IMPACT OF ADDING NKL AND FERMIPAN YEAST: MICROBIAL POPULATION AND DISCOLORATION OF COCOA BEAN "ASALAN" CHIPS DURING FERMENTATION

Dampak Penambahan Ragi NKL dan Fermipan: Perubahan Populasi Mikrobia dan Warna Biji Selama Fermentasi

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Abstract*: The study aims to determine the effect of adding microbes to the fermentation process of "Asalan" cocoa beans on their quality. Three treatments were applied: the first treatment (A0) served as the control without any microbe addition; the second treatment (A1) involved the addition of NKL and fermipan yeasts at the beginning of fermentation; and the third treatment (A2) included the addition of 1% fermipan yeast initially, followed by 1% NKL yeast after 24 hours. Seed discoloration was evaluated using the Cut Test, while microbial populations were analyzed using the Pour Plate Method. The results showed that at 24, 48, and 72 hours of fermentation, treatment A2 had the highest populations of S. cerevisiae (12.55 x 10^{-7} cfu/g), L. lactis (12.53 x 10^{-7} cfu/g), and A. aceti (12.13 x 10^{-7} cfu/g) among the three treatments. The highest percentage of cocoa bean chips (97.01%) was also observed in treatment A2. Based on the findings, it can be concluded that treatment A2 enhances the population growth and induces a brown color change in cocoa beans.*

Keywords*: Asalan cocoa beans, fermipan, NKL yeast*

Abstrak: *Biji kakao "asalan" didefinisikan sebagai tidak difermentasi, memiliki kadar air yang tidak diketahui, dan dipasarkan tanpa mempertimbangkan kualitas. Penelitian ini bertujuan untuk mengetahui bagaimana penambahan mikrobia pada fermentasi biji kakao asalan mempengaruhi mutu biji kakao. Percobaan menggunakan tiga perlakuan: perlakuan pertama (A0) tidak ditambahkan mikrobia (kontrol), perlakuan kedua (A1) ragi NKL dan fermipan ditambahkan pada awal fermentasi, dan perlakuan ketiga (A2) memiliki fermipan 1% fermentasi awal diikuti oleh 1% ragi NKL ditambahkan pada 24 jam setelahnya. Cut Test digunakan untuk mengevaluasi perubahan warna biji, sedangkan Metode Pour Plate digunakan untuk menganalisis populasi mikroba. Dalam studi tersebut, pada 24, 48, dan 72 jam fermentasi, ditemukan bahwa jumlah S. Cerevisiae 12.55 X 10-7 (cfu/g), L. Lactis 12.53 x 10-7 (cfu/g), dan A. aceti 12,13 x 10-1 (cfu/g) pada ketiga perlakuan tersebut terbesar pada perlakuan (A2). Persentase keripik biji coklat (Ao), A1, dan A2 tertinggi terdapat pada kelompok perlakuan (A2) yang memiliki persentase 97,01%. Berdasarkan hasil penelitan dapat disimpulkan perlakuan (A2) dapat meningkatkan populasi dan perubahan warna coklat pada biji kakao.*

Kata kunci*: Biji kakao asalan, fermipan, ragi NKL*

INTRODUCTION

Theobroma cacao plants exhibit optimal growth in tropical climates. Indonesia ranks third in global cocoa production, following Ghana and Côte d'Ivoire. According to Febrianto and Zhu (2022), cocoa production in Indonesia amounts to 657,100 tonnes. Despite the fact that Indonesia still produces a small quantity of low-quality cocoa beans, the price of the commodity has decreased.

Indonesia is a significant global producer of cocoa. In Indonesia, the majority of cocoa beans, specifically over 90%, are cultivated without undergoing the process of fermentation. According to Apriyanto et al. (2017), freshly harvested Indonesian cocoa beans are highly prized for their butter content. To preserve this quality, the beans are sun-dried to prevent fermentation. Despite the fact that research institutions have developed methods to adjust fermentation procedures to accommodate the quantity of cocoa beans in the field,

government organizations have endeavored to promote the adoption of fermentation among farmers. The availability of fermented cocoa beans in supply chains is limited due to the high demand for unfermented cocoa beans, as noted by Nurhayati and Apriyanto (2021).

To eliminate the pulp from fresh cocoa beans, it is common practice among cocoa farmers to soak them in water before exposing them to sunlight for drying (Tunjung Sari *et al*. 2023; Apriyanto, Riono, and Rujiah 2020). Unfermented cocoa beans, also referred to as cocoa pulp, are characterized by their freshness, cleanliness, and dryness after being recently harvested and processed. According to Cempaka *et al*. (2021), "asalan" cocoa beans are categorized as non-fermented cocoa beans that are sold without consideration for their moisture content and dry bean characteristics, indicating a lack of attention to quality control in the marketing process. In the absence of fermentation, cocoa beans are cultivated with bitter beans that lack the distinctive aroma associated with cocoa. According to Dang and Nguyen (2019), the fermentation of cocoa beans comprises two distinct phases. The first phase involves the removal of pulp from the surface of the beans, while the second phase involves a hydrolytic process that occurs within the beans' cotyledons.

According to Vuyst and Leroy (2020) research, the failure of fermentation can be attributed to a lower number of microbes that ferment substrates, resulting in suboptimal microbial succession. Additionally, the moisture content of the substrate may not be conducive to microbial growth during the fermentation process. According to Vuyst and Leroy (2020) assertion, re-fermentation can enhance the quality of low-grade cocoa beans.

The cocoa beans variety "Asalan" exhibits a low pulp moisture content, resulting in a limited presence of natural microbes. To ensure successful fermentation, the pulp moisture content is adjusted to approximate that of fresh cocoa bean pulp, and the number of microbes is increased through addition. The objective of this study is to investigate the impact of microbial supplementation on the quality of cocoa beans during the fermentation process..

METHOD

The present study was conducted at the Food Technology Laboratory of Indragiri Islamic University between August and September 2022. The harvesting of bulk cocoa fruit took place in Sibuak Jaya Village, located in the Tapung District of Kampar Regency, situated in the Riau Province. The fruit exhibits mature characteristics such as a length of 15 cm, diameter of 8 cm, orange skin color when fully ripe, and 35 seeds per pod. The type of ragi utilized in this context is yeast (Na Kok Liong), while yeast (Fermipan) can be procured within the confines of Indragiri Hilir city.

Preparation of Asalan cocoa beans for research purposes

The production of cocoa beans involves a process of sun-drying fresh cocoa beans for a period of 3 to 5 days, typically between the hours of 9 am and 4 pm. The duration of the drying process and the moisture level of cocoa beans are simulated using the same methodology as those employed in previous studies. Cocoa beans cultivated by farmers typically exhibit moisture levels of 5%, 10%, or 15%. Cocoa beans are dried on a longitudinal drying floor that measures 100 cm in height, spanning from east to west. Figure 1 displays an image of the drying floor.

After the drying process, cocoa beans undergo a rehydration process to attain the same moisture content as that of fresh cocoa bean pulp, which is recorded at 87.96 percent. Moreover, there are rehydrated dry cocoa beans weighing 100 g each, with varying moisture levels of 5%, 10%, and 15% that are currently accessible. The process involves the rehydration of dried

cocoa beans, which are subsequently placed in a sample bottle and left to ferment at ambient temperature. The optimal specimen for subsequent analysis comprised of dehydrated cocoa beans of the "asalan" variety, exhibiting a pulp moisture content of 15%. Table 1 displays the moisture content of dried cocoa beans that meet the criteria of being "asalan" enough to undergo fermentation.

Figure 1. Displays freshly harvested cocoa beans that have undergone the drying process. Source : research document

Table 1. The moisture content of cocoa beans of dry origin that is appropriate for fermentation.

The initial moisture condition expressed as a percentage.	The percentage of moisture content after immersion	The condition of seeds after 120 hours of post-fermentation.
5	82,5 %	Black, Moldy, Rotten
10	80,6 %	Black, Moldy, Rotten
15	83,5 %	Partially brown seed chips

Fermentation Process of Cocoa Beans

The cocoa beans referred to as "asalan" are utilized in the production of three distinct categories of fermented cocoa beans. The fermentation box employed in this investigation is constructed from styrofoam and possesses dimensions of 75 cm x 40 cm x 32 cm. The container possesses a capacity of approximately 40 kg for storing wet cocoa beans. The initial fermentation process employed in this study is spontaneous (control) (A0), without the use of yeast. In the realm of fermentation techniques, the second approach (A1) entails the deliberate amalgamation of NKL yeast and fermipan yeast, accompanied by a regulated fermentation process and a 1% addition ratio of the two yeast strains. The third fermentation method (A2) involves an inoculation process that requires the addition of predetermined quantities of fermipan yeast and NKL yeast at various stages. The experimental procedure involves the addition of 1% fermipan yeast during the initial 24-hour period, followed by the introduction of 1% tape yeast at the onset of the subsequent 24-hour period. Subsequently, a composite of baker's yeast and tape yeast in a 1:1 proportion, constituting 1% of the mixture, is introduced at the onset of the third 24-hour period. The quantity of cocoa beans employed in the process of fermentation amounts to 40 kilograms. The process of fermentation necessitates a duration of 120 hours. Moreover, a comprehensive analysis of sample cocoa beans was carried out during the fermentation process.

In terms of the moisture content and properties of dry beans, "asalan" cocoa beans are categorized as

unfermented, with an unspecified level of moisture, and are marketed without consideration for their grade.

The parameters that were measured

The population dynamics of S. Cerevisae, L. Lactis, and A. Aceti during the process of fermentation.

The present study conducted an analysis on the microbial population of dried cocoa bean pulp using the pour plate method as described by Apriyanto et al. (2016). Specifically, 1 g of the sample was homogenized with a stomaker for 15 minutes after the addition of 9 mL of 0.85 percent NaCl solution (Merck, Germany). A 9 mL solution of NaCl at a concentration of 0.85% was prepared, followed by the creation of one 1 mL blue tip, seven petri dishes, and seven reaction tubes for the purpose of growing media.

The Lactococcus lactis medium comprises 20 grams of glucose, 4.5 grams of yeast extract, and 7.5 grams of peptone, which is to be diluted in 1 liter of water. All the components were procured from Oxoid, UK. In order to eradicate any residual bacteria, the aforementioned mixture is subsequently subjected to autoclaving at a temperature of 121°C for a duration of 15 minutes. One milliliter (mL) of the liquid sample was extracted using a measuring pipette and transferred to the diluent solution in a separate test tube. The mixture was then homogenized using a vortex. Subsequently, 1 mL of the homogeneous solution 1 should be added to the prepared test tube. The aforementioned procedure is executed within the sixth test tube.

The initial dilution involves a ten-fold reduction, resulting in a 10-7 dilution. Subsequently, a volume of 1 mL is extracted from the third to the seventh test tube and transferred into a petri dish. Subsequently, a volume of 10-15 mL of chilled medium is introduced into each petri dish, and the contents of the test tube are gently agitated to ensure uniform mixing. Furthermore, the sample was incubated in a petri dish at a temperature of 35°C for a duration of three days. The quantification of microbial colonies that were formed was conducted. The enumeration of colonies can be accomplished through the utilization of the Quebec Colony Counter (QCC) developed by Reichert. In order to cultivate cells, a range of 30-300 colonies are typically introduced to the growth medium within a petri dish. The microbial populations were quantified at specific time points during the fermentation process, including 0, 12, 24, 36, 48, 60, 72, 84, 96, 108, and 120 hours. The assessment of microbial populations can be conducted using fermentation fluids, as indicated by a recent study conducted by Obinze, Ojimelukwe, and Eke (2022). Drying a sample of dried cocoa beans by 50 grains reveals the colour of the beans (random collection). Cocoa beans are split longitudinally with a cutter knife, and the split beans are then individually examined to see if they are brown, brownish purple, or slaty. After fermentation, cocoa beans' colour is evaluated. Figure 2 shows the research flow diagram.

The process of inducing discoloration in cocoa beans during fermentation, as described by Hernani, Hidayat, and Mulyawanti (2019), was implemented with minor modifications.

The efficacy of fermentation can be determined by the percentage of brown bean chips produced. The test is conducted through visual and subjective observation of color changes. A random sample of 50 fermented dried cocoa beans should be taken for analysis. The process of assessing the quality of cocoa beans involves longitudinal cutting of the beans using a cutter knife, followed by individual observation of the split beans. The beans are then categorized based on their condition as either brown, brownish-purple, or slaty. The present study provides information regarding the characteristics of seeds as determined by the seed chip color test.

1. Non-fermented cocoa beans are commonly referred to as slaty beans due to their distinct appearance. When sliced, at least half of the surface of the seed pieces exhibit a grayish color similar to slate or grayish-blue. Additionally, these beans possess a dense and steep texture.
2. Under-fermented seeds refer to seeds that have not undergone complete fermentation. These seeds exhibit a dense and steep texture on at least 3/4 of the surface of the purple seed chip slices.
3. Fermented cocoa beans are characterized by their brown seed coat that encompasses the entire bean. This section outlines the procedure for reporting the outcomes of the cocoa bean color test, which were determined using Equations (1-3).

$$\%\ \text{"Slaty Seeds"} = \sum \frac{\text{"slaty colored seed halves"}}{\text{"total halves of cocoa beans"}} \times 100\% \quad \text{.....(1)}$$

$$\%\ \text{"Purple Brown Seeds"} = \sum \frac{\text{"purple seeds"}}{\text{"total halves of cocoa beans"}} \times 100\% \quad \text{..... (2)}$$

$$\%\ \text{"Brown Seeds"} = \sum \frac{\text{"brown seeds"}}{\text{"total halves of cocoa beans"}} \times 100\% \quad \text{..... (3)}$$

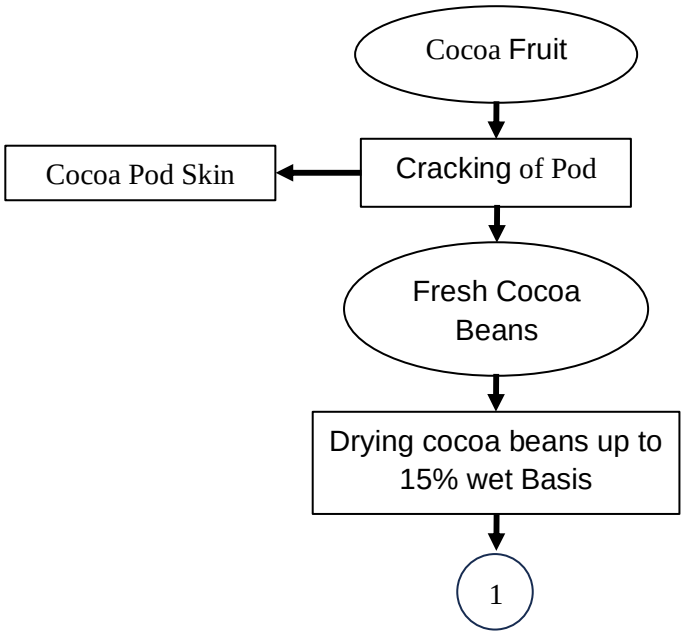
The present study involves an analysis of data.

The study employed a complete randomized trial design and utilized three distinct tests for data collection. To ascertain the effectiveness of the treatment, an improved LSD test was carried out using the SPSS 17 software. The output data of the statistical test employed a one-way diversity analysis with a significance level of 95%.

RESULT AND DISCUSSION

The Relationship Between Microbial Activity During Fermentation and Seed Chip Color

The investigation yielded results indicating that the initial populations of S. cerevisiae, L. lactis, and A. aceti in the control treatment were 5.55, 6.66, and 4.65 log (cfu/g) at the onset of fermentation (A0). During the 24-hour fermentation, the populations of S. cerevisiae, L. lactis, and A. aceti underwent changes, with corresponding values of 7.24, 6.70, and 6.71 logs (cfu/g). According to Chu *et al*. (2022), the populations of S. cerevisiae, L. lactis, and A. aceti during the initial 48 hours of fermentation were 5.88, 8.66, and 6.64 logs (cfu/g), respectively.



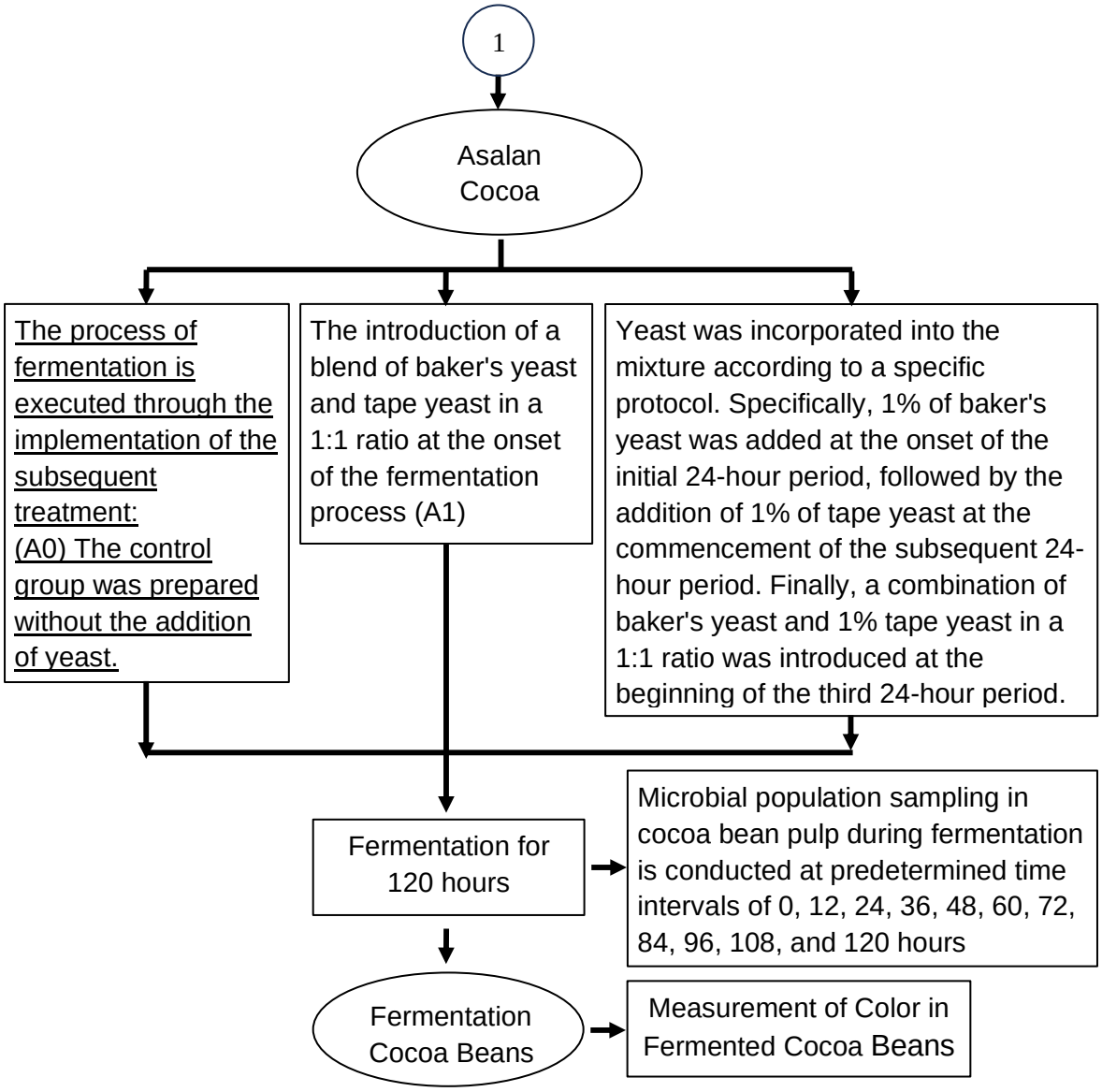


Figure 2. Research Scheme

The populations of S. cerevisiae, L. lactis, and A. aceti were determined to be 4, 22, 8, 43, and 6, 11 log (cfu/g) at 72 hours of fermentation. The average population of S. cerevisiae was observed to be . The colony-forming unit per gram (cfu/g) values for S. cerevisiae treated with A0, A1, and A2 were 4.93 log, 5.86 log, and 5.66 log, respectively. The results of the one-way diversity analysis indicated that there was no significant difference between these values. It is expected that the regulation of ambient temperature will ultimately prove to be optimal for the growth of S. cerevisiae. The control treatment, which involved the simultaneous addition of inoculum at 6.90, 8.60, and 8.55 logs (cfu/g), as well as the control treatment itself, exhibited a statistically significant difference from the other treatments in terms of the average population of L. lactis (Lee *et al*. 2019).

The mean population of A. aceti was 5.57 log (cfu/g) in the control treatment, while it was 7.78 log (cfu/g) and 8.74 log (cfu/g) when the inoculum was added simultaneously and gradually, respectively. The results of multiple tests indicate that there was a statistically significant difference ($p < 0.05$) between the control treatment and

the treatment that added inoculum both simultaneously and progressively. The growth of yeast, lactic acid bacteria, and acetic acid bacteria during the fermentation of fresh cocoa beans in Indonesia was investigated by Schlüter *et al*. (2022), as reported by their research findings. The populations of S. cerevisiae, L. lactis, and A. aceti were found to have undergone changes in response to the control treatment, as discovered by the researchers.

Based on the findings of the present study on the effects of treatment (A1) during the specified period, the populations of S. cerevisiae, L. lactis, and A. aceti were recorded as 8.28 log (cfu/g), 8.58 log (cfu/g), and 8.57 log (cfu/g), respectively, at the onset of the fermentation process. Following 24 hours of fermentation, the population of S. cerevisiae demonstrated a significant increase, reaching 12.41 log (cfu/g), 8.39 log (cfu/g), and 8.38 log (cfu/g), respectively. During the final 48 hours of fermentation, when populations of S. cerevisiae, L. lactis, and A. aceti were at 5.88, 11.82, and 8.82 log (cfu/g), respectively, the population of L. lactis reached its peak (Anggraini *et al*. 2021). The correlation between microbial populations and seed chip colours is illustrated in Figures 3, 4, and 5.

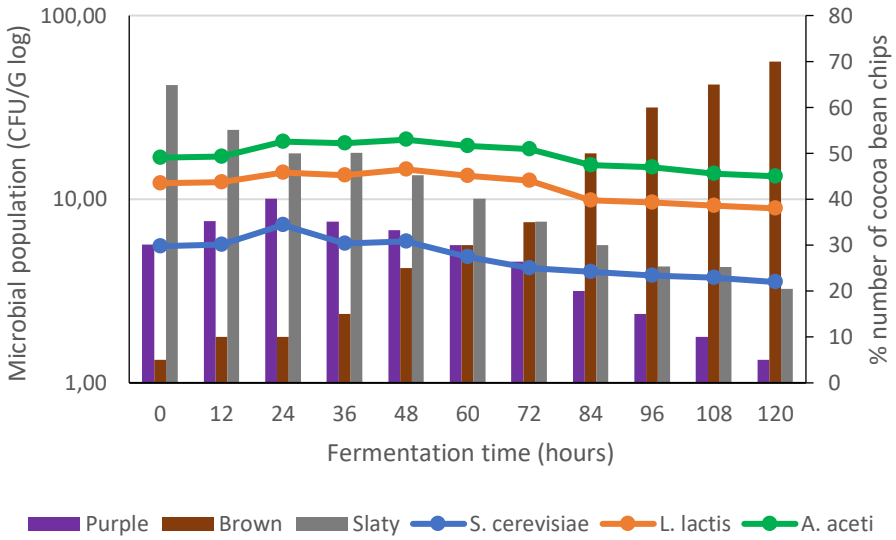


Figure 3. This study examines the correlation between the quantity of slaty, purple, and brown fragments of cocoa bean and the percentage variation in populations of S. cerevisiae, L. lactis, and A. aceti following fermentation with the A0 treatment.

During a 72-hour fermentation period, the population of A. aceti reached its peak. At this point, the populations of S. cerevisiae, L. lactis, and A. aceti were 4, 22, 9, and 10 log (cfu/g), respectively. Sequential reductions of 2.5, 6.39, and 5.45 log (cfu/g) were observed in the populations of S. cerevisiae, L. lactis, and A. aceti, respectively. The findings of Hernani, Hidayat, and Mulyawanti. According to a study conducted in 2019, the incorporation of cocoa beans into the fermentation process of fresh lindak type cocoa beans resulted in a significant increase in the populations of S. cerevisiae, L. lactis, and A. aceti.

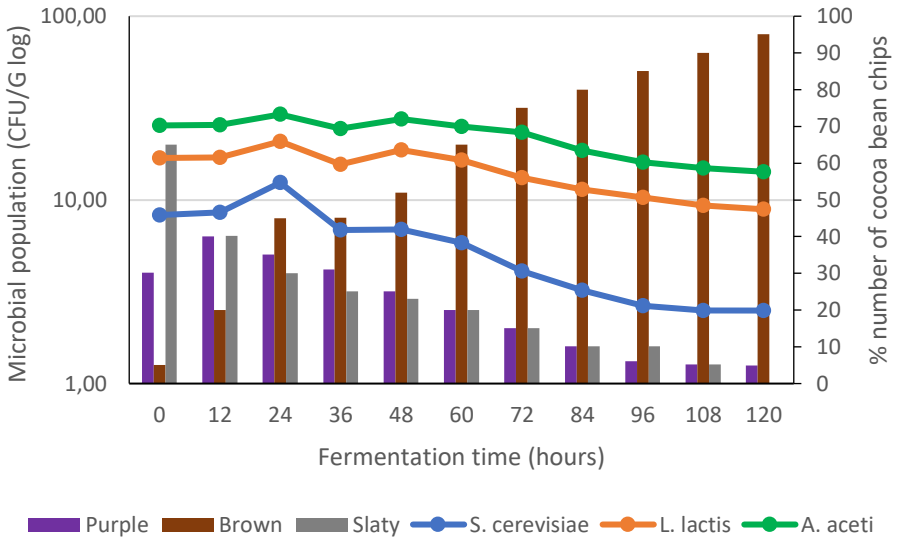


Figure 4. The present study investigates the impact of the A1 treatment on the cocoa bean fermentation process, specifically examining the quantity of slaty, purple, and brown bits of cocoa bean and the percentage change in populations of S. cerevisiae, L. lactis, and A. aceti.

The findings of the analysis indicate that during the initial stages of the fermentation process, the populations of S. cerevisiae, L. lactis, and A. aceti were subjected to treatments of 8.66, 6.64, and 4.67 log (cfu/g), respectively. The populations of S. cerevisiae, L. lactis, and A. aceti exhibited significant changes, with a respective increase of 6.54, 12.53, and 8.73 log (cfu/g) after 48 hours of fermentation. After a 24-hour period of fermentation, the populations of the three aforementioned species were recorded as 12.55, 8.23, and 5.53 logs, respectively. After 72 hours of fermentation, the populations of S. cerevisiae, L. lactis, and A. aceti were determined to be 3.87, 10.64, and 12.13 logs (cfu/g), respectively. Following a 24-hour fermentation period, the temperature and pH of the fermentation environment rise, creating an optimal condition for the activation of polygalacturonase (PG) enzyme and enhancing the production of ethanol (Rottiers *et al.* 2019; Yan et al. 2021). This renders S. cerevisiae ineffective and replaces it with L. lactis. The ideal conditions for A. aceti are achieved after 48 hours of fermentation, characterized by a high concentration of ethanol, improved aeration, and a relatively low level of sugar substrate (Afoakwa et al. 2014). There were observable differences in the proportion of cocoa bean chip slaty colour between the A0 and A2 treatments. The average percentage of cocoa bean chips exhibiting a slaty color after treatment with A0, A1, and A2 was 40.15%, 22.19%, and 17.78%, respectively (Lee *et al*. 2019b). Following a 120-hour fermentation period, the color of the cocoa bean chips treated with A0, A1, and A2 decreased from 65% to 20.53%, 0%, and 0%, respectively.

The results of the one-way diversity analysis indicated that the color of cocoa bean chips, specifically the brownish-purple hue, remained consistent across the different fermentation processes and was not significantly impacted (Utrilla-Vázquez *et al*. 2020). The chromaticity of the "asalan" cocoa bean chips, characterized by a brownish-purple hue, did not exhibit any significant variation among the different treatments. The average color of cocoa bean chips was controlled by injecting inoculum at rates of 25.49%, 20.25%, and 17.70% concurrently and progressively. At the beginning of the A0, A1, and A2

treatments, the proportion of cocoa bean chips exhibiting a brownish-purple hue were 30%, 11%, 30%, and 9%, respectively. Following the fermentation process, it was observed that treatments A0, A1, and A2 experienced a gradual reduction in the percentage of brownish-purple color, with decreases of 5.03%, 4.98%, and 3.02%, respectively.

The control treatment's dark cocoa bean chips and the gradual addition of the inoculum did not exhibit any noticeable distinction, as indicated by the lack of discernible difference (Chu *et al*. 2022). In the control group, the inoculum was added in a simultaneous and progressive manner, resulting in an average percentage of brown seed pieces of 34.36%, 57.56%, and 64.52% for each respective trial. The inoculum was administered simultaneously and progressively at the onset of three consecutive fermentations, each with initial concentrations of 5.02, 5.02, and 5.01 percent. At the conclusion of the fermentation process, the control group exhibited a gradual increase in the proportion of brown seed chips, with increments of 70.01, 95.03, and 97.01 percent.

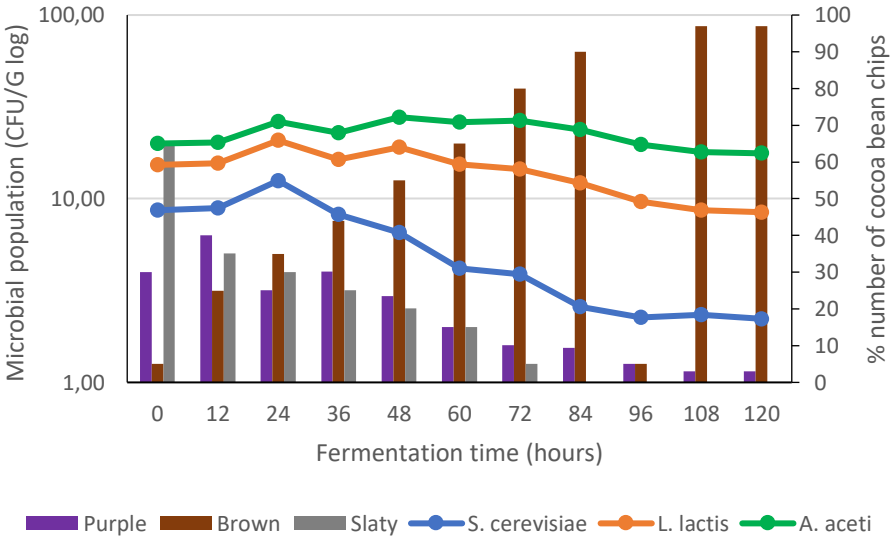


Figure 5. This study examines the correlation between the quantity of slaty, purple, and brown fragments of cocoa bean and the percentage variation in populations of S. cerevisiae, L. lactis, and A. aceti following fermentation with the A2 treatment.

CONCLUSION

The assessments conducted on the aforementioned characteristics concluded that the therapy involving the addition of yeast exhibited superior efficacy compared to the other two treatments. The findings of the study indicate that the rehydration of dried cocoa bean pulp has the potential to improve its composition as a substrate for fermentation. Altering the parameters for microbial population changes during fermentation results in an increase in the fraction of dark cocoa beans.

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