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Cocoa Bean During Fermentation: A Meta-Analysis

Biji Kakao Selama Fermentasi: Analisis Meta

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ABSTRAK

Latar Belakang: Fermentasi kemampuannya untuk menghancurkan kotiledon dan menghasilkan prekursor aroma, fermentasi sangat penting. Sebaliknya, petani Indonesia menunjukkan keengganan terhadap fermentasi karena durasi proses fermentasi yang berlarut-larut, yang berlangsung dari 5 hingga 7 hari. Tujuan: Meta-analisis dilakukan untuk menentukan kemanjuran komparatif kultur starter yang digunakan dalam proses fermentasi biji kakao. Metode: Artikel yang diterbitkan dalam jurnal peer-review mengenai fermentasi kakao digunakan untuk menyusun database. Dengan memanfaatkan platform openknowledgemaps.org, yang mengintegrasikan Pubmed (ilmu hayati) untuk menemukan artikel teks lengkap yang diterbitkan dalam bahasa Inggris dan Indonesia dari Januari 2014 hingga Januari 2024, pencarian literatur yang komprehensif dilakukan. Metode analisis statistik deskriptif digunakan dalam penelitian ini, memanfaatkan aplikasi OpenMEE untuk analisis data. Hasil: Temuan menunjukkan perubahan ragi, bakteri asam laktat, dan bakteri asam asetat selama proses fermentasi. Ukuran efek ragi, bakteri asam laktat, dan bakteri asam asetat masing-masing memiliki nilai -8,061, -0,148, dan 0,020, memenuhi kriteria sederhana Kesimpulan: Berdasarkan analisis meta-analisis, perlakuan eksperimental pertumbuhan ragi, BAL, dan BAA selama fermentasi memiliki efek yang seragam

Kata Kunci: Bakteri asam asetat, Bakteri asam laktat, Meta-Analisis, Ragi.

ABSTRACT

Background: Due to its ability to destroy cotyledons and generate precursors of scent, fermentation is crucial. In contrast, Indonesian farmers exhibit reluctance towards fermenting due to the protracted duration of the fermentation process, which spans from 5 to 7 days. Objectives: A meta-analysis was conducted to determine the comparative efficacy of starter cultures utilised in the cocoa bean fermentation process. Methods: Articles published in peer-reviewed journals regarding cocoa fermentation were utilised to compile the database. By utilising the openknowledgemaps.org platform, which integrates Pubmed (life sciences) to locate full-text articles published in both English and Indonesian from January 2014 to January 2024, a comprehensive literature search was performed. The descriptive statistical analysis method was employed in this study, utilising the OpenMEE application for data analysis. Results: The findings indicated alterations in yeast, lactic acid bacteria, and acetic acid bacteria throughout the fermentation process. The yeast effect size, lactic acid bacteria, and acetic acid bacteria have values of -8.061, -0.148, and 0.020, respectively, meeting the modest criterion. Conclusions: Based on meta-analysis analysis, experimental treatment of yeast, BAL, and BAA growth during fermentation has a uniform effect.

***Keyword**: Acetic acid bacteria, A Meta-Analysis, Lactic acid bacteria, Yeast.*

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INTRODUCTION

Microorganisms are utilised in the food industry during fermentation, among other refining methods. Cocoa beans are a product that undergoes fermentation during its manufacturing. This method can stimulate the development of flavour precursors and the alteration of colour in cocoa beans that are created during the fermentation process (Apriyanto & Novitasari, 2021; Febrianto *et al*., 2022). Following the harvesting of cocoa fruit, the fermentation of cocoa beans occurs. Typically, cocoa beans are extracted from cocoa fruit and undergo natural fermentation in a wooden container that is sealed with banana leaves for a period of 5-7 days. Activity of microorganisms such as yeast, lactic acid bacteria (BAL), and acetic acid bacteria (BAA) in the pulp during fermentation causes alcohol and acid to diffuse into the seeds, resulting in their death and the formation of flavour compounds, aromas, and colour changes via enzymatic reactions (Díaz-Muñoz, 2022; Mota-Gutierrez & Cocolin, 2021).

Following the completion of fermentation, the subsequent step involves the dehydration of cocoa beans. Drying serves the objective of halting the fermentation process and reducing the moisture content. The duration of fermentation varies between 5 to 7 days, depending on the specific type of cocoa beans utilised. Drying is continued until the moisture content reaches a level of 6-7% (Apriyanto & Umanailo, 2019; Saunshia *et al*., 2018). After undergoing maillard reactions, caramelization, protein degradation, and compound synthesis, the precursor compounds of flavour and fragrance that were formed during fermentation will be altered as the cocoa beans are roasted (Figueroa-Hernández *et al*., 2019; Hernani *et al*., 2019; Misbakh *et al*., 2022). A considerable number of cocoa bean producers in Indonesia opt not to engage in the fermentation process due to its perceived time-consuming nature. Consequently, cocoa pods produced for low selling prices are of inferior final quality. One approach to reduce the duration of fermentation involves incorporating starter cultures (Saunshi *et al*., 2020).

Fermentation can be accelerated and enhanced by adding starter cultures, which are materials rich in related microorganisms (Sabahannur *et al*., 2018). It is anticipated that the incorporation of starter culture into the fermentation procedure will increase the quality of cacao beans and accelerate the reaction time. The inclusion of liquid starter cultures comprising yeast, BAL, and BAA in the fermentation process has been shown to increase total acid, fermentation temperature, and yeast growth profile, while also reducing pH, according to research conducted by (Misgiyarta & Winarti, 2023). A substantial body of research has been dedicated to investigating the effects of incorporating starter culture into the cocoa bean fermentation process (Mardesci *et al*., 2023; Vanderschueren *et al*., 2023; Wahyuni *et al*., 2022). Regarding the impact of starter culture addition on the profiles of microorganisms and metabolites derived from all conducted studies, however, no one has examined meta-analyses of this nature. This is beneficial for assessing the correlation between the incorporation of starting culture and the efficiency of fermentation duration, as well as enhancing the calibre of cocoa beans. When choosing a starter culture for cocoa bean fermentation, it is crucial to consider the desired quality characteristics of the end product and its impact on the fermentation process. In order to identify the appropriate starter culture, additional research is required to compare the starter cultures in terms of their impact on the metabolite profile during fermentation and the resulting features of cocoa bean products.

Open Science and Technology
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The objective of this study was to assess the impact of different types of starting cultures on the composition of microorganisms and metabolites generated throughout the fermentation process by meta-analysis).

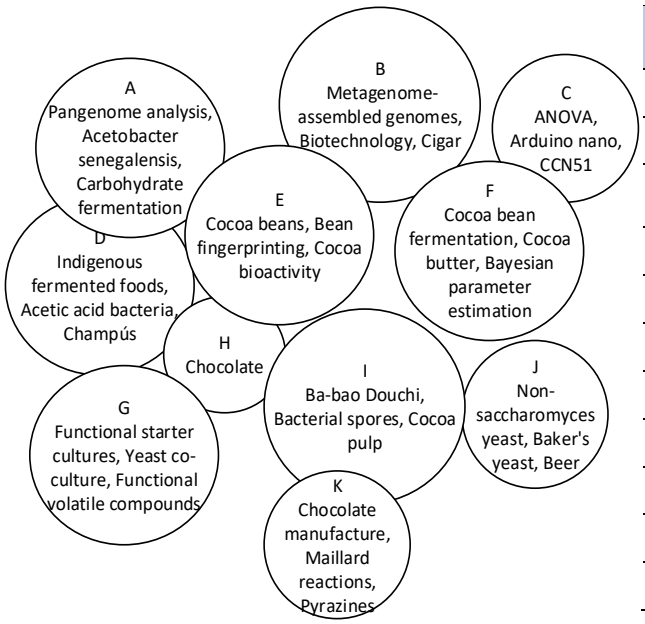
RESEARCH METHODOLOGY

Articles published in peer-reviewed journals regarding cocoa fermentation were utilised to compile the database. A literature search was performed utilising the openknowledgemaps.org platform, which integrates Pubmed (life sciences) to identify comprehensive articles published from January 2014 to January 2024 in both English and Indonesian. Automated generation of up to one hundred article papers categorised according to the search term "fermented cocoa" will occur on the website. The inclusion criteria include selecting studies published between 2014 and 2024 to ensure the research undertaken is recent and innovative. The fermentation of cocoa beans with the addition of starter cultures has been the subject of research in the literature; the study provides the mean value plus or minus one standard deviation or error; and a control was utilised for comparison. In the absence of these components, a study will be disqualified from the screening phase. Using the OpenMEE tool, which emphasises descriptive statistical analysis, data analysis entails the examination and interpretation of data.

RESULT AND DISCUSSION

Search Results

On openknowledgemaps.org, eleven categories were applied to the selected publications that appeared on PubMed between 2014 and 2024. As shown in Figure 1, the group with the highest accumulation of articles was Cocoa bioactivity (E), which comprised 21 articles.



Group code	Explanation
A	Acetobacter senegalensis
B	Metagenome-assembled genomes
C	Cocoa clone Cacao Castro Naranjal 51 (CCN 51)
D	Acetic acid bacteria
E	Cocoa bioactivity
F	Cocoa bean fermentation
G	Yeast co-culture
H	Chocolate
I	Cocoa pulp
J	Baker's yeast
K	Pyrazines

Figure 1. Openknowledgemaps.org-generated map of cocoa fermentation research from 2018 to 2024; all content retrieved from PubMed.

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Yeast co-culture (G)-11, cocoa bean fermentation (F)-16, and acetic acid bacteria (B)-15 are among the many publications authored by the aforementioned groups. The fermentation process of cocoa is predominantly examined in relation to yeast, although starter cultures, Yeast, Lactobacillus species, and Acetobacter aceti are also being investigated. In accordance with the PRISMA diagram, which is illustrated in Figure 2, the attributes or categories of each article have been modified.

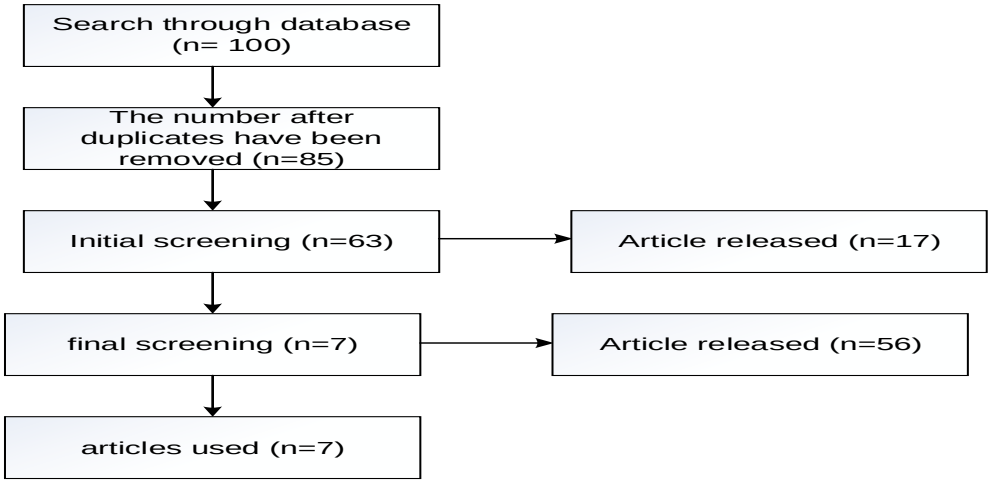

Figure 2. PRISMA literature filtering flowchart

Types of Cocoa Beans

Criollo, Forastero, and Tinitario are the three primary classifications of cocoa bean varieties. Out of the 7 literature sources analysed in this study, 5 sources specifically focus on the use of Forastero cocoa bean varieties. The remaining 2 sources examine different types of cocoa beans, and 1 source does not specify the type of cocoa bean utilised. With an approximate 90-95% share of global cocoa production, Forastero cocoa beans are the most extensively cultivated variety among cocoa beans (Gil *et al*., 2021; Tunjung Sari *et al*., 2023).

In Indonesia, Forastero is frequently considered a variety of lindak and is characterised by the production of cocoa beans of average quality (bulk cocoa). This variety of cocoa bean has the following qualities: green fruit, thick epidermis, thin seed cotyledons that turn purple when wet, and fruit seeds that are thin in form (Koné *et al*., 2021; Santos, 2020). The seeds acquire their purple hue as a result of the presence of anthocyanin (Castro-Alayo *et al*., 2019). Variations in flavour and other parameters will result from the utilisation of distinct varieties of cocoa beans during the fermentation process. The content of the seeds, the place and origin of growth, and the environmental factors all contribute to this variation, according to (Castro-Alayo *et al*., 2019), Additionally, according to (Vuyst & Leroy, 2020), the quality, flavour, and composition of fermented cocoa beans are influenced by the variety of cocoa beans utilised, which in turn affects crop yield and resistance to pests. To differentiate the aromas generated by various cocoa bean varieties, a number of studies that examine the impact of cocoa bean variety on the final product quality place greater emphasis on volatile compounds. (Rojo-Poveda *et al*., 2020) propose that the Forastero variation has a reduced concentration of volatile chemicals compared to other varieties.

Profile of Yeast, Lactic Acid Bacteria and Acetic Acid Bacteria

There are numerous techniques for fermenting cocoa seeds, including modified and conventional techniques. Typically, traditional cocoa bean fermentation occurs naturally within wooden containers enclosed with banana leaves for a duration of 5-7 days. Differentiating the seven works of literature utilised in this investigation by container and fermentation time are a number of distinct techniques. The majority of studies employ techniques that are comparable to those used in the past, namely wooden crates. (Mota-Gutierrez *et al*., 2019) asserted that variations in fermentation techniques significantly influence the growth characteristics of microorganisms and the composition of metabolite chemicals generated during the fermentation process. The incubator media exhibits the highest value in the yeast profile assessment. Utilising hardwood boxes resulted in a higher ethanol level in comparison to the control, whilst the utilisation of plastic yielded a lower ethanol content. Regarding lactic acid levels, the utilisation of plastic and leaf media yields values that are comparable to the control group, whereas the utilisation of other media results in lactic acid levels exceeding those of natural fermentation. Wooden boxes are commonly used due to their straightforward technicalities. Nevertheless, it is necessary to rotate or flip the use of this medium while the fermentation process is taking place. It is necessary to perform this task in order to ensure that the fermentation process proceeds uniformly (Ramos-Escudero *et al*., 2021).

During fermentation, the growth profiles of yeast, lactic acid bacteria, and acetic acid bacteria are depicted in Figure 3. According to (Díaz-Muñoz & De Vuyst, 2022), the ideal yeast growth occurs when there is a density of 7–9 log cfu.g-1 at the 24-hour mark of fermentation. Figure 3 demonstrates that the fermentation process, when supplemented with a starter culture, yields a yeast profile value that is on average comparable to both the control and the addition of the starter. Regardless of the specific methods used to increase starter cultures, the yeast profile remains effectively regulated. S. cerevisiae is employed as a starter culture due to its notable attributes, including a significant capacity to withstand ethanol (Gutiérrez-Ríos *et al*., 2022) and its ability to generate the enzyme invertase (Ganda-Putra *et al*., 2019). The incorporation of starting culture leads to variations in the concentration of metabolites generated, contingent upon the metabolic activity of microorganisms during fermentation. This can enhance the initial yeast profile in the fermentation process, hence increasing its value.

As fermentation continues, the quantity of acetic acid produced from ethanol, a yeast metabolite converted by BAA, is consumed by aerobic yeast, or is lost through transpiration or perspiration (Comasio *et al*., 2019). As the process of fermentation advances, the quantity of yeast diminishes as a consequence of elevated pH, increased air content in the legumes, and elevated temperature. Consequently, BAL and BAA assume the functions previously performed by yeast.

Profiles of the growth of lactic acid bacteria on three distinct initial cultures. According to (Chagas Junior *et al*., 2021), the ideal BAL profile value is 7-9 log cfu.g-1 after 48 hours of fermentation. Based on the acquired data, the overall fermentation process utilising a starter culture exhibits a worse outcome compared to the control group. The incorporation of starter culture results in a significant increase in the BAL profile value, primarily because of the inclusion of lactic acid starter cultures like Lactobacillus plantarum and Lactobacillus lactis. Consequently, the quantity is significantly greater in both treatments when compared to the treatment that alone

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introduces the starter culture yeast Saccharomyces cerevisiae into the fermentation procedure.

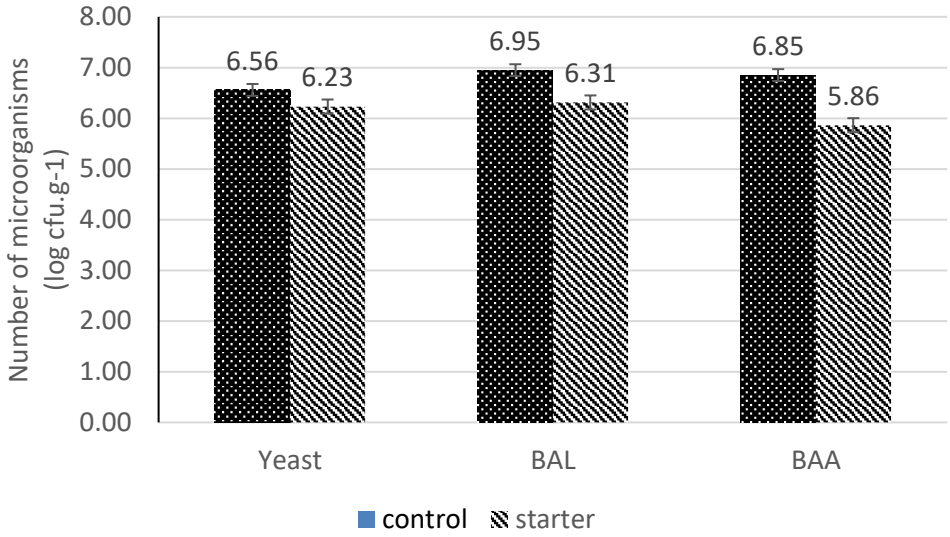

Figure 3. Average amount of yeast, BAL and BAA during fermentation.

In terms of enhancing or diminishing the BAL profile, the culture as a whole had almost no impact. Optimally occurring between 48 and 96 hours after fermentation, microarobic enterobacterial phase (BAL) proliferation transforms sugar and certain organic acids into lactic acid (Agostini *et al*., 2021). During this stage, sugars and organic acids undergo a conversion into lactic acid through the action of BAL (Ramos-Escudero *et al*., 2021). According to (Ooi *et al*., 2020; Viesser *et al*., 2021), citric acid will be decomposed by BAL into pyruvic acid, acetic acid, and lactic acid during fermentation.

The reduction in BAL and lactic acid levels during fermentation is attributed to the depletion of energy sources, elevated ethanol concentrations, and increased temperatures.

Fermentation time can be accelerated by incorporating BAL and BAA starter cultures, which facilitate the conversion of citric acid and lactic acid more rapidly (Devaki & Premavalli, 2019). The reduction in BAL (base acidity level) and BAA (base acid acceptance) profile values will correspond to the heightened oxidation of acetic acid, thereby diminishing the acidity measurement of the seeds. Figure 6 displays the growth profile values of acetic acid bacteria in 5 distinct starter cultures and the overall starter. According to (Comasio *et al*., 2021), the ideal BAA profile value is between 5 and 8 log cfu.g-1 after 72 hours of fermentation.

Starting cultures, including Acetobacter aceti, exhibit a significant increase in BAA profile value when acetic acid is added. During its aerobic phase or growth, BAA transforms ethanol into acetic acid (Ale *et al*., 2020). At this stage, BAA such as Acetobacter predominate and are capable of oxidising lactic acid to acetic acid and ethanol to acetic acid. Successful BAA activity is achieved through the reduction of ethanol and lactic acid concentrations (Gamero *et al*., 2022). The reduction in BAA levels is attributed to elevated fermentation temperatures, leading to evaporation and reduced ethanol concentrations, ultimately halting the fermentation process.

This observation suggests that fluctuations in the BAA profile value throughout fermentation are influenced by the introduction of starter culture. The inclusion of similar cultures has greatly reduced the profile of BAA.

Meta Analysis

Table 1 contains a summary of the principal findings of this meta-analysis. The analysis conducted utilising a random effect model yielded the following results: the mean effect size for the yeast variable across 43 studies was -8.061 (p <.001). A 95% confidence interval spanning -9.8730 to -6.249 was established. The results indicated a substantial influence of yeast on the control group in comparison to the treatment group. The positive effect size indicates that the experimental treatment has a bigger effect than the control, while a negative effect size indicates the opposite. The impact of size on the yeast variable exhibits a negative value, indicating that the control exerts a stronger influence than the experimental treatment. Effect sizes of 0.80, 0.50, and 0.20 indicate significant, moderate, and minor effects, respectively. Hence, it can be inferred that the impact of yeast addition during fermentation treatment is minimal.

Table 1. Growth of yeast, BAL and BAA during fermentation

Variabel	k	Mean EZ	SE	95%CI	df	Q	P value
Yeast	43	-8.061	0.924	(-9.873:-6.249)	42	314.386	< 0.001
BAL	47	-0.148	0.356	(-0.846; 0.549)	46	211.859	< 0.001
BAA	47	0.020	0.391	(-0.746; 0.787)	46	236.462	< 0.001

The results of the heterogeneity test (refer to Table 1) indicated that the effect sizes of yeast were heterogeneous across 43 independent samples (Q = 314.386, df = 42, p <.001). The variability of the effect sizes utilised in this investigation appears to be substantial, as indicated by these results.

The random effect model analysis yielded the following results: the mean effect size for the BAL 47 variable study was -0.148 (p <.001), with a 95% confidence interval spanning from -0.846 to 0.549. A significant difference was observed between the effects of BAL on the control group and the treatment group, according to these findings. The positive effect size number indicates that the experimental treatment has a bigger effect than the control, whereas a negative value indicates the opposite. The impact of size on the variable BAt exhibits a negative value, leading to the conclusion that control exerts a bigger influence than the experimental treatment. The heterogeneity test results (refer to Table 1) indicate that the effect sizes of yeast, derived from 47 separate samples, exhibit heterogeneity (Q = 211.859, df = 46, p < .001). These findings indicate that the variability in impact sizes utilised in this investigation is substantial. Effect sizes of 0.80, 0.50, and 0.20 indicate significant, moderate, and minor effects, respectively. Hence, it can be inferred that the impact of including yeast during fermentation treatment is exceedingly negligible.

The analysis utilising a random effect model revealed that the average impact size of the BAA 47 study variable was -0.148 (p p < .001), with a 95% confidence interval ranging from -0.746 to 0.787. The results indicated a notable influence of BAL on the control group in comparison to BAA on the treatment group. The positive impact size indicates that the experimental treatment has a bigger effect than the control, while a

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ISSN (Online) :2776-1681

negative effect size suggests the opposite. The negative value of the variable BAA indicates that size has a detrimental impact. Consequently, it may be inferred that the control factor exerts a more significant influence compared to the experimental treatment. The results of the heterogeneity test (refer to Table 1) indicate that the effect sizes of yeast are heterogeneous across 47 independent samples (Q = 236.462, df = 46, p <.001). These findings indicate that the variability of effect sizes employed in this study is substantial. Effect sizes of 0.80, 0.50, and 0.20 correspond to significant, moderate, and minimal effects, respectively. Hence, it can be inferred that the impact of yeast addition during fermentation treatment is negligible.

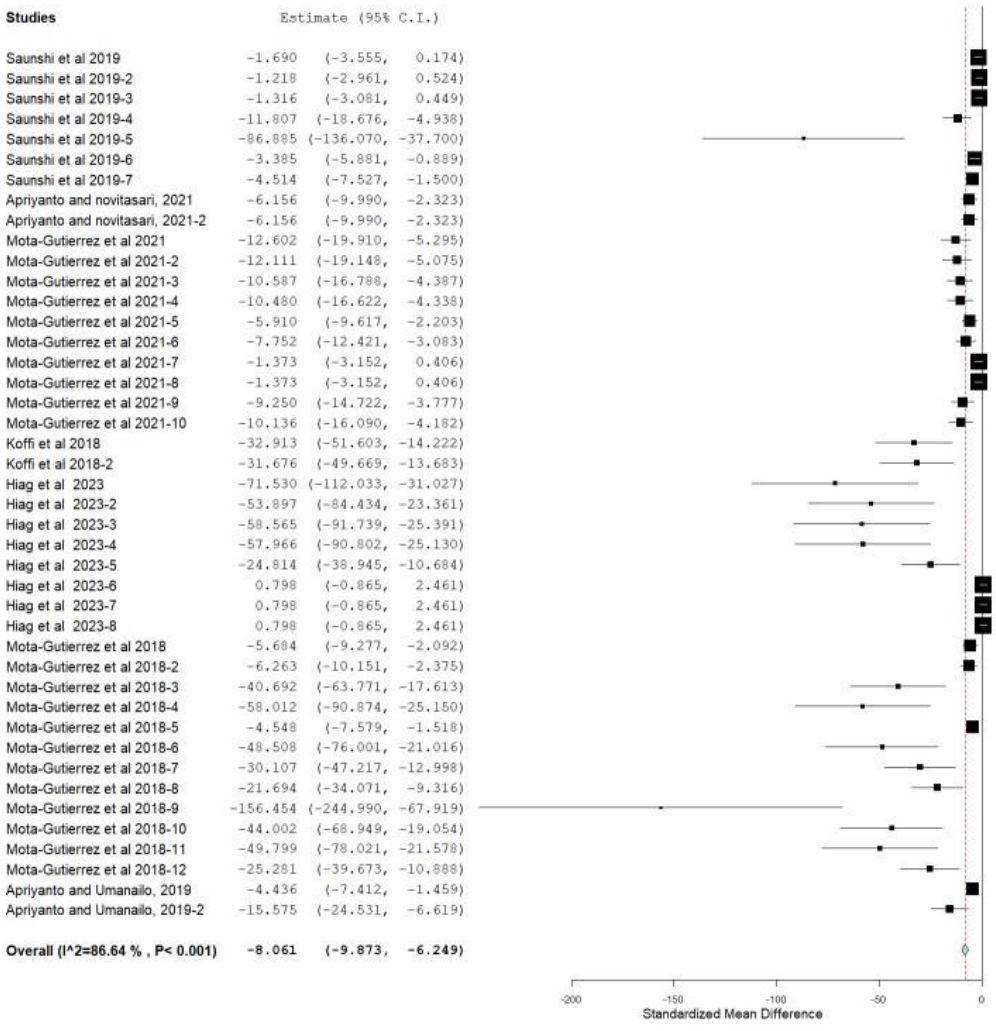

Figure 4. Forest Plot yeast with 43 samples

Figure 4 displays forest plots derived from the analysis of 43 papers utilising a random effect model. In the forest plot effect size, each research is represented by a black square, while the horizontal lines on both sides of the square symbolise the estimated confidence interval. The forest plots revealed a wide range of impact sizes across 43 research, ranging from a minimum of -156,454 to a maximum of 0.798. Several studies have shown adverse size effects, suggesting that yeast growth in

Open Science and Technology
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ISSN (Online) :2776-1681

experimental treatments is not significantly more effective than yeast growth in control groups during fermentation. Overall, forest plots reveal that the majority of the analysed research have a moderate impact size. However, there are certain studies, such as (Hiag *et al*., 2023), that demonstrate a medium effect size. These results suggest that yeast growth is more efficient in the control treatment compared to the experimental treatment during fermentation.

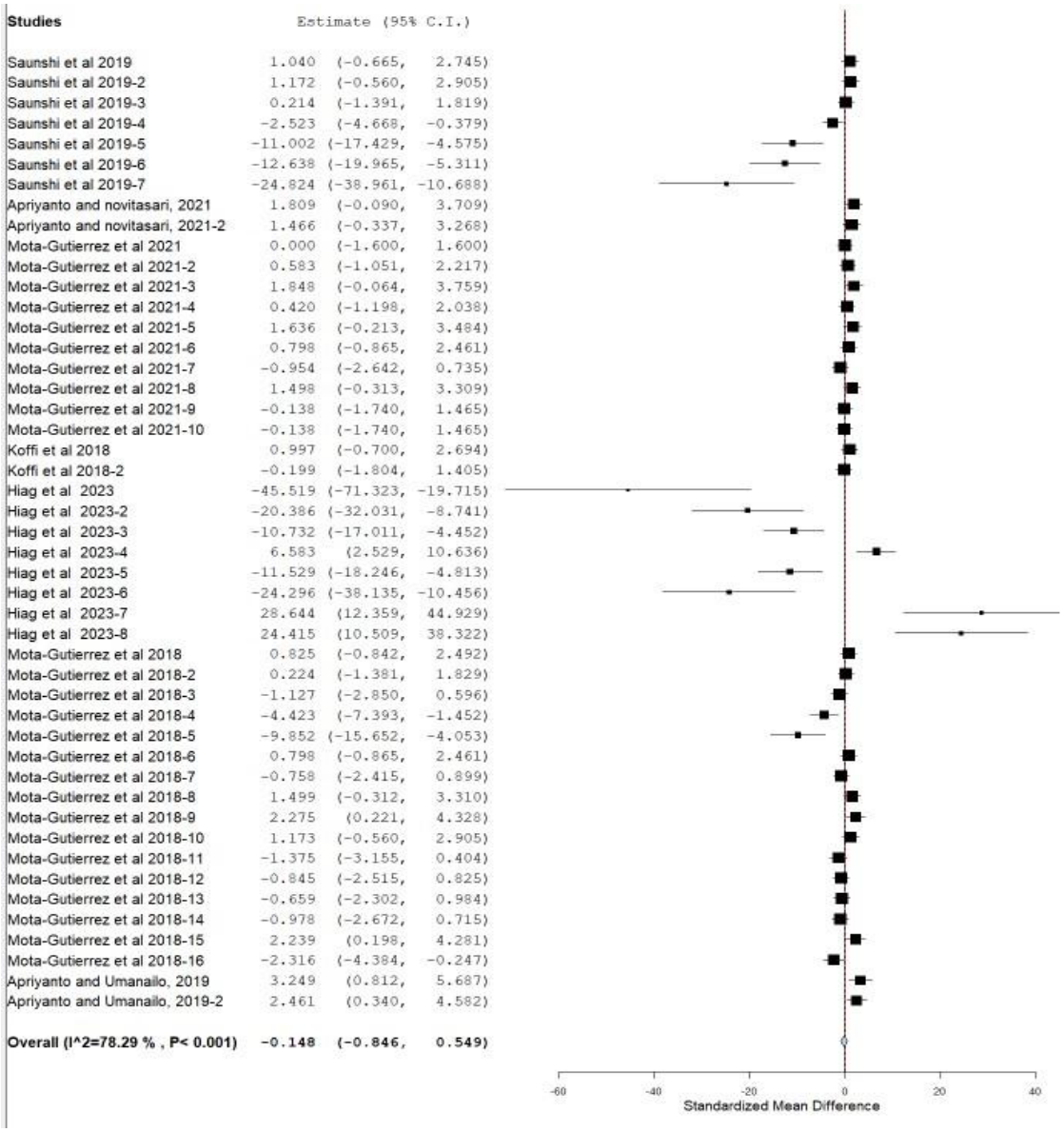

Figure 5. BAL Forest Plot with 47 samples

Figure 5 shows forest plots from 47 studies analyzed using a random effect model. In the forest plot effect size, each study is symbolized by a black square (square dot), while the horizontal lines on both sides of the squared dot indicate the estimated confidence interval.

Forest plots showed that the effect sizes of 47 studies were quite diverse with the lowest effect size at -45,519 and the highest at 28,644. Several studies had negative size

Open Science and Technology
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ISSN (Online) :2776-1681

effects indicating that BAL growth in confirmed trial treatments was no more effective than BAL growth in controls during fermentation. However, in general, it can be seen in forest plots that most of the studies analyzed have a small size effect but there are studies included in the medium size effect, namely (Mota-Gutierrez & Cocolin, 2022) and large size effects, namely (Ooi *et al*., 2020). These studies indicate that BAL growth in control treatment is more effective than experimental treatment during fermentation.

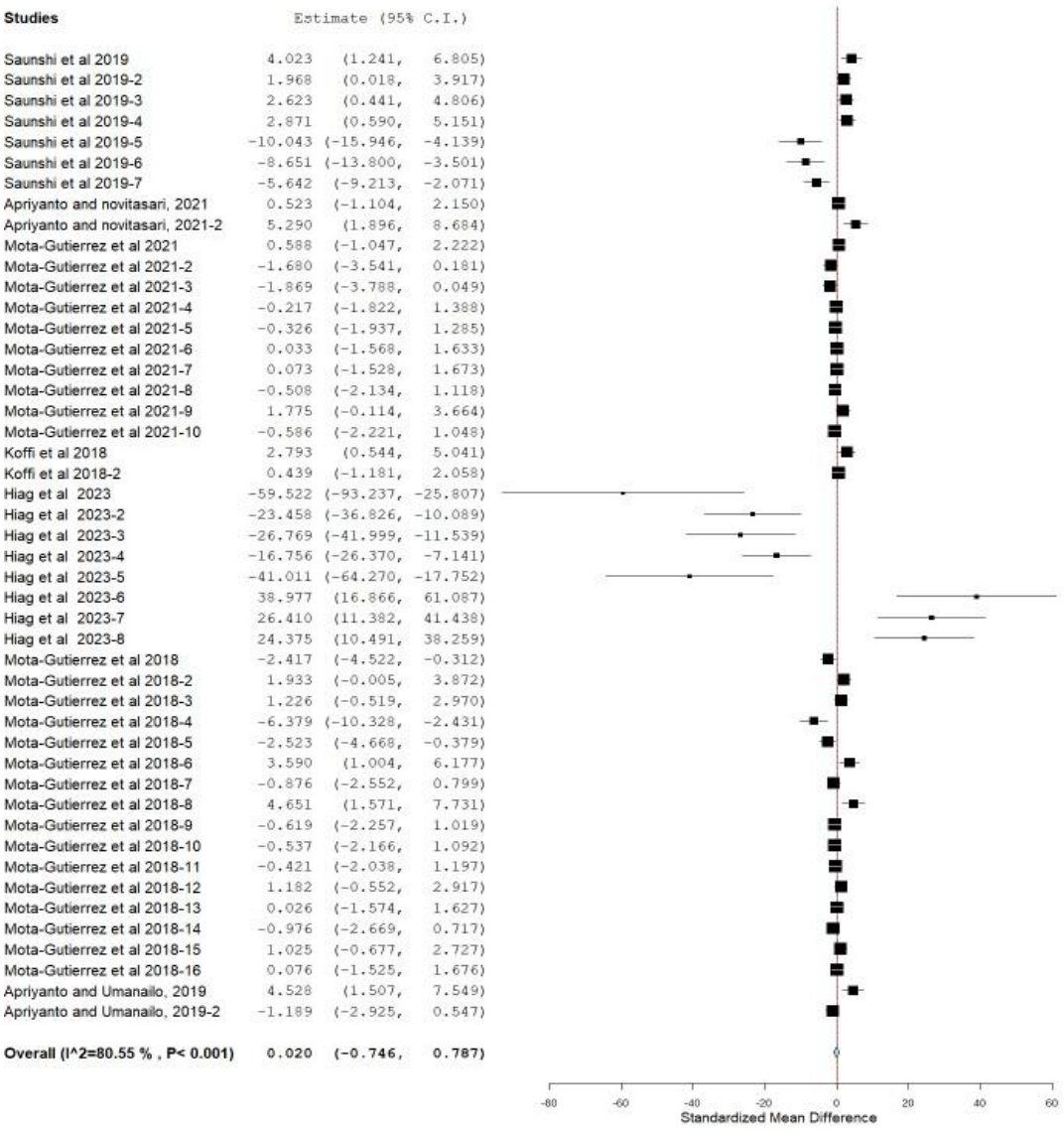

Figure 6. Forest Plot BAA with 47 samples.

Figure 6 shows forest plots from 47 studies analyzed using a random effect model. In the forest plot effect size, each study is symbolized by a black square (square dot), while the horizontal lines on both sides of the squared dot indicate the estimated confidence interval. Forest plots show that the average effect size is 0.02, from 47 studies quite diverse with the lowest effect size at -59,522 and the highest at 38,977. Several studies have negative size effects indicating that BAA growth in confirmed trial

treatments is no more effective than BAA growth in controls during fermentation. However, in general, it can be seen in the forest plot that most of the studies analyzed have a small size effect, namely the (Hernandez & Granados, 2021) study, but there are studies included in the medium size effect, namely (Apriyanto & Novitasari, 2021; Mota-Gutierrez & Cocolin, 2022) the large size effect, namely (Hiag *et al*., 2023). These studies indicate that the growth of BAA in control treatments is more effective than experimental treatments during fermentation.

CONCLUSION

The fermentation process of cocoa beans involves microorganisms such as yeast, BAL, and BAA. Although naturally, these microorganisms will be present during the fermentation process, the addition of starter culture will affect the fermentation process and the final product produced. Based on meta-analysis analysis, experimental treatment of yeast, BAL, and BAA growth during fermentation has a uniform effect. The growth of yeast, BAL, and BAA during fermentation in the experimental treatment was not effective compared to the control treatment. In general, experimental treatment of yeast, BAL, and BAA growth during fermentation has a significant effect.

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