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# Significant potential of tape yeast and plant leaves as a cover for fermented cocoa (*Theobroma cacao* L.) beans to generate qualities of dry cocoa beans

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**Abstract**

The purpose of this study was to determine the response to the addition of tape yeast and various cover leaves in the fermentation process of quality cocoa beans (*Theobroma cacao* L.) according to the Indonesian National Standard (SNI). This research was conducted at PT Perkebunan Nusantara (PTPN) XII, Ngrangkah Pawon plantation, Kediri Regency and Laboratory of Agrotechnology, Universitas Darussalam Gontor in April-August 2020. The research design used a factorial completely randomized design (CRD). The first factor was the addition of tape yeast (R) which consisted of 5 levels, namely: no yeast (R0), the addition of 0.5% tape yeast (R1), 1.0% (R2), 1.5% (R3), and 2.0% (R4). The second factor was plant leaves (D) which consisted of 5 levels, namely: D1 (banana leaves), D2 (teak leaves), D3 (taro leaves), D4 (papaya leaves) and D5 (bamboo leaves). Observation parameters included fermentation temperature of cocoa beans, pulp acidity (pH) of cocoa beans, the account of cocoa beans per 100 g, moisture content, sugar content, cut test and, phytochemical qualitative test. Observation data were analyzed by using analysis of variance (ANOVA) and further tested with LSD 5%. The addition of tape yeast and plant leaves as a cover for fermented cocoa beans improved the quality of the dry cocoa beans. There was no interaction between the addition of tape yeast and plant leaves as a cover for fermented cocoa beans in all observations of the quality of dry cocoa beans. The addition of 1% tape yeast improved the quality of fermented cocoa beans on the parameters of fermentation temperature on day 2 (43.2°C), the account of cocoa beans per 100 g (93.8 cocoa beans), sugar content (2.8°Bx) and brown seed color (99.2%). Teak leaves, taro leaves, papaya leaves and bamboo leaves can be used as a cover for fermenting cocoa beans such as banana leaves which are commonly used by farmers.

## 1. Introduction

Unfermented cocoa beans are low quality as raw material for processed foods. Unfermented cocoa beans contain higher polyphenols and lower amino acid content than fermented cocoa beans (Rosniati and Kalsum, 2012). The bitter and astringent taste of cocoa beans can be reduced and eliminated through the fermentation process during post-harvest handling (Kongor *et al*., 2016). Fermentation can improve the quality of cocoa beans and meet the quality requirements as raw materials for making chocolate. Fermentation of cocoa beans serves to decompose the pulp of cocoa beans, improve the color and taste of cocoa, and reduce the bitter and astringent taste and form cavities in the cotyledons of cocoa beans (De Vuyst and Weckx, 2016). The aroma component strengthens and permeates the cotyledon tissue of the cocoa bean during fermentation. Precursors of the characteristic aroma and taste of chocolate are formed in cocoa beans during the fermentation process (Kadow *et al*., 2013).

Fermentation is often described as the process of sugar catabolism in fruit extracts and seeds by yeasts which ultimately produces ethanol (Stanbury *et al*., 2017). Pulp is rich in sugar content and decreases even almost completely loses sugar during the fermentation of cocoa beans (Purwanto *et al*., 2019). During the fermentation process, the diversity of microorganisms involved in the fermentation of cocoa beans is very high. Greater diversity of yeasts when compared to bacteria involved in fermentation (Figueroa-Hernández *et al*., 2019). Yeast plays an important role in the early fermentation of cocoa beans and the decomposition of the pulp (De Vuyst and Weckx, 2016). The quality of cocoa beans can be increased by the presence of yeast

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activity (Santos *et al*., 2020). The presence of yeast during fermentation makes the processed chocolate taste more delicious and forms cocoa beans that are browner (Ho *et al*., 2014).

Microorganisms that are often used in the fermentation process are yeast *Saccharomyces cerevisiae*. Some of the advantages of *S. cerevisiae* are fast growth, tolerance to low pH, tolerance to acetic acid and tolerance to high temperatures (Beato *et al*., 2016). *Saccharomyces cerevisiae* improves the quality of processed food and beverage products from fermented cocoa beans. *Saccharomyces cerevisiae* influences the formation of chocolate taste sensors in cocoa beans when fermented.

On average, tape yeast contains molds, yeasts and bacteria (Muhiddin *et al*., 2019). Tape yeast can be used as a substitute for fermenters in fermenting milk into yogurt (Oktaviana *et al*., 2015). The addition of dry bread yeast improved the quality of the cut test of cocoa beans but there were still some seeds that germinated were moldy and were attacked by insects (Patty, 2019). Tape yeast has the opportunity to accelerate the fermentation of cocoa beans. Purwanto *et al*. (2019) stated that the addition of tape yeast accelerated the fermentation process of cocoa beans, on the 3$^{rd}$ day the fermentation process went perfectly with the indicated fermentation index of ≥1, while natural fermentation on the 3$^{rd}$ day had a fermentation index of ≤1. Yeast significantly improves the pulp removal process of cocoa beans while producing more metabolic products leading to an increase in the fermentation index (Cempaka *et al*., 2014).

The results of Kristanto *et al*. (2017) study of the temperature of 5 kg wet cocoa beans added with 5 g yeast was higher than 3 g yeast, but the addition of 3 g and 5 g yeast had not been able to produce grade A dry cocoa beans. This study aimed to determine the optimal percentage of tape yeast addition to improve the quality of cocoa beans during the fermentation process. Through a clear comparison between the amount of tape yeast that must be given and the number of wet cocoa beans to be fermented, it will be easier for farmers to add tape yeast.

Some farmers prefer to sell wet cocoa beans and are not used to fermentation to produce quality cocoa beans (Manalu, 2018). Middleman or local markets give the price of dry fermented and unfermented cocoa beans a small difference at the farmer level. Many farmers choose to sell dry cocoa beans without fermentation (Bachtiar *et al*., 2019; Pradnyawathi *et al*., 2019). In general, smallholders do not ferment cocoa beans because they only get a few cocoa pods that are harvested (Pradnyawathi *et al*., 2019). Long dry seasons often result in reduced yields of cocoa pods and fewer beans, so there is not much of it to ferment. Farmers can store cocoa pods until it is felt that the number of beans obtained is sufficient for fermentation. The disadvantage of storing cocoa pods: if they are stored for more than 7 days, it causes some cocoa beans to germinate and if stored for more than 21 days, it will cause the cocoa beans to become moldy (Emmanuel *et al*., 2012).

The room temperature of the fermenter or fermentation box affects the change in the fermentation temperature of cocoa beans (Hartuti *et al*., 2018). The small volume of fermented cocoa beans makes the heat generated during fermentation low and makes the fermentation yield low. The larger the volume of fermented cocoa beans (1 ton) can make the fermentation yield low (Hernández-Hernández *et al*., 2016). Fermentation containers affect the quality of fermented cocoa beans (Aryani *et al*., 2018). The most commonly used fermentation method is carrying out the fermentation of cocoa beans using wooden boxes, followed by the pile method without a special container (Figueroa-Hernández *et al*., 2019). Fermentation of cocoa beans is simply done by stacking 50 kg of cocoa beans and then covering them with banana leaves (Tarigan and Iflah, 2017).

*Saccharomyces cerevisiae* has the potential to be developed as a starter for cocoa bean fermentation (De Almeida *et al*., 2019). Tape yeast contains *S. cerevisiae* as the yeast that dominates the population. Farmers are more familiar with tape yeast which is sold freely in the market as a microbe to make fermented food. The use of tape yeast as a stimulant for cocoa bean fermentation makes it easier for farmers to carry out fermentation. The styrofoam box is used as a fermenter to maintain the warmth of the fermenter temperature because the amount of wet fermented cocoa beans is small (4 kg). Before the styrofoam box is closed, various plant leaves are covered over the wet cocoa beans to maintain humidity and temperature during fermentation. This fermentation method requires proof through experimentation in order to be a solution for small farmers in carrying out fermentation to produce quality cocoa beans. This study aimed to determine the response to the addition of tape yeast and cover leaves for small-scale cocoa bean fermentation in styrofoam boxes.

## 2. Materials and methods

### *2.1 Source of sample*

Cocoa pods were sourced from PT Perkebunan Nusantara (PTPN) XII, Ngrangkah Pawon plantation, Kediri Regency, East Java, Indonesia.

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### 2.2 Research methods

The experiment in this study used a two-factor completely randomized design (CRD). The first factor is the addition of tape yeast (R) which consists of 5 levels, namely: without tape yeast (R0), the addition of 0.5% tape yeast (R1), 1.0% (R2), 1.5% (R3), and 2.0% (R4). The second factor is leaf cover (D) which consists of 5 levels, namely: D1 (banana leaves), D2 (teak leaves), D3 (taro leaves), D4 (papaya leaves) and D5 (bamboo leaves). The combination of the two treatment factors obtained 25 treatment combinations. Each treatment combination was repeated 2 times, based on the implementation time, so that 50 experimental units were obtained.

The styrofoam box used is 34 cm × 25 cm × 30 cm (Figure 1). Each side of the styrofoam box is perforated with a diameter of 1 cm, with as many as 8 holes on each side of the box. The top side of the box or box lid is not perforated so there are five perforated sides of the box. The styrofoam box is placed on a wooden block.

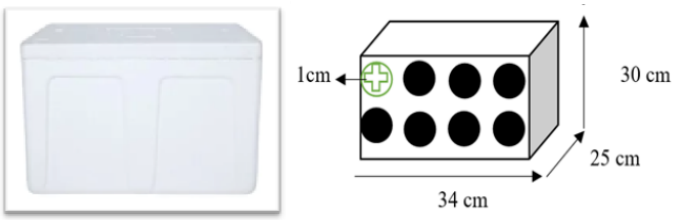


Figure 1. The styrofoam fermentation box.

### 2.3 Cocoa bean fermentation process

The samples of cocoa beans used in this research were 4 kg × 50 boxes of fermentation = 200 kg of wet cocoa beans. Which is fermented for 4 days in a fermentation box. The process of fermenting cocoa beans follows the flow chart in Figure 2.

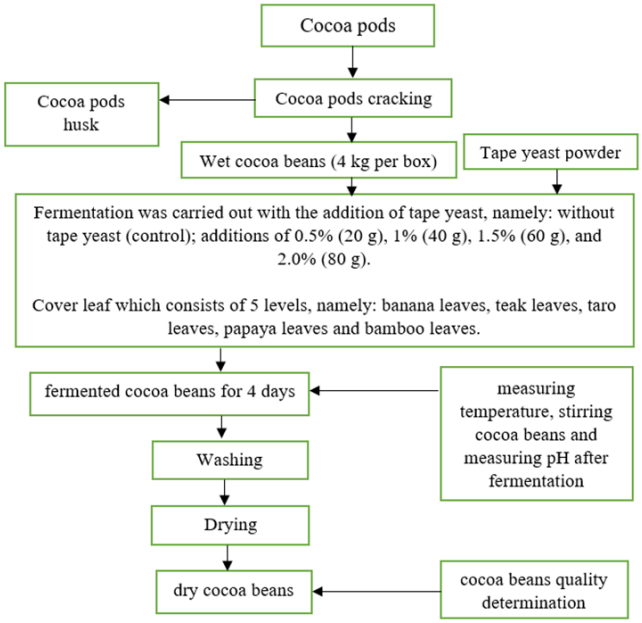


Figure 2. The process of fermenting cocoa beans.

### 2.4 Cocoa beans quality determination

#### 2.4.1 Temperature

The measurement of the temperature of wet cocoa beans is carried out during the fermentation process every 12 hrs. Temperature measurement was done using a thermometer.

#### 2.4.2 pH of cocoa beans pulp

The measurement of the external pH of cocoa beans was carried out before and after fermentation using a pH meter. Measurements were carried out by dissolving 10 g of wet cocoa beans into 10 mL of distilled water and then inserting the pH meter into the cocoa bean solution.

#### 2.4.3 Account of cocoa beans per 100 g

Measurement of the number of beans per 100 g was done by weighing 100 g of dry cocoa beans and then counting the number of beans contained in the 100 g (Bada Standarisasi Nasional, 2008). Grade AA if the maximum number of cocoa beans is 85 beans per 100 g. Grade A if the number of cocoa beans is 86-100 beans per 100 g. Grade B if the number of cocoa beans is 101-110 beans per 100 g. Grade C if the number of cocoa beans is 111-120 beans per 100 g. Grade S if the number of cocoa beans is more than 120 beans per 100 g.

#### 2.4.4 Moisture content

Measurement of water content begins with drying the empty petri dish for 10 mins. Weigh the empty petri dish (M0). Dry cocoa beans were broken and weighed as much as 3 g and then put into a petri dish. Counting the weight of the petri dish and the weight of cocoa beans (M1). The petri dish containing cocoa beans was placed in an oven at a temperature of (103±2°C) for 16 hrs, then put in a desiccator and weighed (M2).

$$\text{Moisture content} = \frac{M1 - M2}{M1 - M0} \times 100\%$$

#### 2.4.5 Sugar content

Measuring the sugar content of dry cocoa beans using a refractometer. Dry cocoa beans were pounded and then dripped with distilled water. Liquid cocoa beans were dropped on a refractometer prism to determine the sugar content on the Brix scale.

#### 2.4.6 Cut test

Dry cocoa beans were split lengthwise in the middle so that they became two halves of the same size. Then, they were observed one by one for the color of the cocoa beans based on their classification. The classification of this test is divided into three classes: slaty, purple and brown. Slaty color is included in the class of unfermented beans. The dominant purple color over

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brown is incorporated into the semi-fermented beans. The dominant brown color is included in the well-fermented beans. Calculate the percentage of seed color using the following formulas:

$$\text{Unfermented cocoa beans} = \frac{\text{number of slaty} - \text{colored cocoa beans}}{\text{total number of cocoa beans}} \times 100\%$$

$$\text{Semi} - \text{fermented coca beans} = \frac{\text{number of purple} - \text{colored cocoa beans}}{\text{total number of cocoa beans}} \times 100\%$$

$$\text{Well} - \text{fermented coca beans} = \frac{\text{number of brown} - \text{colored cocoa beans}}{\text{total number of cocoa beans}} \times 100\%$$

The cut test can be used to calculate the number of cocoa beans damaged by mold, insect-infested and germination.

*2.4.7 Phytochemical qualitative test*

The alkaloid test was carried out by extracting 0.5 g of cocoa bean powder with 3 mL of 95% ethanol and then filtered using Whatman filter paper.

Alkaloids. A total of 2 mL of cocoa bean extract was added to 1 mL of HCl and 1 mL of distilled water, then reacted with 2 drops of Dragendorff's reagent. If the solution changes color to orange, it indicates that the cocoa beans contain alkaloids (Kayaputri *et al.,* 2014).

Polyphenol. A total of 1 mL of cocoa bean extract was reacted with a 5% $FeCl_3$ solution. If the solution changes color to blackish green, it indicates that the cocoa beans contain polyphenols (Adhayanti *et al.,* 2018).

Flavonoid. A total of 1 mL of cocoa bean extract was mixed with 0.1 g of Mg powder and 1 mL of HCl, and then the solution was heated for 10 mins. If the solution changes color to red, it indicates that the cocoa bean contains flavonoids (Ergina and Pursitasari, 2014).

Tannin. A total of 1 mL of cocoa bean extract is mixed with 2 mL of hot water, 1 mL of 10% NaCl, and 1 mL of 2% gelatin solution. If a precipitate forms, it shows that the cocoa beans contain tannin (Ikalinus *et al.,* 2015).

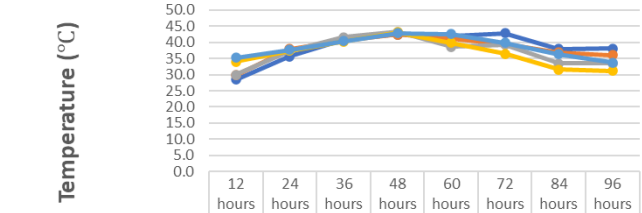
*2.5 Data analysis*

Observation data were analyzed by analysis of variance (ANOVA) through Microsoft Excel if there was a significant effect because the treatment would be continued with the Least Significance Different test (LSD) 5%.

## 3. Results and discussion

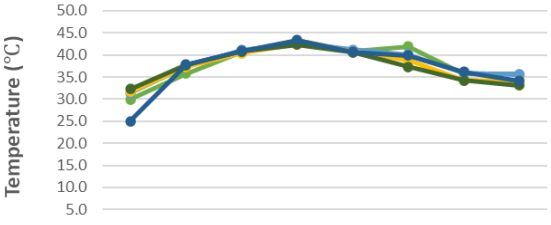
*3.1 Temperature*

Temperature is one of the parameters of the observations made to determine the occurrence of the fermentation process. The addition of tape yeast and leaf cover simultaneously did not affect the fermentation temperature. ANOVA test results indicate that the treatment with the addition of tape yeast affects the fermentation temperature of cocoa beans when observed at 12 hrs, 60 hrs, 72 hrs, 84 hrs, and 96 hrs after fermentation. At 12 hrs of fermentation, the temperature started to increase and at 60 hrs of fermentation, the temperature began to decrease (Figure 3). The dose of tape yeast that was added significantly increased the fermentation temperature at 12 hrs of fermentation. The addition of 2% and 1.5% tape yeast resulted in temperatures of 35.3°C and 34.0°C which were significantly different at 5% LSD (4.45) with the treatment temperature without the addition of tape yeast (28.4°C). Fermentation of cocoa beans without the addition of tape yeast experienced a maximum temperature at 72 hrs of fermentation, while the addition of 0.5%, 1%, 1.5% and 2% tape yeast experienced a maximum temperature at 48 hrs of fermentation (Figure 3). The results of the ANOVA showed that the leaf cover treatment independently had no effect on the fermentation temperature at all observations of fermentation time. At 12 hrs of fermentation, the temperature on the treatment of bamboo leaves as cocoa bean cover produced the lowest temperature (25°C) which was not significantly different from that of banana leaves, teak leaves, taro leaves and papaya leaves on the 5% LSD test. The maximum temperature in all leaf cover treatments occurred at 48 hrs of fermentation and the highest temperature was found in the cover leaves from




| | 12 hours | 24 hours | 36 hours | 48 hours | 60 hours | 72 hours | 84 hours | 96 hours |
|---|---|---|---|---|---|---|---|---|
| R0 (without tape yeast) | 28.4 | 35.6 | 40.9 | 42.4 | 42.0 | 42.9 | 38.0 | 38.1 |
| R1 (0.5% tape yeast) | 29.8 | 37.9 | 40.6 | 42.5 | 41.1 | 39.5 | 36.9 | 36.1 |
| R2 (1% tape yeast) | 29.9 | 37.4 | 41.6 | 43.2 | 38.6 | 39.3 | 33.5 | 33.5 |
| R3 (1.5% tape yeast) | 34.0 | 37.4 | 40.3 | 43.1 | 39.8 | 36.5 | 31.6 | 31.2 |
| R4 (2% tape yeast) | 35.3 | 37.7 | 40.4 | 42.9 | 42.6 | 39.9 | 36.3 | 33.7 |

Figure 3. Fermentation temperature of cocoa beans due to the effect of adding tape yeast.




| | 12 hours | 24 hours | 36 hours | 48 hours | 60 hours | 72 hours | 84 hours | 96 hours |
|---|---|---|---|---|---|---|---|---|
| D1 (Banana leaves) | 29.9 | 35.8 | 40.6 | 43.3 | 40.8 | 41.9 | 35.8 | 35.7 |
| D2 (Teak leaves) | 31.4 | 37.4 | 41.1 | 42.7 | 41.2 | 40.1 | 35.9 | 35.6 |
| D3 (Taro leaves) | 31.7 | 37.3 | 40.4 | 42.4 | 40.8 | 38.9 | 34.2 | 34.1 |
| D4 (Papaya leaves) | 32.3 | 37.7 | 40.8 | 42.3 | 40.6 | 37.3 | 34.2 | 33.1 |
| D5 (Bamboo leaves) | 25.0 | 37.8 | 40.9 | 43.4 | 40.7 | 39.9 | 36.2 | 34.1 |

Figure 4. Fermentation temperature due to the effect of plant leaves as a cover for cocoa beans.

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bamboo and banana leaves (43.4℃ and 43.3℃) (Figure 4). The pattern of changes in the temperature of cocoa bean fermentation from 12 hrs to 96 hrs increases until it reaches the peak then the temperature decreases, after decreasing it experiences ups and downs, but the increase does not exceed the maximum temperature (Figures 3 and 4).

Yeast is a microorganism that dominates the fermentation process on the first and second days of fermentation. Yeast decomposes the cocoa bean pulp, increases evaporation, and reduces the viscosity of the pulp, thereby allowing air to enter the cocoa beans (De Vuyst and Weckx, 2016). There is an increase in the average temperature starting on the first day of fermentation *(Pereira et al*., 2020). The highest yeast abundance was observed in the first 24 hrs, yeast dominated the fermentation and produced ethanol (Pereira *et al*., 2020; Lima *et al*., 2021). Yeast produces ethanol at a temperature of 25-35℃ (Hernández-Hernández *et al*., 2016). The addition of tape yeast increases the amount of ethanol (Cempaka *et al*., 2014; Kurniawan *et al*., 2014; Hatmi *et al*., 2015). The highest ethanol concentration was detected at 48 hrs of fermentation (Lima *et al*., 2021). Ethanol triggers an increase in fermentation temperature (De Vuyst and Weckx, 2016). On the 2$^{nd}$ day of fermentation, the temperature can reach 45℃ (Santos *et al*., 2020). The addition of yeast triggers the achievement of high temperatures faster at the beginning of fermentation (Chagas Junior *et al*., 2021). Natural fermentation without the addition of microbes, the highest temperature on the 3$^{rd}$ to 5$^{th}$ day then the temperature decreases, with the maximum temperature reaching 40-50℃ (Hernández-Hernández *et al*., 2016; Deus *et al*., 2021; Bobiles *et al*., 2022). The optimum temperature for cocoa bean fermentation is ~40°C (Hernani *et al*., 2019). There was no significant difference between the yeast population in natural fermentation and the addition of yeast during fermentation, but the addition of yeast increased the yeast population at the beginning of the fermentation (Peralta *et al*., 2021). Fermented cocoa beans with tape yeast reached the maximum temperature (42.5-43.2℃) on the 2$^{nd}$ day and those without tape yeast reached the maximum temperature (42.9℃) on the 3$^{rd}$ day. Therefore, the addition of tape yeast is recommended to accelerate the achievement of the maximum temperature in the cocoa bean fermentation process.

Temperature can affect the fermentation time to be faster (Sari *et al*., 2021). The room temperature of the fermenter or fermentation box affects the fermentation temperature of the cocoa beans (Hartuti *et al*., 2018). The styrofoam box increases the fermentation temperature of the cocoa beans and shortens the time required for fermentation (Rahmi *et al*., 2017). The temperature in the styrofoam box is maintained so that the fermentation temperature can be as high as 42.3-43.4℃ (Figures 3 and 4), even though the number of wet fermented cocoa beans is only 4 kg. Temperature affects the growth of *S. cerevisiae* compared to pH, ethanol and free oxygen factor. *Saccharomyces cerevisiae* grows optimally at a temperature of 30°C to 35°C and a maximum temperature of 45°C, at a temperature of 42°C its growth stops (Kouamé *et al*., 2021). At a temperature of >40°C the yeast suspected to be *S. cerevisiae* was quite high and still persisted until the cocoa bean fermentation was complete (Purwanto *et al*., 2019). Yeast inoculation and increasing temperature affect yeast growth during the fermentation process. The presence of pH, ethanol and nitrogen had no significant effect on yeast growth. The addition of high enough yeast coupled with a low temperature increase will make glucose run out quickly. On the other hand, if the addition of a small amount of yeast is accompanied by a high enough temperature increase, the yeast growth will stop before the glucose runs out (Kouamé *et al*., 2021).

Basically, all treatments of plant leaves as a cover for fermented cocoa beans are able to maintain the warmth of the fermentation temperature of 4 kg of wet cocoa beans. Treatment of banana leaves and bamboo leaves was able to produce the highest temperature of 43℃ which was not significantly different from teak leaves, taro leaves and papaya leaves (Figure 4). Natural fermentation of wet cocoa beans ≤ 5 kg can reach the maximum temperature of ≥ 40℃, because the heat loss of fermentation is prevented by cheesecloth wrapping the cocoa beans (Bobiles *et al*., 2022). All types of leaf-covered fermented cocoa beans were able to reach the maximum temperature (42.3-43.4℃) on the 2$^{nd}$ day. So that in addition to using banana leaves, fermented cocoa beans can be covered using teak leaves, taro leaves, papaya leaves and bamboo leaves.

The aroma and taste precursors of chocolate are formed as the fermentation temperature increases. The enzymatic browning reaction and the formation of the characteristic brown color of cocoa beans are driven by polyphenol oxidase (PPO) activity. The temperature of 42°C is ideal for supporting PPO activity in influencing the color, bitterness and astringency of chocolate (Chagas Junior *et al*., 2021). Increased temperature supports proteolytic activity. The cocoa flavor is formed at high temperatures ranging from 34.6-46.6℃ with a high hydrophobic amino acid content (Deus *et al*., 2021). Amino acid biosynthesis can be affected by the presence of *S. cerevisiae* (Almeida *et al*., 2020). The maximum temperature of cocoa bean fermentation achieved on day 2 was 42.5-43.4°C (Figures 3 and 4) and the application

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of tape yeast containing *S. cerevisiae* was thought to be able to make cocoa beans ferment perfectly.

### *3.2 pH of cocoa beans pulp*

The pH of the cocoa bean pulp before fermentation was 4.3 and after fermentation for 4 days, there was an increase in pH to 5.75-6.14 (Figure 5). The combination of additional treatment of tape yeast and cover leaf fermented cocoa beans did not significantly affect the pH increase of cocoa beans pulp. Separately, the addition of tape yeast and leaf cover of cocoa bean fermentation significantly affect the increase in pH of cocoa bean pulp. With a significant difference test at 5% LSD (0.23) fermentation that was not given tape yeast produced the lowest pH (5.75) and the pH of cocoa beans due to treatment with 0.5-2% tape yeast dose was not significantly different (5.93-6.14). The leaf cover treatment using taro leaves and papaya leaves produced the lowest pH (5.77 and 5.82) and was significantly different from the treatment of bamboo leaves, teak leaves and banana leaves (6.05, 6.05 and 6.20).

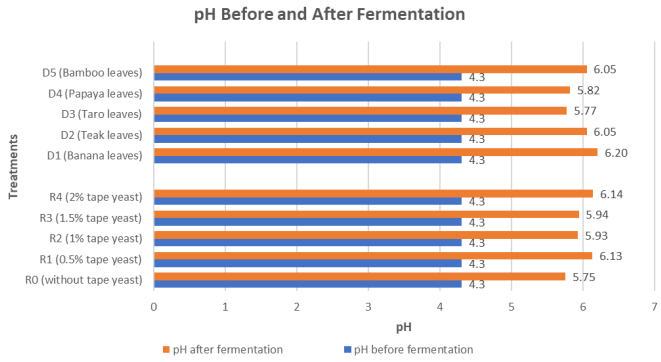


Figure 5. pH of cocoa beans pulp after fermentation due to the application of tape yeast and the leaf cover.

There was an increase in the pH of the cocoa bean pulp every day during fermentation. The addition of tape yeast in the laboratory-scale fermentation of cocoa beans has an effect on the increase in pH (Purwanto *et al*., 2019). The growth of *S. cerevisiae* can be affected by pH, its growth decreases at pH 6.5 and its growth stops at pH 8 (Kouamé *et al*., 2021). The presence of *S. cerevisiae* continues to degrade the cocoa bean pulp and the formation of organic acids required by further bacteria. Decomposition of organic acids during fermentation which makes the pH increase. The pH of the pulp is inversely proportional to the pH of the cocoa bean. The pH of cocoa beans decreased during fermentation and the addition of lactic acid bacteria (LAB) significantly reduced the acidity of the cocoa beans (Apriyanto, 2017; Miguel *et al*., 2017).

According to the research by Hernani *et al*. (2019), the pH of cocoa bean pulp that reaches >5 during fermentation can improve the quality of cocoa beans. In all treatments, the pH of the cocoa bean pulp was >5 (5.75-6.14) after 4 days of fermentation in a styrofoam box. Proving that fermented cocoa beans in small quantities of 4 kg covered with leaves in a styrofoam box are able to produce an optimal pH of cocoa bean pulp.

### *3.3 Account of cocoa beans per 100 g*

The number of dry cocoa beans after fermentation from all treatments was 92.7-103.7 beans per 100 g (Figure 6). The addition of tape yeast had a significant effect on the number of 100 cocoa beans, while the fermentation cover had no significant effect on the number of 100 cocoa beans. The addition of 2% tape yeast resulted in the highest number of cocoa beans and was significantly different from the 5% LSD test level (5.33). In 100 g of dry cocoa beans as a result of treatment with 2% tape yeast, there are 103.7 cocoa beans. The application of tape yeast 0-1.5% produced dry cocoa beans as much as 93.3-96.8 cocoa beans per 100 g. The leaf cover treatments resulted in 92.7-99 dry cocoa beans per 100 g. According to Badan Standarisasi Nasional (2008) in the Indonesian National Standard (SNI 2323:2008), the quality of fermented cocoa beans is classified into grades AA, A, B, C and S. The fermentation of cocoa beans from research results can be classified into quality levels A and B. The number of cocoa beans per 100 g is significantly affected by the fermentation time (Aryani *et al*., 2018). Fermented cocoa beans produce dry cocoa beans of groups AA, A and B (Aryani *et al*., 2018; Hartuti *et al*., 2018). Dry fermented cocoa beans have the opportunity to get a high selling value. The quality of cocoa beans determines the selling value and different places of sale. Grades A and B can be sold to chocolate processing companies, while grades C and S are sold to middlemen and local markets (Bachtiar *et al*., 2019).

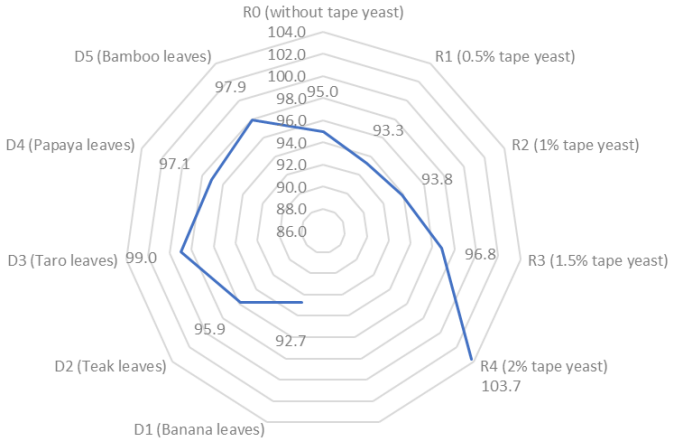


Figure 6. Account of cocoa beans per 100 g due to application of tape yeast and the leaf cover.

### *3.4 Cocoa beans moisture content*

The moisture content of fermented cocoa beans after sun drying in all treatments was 8-5.5% (Figure 7). The

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moisture content of the cocoa beans has not met the requirements of the Indonesian national standard which requires the maximum moisture content of dry cocoa beans to be 7.5% (Badan Standarisasi Nasional, 2008). Several researchers (Hayati *et al*., 2012; Baihaqi *et al*., 2016); Nizori *et al*., 2021) reported that the drying technique affects the moisture content of cocoa beans. Drying in the sun tends to produce high moisture content. A temperature of 50-70℃ is the optimal temperature for drying cocoa beans in an oven. Drying cocoa beans in the sun has various obstacles such as rainy and cloudy days that can hinder the drying process. To achieve the moisture content of cocoa beans according to quality standards, it is recommended that after drying in the sun, they are dried in an oven to uniform the moisture content of the cocoa beans.

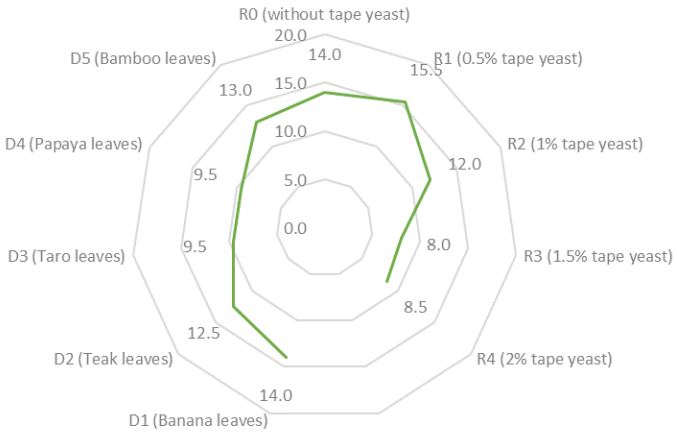


Figure 7. Cocoa beans moisture content due to application of tape yeast and the leaf cover.

The addition, of the presence of *S. cerevisiae* at the beginning of the fermentation resulted in a lower moisture content of dry cocoa beans (Baihaqi *et al*., 2016). The results of this study indicate that increasing the dose of tape yeast tends to produce a lower moisture content of dry cocoa beans. Application of 1.5% and 2% tape yeast resulted in lower moisture content of cocoa beans than 0.5% and 1% tape yeast.

### *3.5 Sugar content*

The total dissolved sugar solids in dry cocoa beans, the results of the study, ranged from 1.8 to 2.8°Bx (Figure 8.). The addition of tape yeast can produce large and small amounts of sugar in cocoa beans. Leaf cover fermented cocoa beans from banana leaves, teak leaves, taro leaves, papaya leaves and bamboo leaves produce soluble sugar in the range of 2.2-2.6°Bx. Cocoa fruit that is ripe when the fruit is opened has a sugar content of ~13.3-20°Bx (Cubillos Bojacá *et al*., 2019; Dulce *et al*., 2021). The sugar content of cocoa beans decreases with the process of fermentation, drying and storage of cocoa pods (Afoakwa *et al*., 2013; Afoakwa *et al*., 2015). The presence of sucrose in dry cocoa beans can be used as an indicator if the fermentation is not perfect. Dried cocoa beans are only glucose and fructose, which is an indication that the fermentation process was successful.

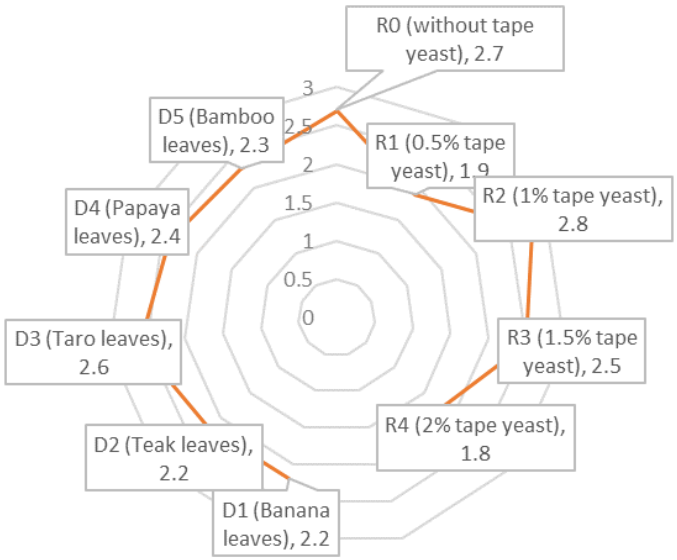


Figure 8. Cocoa beans sugar content due to application of tape yeast and the leaf cover.

### *3.6 Cut test*

Changes in the color of cocoa beans inside and outside the beans can be used as an indication of the success of fermentation. Color changes in cocoa beans can be tested by cut test. The results of the dry cocoa bean cutting test after 4 days of fermentation showed that the number of cocoa beans that were brown was ≥88% of the cocoa beans were brown (Figure 9). Brown cocoa beans indicate that the cocoa beans are well fermented. The addition of 1% tape yeast significantly increased the number of well-fermented cocoa beans by 11.29% from the control (cocoa beans without tape yeast). Cocoa beans that were not added with tape yeast produced 11.4% purple cocoa beans. Purple cocoa beans indicate that the fermentation process is not yet complete (semi-fermentation). Banana leaves, teak leaves, taro leaves, papaya leaves and bamboo leaves have the potential to be used as a cover for fermenting cocoa beans to improve their quality. From all fermentation cover leaf treatments, it was found that slaty colored cocoa beans or unfermented cocoa beans were only around 0-2.8%. Wet cocoa beans weighing <5 kg can be fermented using a Styrofoam box. Before the Styrofoam box is closed, it is best to cover the pile of cocoa beans with leaves (banana, teak, taro, papaya and bamboo).

Tape yeast contains yeast and bacteria (Ninsix, 2013). The addition of tape yeast accelerated the growth of yeast, LAB and BAA at the beginning of the fermentation so as to accelerate the fermentation of cocoa beans. Yeast produces ethanol and pectinolytic enzymes which contribute to the physicochemical

https://doi.org/10.26656/fr.2017.8(S2).50 

changes of cocoa beans during fermentation (Pereira *et al*., 2020; Santos *et al*., 2020; Dulce *et al*., 2021). In the cut test, cocoa beans were classified according to the color change of the cotyledons into slaty, purple and brown colors as the degree of success of fermentation. Color as a measure of the anthocyanin oxidation process in cocoa beans. Slaty color as an indicator of unfermented and purple as an indicator of semi-fermented cocoa beans, while the brown color indicates well-fermented cocoa beans (Hernández-Hernández *et al*., 2016). The entry of acid into the cotyledons during fermentation triggers the cells containing anthocyanins (purple pigments) to rupture, causing the cotyledons to turn purple. The polyphenol oxidase enzyme causes the oxidase of anthocyanins so that the cotyledons turn brown (Rahardjo *et al*., 2022).

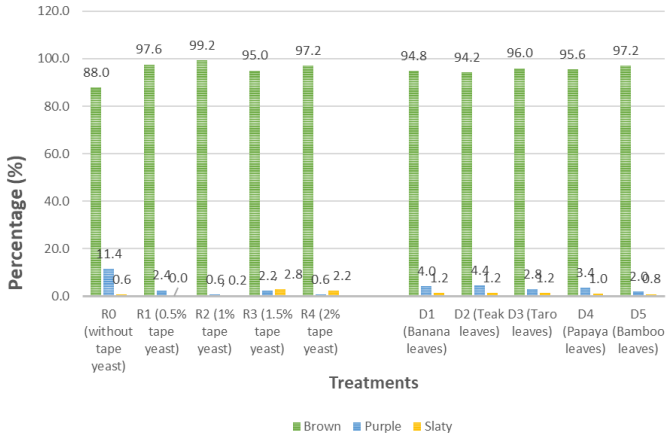


Figure 9. Percentage of cotyledon color from cut test results.

The amount of fermented cocoa beans increases with the duration of fermentation. In the first 12 hrs of fermentation, there were 20% fermented cocoa beans and at 72 hrs of fermentation, there were 70% fermented cocoa beans (Apriyanto *et al*., 2020). Improving the quality of cocoa beans can be done by adding *S. cerevisiae* and stirring or giving aeration during fermentation (Hanny and Kresnowati, 2015; Sabahannur, 2017). The addition of 0.2% tape yeast increased the fermentation index of cocoa beans on a laboratory scale and accelerated the fermentation process with an indication that on the 3rd day the fermentation index had reached >1 (Purwanto *et al*., 2019).

The results of this study did not find moldy and germinated cocoa beans. A fermentation temperature of >40°C suppresses fungal growth on cocoa beans (Hatmi *et al*., 2015). The fermentation process that is not stirred can cause the cocoa beans in the top pile to become moldy (Hartuti *et al*., 2018). Germination can be caused by the storage of cocoa pods for too long (~10 days) before the cocoa beans are fermented (Kongor *et al*., 2013). All treatments in this study could reach a maximum temperature of 42.3-43.4°C so that no seeds were damaged due to fungal attacks or seeds germinated.

### *3.7 Phytochemical qualitative*

In the phytochemical test of dry cocoa beans after fermentation, there were still alkaloids, polyphenols, flavonoids and tannins (Table 1). All fermentation treatments in this study did not eliminate the content of alkaloids, polyphenols, flavonoids and tannins, although they are present in small amounts. These compounds are included in secondary metabolites of cocoa beans that humans need. Wet cocoa beans contain high levels (15%) of polyphenols (Kadow *et al*., 2013). Post-harvest activities such as fermentation, drying, and roasting can reduce the polyphenol content of cocoa beans (Atmaja *et al*., 2016; Apriyanto *et al*., 2020; Lima *et al*., 2021). During fermentation, polyphenols are oxidized, so that the astringent and bitter taste of cocoa beans is reduced and the taste of chocolate is increased (Kongor *et al*., 2016). *Saccharomyces cerevisiae* was able to reduce phenolic compounds by 26 mg ECE/g (Chagas Junior *et al*., 2021).

Table 1. Phytochemical qualitative of fermented cocoa beans due to application of tape yeast and the leaf cover.

| Treatments | Phytochemical Qualitative | | | |
|---|---|---|---|---|
| | Alkaloid | Polyphenol | Flavonoid | Tannin |
| R0 (without tape yeast) | + | + | + | + |
| R1 (0.5% tape yeast) | + | + | + | + |
| R2 (1% tape yeast) | + | + | + | + |
| R3 (1.5% tape yeast) | + | + | + | + |
| R4 (2% tape yeast) | + | + | + | + |
| D1 (Banana leaves) | + | + | + | + |
| D2 (Teak leaves) | + | + | + | + |
| D3 (Taro leaves) | + | + | + | + |
| D4 (Papaya leaves) | + | + | + | + |
| D5 (Bamboo leaves) | + | + | + | + |

Cocoa beans as raw material for processed foods such as chocolate should be fermented to reduce polyphenols. Polyphenols in cocoa beans are included in antioxidant compounds. Cocoa beans for cosmetic and medicinal raw materials require large amounts of polyphenols. Efforts that can be made to increase and maintain the polyphenol content can be made by blanching technique before fermentation and the addition of cysteine during fermentation (Arizona *et al*., 2013; Nurhayati *et al*., 2018; Nurhayati *et al*., 2022). Apart from post-harvest handling techniques, the content of secondary metabolites of cocoa beans such as flavonoids is influenced by environmental and climatic conditions where the plant grows (Agudelo *et al*., 2022).

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## 4. Conclusion

The addition of tape yeast which is sold freely in the market can increase the number of perfectly fermented cocoa beans. Tape yeast increases the fermentation temperature of cocoa beans, the account of cocoa beans per 100 g, the sugar content, and the number of brown cocoa beans. The best dosage of tape yeast for fermentation is 1% of the wet weight of the wet cocoa beans. During fermentation, teak leaves, taro leaves, papaya leaves, and bamboo leaves have the same role as banana leaves in maintaining the temperature and humidity of fermenting cocoa beans, so these leaves can be used as a cover for fermenting cocoa beans like banana leaves, which are usually used by farmers.

## Conflict of interest

The authors declare no conflict of interest.

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## References

Adhayanti, I., Abdullah, T. and Romantika, R. (2018). Uji kandungan total polifenol dan flavonoid ekstrak etil asetat kulit pisang Raja (*Musa paradisiac*a var. *sapientum*). *Media Farmasi*, 17(1), 146-152. https://journal.poltekkes-mks.ac.id/ojs2/index.php/mediafarmasi/article/view/84/47 [In Bahasa Indonesia]

Afoakwa, E.O., Kongor, J.E., Takrama, J. and Budu, A.S. (2013). Changes in nib acidification and biochemical composition during fermentation of pulp pre-conditioned cocoa (*Theobroma cacao*) beans. *International Food Research Journal*, 20(4), 1843-1853.

Afoakwa, E., Ofosu-Ansah, E., Budu, A., Mensah-Brown, H. and Takrama, J. (2015). Changes in some biochemical qualities during drying of pulp pre-conditioned and fermented cocoa (*Theobroma cacao*) beans. *African Journal of Food, Agriculture, Nutrition and Development*, 15(68), 9651-9670. https://doi.org/10.18697/ajfand.68.13695

Agudelo, C., Acevedo, S., Carrillo-Hormaza, L., Galeano, E. and Osorio, E. (2022). Chemometric classification of Colombian cacao crops: Effects of different genotypes and origins in different years of harvest on levels of flavonoid and methylxanthine metabolites in raw cacao beans. *Molecules*, 27(7), 2068. https://doi.org/10.3390/molecules27072068

Almeida, O.G.G., Pinto, U.M., Matos, C.B., Frazilio, D.A., Braga, V.F., von Zeska-Kress, M.R. and De Martinis, E.C.P. (2020). Does quorum sensing play a role in microbial shifts along spontaneous fermentation of cocoa beans? An in silico perspective. *Food Research International*, 131, 109034. https://doi.org/10.1016/j.foodres.2020.109034

Apriyanto, M. (2017). Perubahan pH, keasaman dan indeks fermentasi biji kakao selama fermentasi hasil biji kakao (*Theobroma cacao*). *Jurnal Teknologi Pertanian*, 6(1), 12-18. [In Bahasa Indonesia]

Apriyanto, M., Mardesci, H. and Rujiah, R. (2020). Change of acetic acid, total polifenol and color of cocoa origin during fermentation. *Jurnal Industri Hasil Perkebunan*, 15(1), 10-16. https://doi.org/10.33104/jihp.v15i1.5573

Arizona, D., Maksum, I.P. and Soedjanaatmadja, R.U.M.S. (2013). Nutritional analysis in fermentation modification cocoa beans with the additional of exogenous *Saccharomyces cerevisiae*, *Lactobacillus plantarum*, *Acetobacter aceti*, bromelain enzyme and cysteine. *Indonesian Journal of Agricultural Science*, 3(1), 42-45. https://jurnal.unpad.ac.id/ijas/article/view/16829/8129

Aryani, N.L.P.N.A., Yulianti, N.L. and Arda, G. (2018). Characteristics of cocoa beans on small capacity fermentation results based on different types of containers and different fermentation lengths. *Jurnal Beta (Biosistem Dan Teknik Pertanian)*, 6(1), 17-24. https://ojs.unud.ac.id/index.php/beta/article/view/35777/22158

Atmaja, M.I.P., Haryadi and Supriyanto. (2016). Quality improvement of non fermented cocoa bean through pre-incubation treatments. *Jurnal Tanaman Industri Dan Penyegar*, 3(1), 11-20. https://doi.org/10.21082/jtidp.v3n1.2016.p11-20

Bachtiar, W.F., Aji, G.K. and Norsita, D.I. (2019). Value chain performance of cocoa beans in Banjaroya, Kulon Progo. *Gontor AGROTECH Science Journal*, 5(1), 2811. https://doi.org/10.21111/agrotech.v5i1.2811

Badan Standarisasi Nasional (BSN). (2008). Biji Kakao (SNI 2323:2008). Retreived from BSN website: http://sispk.bsn.go.id/SNI/DetailSNI/7490 [In Bahasa Indonesia]

Baihaqi, Hayati, R. and Abubakar, Y. (2016). Effects of various fermentation facilitators and drying temperature on the quality of cocoa beans. *Jurnal Floratek*, 11(2), 134-142.

Beato, F.B., Bergdahl, B., Rosa, C.A., Forster, J. and Gombert, A.K. (2016). Physiology of

 

*Saccharomyces cerevisiae* strains isolated from Brazilian biomes: New insights into biodiversity and industrial applications. *FEMS Yeast Research*, 16(7), fow076. https://doi.org/10.1093/femsyr/fow076

Bobiles, S.C., Elegado, F.B., Millena, C.G. and Merca, F.E. (2022). Small-scale cacao (*Theobroma cacao* L.) fermentation process utilizing cacao pod husk. *Food Research*, 6(4), 236-245. https://doi.org/https://doi.org/10.26656/fr.2017.6(4).502

Cempaka, L., Aliwarga, L., Purwo, S. and Penia Kresnowati, M.T.A. (2014). Dynamics of cocoa bean pulp degradation during cocoa bean fermentation: Effects of yeast starter culture addition. *Journal of Mathematical and Fundamental Sciences*, 46(1), 14-25. https://doi.org/10.5614/j.math.fund.sci.2014.46.1.2

Chagas Junior, G.C.A., Ferreira, N.R., Gloria, M.B.A., Martins, L.H. da S. and Lopes, A.S. (2021). Chemical implications and time reduction of on-farm cocoa fermentation by *Saccharomyces cerevisiae* and *Pichia kudriavzevii*. *Food Chemistry*, 338, 127834. https://doi.org/10.1016/j.foodchem.2020.127834

Cubillos Bojacá, A.F., García Muñoz, M.C., Calvo Salamanca, A.M., Carvajal Rojas, G.H. and Tarazona-Díaz, M.P. (2019). Study of the physical and chemical changes during the maturation of three cocoa clones, EET8, CCN51, and ICS60. *Journal of the Science of Food and Agriculture*, 99(13), 5910-5917. https://doi.org/10.1002/jsfa.9882

De Almeida, S.D.F.O., Silva, L.R.C., Junior, G.C.A.C., Oliveira, G., da Silva, S.H.M., Vasconcelos, S. and Lopes, A.S. (2019). Diversity of yeasts during fermentation of cocoa from two sites in the Brazilian Amazon. *Acta Amazonica*, 49(1), 64-70. https://doi.org/http://dx.doi.org/10.1590/1809-4392201703712

De Vuyst, L. and Weckx, S. (2016). The cocoa bean fermentation process: From ecosystem analysis to starter culture development. *Journal of Applied Microbiology*, 121(1), 5-17. https://doi.org/10.1111/jam.13045

Deus, V.L., Bispo, E.S., Franca, A.S. and Gloria, M.B.A. (2021). Understanding amino acids and bioactive amines changes during on-farm cocoa fermentation. *Journal of Food Composition and Analysis*, 97, 103776. https://doi.org/10.1016/j.jfca.2020.103776

Dulce, V.R., Anne, G., Manuel, K., Carlos, A.A., Jacobo, R.C., Sergio de Jesús, C.E. and Eugenia, L.C. (2021). Cocoa bean turning as a method for redirecting the aroma compound profile in artisanal cocoa fermentation. *Heliyon*, 7, e07694. https://doi.org/10.1016/j.heliyon.2021.e07694

Emmanuel, O.A., Jennifer, Q., Agnes, S.B., Jemmy, S.T. and Firibu, K.S. (2012). Influence of pulp-preconditioning and fermentation on fermentative quality and appearance of Ghanaian cocoa (*Theobroma cacao*) beans. *International Food Research Journal*, 19(1), 127-133.

Ergina, S.N. and Pursitasari, I.D. (2014). Qualitative test of secondary metabolites compounds in Palado leaves (*Agave angustifolia*) extracted with water and ethanol. *Jurnal Akademi Kimia*, 3(3), 165-172.

Figueroa-Hernández, C., Mota-Gutierrez, J., Ferrocino, I., Hernández-Estrada, Z.J., González-Ríos, O., Cocolin, L. and Suárez-Quiroz, M.L. (2019). The challenges and perspectives of the selection of starter cultures for fermented cocoa beans. *International Journal of Food Microbiology*, 301, 41-50. https://doi.org/10.1016/j.ijfoodmicro.2019.05.002

Hanny, M.P.F. and Kresnowati. (2015). Mapping the effects of starter culture addition on cocoa bean fermentation. *ASEAN Engineering Journal*, 5(1), 25-37. https://journals.utm.my/aej/article/view/15465/6974

Hartuti, S., Bintoro, N., Karyadi, W.N.J. and Pranoto, Y. (2018). Isothermal fermentation of cocoa beans (*Theobroma cacao*. L) with controlled aeration system. *Agritech*, 38(4), 364-374. http://doi.org/10.22146/agritech.32162

Hatmi, R.U., Kobarsih, M. and Cahyaningrum, N. (2015). Fungi level analysis of cocoa beans based on fermentation box type and duration. *Procedia Food Science*, 3, 371-382. https://doi.org/10.1016/j.profoo.2015.01.041

Hayati, R., Yusmanizar, Y., Mustafril, M. and Fauzi, H. (2012). Study of fermentation and drying temperature in cacao quality (*Theobroma cacao* L.). *Jurnal Keteknikan Pertanian*, 26(2), 129-135.

Hernández-Hernández, C., López-Andrade, P. A., Ramírez-Guillermo, M.A., Guerra Ramírez, D. and Caballero Pérez, J.F. (2016). Evaluation of different fermentation processes for use by small cocoa growers in Mexico. *Food Science and Nutrition*, 4 (5), 690-695. https://doi.org/10.1002/fsn3.333

Hernani, Hidayat, T. and Mulyawanti, I. (2019). The usage of dried starter for re-fermentation of unfermented cocoa beans. *IOP Conference Series: Earth and Environmental Science*, 309, 012061. https://doi.org/10.1088/1755-1315/309/1/012061

Ho, V.T.T., Zhao, J. and Fleet, G. (2014). Yeasts are essential for cocoa bean fermentation. *International Journal of Food Microbiology*, 174, 72-87. https://doi.org/10.1016/j.ijfoodmicro.2013.12.014

Ikalinus, R., Widyastuti, S. and Eka Setiasih, N. (2015).

 

Phytochemical screening ethanol extract skin stem moringa (*Moringa oleifera*). *Indonesia Medicus Veterinus*, 4(1), 71-79.

Kadow, D., Bohlmann, J., Phillips, W. and Lieberei, R. (2013). Identification of main fine or flavour components in two genotypes of the cocoa tree (*Theobroma cacao* L.). *Journal of Applied Botany and Food Quality*, 86(1), 90-98. https://doi.org/10.5073/JABFQ.2013.086.013

Kayaputri, I.L., Sumanti, D.M., Djali, M., Indiarto, R. and Dewi, D.L. (2014). Phytochemical study of cocoa bean shell extract (*Theobroma cacao* L.). *Chimica et Natura Acta*, 2(1), 83-90. https://doi.org/10.24198/cna.v2.n1.9140

Kongor, J.E., Felix Takrama, J., Simpson Budu, A., Mensah-Brown, H. and Afoakwa, E.O. (2013). Effects of fermentation and drying on the fermentation index and cut test of pulp pre-conditioned Ghanaian cocoa (*Theobroma cacao*) beans. *Journal of Food Science and Engineering*, 3, 625-634. https://ir.mug.edu.gh/xmLui/handle/123456789/161

Kongor, J.E., Hinneh, M., de Walle, D. Van, Afoakwa, E.O., Boeckx, P. and Dewettinck, K. (2016). Factors influencing quality variation in cocoa (*Theobroma cacao*) bean flavour profile-A review. *Food Research International*, 82, 44-52. https://doi.org/10.1016/j.foodres.2016.01.012

Kouamé, C., Loiseau, G., Grabulos, J., Boulanger, R. and Mestres, C. (2021). Development of a model for the alcoholic fermentation of cocoa beans by a *Saccharomyces cerevisiae* strain. *International Journal of Food Microbiology*, 337, 108917. https://doi.org/10.1016/j.ijfoodmicro.2020.108917

Kristanto, W.H., Tamrin and Erna, M. (2017). The influence of yeast (*Saccharomyces cerevisiae*) addition and the number of box's holes in cocoa (*Theobroma cacao* L) fermentation on the quality of dried cocoa beans. *Jurnal Teknik Pertanian Lampung*, 6(1), 1-10. [In Bahasa Indonesia].

Kurniawan, T.B., Bintari, S.H. and Susanti, R. (2014). Interaction effects tape and bread yeast on the level of bioethanol cassava (*Manihot utilissima*, Pohl) Mukibat varieties. *Biosaintifika*, 6(2), 152-160. https://doi.org/10.15294/biosaintifika.v6i2.3783

Lima, C.O. de C., Vaz, A.B.M., Castro, G.M. De, Lobo, F., Solar, R., Rodrigues, C., Pinto, L.R.M., Vandenberghe, L., Pereira, G., Costa, A.M. da, Benevides, R.G., Azevedo, V., Uetanabaro, A.P.T., Soccol, C.R. and G´oes-Neto, A. (2021). Integrating microbial metagenomics and physicochemical parameters and a new perspective on starter culture for fine cocoa fermentation. *Food Microbiology*, 93, 103608. https://doi.org/10.1016/j.fm.2020.103608

Manalu, R. (2018). Pengolahan Biji Kakao Produksi Perkebunan Rakyat untuk Meningkatkan Pendapatan Petani. *Jurnal Ekonomi Dan Kebijakan Publik*, 9(2), 99–111. https://doi.org/10.22212/jekp.v9i2.1006 [In Bahasa Indonesia].

Miguel, M.G. da C.P., Reis, L.V. de C., Efraim, P., Santos, C., Lima, N. and Schwan, R.F. (2017). Cocoa fermentation: Microbial identification by MALDI-TOF MS, and sensory evaluation of produced chocolate. *LWT-Food Science and Technology*, 77, 362-369. https://doi.org/10.1016/j.lwt.2016.11.076

Muhiddin, N.H., RamLawati, R., Yanti, N.A. and Mun'im, A. (2019). The quantitative analysis of microorganisms in local ragi tape and palatability of produced "Tape Jusinta." *BioWallacea : Jurnal Penelitian Biologi*, 6(2), 1007-1016. https://doi.org/10.33772/biowallacea.v6i2.8950

Ninsix, R. (2013). Pengaruh konsentrasi ragi merk NKL terhadap mutu tape yang dihasilkan. *Jurnal Teknologi Pertanian*, 2(2), 1-11. [In Bahasa Indonesia]

Nizori, A., Tanjung, O.Y., Ulyarti, U., Arzita, A., Lavlinesia, L. and Ichwan, B. (2021). The effect on time of cacao fermentation in the physico-chemical and organoleptic (*Theobroma cacao* L.) properties of cocoa powder. *Jurnal Pangan Dan Agroindustri*, 9 (2), 129-138. https://doi.org/10.21776/ub.jpa.2021.009.02.7

Nurhayati, D., Mulia, Y.S. and Azhiima, A.F. (2022). The effect of cysteine inhibitors on cocoa seed fermentation on the content of polyphenol. *Jurnal Riset Kesehatan*, 14(1), 191-196. https://doi.org/https://doi.org/10.34011/juriskesbdg.v14i1.2021 191

Nurhayati, N., Marseno, D.W., Setyabudi, F.S. and Supriyanto, S. (2018). Steam blanching effect on polyphenol oxidase activity, total polyphenol content, and antioxidant activity of cocoa bean. *Jurnal Aplikasi Teknologi Pangan*, 7(3), 95-103. https://doi.org/10.17728/jatp.2314

Oktaviana, A.Y., Suherman, D. and Sulistyowati, E. (2015). Effect of yeast on pH, lactate bacteria, and lactose of yogurt. *Jurnal Sain Peternakan Indonesia*, 10(1), 22-31.

Patty, A. (2019). Analysis of physical properties of raw cocoa beans under various fermentation methods and Fermipan concentrations. *Jurnal Hutan Pulau-Pulau Kecil*, 3(1), 13-24. https://doi.org/10.30598/jhppk.2019.3.1.13

Peralta, J.G.B., Elegado, F.B., Simbahan, J.F., Pajares, I.G. and Dizon, E.I. (2021). Microbial and

 

metabolite profiles of spontaneous and adjunct-inoculated cacao (*Theobroma cacao* L.) fermentation. *Food Research*, 5(2), 331-339. https://doi.org/10.26656/fr.2017.5(2).526

Pereira, A.P.M., Stellari, H.A., Vilela, L.F., Schwan, R.F. and Sant'Ana, A.S. (2020). Dynamics of *Geobacillus stearothermophilus* and *Bacillus cereus* spores inoculated in different time intervals during simulated cocoa beans fermentation. *LWT-Food Science and Technology*, 120, 108941. https://doi.org/10.1016/j.lwt.2019.108941

Pradnyawathi, N.L.M., Wijaya, I.K.A., Sutedja, I.N. and Astiningsih, A.A.M. (2019). Study of assorted fermentation methods conducted by local farmers on the quality of cocoa beans (*Theobroma cacao* L.). *Agrotrop: Journal on Agriculture Science*, 8(2), 189-196. https://doi.org/10.24843/ajoas.2018.v08.i02.p10

Purwanto, E.H., Setyabudi, S. and Supriyanto. (2019). Microbial activities in cocoa (*Theobroma cacao* L.) pulp during fermentation with Ragi Tape addition. *Journal of Industrial and Beverage Crops*, 6(1), 21-32. https://doi.org/10.21082/jtidp.v6n1.2019.p21-32

Rahardjo, Y.P., Rahardja, S., Samsudin, Saidah, Dalapati, A., Amalia, A.F., Purwaningsih, H. and Syamsu, K. (2022). A literature review on cocoa fermentation techniques to shorten fermentation time. *IOP Conference Series: Earth and Environmental Science*, 974, 012111. https://doi.org/10.1088/1755-1315/974/1/012111

Rahmi, F., Zulfahrizal, Z. and Siregar, K. (2017). Analysis of transfer heat room of cocoa seed fermentation (*Theobroma cacao* L.) using wood box and styrofoam. *Rona Teknik Pertanian*, 10(1), 34-45. https://doi.org/10.17969/rtp.v10i1.7448

Rosniati and Kalsum. (2012). Processing of cocoa powder from fermented and unfermented cocoa beans as a supply of functional food. *Balai Besar Industri Hasil Perkebunan*, 13(2), 107-116. DOI:10.33104/jihp.v13i2.4157

Sabahannur, N. (2017). Study of the effect of number of cocoa beans per box and stirring time on the success of the fermentation process. *Jurnal Pendidikan Matematika dan IPA*, 8(2), 18-30. https://doi.org/10.26418/jpmipa.v8i2.21172

Santos, D.S., Rezende, R.P., Santos, T.F. dos, Marques, E.de L.S., Ferreira, A.C.R., Silva, A.B.de C.e., Romano, C.C., Santos, D.W.da C., Dias, J.C.T. and Tavares Bisneto, J.D. (2020). Fermentation in fine cocoa type Scavina: Change in standard quality as the effect of use of starters yeast in fermentation. *Food Chemistry*, 328, 7-12. https://doi.org/10.1016/j.foodchem.2020.127110

Sari, R.S., Nuryanto and Widiyanto, A. (2021). Temperature and humidity control system for Tempe Gembus fermentation process based on internet of things. *Urecol Journal. Part G: Multidisciplinary Research*, *1*(1), 39-45. https://doi.org/https://doi.org/10.53017/ujmr.63

Stanbury, P.F., Whitaker, A. and Hall, S.J. (2017). Principles of fermentation technology. 3rd ed. United Kingdom: Butterworth-Heinemann

Tarigan, E.B. and Iflah, T. (2017). Some physicochemical cocoa fermentation and non fermentation. *Jurnal Agroindustri Halal*, 3(1), 48-62. https://doi.org/10.30997/jah.v3i1.687

 