed, so the hydrolysis and oxidation process of compound taste, color, and the smell of cocoa will happen there. The content of water required in cocoa fermentation is more than 35%. The substrate is the material that is recognized by microbes during the fermentation process. Substrates in fermenting cocoa seeds are sucrose and citric acid that contained in the pulp [6]. During the process of fermentation, the pulp will be transformed by microbes into organic acid. Acids will diffuse into the beans and induce enzymatic reactions to form the compounds of taste, aroma, and color [7]. To optimize the process of fermentation, it is required conditions that help the process of fermentation such as dissolved substrates and microbes.

Therefore, it is necessary to research about the use of bread yeast and sucrose that is more applicable so that optimization efforts can be implemented mainly by the farmers. Successful fermentation of cocoa beans using bread yeast, and sucrose is determined by its concentration. The Research Objective is to study the influence of bread yeast with sucrose in optimizing the process of fermentation of cocoa beans.

2. Research Method

The experiment was conducted by applying Complete Randomized Design (CRD) with a factorial pattern in two (2) factors. The first factor was sucrose (P) with concentration: 0% (p0), 1% (p1), 2% (p2), 3% (p3). The second factor of bread yeast (R) is 0.5% (r1), and 1% (r2). The total of eight treatment combinations was repeated twice, so there were 16 experimental boxes.

2.1. Materials and Tools

The ingredients used in the study are: Lindak type cocoa, bread yeast (fermipan), sucrose, fermentation box size 40 cm x 30 cm x 30 cm, oven, thermometer, and gunny sack. Chemicals for analysis include: ethanol 70%, N-hexane, methanol 90%, and NaOH 0.1 N. The tools used are: pH-meter, blender, analytical scale, measuring flask, UV-Vis Type spectrophotometer, measuring cylinder, electric oven, pipette, Petridis plate, magnetic stirrer, Whatman 42 filter paper, test tube, Erlenmeyer, and glassware.

2.2. Observation

2.2.1. The Fermentation Index

The dried cocoa beans are measured and sieved with size > 35 mesh, then weighed as much as 0.5 grams. The sample was put into a 100 ml breaker glass, add a fermentation index solution (FI) (97 parts of methanol ±three parts 30% (HCl) of 50 ml. The FI solution obtained from 3 ml of HCl was inserted into a 100 ml measuring flask, then added methanol to the limit and shaken until homogeneous. 100 ml breaker glass containing cocoa samples and FI solution covered by aluminum foil for refrigeration for one night after storage. The solution is filtered with filter paper to be separated by the raffinate, and the filter is prepared in cuvette then measured. The absorbance value was measured by using a spectrophotometer at a wavelength of 460 nm and 530 nm to calculate its fermentation index.

\[
FI = \frac{\text{abs } \lambda \text{ 460 nm}}{\text{abs } \lambda \text{ 530 nm}}
\]
2.2.2. Salty beans with the cut test method
The test is done by observing the changes color visually and subjectively. A total of 100 cocoa beans were split longitudinally right in the middle into two (2). From 100 parts of the beans were observed one by one the color of cocoa beans based on its classification [8]. In this study, the classification classified into three (3) classes where the slaty color was inserted into unfermented beans classes, the dominant purple to brown color is classified into the underfermented bean’s class, and the dominant brown enters the fermented bean’s class. The percentage of the three (3) classifications were slaty, purple, and brown calculated the percentage with the following formula (9,10):

\[
\% \text{ Unfermented beans} = \frac{\text{total part of cocoa beans}}{\text{slices of slaty beans} \times 100}\%
\]

\[
\% \text{ Underfermented beans} = \frac{\text{total of cocoa beans}}{\text{slices of purplr beans} \times 100}\%
\]

\[
\% \text{ Fermented beans} = \frac{\text{total of cocoa beans}}{\text{slices of chocolate beans} \times 100}\%
\]

2.2.3. pH of dried beans
Implementation procedure, sample test is taken as many as 12 beans up to 20 beans, outer skin separated, then milled using a blender. The test sample is weighed 10 g into the cup glass, add 90 ml of hot distilled water (70° C to 80° C), stir slowly until a suspension is formed which must be free of lumps. The filtrate is filtered and cooled till the room temperature is (27-29)° C and the filtrate pH is determined as soon as possible at that temperature. The results are expressed according to the readings indicated by the pH-meter for the filtrate.

3. Result and Discussion

3.1. Result
Fermentation Index. Based on the result of research showed that giving sucrose had a significant effect, while the addition of bread yeast and the interaction of sucrose and yeast did not significantly affect the fermentation index. The average fermentation index at various concentrations of sucrose is presented in Table 1.

| Sucrose Concentration | Bread yeast Concentration | Average | HSD 0.05 |
|-----------------------|---------------------------|---------|----------|
|                       | r1 (0.5%)                 | r2 (1%) |          |
| p0 (control)          | 1.71                      | 1.73    | 1.72a    | 0.23     |
| p1 (1%)               | 1.67                      | 1.33    | 1.50a    |          |
| p2 (2%)               | 1.15                      | 1.14    | 1.14b    |          |
| p3 (3%)               | 1.10                      | 1.10    | 1.10b    |          |
| **Average**           | **1.41**                  | **1.33**|          |

Note: the number in the line followed by different letters (a,b,c) means significantly different with the Honestly significance Difference (HSD) 0.05

Based on the Honestly Significance Difference (HSD), results on Table 1 shows the index fermentation value in the treatment of the average addition of sucrose >1. The treatment without the
addition of sucrose, fermentation index value was 1.72 but not significantly different from the sucrose concentration of 1% (1.50), and it was significantly different in 2% and 3% (1.14) of the sucrose concentration with 1.10 of fermentation index.

3.1.1. Slaty Beans
Based on the research result showed that sucrose adding was not significant. While the bread yeast is adding and the interaction of sucrose and bread yeast had a very significant effect on the number of slaty beans. The average of slaty beans in various concentrations of sucrose and yeast is presented in Table 2.

Table 2. The average of the number of slaty beans

| Sucrose Concentration | Bread Yeast Concentration | Average | HSD 0.05 |
|-----------------------|---------------------------|---------|----------|
|                       | r1 (0.5%) | r2 (1%) |          |
| p0 (control)          | 2.70       | 1.56    | 2.13     |
| p1 (1%)               | 1.40       | 1.70    | 1.55     |
| p2 (2%)               | 1.70       | 1.20    | 1.45     |
| p3 (3%)               | 1.60       | 1.70    | 1.65     |
| Average               | 1.85       | 1.54    |          |

Note: The numbers on the line followed by different letters (a,b) means significantly Honestly significance Difference (HSD) 0.05)

Based on the result of Honestly Significance Difference (HSD) results in Table 2 shows that interaction of 2% sucrose treatment with 1% bread yeast addition resulted in the lowest slaty beans of 1.20 significantly different from the addition of 0.5% bread yeast in all sucrose concentrations. The average number of most slaty beans obtained in cocoa beans that are not given sucrose with only 0.5% of bread yeast was 2.70.

3.1.2. pH Cocoa beans
The results showed that the addition of sucrose had no significant effect, while the addition of bread yeast had a significant effect and the yeast and sucrose interaction had a very significant effect on the pH of cocoa beans. The average pH of cocoa beans at various concentrations of bread yeast and sucrose is presented in Table 3.

Based on the result of HSD 0.05 in Table 3 shows that 1% of sucrose addition and 0.5% of yeast bread added result lower pH (4.50) of cocoa beans significantly different with 1% of bread yeast concentration in (5.45). Higher average pH values were obtained in non-sucrose cocoa beans in either 0.5% or 1% of bread yeast with values of 5.20 and 5.30 respectively.
Table 3. The pH average of Cacao beans

| Sucrose Concentration | Bread Yeast Concentration | Average | HSD 0.05 |
|----------------------|---------------------------|---------|----------|
|                      | r1 (0.5%)                 | r2 (1%) |          |
| p0 (control)         | 5.20 \( \text{a} \)      | 5.30 \( \text{a} \) | 5.25     |          |
| p1 (1%)              | 4.50 \( \text{a} \)      | 5.45 \( \text{a} \) | 4.98     | 0.49     |
| p2 (2%)              | 5.15 \( \text{b} \)      | 4.90 \( \text{b} \) | 5.03     |          |
| p3 (3%)              | 4.95 \( \text{a} \)      | 5.05 \( \text{a} \) | 5.00     |          |
| Average              | 4.95                      | 5.18    | 5.06     |          |

Note: The numbers on the line followed by different letters (a, b) means very different in Honestly Significance Difference (HSD) (0.05).

3.2. Discussion

The purpose of fermentation is to release the pulp from the seeds to facilitate the drying process, and the seed shell is easily removed from seed pieces. Also, fermentation aims to kill seeds and provide opportunities for the process leading to the formation of colors, flavors, and aroma, and lower levels of non-fat ingredients so that the relative fat levels will increase [11].

The cocoa seeds fermentation process generally takes place naturally by microorganisms present in the fermentation atmosphere which lasts for six days with reversals on day two and at every 24 hours [12, 13, 14, 7]. At the beginning of 24 hours, bread yeast fermentation dominates the fermentation that will break the sugar component inside the pulp into alcohol [5, 12].

The results showed that the addition of sucrose and bread yeast at the beginning of fermentation of cocoa seeds significantly affect the fermentation index, slaty seed, and pH of cocoa beans. The addition of sucrose and bread yeast at the beginning of fermentation yields cocoa seeds with a fermentation index greater than 1 (Table 1). This shows that cocoa seeds have complete fermentation due to the addition of yeast at the beginning of fermentation. The cocoa fermentation process generally takes place naturally by microorganisms found in the fermentation atmosphere [12, 13, 14, 7]. At the time the fruit is broken, the pulp is immediately contaminated by microorganisms in the surrounding air so that the pulp fermentation process will happen soon. High sugar content with high acidity (pH 3.5) because the content of citric acid is an ideal condition for the growth of microorganisms.

Dry cocoa beans with FI values (fermentation index) equal to one and above one (FI ≥1) are considered perfectly fermented [15, 16]. Fermentation of cocoa beans with fermentation index values below one (FI<1) indicates imperfect fermentation. The fermentation index is a brownish size of the cocoa beans to ensure the level of fermentation of the beans [17]. According to Kongor et al., the potential of cocoa beans flavor can be recognized from the quality of fermentation through a color index called fermentation index [18]. Qualitatively the perfection of the fermentation process can be seen from the color change of cocoa bean pieces. The measurement of fermentation index by spectrophotometry is the ratio of brown pigment absorbance (\( \lambda \): 460 nm) and purple (\( \lambda \): 530 nm). The measurements were based on flavonoid levels that gave brown color with anthocyanin giving purple color [15, 19].

Table 2 shows that there is an interaction between sucrose and bread yeast with the number of slaty seeds. By adding 2% of sucrose and 1% of bread, yeast is the best treatment for the number of slaty seeds because the lowest value is 1.20. This happens because the addition of 2% sucrose can provide energy for yeast growth to perform activities optimally during the fermentation process. According to
the Indonesian National Standard (SNI 2323-2008), the number of slaty beans is a maximum of 3% (quality I-B) [8]. Slaty beans meant unfermented beans showed half or more slices of grayish-gray slices, such as; slate or grayish-blue and textured stable and reliable like cheese. Slaty beans will give astringent flavor and excessive bitter and low taste of cocoa beans [20]. Polyphenol compounds are the determinants of the color of the beans. The presence of polyphenols is not only responsible for the formation of bitter taste and astringent flavor but also causes the characteristic of brown color from fermented cocoa beans [20].

The quality of fermented cocoa beans is determined mainly by the acidity (pH). The cocoa processing industry requires seed pH between 5.2-5.8 to produce quality cocoa butter [21]. The results of the research on the pH of dry cocoa beans showed that there was an interaction between giving bread yeast and sucrose to the pH of the cocoa beans. The highest average pH value of 5.45 is found in the addition of 1% sucrose and 1% yeast. The excellent pH value of cacao beans is close to neutral (pH> 6) so that Brown-specific compounds can form intensively and acidic beans have a pH <5.0 [22]. The fermentation process made a pH change of 3.5 on fresh beans before fermentation to about 4.8 on the 3rd day of fermentation and eventually to about 5.5 in dried beans. The addition of yeast increases the number of microbes in the seed pulp, thus increasing the ability of yeast to break down sucrose into alcohol. The higher the concentration of yeast added the more carbohydrates are converted into glucose, alcohol, lactic acid, and other compounds. pH is closely related to total acid since in general the increase in total acid is followed by a decrease in pH.

4. Conclusion
Based on the result of the research, it can be concluded that there is an interaction between sucrose and bread yeast with the success of the cocoa beans fermentation process. The treatment without sucrose and the addition of 1% bread yeast had a better effect on the fermentation index value of 1.72, the number of slaty beans was 1.56, and the pH of the beans was 5.30.

Suggestion
To optimize the process of fermentation of small-scale cocoa beans it is recommended to add bread yeast at the beginning of fermentation with a concentration of 1%, but still, it needs to do more research by increasing the yeast concentration above 1% to obtain optimal concentration.

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