International Journal of Agricultural Technology 2026 Vol. 22(2):663-682
DOI: https://doi.org/10.63369/ijat.2026.22.2.663-682
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Utilization of mixed dry starter culture of yeast, lactic acid bacteria and acetic acid bacteria in cocoa bean fermentation

Gunawan, E. C., Nurmalela, I. P., Halim, Y.*, Natania, Handayani, R. and Cornelia, M.

Food Technology Department, Faculty of Science and Technology, Universitas Pelita Harapan, Banten, Indonesia.

Gunawan, E. C., Nurmalela, I. P., Halim, Y., Natania., Handayani, R. and Cornelia, M. (2026). Utilization of mixed dry starter culture of yeast, lactic acid bacteria and acetic acid bacteria in cocoa bean fermentation. International Journal of Agricultural Technology 22(2):663-682.

Abstract The starter culture of *Saccharomyces cerevisiae, Lactobacillus plantarum,* and *Acetobacter pasteurianus* was made into a dried form to improve the cocoa bean fermentation. Rice flour was added as a carrier material at concentration of 30%, 40%, 50%, and 60%. The best concentrations of coating material in each dry starter culture were 50% for *S. cerevisiae* and *L. plantarum* and 60% for *A. pasteurianus*. The dry starter culture was then mixed in different ratio (1:1: 1, 2:1:1, 1:2:1, 1:1:2) and used in the cocoa fermentation. The fermentation results showed that the addition of mixed dry starter culture with a ratio of 1:2:1 gave a better fermentation result, after 5 days of fermentation, compared to spontaneous fermentation, in terms of temperature during cocoa bean fermentation, pH of pulp and cocoa beans, and fermentation index.

Keywords: *A. pasteurianus*, Cocoa beans, Controlled fermentation, Fermentation time, *L. fermentum*, *S. cerevisiae*

Introduction

Indonesia is the fifth largest cocoa producer in the world, with a cocoa production of 240,000 tons per year (Muhardi *et al.*, 2020). Cocoa derivatives are also very popular due to consumer preferences for their colour, aroma, and taste (Muktiningrum *et al.*, 2022). One of the challenges in cocoa cultivation in Indonesia is the quality of the cocoa beans produced (Muhardi *et al.*, 2020). According to the Quality Testing and Certification Institute of the South Sulawesi Department of Trade, 86.09% of the cocoa beans produced in Indonesia are classified as low quality and receive a price reduction of 10-15% from the market price. One reason for this is the high variation in the quality of cocoa beans in Indonesia, stemming from the agroecological conditions of cocoa cultivation. It is known that the chemical and sensory composition of cocoa beans is significantly influenced by their quality, especially taste and aroma.

* **Corresponding Author:** Halim, Y.; **Email:** yuniwaty.halim@uph.edu

Environmental factors (climate, rainfall, sunlight, and soil), genetics, and post-harvest handling (ripening, harvesting, fermentation, and drying) contribute to the chemical content of cocoa beans (Sari *et al.*, 2023).

Most of Indonesia's cocoa production is exported as raw cocoa beans (Praseptiangga *et al.*, 2020). The beans are typically processed only through drying in the sun without undergoing fermentation. However, fermentation is a crucial step in developing the characteristic flavour of cocoa beans (Apriyanto *et al.*, 2018; Praseptiangga *et al.*, 2020; Wahyuni *et al.*, 2021). During fermentation, polyphenolic components such as epicatechin, catechin, procyanidin, cyanidin, and leucocyanidin break down, influencing colour changes as well as the bitterness and astringency of the cocoa beans (Calvo *et al.*, 2021). Unfermented cocoa beans have a strong bitter and astringent taste but lack the aroma precursors found in fermented beans (Fang *et al.*, 2020). Fermentation with a final pH of around 5.0 produces high-quality cocoa beans with a distinct aroma, while cocoa beans with a pH below 5.0 are considered lower quality due to excessive sourness. Beans with a higher pH can produce less acidic chocolate, which is a characteristic of chocolate with a good quality (Hartuti *et al.*, 2019; Oussou *et al.*, 2022).

Cocoa farmers in Indonesia often skip the fermentation process due to narrow farming land, leading to low production capacity (Leksono *et al.*, 2021). Natural fermentation usually requires around 40 kg of fresh cocoa beans to achieve ideal temperature conditions. Additionally, the mixing technique and relatively long fermentation time of 5-7 days often lead to inconsistent fermentation results (Jamili *et al.*, 2016; Rachmatullah *et al.*, 2021). The fermentation process is often spontaneous, resulting in a diverse number of microorganisms, which in turn leads to various metabolites responsible for the unique characteristics of the produced chocolate (De Vuyst and Weckx, 2016).

One example is that more than half of the cocoa plantations in East Java sell unfermented cocoa products, and over 76% of cocoa farmers lack sufficient education (Praseptiangga *et al.*, 2020). On the other hand, the demand for fermented cocoa beans in the cocoa processing industry is increasing with the establishment of cocoa processing facilities. However, domestic cocoa production has not been able to meet the target quantity and quality of fermented cocoa beans in the industry. This has led the processing industry to blend imported fermented beans with domestic unfermented beans to provide alternative raw materials for the chocolate industry (Rahardjo *et al.*, 2022).

One alternative to minimize quality variation in fermented beans is the addition of starter cultures during the fermentation process. In this process, potential microorganisms synthesize flavour compounds and their derivatives during fermentation, thereby reducing the time required for controlled cocoa fermentation (Balcázar-Zumaeta *et al.*, 2023; Sandhya *et al.*, 2016). The addition of starter cultures from microbial species can control the fermentation process, resulting in the desired chocolate flavour (Tsaaqifah *et al.*, 2023). Previous studies have reported that the addition of microorganism starters can accelerate fermentation (Bagus *et al.*, 2021), increase fermentation temperature, and enhance the acidity levels of cocoa beans (Apriyanto *et al.*, 2018).

Cocoa bean fermentation generally involves a consortium of microorganisms, including yeasts, lactic acid bacteria, and acetic acid bacteria (Apriyanto *et al.*, 2018; Díaz-Muñoz *et al.*, 2023; Lee *et al.*, 2019; Lefeber *et al.*, 2011; Tsaaqifah *et al.*, 2023). Some microorganisms known to play a dominant role in the cocoa bean fermentation process include *Saccharomyces cerevisiae, Lactobacillus fermentum* (Oussou *et al.*, 2022), *Acetobacter tropicalis, Acetobacter pasteurianus* (Soumahoro *et al.*, 2020), *Lactobacillus plantarum*, and *Lactobacillus lactis* (Ordoñez-Araque *et al.*, 2020).

Some prior studies have introduced starter cultures of lactic acid bacteria, acetic acid bacteria, or single yeasts to accelerate fermentation; however, there has yet to be research on the development of standardized dry starter cultures. It was expected that the addition of a mixed starter culture can reduce fermentation time and control the types of microorganisms present, leading to more uniform fermentation quality. Furthermore, creating a microorganism consortium in the form of dry starter cultures would extend shelf life and ease application for cocoa farmers.

The aims of this research were to determine the effect of rice flour concentration as the carrier material on the viable microbial count and yield and to determine the best ratio of the microbial dry starter culture used in controlled fermentation.

Materials and methods

The materials used in this research include ripe cocoa pods var. Forastero grown in Pangandaran, West Java, Indonesia. Yeast Extract Peptone Dextrose (YEPD) broth, Yeast Glucose Chloramphenicol Agar (YGCA), De Man–Rogosa–Sharpe agar and broth (MRSA and MRSB), Glucose Yeast Peptone (GYP) agar and broth, Acetobacter agar, 0.85% NaCl solution, pure microbial culture stocks of S*accharomyces cerevisiae, Lactobacillus plantarum*, and *Acetobacter pasteurianus* obtained from Laboratory of Indonesian Culture Collection (InaCC), 'Rose Brand' rice flour, banana leaves, and 15 cm x 15 cm x 5 cm bamboo fermentation container.

Determination of microbial growth curve

Determination of microbial growth curve followed the method of Apriyanto *et al.* (2016). To prepare the intermediate culture, one inoculating loop from each microbial stock culture was transferred to 10 mL of their respective broth media in screw-cap test tubes: *S. cerevisiae* in YEPD broth, *L. fermentum* in MRS broth, and *A. pasteurianus* in GYP broth. The resulting intermediate culture tubes were incubated at 37°C for *S. cerevisiae* and *L. fermentum*, and at 30°C for *A. pasteurianus*. Each microbial stock culture was also taken for methylene blue staining for *S. cerevisiae* and Gram staining for *L. fermentum* and *A. pasteurianus.*

After each intermediate microbial culture reached an OD600 of ~0.25, 1 mL was transferred into their respective broth media (80 mL) in the 100 mL sterile dilution bottle. This was the working culture for determining the growth curves. A serial dilution for every several hours, starting from 0 h, of the growth curve incubation period was done starting by inoculating 1 mL of the working culture to 9 mL of 0.85% NaCl solution. This was further diluted until the desired dilutions and pour plate method was done for the selected serial dilution to their respective agar media on the Petri plates: *S. cerevisiae* in YGCA, *L. plantarum* in MRSA, *A. pasteurianus* in GYP agar. After the agar medium was set, the Petri plates were incubated upside down at their respective incubation temperature. The growth curves were determined by plotting the log of microbial count on the y-axis against the incubation time (hours) on the x-axis.

Preparation of dry starter culture

Preparation of dry starter culture followed the method of Abubakar *et al*. (2022), and Rukmi *et al*. (2012). The microbial culture for the starter culture was taken from the working culture that was incubated until their respective log phase based on the growth curve determination. This liquid starter culture was analyzed for the yeast and Gram staining. For the adaptation with the carrier materials, 20 mL of liquid starter culture from each microbe was mixed with 4 g of rice flour in 200 mL of sterilized distilled water in a 500 mL dilution bottle and incubated at room temperature for *S. cerevisiae,* at 37°C *L. fermentum,* and at 30°C for *A. pasteurianus*. for 24 hours. The remaining rice flour based on the respective treatment group with different concentrations (30%, 40%, 50%, 60%w/v) was then mixed with the microbial culture by stirring them with sterile stirring rod. The mixture was then poured into the stainless tray lined with baking paper and was dried with a dehydrator at 40-45°C for 4-6 hours and stored in plastic bags added with silica gels at room temperature until further use. The moisture content, yield (Chandralekha *et al.*, 2016), and viable microbial count (Erdiandini *et al.*, 2015) were observed on the resulting microbial dry starter culture.

Cocoa bean fermentation process using dry starter culture

Before the fermentation, the surface of cocoa pod was cleaned and then cut into 2 parts using a clean knife. The cocoa beans with the pulp attached (wet cocoa beans) were scooped manually out of the pod. The placenta inside the pod along with the pod shell/husk were removed. The wet cocoa beans weighed 800 g and were transferred into the fermentation container, which was bamboo box lined with clean banana leaves (Abubakar *et al.*, 2022; Bobiles *et al.*, 2022; Hernani *et al.*, 2019).

The pre-mixed dry starter culture of *S. cerevisiae: L. fermentum: A. pasteurianus* with different ratios (1:1:1, 2:1:1, 1:2:1, 1:1:2) was added 3% (w/w) of wet cocoa bean mass into the respective boxes. The formulation of dry starter culture addition in the fermentation can be seen on Table 1.

Table 1. Formulation of dry starter culture addition

Ratio	**Weight (g)**			
	S. cerevisiae	***L. plantarum***	***A. pasteurianus***	**Rice flour**
1:1:1	6	6	6	6
2:1:1	12	6	6	0
1:2:1	6	12	6	0
1:1:2	6	6	12	0

Notes: the amount added was 3% from 800 g of cocoa bean

Spontaneous cocoa bean fermentation without the addition of a dry starter culture was also conducted as the control. A sterile spoon was used to mix the dry starter culture with the wet cocoa beans before the fermentation starts as well as for every 24 hours to provide aeration. The fermenting cocoa mass was analyzed for the viable microbial count (yeast, LAB, AAB) (Erdiandini *et al.*, 2015), pH using pH meter (Apriyanto *et al.*, 2016; Bobiles *et al.*, 2022), temperature (Bobiles *et al.*, 2022), and fermentation index (Hernani *et al.*, 2019; Riza *et al.*, 2023) every 24 hours throughout the 5-day fermentation period.

Statistical analysis

To determine the effect of rice flour concentration as the carrier material on the first stage of this research, the experimental design used was a Completely Randomized One-Factor Design with 3 (three) replications (Duplo). The factor used was concentration of rice flour, *i.e.,* 30%, 40%, 50%, and 60%.

Furthermore, to determine the best ratio of the microbial dry starter culture used in controlled fermentation, the experimental design used was a Completely Randomized One-Factor Design with 2 (two) replications (Duplo). The factor used was ratio of *Saccharomyces cerevisiae: Lactobacillus plantarum: Acetobacter pasteurianus* of 1:1:1, 2:1:1, 1:2:1, 1:1:2, and control (spontaneous fermentation). All statistical analyses in this research were done using SPSS Statistics version 25.0 using One-way ANOVA with a 5% significance level.

Results

Microbial growth curve

This research was started by determining the microbial growth curve of microorganisms that would be used for cocoa bean fermentation. The results can be seen in Figure 1.

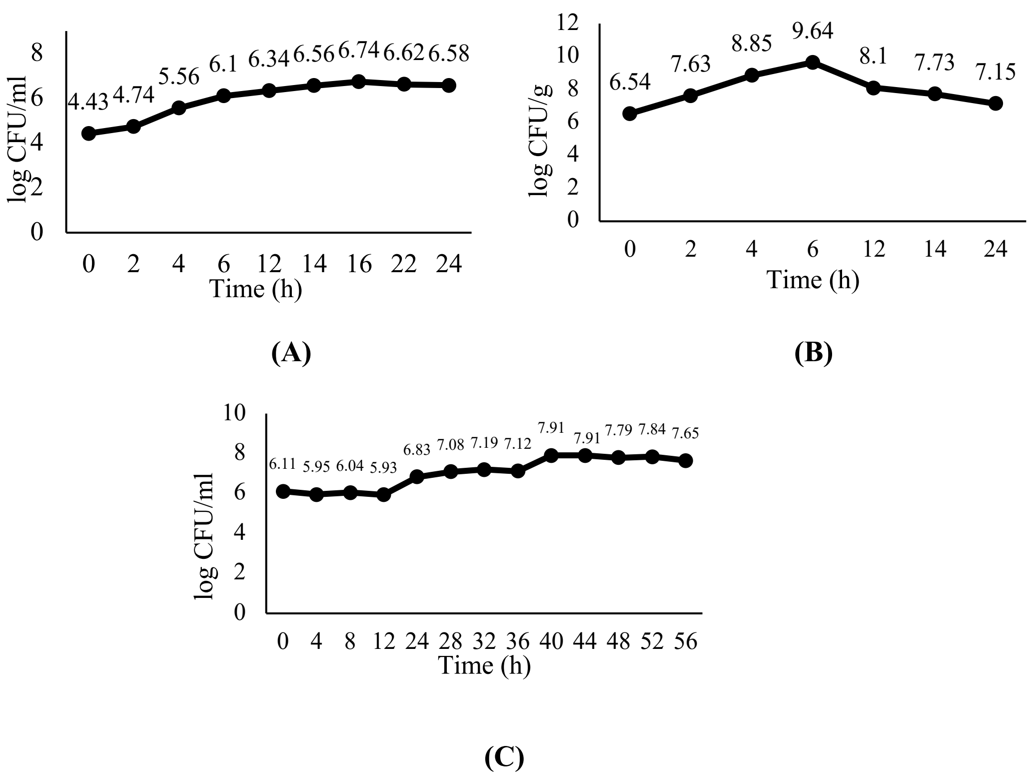

Figure 1. Microbial growth curve
Notes: (A) S. *cerevisiae*; (B) *L. plantarum*; (C) *A. pasteurianus*

Characteristics of dry starter culture

The dry starter culture obtained was analyzed for its viability, yield, and moisture content. Statistical analyses using One-Way ANOVA showed the there were no significant effects (p>0.05) of different rice flour concentration as a carrier material on the viability and moisture content of dry starter culture obtained. However, there was a significant effect (p>0.05) of different rice flour concentration as an carrier material on the yield of dry starter culture obtained (Tables 2, 3, and 4).

Table 2. Viability of dry starter culture

Concentration of rice flour added	Viability (log CFU/g)		
	S. cerevisiae	***L. plantarum***	***A. pasteurianus***
30%	8.03±0.52[a]	9.13±0.44[a]	8.88±0.83[a]
40%	7.98±0.54[a]	9.59±0.27[a]	8.70±0.57[a]
50%	7.83±0.13[a]	9.27±0.18[a]	9.36±0.89[a]
60%	7.73±0.18[a]	8.96±0.21[a]	8.83±0.56[a]

Notes: different letter notation on the same column indicates a significant difference (p≤0,05)

Table 3. Yield of dry starter culture

Concentration of rice flour added	Yield (%)		
	S. cerevisiae	***L. plantarum***	***A. pasteurianus***
30%	26.92±1.51[a]	27.39±2.69[a]	28.11±1.97[a]
40%	35.16±2.58[b]	36.46±1.80[b]	35.30±2.94[b]
50%	45.32±0.97[c]	44.83±1.51[c]	43.07±3.65[c]
60%	46.20±3.18[c]	46.55±2.90[c]	49.90±0.47[d]

Notes: different letter notation on the same column indicates a significant difference (p≤0,05)

Table 4. Moisture content of dry starter culture

Concentration of rice flour added	Moisture content (%)		
	S. cerevisiae	***L. plantarum***	***A. pasteurianus***
30%	25.05±6.06[a]	27.91±3.57[a]	26.76±5.06[a]
40%	27.05±4.27[a]	31.59±3.36[a]	29.29±6.71[a]
50%	34.18±1.14[a]	34.52±2.00[a]	32.76±2.14[a]
60%	27.37±6.67[a]	28.33±2.65[a]	27.91±3.57[a]

Notes: different letter notation on the same column indicates a significant difference (p≤0,05)

Growth of microorganisms during cocoa bean fermentation

During the cocoa bean fermentation process, there is activity from three microorganisms, namely yeast, lactic acid bacteria (LAB), and acetic acid bacteria (AAB). The activity of these microorganisms occurs in the pulp which causes the diffusion of acid and alcohol into the beans; therefore, an enzymatic reaction will occur in the beans that form flavour compounds, colour changes, and aroma (Kongor *et al.,* 2016). Figure 2 shows that the addition of dry starter culture results in higher number of microorganisms compared to control (without addition of dry starter culture).

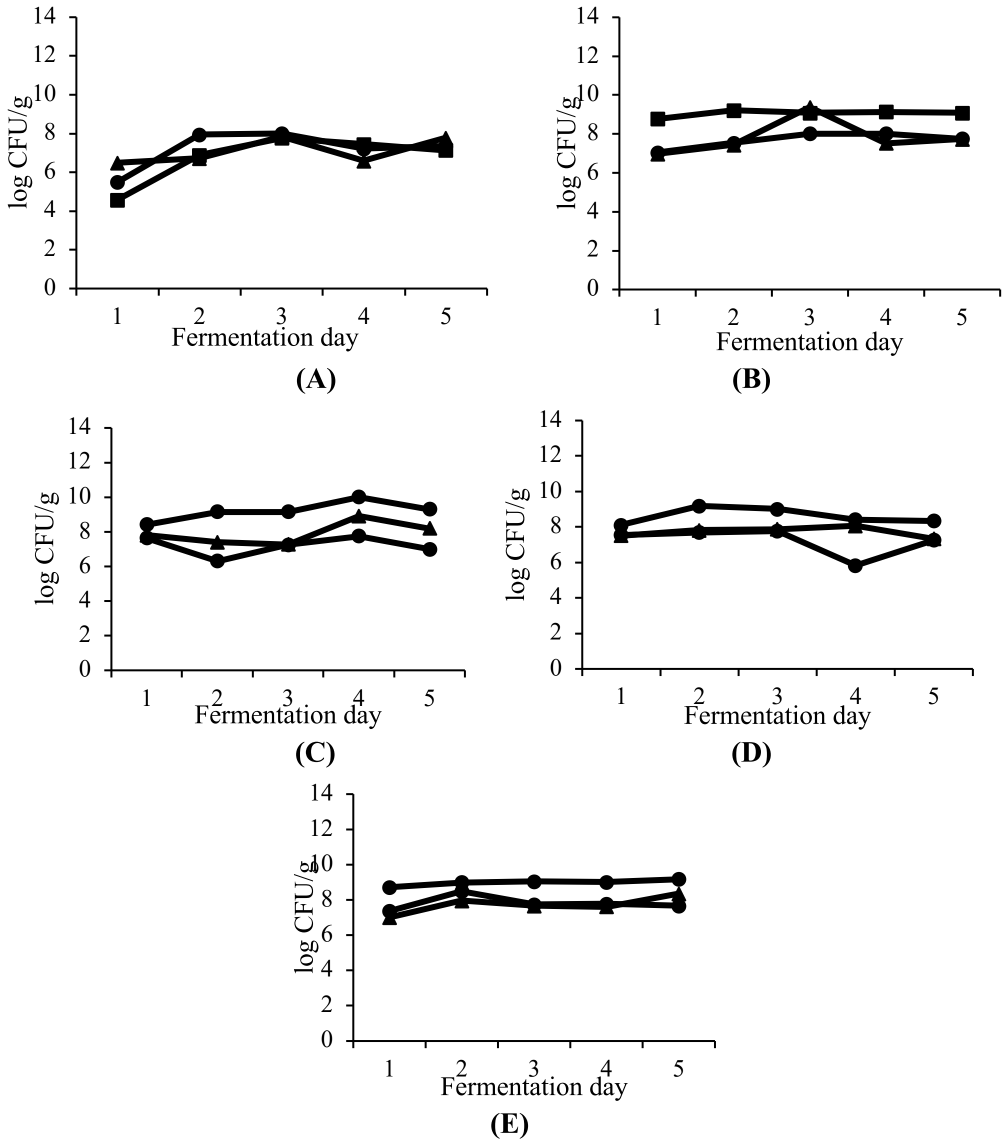

Figure 2. Total yeast (●), lactic acid bacteria (LAB) (▲), and acetic acid bacteria (AAB) (■) during fermentation with different ratios of dry starter culture, control (a), 1:1:1 (b), 2:1:1 (c), 1:2:1 (d), and 1:1:2 (e)

Temperature during cocoa bean fermentation

In this research, the temperature during fermentation was measured every day for 5 days using a thermometer at two points on the fermented cocoa beans. The results of the statistical analysis using One-Way ANOVA showed that different ratios of dry starter culture addition significantly affected ($p \leq 0.05$) the fermentation temperature on days 1, 2, 3, and 5. However, on the fourth day, the fermentation temperature between treatments was not significantly different ($p > 0.05$). The temperature changes during cocoa bean fermentation can be observed in Figure 3.

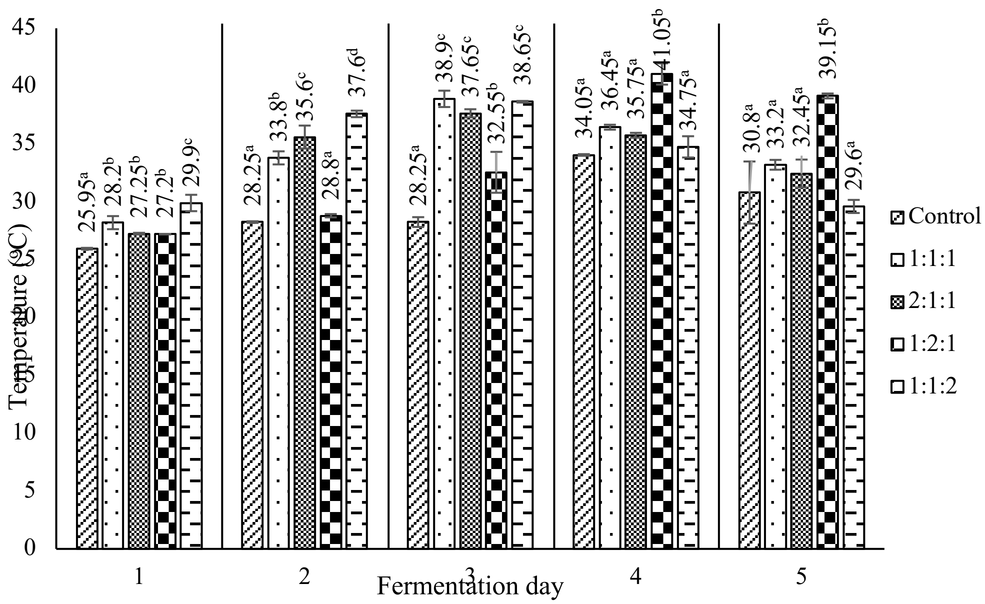

Figure 3. Effect of different ratio of dry starter culture on the temperature during cocoa bean fermentation

Notes: different letter notation on the same fermentation day indicates a significant difference ($p \leq 0,05$)

Fermentation index of cocoa bean fermentation

Fermentation index measurement was carried out daily to determine the progress of the fermentation process based on changes in the colour of cocoa beans (Figure 4). The different ratios of dry starter culture significantly affected ($p \leq 0.05$) the cocoa bean fermentation index during the five days of fermentation. Furthermore, fermentation index value has not reached 1.0 in all treatments, indicating that fermentation was incomplete.

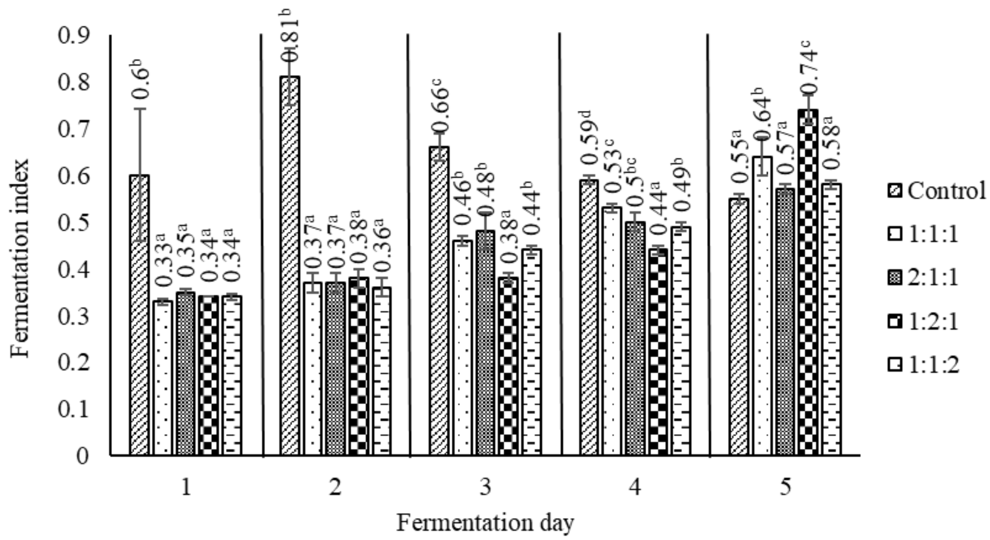

Figure 4. Effect of different ratio of dry starter culture on the fermentation index during cocoa bean fermentation

Notes: different letter notation on the same fermentation day indicates a significant difference (p≤0,05)

pH of cocoa pulp and bean during fermentation

In this research, pH measurements were carried out to determine the acidity level of pulp and cocoa beans during the fermentation process. During fermentation, there is a change in the pH value of the cocoa pulp and cocoa beans. The pH of the cocoa pulp was slowly increased until completely fermentation, whereas the pH of cocoa bean decreases.

The different mixed starter culture ratios significantly affected ($p \leq 0.05$) the pH of the cocoa pulp during five days of fermentation. Overall, pulp pH tends to increase from the first day to the fifth day with a pH range of 3.0 - 6.0. Furthermore, the pH of the pulp with the addition of mixed dry starter culture was higher compared to the control (Figure 5). Figure 6 shows that different mixed starter culture ratios significantly affected ($p \leq 0.05$) the pH of cocoa beans on days 1, 2, 3, and 5. In general, the pH of cocoa beans decreased after 5 days of fermentation.

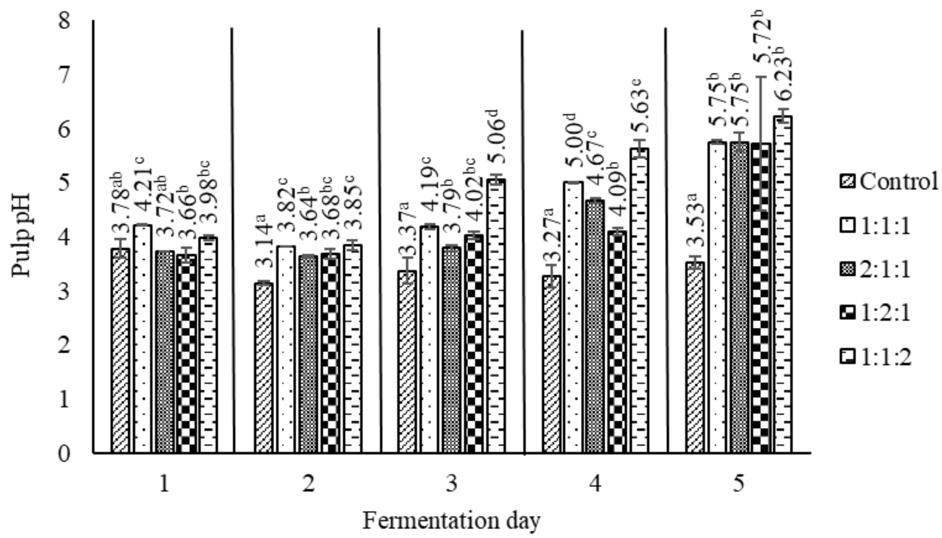

Figure 5. Effect of different ratio of dry starter culture on the pulp pH during cocoa bean fermentation

Notes: different letter notation on the same fermentation day indicates a significant difference (p≤0,05)

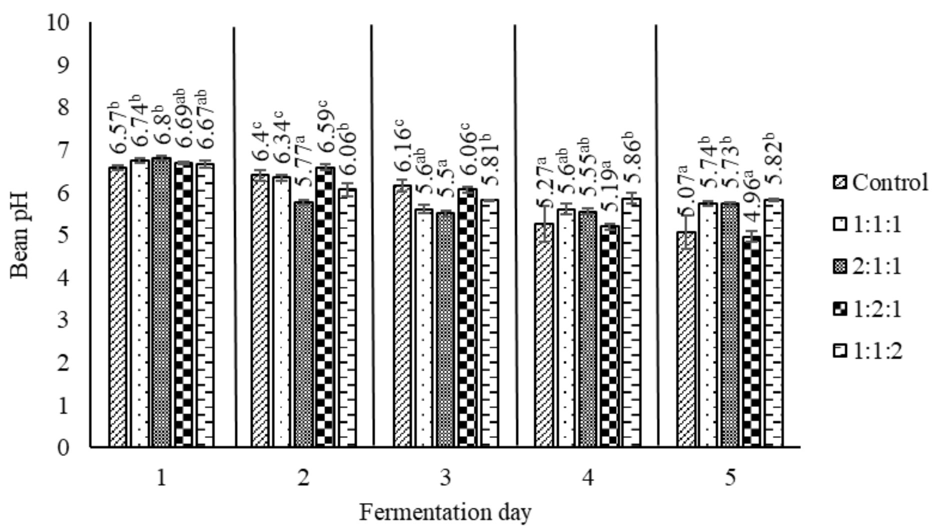

Figure 6. Effect of different ratio of dry starter culture on the bean pH during cocoa bean fermentation

Notes: different letter notation on the same fermentation day indicates a significant difference (p≤0,05)

Discussion

The exponential phases of each microorganism were 16 hours for *S. cerevisiae*, 6 hours for *L. plantarum,* and 40 hours for *A. pasteurianus.* The exponential phase was used for preparing the dry starter culture as in this phase, the microbial cells are in the best physiological condition (Wijaya and Prabaningtyas, 2024), therefore it was expected that these microbial cells would have the best survival rate during drying. Consequently, dry starter culture produced later would contain cells with high viability.

Effect of rice flour concentration on characteristics of dry starter culture

Dry starter culture from the three microorganisms showed a number of cells of 7-9 log CFU/g. The minimum viability of starter culture for fermentation process is around 6 log CFU/ml (Hernani *et al.*, 2019). These results indicated that the dry starter culture can be used in the fermentation in the next stage of this research. These results are found in accordance with previous findings that carrier materials can protect the microbial cells from external stressors, thus preserving microbial quality during drying (Abubakar *et al.*, 2022; Nurhasna *et al.*, 2023; Marwati *et al.*, 2018). A good carrier material could protect the cells during drying, however if the concentration is too high, it could cause swelling or blistering and particle cracking which can reduce retention of the cell nucleus material (Sumanti *et al.*, 2016).

The higher the concentration of rice flour added, the higher the yield of the dry starter culture produced. This is because rice flour can increase the volume and increase the total solids of the material, therefore it can increase the yield. The increase in yield is also influenced by the amount of rice flour added, because the more rice flour added, the greater the total solids obtained. Moreover, the addition of rice flour can also increase the viscosity of the liquid starter culture. It is due to rice flour contains starch molecules that can bind water. The higher the viscosity, the faster the layer will surround and protect the cell nucleus. Thus, increasing the yield of the dry starter culture produced (Mumtazah and Suharto, 2021; Sumanti *et al.*, 2016). However, in this research, the addition of 50% and 60% rice flour did not significantly affect the yield of *S. cerevisiae* and *L. plantarum* dry starter, suggesting that 50% rice flour is sufficient to obtain the maximum yield.

The range of moisture content of dry starter culture in this research is about 25-35%. It suggested that the moisture content of dry starter culture is still too high for long-term shelf life. Based on the previous research, the moisture content of dry starter culture of yeast or lactic acid bacteria is usually less than 10% (Abubakar *et al.*, 2022; Tsaousi *et al.*, 2008).

Storage with airtight conditions is able to maintain the moisture content in dry starters and protect them from excessive oxygen exposure, thereby reducing the occurrence of lipid and protein oxidation in cells (Erdiandini *et al.*, 2015). However, too low moisture content can decrease the viability of the resulting starter culture (Novelina *et al.*, 2005).

Based on the analyses done, the chosen concentrations of rice flour added for dry starter culture preparation were 50% for *S. cerevisiae* and *L. plantarum*, and 60% for *A. pasteurianus*. This decision was based on the yield result, because there was no significant difference in moisture content and viability.

Effect of different ratio of dry starter culture on growth of microorganisms during cocoa bean fermentation

The first phase in the cocoa bean fermentation process is the aerobic phase. In this phase, yeast growth occurs predominantly in the first 24-48 hours of fermentation. Yeast will convert sugar (glucose, sucrose, or fructose) into alcohol (ethanol) in anaerobic, high carbohydrates and acidic conditions. The highest total yeast count was found in the treatment with a ratio of 1:1:2 on the second day of fermentation, then decreased the following day. Gutiérrez-Ríos *et al.* (2022) also stated that the number of yeasts decreases as fermentation progresses. This is because the amount of air, pH and temperature increases. Consequently, the role of yeast is replaced by lactic acid bacteria and acetic acid bacteria. The cocoa bean fermentation added with mixed dry starter culture with a ratio of 1:2:1, decreased on the second day of fermentation, namely 6 log CFU/g and continued to increase the following day. This might be caused by the anaerobic condition of fermentation was disrupted during sampling taken on the second day of fermentation.

The next phase is the LAB growth phase which converts sugar and some organic acids into lactic acid which is dominant in 48-96 hours of fermentation. In this phase, LAB converts citric acid into lactic acid and oxaloacetic acid (which is further converted into pyruvate) (De Vuyst and Leroy, 2020). The treatment with a ratio of 2:1:1 which was increased in the number of LAB from the second to the fourth day and reached the highest number of LAB, which reached 10 log CFU/g. In the treatment with a ratio of 1:2:1, it increased in the total LAB until the third day of fermentation by 9 log CFU/g and decreased the following day to 8 log CFU/g. These results are in line with previous research (Abubakar *et al.*, 2022) which stated that at the beginning of fermentation, LAB growth was low, then increased to around 6 log CFU/g and remained high on the third day and then decreased on the fourth and fifth days. On the second day of fermentation, the number of LAB increased due to the activity of pectinolytic enzymes which provided enough space for aeration. LAB and yeast degrade sugar until the sugar content in the pulp runs out on the third day of fermentation, so that LAB activity decreases and begins to be dominated by AAB activity. Particularly, LAB utilizes glucose to produce lactic acid (Gutiérrez-Ríos *et al.,* 2022), which subsequently affects the acidity and quality of cocoa beans.

The last phase in cocoa bean fermentation is the growth of AAB which converts alcohol (ethanol) into acetic acid. At this stage, AAB oxidizes alcohol into acetic acid and oxidizes lactic acid into acetic acid. In the treatment with a ratio of 1:1:2, it increased to 8 log CFU/g on the fifth day of fermentation. The highest total AAB was found in the treatment with a ratio of 1:1:1 on the third day of fermentation, reaching 9 log CFU/g. The treatment with a ratio of 1:1:1 is in line with previous research (Apriyanto *et al.*, 2017), which stated that the AAB population at the beginning of fermentation was quite low and then increased to 7 log CFU/g on the third day, then decreased to 4 log CFU/g until the end of fermentation. The decrease in the amount of AAB is caused by the high fermentation temperature (De Vuyst and Leroy, 2020).

Effect of different ratio of dry starter culture on temperature during cocoa bean fermentation

The cocoa bean fermentation process is considered well if the oxygen available is sufficient and generates heat due to an increase in temperature. The increase in temperature is the result of the oxidation of sugar compounds found in the cocoa bean pulp (Rachmatullah *et al.*, 2021).

In this research, the temperature of cocoa beans during fermentation tended to increase on the third day and begins to decrease on the fourth day of fermentation. However, in the treatment of adding mixed dry starters with a ratio of 1:2:1, the temperature increases on the fourth day and decreases on the fifth day. In general, the fermentation temperature with the addition of starter culture was slightly higher than the control (without the addition of starter culture). This is due to the influence of *S. cerevisiae* yeast in the first phase of fermentation, which degrades sugar in the pulp into ethanol and the enzymatic reaction that occurs in cocoa beans. The activity of these yeasts will release heat due to exothermic reactions (Abubakar *et al.*, 2022). Furthermore, at the last fermentation stage, the oxidation of ethanol produced by the yeast to acetic acid by AAB also increases the temperature during fermentation (Okonkwo and Igwilo, 2022).

However, in this research, the temperature of each treatment has not reached the optimum fermentation temperature. The optimum fermentation temperature in cocoa beans is 44 - 48˚C (Abubakar *et al.*, 2022) or 45-52ºC (Viesser *et al.*, 2021). The treatment closest to the optimum fermentation temperature is the addition of starter with a ratio of 1:2:1 on the fourth day, which was (41.05 ± 0.92)˚C. This can occur because in this research, the amount of cocoa bean used in this fermentation was not sufficient (800 g per treatment) and decreased as fermentation progressed due to pulp leach and sampling every 24 hours. It was reported that a fermenting mass of less than 5 kg could not maintain the temperature increase due to insufficient pulp substrate (Sari *et al.*, 2023).

Effect of different ratio of dry starter culture on fermentation index during cocoa bean fermentation

The fermentation index in all treatments tends to increase with the length of fermentation time. except in the control, in which the fermentation index value has fluctuating results and the largest standard deviation. The fluctuating results and large standard deviation can be caused by fermentation that was not even and not well-controlled. The fermentation process with the addition of a dry starter culture with a ratio of 1:2:1 has the highest fermentation index value, reaching (0.74 ± 0.03) on the fifth day. The addition of LAB starter culture can accelerate the increase in the fermentation index, so that it can accelerate the fermentation of cocoa beans and shorten the fermentation time (Kresnowati *et al.*, 2013). The fermentation index value that was not achieved can be caused by insufficient fermentation temperature increase.

Effect of different ratio of dry starter culture on pH of bean dan pulp during cocoa bean fermentation

In the treatment of adding starters with a ratio of 1:1:2, the pulp pH is higher compared to other treatments, as this treatment, the amount of AAB added was higher compared to other treatments. At the end of fermentation, volatile organic acids produced by the microorganisms' activities have evaporated, causing the fermentation conditions to become more alkaline (Abubakar *et al.*, 2022). In addition, the increase in pulp pH is also caused by acetic acid produced by AAB that diffuses into the cotyledon (Streule *et al.,* 2024). At the beginning of fermentation, the pulp pH has a low value of pH 3.5 because it contains citric acid. Yeast and LAB will utilize the citric acid to grow, which will cause a decrease in citric acid in the pulp. This condition causes the pulp pH to increase at the end of fermentation (Palupi and Susanti, 2024).

In addition, during fermentation, changes occur in cocoa beans, such as increasing temperature, conversion of sugar to alcohol, and decreasing or increasing pH of cocoa beans. The treatment with the lowest pH of beans was in the 1:2:1 ratio, *i.e.,* from pH of (6.69 ± 0.04) to pH of (4.95 ± 0.12). These results are in line with previous research (Lee *et al.*, 2019), which stated that the acidity level of cocoa beans during fermentation decreases from 6.3–6.5 to 5.0–5.1. The decrease in the pH value of the beans is due to the production of acid by LAB and AAB which diffuse into the beans so that the pH of the beans becomes increasingly acidic. At the beginning of fermentation, the pH of the beans is higher than the pH of the pulp, because there is acid content in the pulp (Palupi and Susanti, 2024).

Different concentrations of rice flour as a carrier material during preparation of dry starter cultures did not affect the viability and moisture content of the dry starter cultures but affected the yield. The chosen concentrations were 50% for *S. cerevisiae* and *L. plantarum*, and 60% for *A. pasteurianus*. The addition of different ratios of mixed dry starter cultures affected the temperature during cocoa bean fermentation, pH of pulp and cocoa beans, and fermentation index. The addition of mixed dry starter culture also gave a better fermentation result compared to control (spontaneous) fermentation. The results in this research showed that a ratio of 1:2:1 had the best fermentation results with temperature of 41.05 ± 0.92 ˚C, bean pH of 4.95 ± 0.12, fermentation index of 0.74 ± 0.03 at the end of fermentation, and total LAB reaching 9 log CFU/g. However, the fermentation index of less than 1.0 in this research indicates inadequate cocoa bean fermentation due to the lack of cocoa fermenting mass in each treatment.

Acknowledgements

The authors would like to thank Ministry of Education, Culture, Research, and Technology of Indonesia for funding this research through Fundamental Regular Research Scheme no. 105/E5/PG.02.00.PL/2024.

Conflicts of interest

The authors declare no conflict of interest.

References

Abubakar, Y., Muzaifa, M., Widayat, H. P. and Martunis, M. (2022). Peningkatan mutu kakao melalui fermentasi menggunakan starter kering bakteri asam laktat dan bakteri asam asetat indigenus kakao Aceh (*Improving the quality of cocoa through fermentation using dry starter of lactic acid bacteria and acetic acid bacteria from indigenous Aceh cocoa*). Agrointek: Jurnal Teknologi Industri Pertanian, 16:84-95.

Apriyanto, M., Sutardi, S., Harmayani, E. and Supriyanto, S. (2017). Perbaikan proses fermentasi biji kakao non fermentasi dengan penambahan biakan murni *Saccharomyces cerevisiae, Lactobacillus lactis* dan *Acetobacter aceti* (*Fermentation process improvement of cocoa beans with addition of non fermentation inoculum Saccharomyces cerevisiae, Lactobacillus lactis and Acetobacter aceti*). Agritech, 36:410-415.

Apriyanto, M., Sutardi, S., Supriyanto, S. and Harmayani, E. (2018). Fermentasi biji kakao kering menggunakan *Saccharomyces cerevisiae, Lactobacillus lactis*, dan *Acetobacter aceti* (*Fermentation of dried cocoa beans using Saccharomyces cerevisiae, Lactobacillus lactis, and Acetobacter aceti*). Agritech, 37:302-311.

Apriyanto, M., Sutardi., Supriyanto. and Harmayani, E. (2016). Study on effect of fermentation to the quality parameter of cocoa bean in Indonesia. Asian Journal of Dairy and Food Research, 35:160-163.

Bagus, I., Gunam, W., Suryanto, A., Ganda Putra, G. P., Kasa, W., Gede, D., Permana, M., Sarjana, P., Wisma, W., Putra, P. and Takama, T. (2021). The effect of mixed inoculum addition concentration and fermentation time on the characteristics of dry cocoa beans *(Theobroma cacao* L.*)*. International Journal of Advance Science, Engineering, Information Technology, 11:272-280.

Balcázar-Zumaeta, C. R., Pajuelo-Muñoz, A. J., Trigoso-Rojas, D. F., Iliquin-Chavez, A. F., Fernández-Romero, E., Yoplac, I., Muñoz-Astecker, L. D., Rodríguez-Hamamura, N., Maza Mejía, I. M., Cayo-Colca, I. S., Chagas-Junior, G. C. A., Maicelo-Quintana, J. L. and Castro-Alayo, E. M. (2023). Reduction in the cocoa spontaneous and starter culture fermentation time based on the antioxidant profile characterization. Foods, 12:3291.

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, 6:236-245.

Calvo, A. M., Botina, B. L., García, M. C., Cardona, W. A., Montenegro, A. C. and Criollo, J. (2021). Dynamics of cocoa fermentation and its effect on quality. Scientific Reports, 11:16746.

Chandralekha, A., Rani, A., Tavanandi, H. A., Amrutha, N., Hebbar, U. and Raghavarao, K. S. M. S. (2017). Role of carrier material in encapsulation of yeast (*Saccharomyces cerevisiae*) by spray drying. Drying Technology, 35:1029-1042.

De Vuyst, L. and Leroy, F. (2020). Functional role of yeasts, lactic acid bacteria and acetic acid bacteria in cocoa fermentation processes. FEMS Microbiology Reviews, 44:432-453.

De Vuyst, L. and Weckx, S. (2016). The cocoa bean fermentation process: from ecosystem analysis to starter culture development. Journal of Applied Microbiology, 121:5-17.

Díaz-Muñoz, C., Van de Voorde, D., Tuenter, E., Lemarcq, V., Van de Walle, D., Soares Maio, J. P., Mencía, A., Hernandez, C. E., Comasio, A., Sioriki, E., Weckx, S., Pieters, L., Dewettinck, K. and De Vuyst, L. (2023). An in-depth multiphasic analysis of the chocolate production chain, from bean to bar, demonstrates the superiority of *Saccharomyces cerevisiae* over *Hanseniaspora opuntiae* as functional starter culture during cocoa fermentation. Food Microbiology, 109:104-115.

Erdiandini, I., Candra Sunarti, T. and Meryandini, A. (2015). Seleksi bakteri asam laktat dan pemanfaatannya sebagai starter kering menggunakan matriks tapioka asam (*Selection of* *lactic acid bacteria and their utilization as dry starter using sour tapioca matrix*). Jurnal Sumberdaya HAYATI, 1:26-33.

Fang, Y., Li, R., Chu, Z., Zhu, K., Gu, F. and Zhang, Y. (2020). Chemical and flavour profile changes of cocoa beans (*Theobroma cacao* L.) during primary fermentation. Food Science and Nutrition, 8:4121-4133.

Gutiérrez-Ríos, H. G., Suárez-Quiroz, M. L., Hernández-Estrada, Z. J., Castellanos-Onorio, O. P., Alonso-Villegas, R., Rayas-Duarte, P., Cano-Sarmiento, C., Figueroa-Hernández, C. Y.and González-Rios, O. (2022). Yeasts as producers of flavour precursors during cocoa bean fermentation and their relevance as starter cultures: A review. Fermentation, 8:331.

Hartuti, S., Bintoro, N., Karyadi, J. N. W. and Pranoto, Y. (2019). Characteristics of dried cocoa beans (*Theobroma cacao* L.) colour using response surface methodology. Planta Tropika: Journal of Agro Science, 7:82-92.

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.

Jamili, Yanti, N. A. and Susilowati, P. E. (2016). Diversity and the role of yeast in spontaneous cocoa bean fermentation from Southeast Sulawesi, Indonesia. Biodiversitas, 17:90-95.

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.

Kresnowati, M. T. A. P., Suryani, L. and Affifah, M. (2013). Improvement of cocoa beans fermentation by LAB starter addition. Journal of Medical and Bioengineering, 2:274-278.

Lee, A. H., Neilson, A. P., O'Keefe, S. F., Ogejo, J. A., Huang, H., Ponder, M., Chu, H. S. S., Jin, Q., Pilot, G. and Stewart, A. C. (2019). A laboratory-scale model cocoa fermentation using dried, unfermented beans and artificial pulp can simulate the microbial and chemical changes of on-farm cocoa fermentation. European Food Research and Technology, 245:511-519.

Lefeber, T., Janssens, M., Moens, F., Gobert, W. and De Vuyst, L. (2011). Interesting starter culture strains for controlled cocoa bean fermentation revealed by simulated cocoa pulp fermentations of cocoa-specific lactic acid bacteria. Applied and Environmental Microbiology, 77:6694-6698.

Leksono, A. S., Mustafa, I., Gama, Z. P., Afandhi, A. and Zairina, A. (2021). Organic cocoa farming in Indonesia: constraints and development strategies. Organic Agriculture, 11:445-455.

Marwati, T., Lamadoken, I. P. P., Djaafar, T. F., Utami, T. and Rahayu, E. S. (2018). Production of dried *Lactobacillus plantarum* HL-15 culture for inhibition growth of mycotoxin producing fungi. Journal of Agricultural Science and Technology B, 8:396-403.

Muhardi, Rahim, A., Effendy, Antara, M., Rauf, R. A., Lamusa, A., Christoporus, Hadayani, Safitri, D. and Mulyo, J. H. (2020). Sustainability of cocoa production in Indonesia. Australian Journal of Crop Science, 14:997-1003.

Muktiningrum, T. A., Fauza, G., Ariviani, S., Muhammad, D. R. A. and Affandi, D. R. (2022). Sensory profile analysis of chocolate drinks using quantitative descriptive analysis (QDA). E3S Web of Conferences, 344:04005.

Mumtazah, S. and Suharto, S. (2021). The effect of concentration and combination of flour type as filling materials on the quality of petis from blue swimming crab (*Portunus pelagicus*) boiled water. Jurnal Ilmu dan Teknologi Perikanan, 3:105-112.

Novelina, Soekarto, S. T., Jenie, B. S. L., Saono, S. and Suhartono, M. T. (2005). Pengeringan kemoreaksi kultur *Saccharomyces cerevisiae* dengan CaO serta pengaruh sorpsi kadar air terhadap stress dan kematian kultur kering (*Chemoreactive drying of Saccharomyces cerevisiae culture with CaO and the effect of water content sorption on stress and death of dry culture*). Jurnal Teknologi dan Industri Pangan,16:71-81.

Nurhasna, A., Meryandini, A. and Sunarti, C. T. (2023). Producing *Lactobacillus plantarum* dry starter using rice bran matrix. Bioeduscience,7:306-316.

Okonkwo, I. F. and Igwilo, C. Q. (2022). Cocoa fermentation: starter addition effect. Agrobiological Records, 9:37-44.

Ordoñez-Araque, R. H., Landines-Vera, E. F., Urresto-Villegas, J. C. and Caicedo-Jaramillo, C. F. (2020). Microorganisms during cocoa fermentation: systematic review. Foods and Raw Materials, 8:155-162.

Oussou, K. F., Guclu, G., Kelebek, H. and Selli, S. (2022). Elucidating the contribution of microorganisms to the spontaneous fermentation and the quality of Ivorian cacao (*Theobroma cacao*) beans: the quality of Ivorian Cacao (*Theobroma cacao*) beans. Quality Assurance and Safety of Crops and Foods,14:23-35.

Palupi, H. T. and Laily Susanti. (2024). Pengaruh lama fermentasi terhadap karakteristik ekstrak pulp biji kakao (*Theobroma cacao* L.) (*The effect of fermentation time on the characteristics of cocoa bean pulp extract (Theobroma cacao L.*). Teknologi Pangan: Media Informasi dan Komunikasi Ilmiah Teknologi Pertanian, 15:155-161. https://doi.org/10.35891/tp.v15i1.4963

Praseptiangga, D., Zambrano, J. M. G., Sanjaya, A. P. and Muhammad, D. R. A. (2020). Challenges in the development of the cocoa and chocolate industry in Indonesia: a case study in Madiun, East Java. AIMS Agriculture and Food, 5:920-937.

Rachmatullah D., Putri D. N., Herianto, F. and Hariani, N. (2021). Karakteristik biji kakao (*Theobroma cacao* L.) hasil fermentasi dengan ukuran wadah berbeda (*Characteristics of cocoa beans (Theobroma cacao L.) fermented in different container sizes*). Jurnal Viabel Pertanian, 15:32-44.

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.

Riza, D. F. Al, Putranto, A. W., Iqbal, Z., Firmanto, H. and Anggraini, C. D. (2023). Prediction of fermentation index and pH of cocoa (*Theobroma cacao* L.) beans based on colour features (cut test) and partial least square regression model. Food Science and Technology (United States), 11:54-62.

Rukmi, W. D., Zubaidah, E. and Maria, M. (2012). Pembuatan *starter* kering kultur campuran bakteri asam laktat dan *Saccharomyces cerevisiae* untuk proses fermentasi produk sereal instan (*Preparation of mixed culture dry starter of lactic acid bacteria and Saccharomyces* *cerevisiae for fermentation process of instant cereal products*). Jurnal Teknologi Pertanian, 4:56-69.

Sandhya, M. V. S., Yallappa, B. S., Varadaraj, M. C., Puranaik, J., Rao, L. J., Janardhan, P. and Murthy, P. S. (2016). Inoculum of the starter consortia and interactive metabolic process in enhancing quality of cocoa bean (*Theobroma cacao*) fermentation. LWT, 65:731-738.

Sari, A. B. T., Fahrurrozi, Marwati, T., Djaafar, T. F., Hatmi, R. U., Purwaningsih, Wanita, Y. P., Lisdiyanti, P., Perwitasari, U., Juanssilfero, A. B. and Rahayu, E. S. (2023). Chemical composition and sensory profiles of fermented cocoa beans obtained from various regions of Indonesia. International Journal of Food Science, 2023:5639081.

Soumahoro, S., Ouattara, H. G., Droux, M., Nasser, W., Niamke, S. L. and Reverchon, S. (2020). Acetic acid bacteria (AAB) involved in cocoa fermentation from Ivory Coast: species diversity and performance in acetic acid production. Journal of Food Science and Technology, 57:1904-1916.

Streule, S., André, A., Freimüller Leischtfeld, S., Chatelain, K., Gillich, E., Chetschik, I. and Miescher Schwenninger, S. (2024). Influences of depulping, pod storage and fermentation time on fermentation dynamics and quality of Ghanaian Cocoa. Foods, 13:2590.

Sumanti, D. M., Lanti, I., Hanidah, I.-I., Sukarminah, E. and Giovanni, A. (2016). Pengaruh konsentrasi susu skim dan maltodekstrin sebagai penyalut terhadap viabilitas dan karakteristik mikroenkapsulasi suspensi bakteri *Lactobacillus plantarum* menggunakan metode *freeze drying* (*The effect of skim milk concentration and maltodextrin as a coating on the viability and characteristics of microencapsulation of Lactobacillus plantarum bacterial suspension using the freeze drying method*). Jurnal Penelitian Pangan (Indonesian Journal of Food Research), 1:7-13.

Tsaaqifah, H., Fahrurrozi, F. and Meryandini, A. (2023). Selection of lactic acid bacteria as starter culture for cocoa fermentation (*Theobroma cacao* L.). Jurnal Penelitian Pendidikan IPA, 9:825-831.

Tsaousi, K., Dimitrellou, D. and Koutinas, A. A. (2008). Low-temperature thermal drying of *Saccharomyces cerevisiae* starter culture for food production. Food Chemistry, 110:547-553.

Viesser, J. A., de Melo Pereira, G. V., de Carvalho Neto, D. P., Favero, G. R., de Carvalho, J. C., Goés-Neto, A., Rogez, H. and Soccol, C. R. (2021). Global cocoa fermentation microbiome: revealing new taxa and microbial functions by next generation sequencing technologies. World Journal of Microbiology and Biotechnology, 37:118.

Wahyuni, N. L., Sunarharum, W. B., Muhammad, D. R. A. and Saputro, A. D. (2021). Formation and development of flavour of cocoa (*Theobroma cacao* L.) cultivar Criollo and Forastero: a review. IOP Conference Series: Earth and Environmental Science, 733.

Wijaya, L. G. C. and Prabaningtyas, S. (2024). Effect of phosphate source on growth of *Chlorella vulgaris* cultured with indole-3-acetic acid-producing bacteria. Jurnal Ilmiah Sains, 24:148-159.

(Received: 17 February 2025, Revised: 15 January 2026, Accepted: 10 March 2026)