A literature review on cocoa fermentation techniques to shorten fermentation time

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Abstract. Indonesia's cocoa processing industry has a high demand for fermented cocoa but only about 49% of the fermented cocoa is available. Fermentation is critical because it kills the cotyledons and generates aroma precursors. Indonesian farmers, on the other hand, are hesitant to ferment due to the lengthy fermentation process (6–7 days). The purpose of this study is to conduct a review of several modified cocoa fermentation techniques to determine how they can be used to shorten the fermentation time. The database was compiled from articles published in peer-reviewed journals on cocoa fermentation. The literature search was conducted using the website openknowledgemaps.org, which utilized PubMed (life science) to discover full-text articles published in English between January 1990 and January 2021. The results indicated that fermentation time could be decreased by (1) adding inoculum, (2) determining the pH and temperature required to activate an intracellular enzyme, (3) addition of external enzymes during fermentation, and (4) reducing the pulp content. Combining these methods to improve fermentation techniques can enhance the quality of farmers' cocoa beans in a shorter processing time.

1. Introduction
Currently, Ivory Coast, Ghana, Indonesia, Nigeria, Cameroon, Brazil, Ecuador, and Mexico account for 93% of global cocoa production [1, 2]. The Ivory Coast and Ghana produce 60 percent of the world's cocoa, and their ability to supply raw materials has an impact on international cocoa prices [2]. World cocoa prices generally decline each month because of the abundance and established contract system. Every year, tropical storms threaten to damage and reduce production on African cocoa plantations, raising cocoa prices [3]. Changes in cocoa prices around the world have a significant impact on domestic prices. Whenever prices rise, farmers in Indonesia typically sell their cocoa beans immediately, without properly processing them, resulting in poor quality beans. Farmers are also still hesitant to ferment cocoa because the natural fermentation process takes 5–7 days, which is a significant amount of time for them.

Several issues contribute to poor quality cocoa beans, including (1) moldy seeds, (2) infected by the Cocoa Fruit Borer (CFB), (3) incomplete fermentation, and (4) the use of a large number of chemical applications. Moldy bean contamination is caused by inadequate drying. A combination of a large number of chemicals used for controlling pests and diseases in fields, as well as improper harvest and post-harvest processes resulting in significant pesticide residues in the final product. This has caused the
U.S. to impose (automatic detention) cuts in Indonesia’s cocoa bean price since 2005. The beans that are infected by the Cocoa Fruit Borer (CFB) become sticky, black, and crumbly when dried. Incomplete fermentation produced high organic acids that results in a high level of acidity in dry cocoa beans.

The government published Permentan 67 of 2014, which instructs farmers on how to ferment cocoa according to SNI 2323:2008/2010. The government in tend to motivate farmers through several activities, including the establishment of a fermentation business unit at the sub-district level, raising the price of fermented cocoa above the domestic price at the local government cocoa processing unit, and locating a cocoa processing business unit near the farming community. However, after two years of ineffective implementation, this Permentan was canceled.

The demand for fermented cocoa beans in the cocoa processing industry is increasing as a result of the construction of new cocoa processing facilities. According to Airlangga Hartato, the total processing capacity of cocoa production is 800,000 tons per year, but only 49% of raw materials are utilized [4]. The domestic production of cocoa beans has not been able to fill orders for cocoa beans in the industry, in terms of either quantity or quality. The amount of cocoa that can be produced by local farmers is maxed at 340,000 tons [5]. The processing industry blends fermented cocoa beans from import with unfermented (domestic) cocoa beans to provide alternative raw materials for the chocolate industry.

The traditional policy strategy for growing the cocoa sector has been to build a cocoa factory (physical), change fiscal policies (export tax and VAT), and create a price difference policy between fermented and unfermented cocoa (market). Despite the availability of many fermentation technologies, there is still a scarcity of studies investigating fermentation methods for small farmer with low cocoa bean production. The purpose of this study was to conduct a literature review to analyze the development of cocoa fermentation technology and the possibility of accelerating the fermentation process.

2. Materials and methods
The database was compiled from articles published in peer-reviewed journals on cocoa fermentation. The literature search was conducted using the website openknowledgemaps.org, which utilized Pubmed (life science) to discover full-text articles published in English between January 1990 and January 2021. The website will automatically generate a maximum of 100 article papers that are organized by keyword. The search term "cacao fermentation" was used. Additionally, article searches on the Scopus website were conducted using the keywords cocoa, fermentation, inoculation, and heap/spontaneous. Manually search focused on keywords cacao, cocoa, fermentation, and aroma. All full-text article that collected in manual and Scopus was between January 2010 and January 2017 including articles in Bahasa. The results of the articles were then classified into two major categories of fermentation processes: spontaneous and inoculation (starter), and the articles' added value was quantified. In this article, which focuses on fermentation technology, the production potential is examined regardless of whether the process is replaced or accelerated. Some articles do not specifically examine fermentation acceleration, but a change in technique can accelerate fermentation.

A number of 76 articles were identified using openknowledgemaps.org and divided into 15 groups (figure 1). In manual search and Scopus website found 62 articles. Due to duplicate content, 7 articles were removed. Following an initial assessment of the title and abstract, 45 references were deleted due to their topic being irrelevant to the investigation. 12 article also remove due topic related to the isolation of microorganisms from spontaneous fermentation. Following an examination of the research finding 74 articles but only 47 articles showed in Table 1, Table 2, and Table 3.

3. Results and discussion
3.1 Study characteristics
The selected articles that listing on Pubmed from 1990 to 2021 were generated to 15 groups on the website openknowledgemaps.org. In Fig. 1, The yeast group (F) that contained 14 articles was high accumulated articles than the other group. There are numerous publications from the following groups: spontaneous fermentation (K)-12, acetic acid bacteria (M)-10, and aroma formation (D)-6. The central group represents the primary group, while the slices connecting it to the other groups represent
relationships. The fermentation of cocoa is primarily investigated using yeast, with acetic acid bacteria, acetic metabolites, *Bacillus Sp*, and starter cultures also being investigated.

![Figure 1. Map of Cocoa Fermentation Research in the period 1990-2021 generated from openknowledgemaps.org[6], all content retrieved from PubMed.](image)

In Figure 1, information is obtained that the cocoa fermentation process is spontaneous and uses a heap of cocoa with fermentation equipment using a fermentation box, basket and other equipment which is very common. At the inoculation stage of fermentation, the stage of spontaneous cocoa fermentation is frequently used as a control. The inoculation of microorganisms during the fermentation stage aims to determine the addition of the starter with specific results, most notably in the taste produced.

### 3.2 Cocoa fermentation acceleration

#### 3.2.1. Addition of inoculums

Microorganism ecology in cocoa fermentation research takes two approaches. 1) Spontaneous fermentation is induced by microorganisms that grow together naturally. However, determining the contribution of each microorganism to fermentation, bean quality, and cocoa yield are difficult. 2) In controlled fermentation, certain bacterial groups are inhibited and regulated. This method uses a mix of yeast, lactic acid, and acetic acid bacteria that have been isolated from naturally occurring fermentations. Adding specific microorganisms as inoculum can improve cocoa quality or speed up decomposition/fermentation. These processes had a similar microbial succession, processing speed, grain and cocoa quality to spontaneous fermentation. So controlled inoculation could be used to mimic spontaneous fermentation. Table 1 showed articles that used inoculum addition in cacao fermentation.

Yeast inoculation can change the microbial profile, which affects the product's volatile chemical and sensory qualities [7]. Despite never being the dominant yeast (*Pichia kluyveri* and *Kluyveromyces marxianus*) during the fermentation process, the flavors of the two inoculation fermentations differed from the spontaneously fermented control [8]. Batista et al. [9] also compared spontaneous fermentation to the use of a yeast consortium (*Saccharomyces cerevisiae UFLA CA11, Pichia kluyveri UFLA YCH194 and Hanseniaspora uvarum UFLA YCH203*). In comparison to normal fermentation, the addition of a starter (yeast consortia) resulted in a higher conversion of sugar to alcohol and acetic acid. This is because the presence of acetate bacteria in the fermentation box/environment has a significant role in the formation of acetate. Additionally to inoculation, the number of microbial inoculations supplied spontaneously is often greater, requiring less time for adaption, particularly under optimal temperature and pH conditions.

The fermentation time was reduced to three days by adding a 10% (w/v) consortium starter inoculum while preserving uniform fermentation and a cocoa flavor [10]. The inoculum utilized was a mix of *Saccharomyces cerevisiae MTCC 173, Lactobacillus plantarum MTCC 5422*, and *Acetobacter aceti*.
MTCC 3347, which were harvested by centrifugation and mixed with TYGKCC medium (containing of tryptone (0.5%), yeast extract (0.5%), D-glucose (0.1%), K2HPO4 (0.1%), CaCO3 (0.1%), and cocoa pulp (2.0%). A liter of inoculum is required for every 10 kg of cocoa beans. The ideal microorganism growth environment includes 42 °C maximum temperature, 5.5 pH, and 30% seed moisture content. Kustyawati and Setyani [11] also agreed that starting the experiment with a mixture of bacteria resulted in a shorter fermentation period than gradually adding Lactobacillus lactis, Saccharomyces cerevisiae, and Acetobacter acetic on days 1, 2, and 3.

Adler et al. [12] using carbon -13 (13C) succeeded in identifying the metabolic flow of sugar consumed by LAB bacteria. Lactobacillus fermentum can turns fructose into mannitol which is rarely utilized by the main route. Lactobacillus fermentum is also able to convert citrate to acetate, resulting in an increase in pH, which encourages the growth of acetic acid bacteria. Beans fermented with LAB contained higher levels of lactic acid (0.6–1.2 mg/g), while higher levels of acetic acid (1.8–2.2 mg/g) were detected in beans inoculated with AAB [13].

### Table 1. Inoculum addition in cacao fermentation.

| No | Subst. | Var. | Mikroorg. | Method | Enzyme | Aroma | Accelerated | Author |
|----|--------|------|-----------|--------|--------|-------|-------------|--------|
| 1  | 1      | 1    | 1         | 1      | 1      | 1     | [14]        |        |
| 2  | 1      | 1    | 1         | 1      | 1      | 1     | [15]        |        |
| 3  | 1      | 1    | 1         | 1      | 1      | 1     | [11]        |        |
| 4  | 1      | 1    | 1         | 1      | 1      | 1     | [10]        |        |
| 5  | 1      | 2    | 10        | 0      | 0      | 5     | 0           | 10     | [16] [17] [18] [19] [20] |
| 6  | 1      | 1    | 1         | 1      | 1      | 1     | [9]         |        |
| No | Summary | Variable - Result | Accelerated | Author |
|----|---------|-------------------|-------------|--------|
| 7  | Acetic acid bacteria (AAB) and lactic acid bacterium (LAB) were added to the fermenting cocoa pulp-bean mass, speeding up the process of citric acid conversion and lactic acid synthesis. | 1 1 | Yes  No | [21] |
| 8  | A blend of *Saccharomyces cerevisiae* and *Pichia kudriavzevii* was shown to be a promising starter fermentation, reducing the time it took to complete and regulating high-quality components. Using combined yeast cuts fermentation time by 24 hours. | 1 1 | Yes  No | [22] |
| 9  | Inoculation of *S. cerevisiae* UFLA CA11 accelerated the fermentation process. Until 36 hours, carbohydrate consumption and high ethanol production inhibit the growth of harmful microbes. The yeast did not affect the final concentration of acetic acid. | 1 1 | Yes  No | [23] |
| 10 | • The cocktail inoculum is composed of five species and is intended to mimic the natural fermentation process. • Increased temperature tolerance and fermentation capacity in selected *Saccharomyces cerevisiae* strains. • The metabolic and niche adaptations of *L. fermentum* 222 and *L. plantarum* 80 dominate the fermentation process. • The molasses and yeast extract culture media facilitated the growth of *Candida* sp. • *L. plantarum* KSL2 is adaptable to low pH, heat, ethanol, and fermentation conditions. • *Lactobacillus plantarum* 80, *Lactobacillus fermentum* 222, and *Lactobacillus pasteurianus* 386B may now be used as a mixed-strain starter culture to improved fermentation process. • 36 yeast strains demonstrated resilience to alcohol and thermal stress (10-12% alcohol and temperatures up to 40°C), while growth was inhibited at 35°C and alcohol concentrations less than 8-10%. • The initial consortium influenced fermentation and cocoa quality. • The metabolic flux pattern of *A. pasteurianus* 386B was simulated during two phases of growth in cocoa fermentation. • *Pichia kudriavzevii* ECA33 caused a significant amount of fatty acid and amino acid-derived volatile organic compounds, whereas *Saccharomyces cerevisiae* induced a greater amount of fatty acid-derived volatile organic compounds. | 2 0 14 0 0 3 0 15 |  | [24] |
| 11 | Researchers have found that the A19 inoculation of fermented cocoa reduced each OTA-producing species. Organic acids affect fungal development and the synthesis of ochratoxin A. | 0 0 2 0 0 0 2 |  | [34] |
### 3.2.2. Intracellular enzymes optimized during fermentation

Aspartic endoprotease, carboxypeptidase, polyphenol oxidase, invertase, α-galactosidase, α-arabinosidase dan α-mannosidase are all significant enzymes that contribute to generated aroma precursor, distinctive taste of chocolate and significantly reduce the astringent and bitter taste [36, 37]. Kadow et al. [38] investigated the cocoa fermentation process in the absence of microbes using temperature changes and a mixture of microorganism metabolites such as acetic acid and alcohol. The results indicated that the experiment produced more free amino acids (15-25 mg/g ffdm) than was spontaneously created (8.0-14.0 mg/g ffdm). Aseptic artificial fermentation at 45°C for 24 hours with 0.4 g/l of the acetic acid solution, they were able to degrade cocoa protein to the point where it could no longer be detected on the SDS page as a fully fermented bean after three days [39]. Table 2 showed articles which studied the effect of intracellular enzymes in cocoa beans that are optimized during fermentation.

| No | Summary | Variable – Result | Accelerated | Author |
|----|---------|-------------------|-------------|--------|
| 1  | Fermentation-like incubation (with a specified amount of acetic acid, a regulated temperature setting, and no microorganisms), is a viable alternative to fermenting cocoa beans. | Subst. | 1 | 1 | [38] |
| 2  | • Researchers simulated selected microbial strains on cocoa pulp media to understand their roles. <br> • The polyphenol content of fermented cocoa beans reduced from 16.11% to 6.01% after 6 days, while the peptide pattern of unfermented and partially fermented bean powder was quite similar to spontaneously well fermented. <br> • To create cocoa-specific precursors, carboxypeptidase and endopeptidase enzymes must cooperate on cacao-seed protein. | Var. | 2 | 4 | 0 | 4 | 2 | 8 | [40] <br> [41] <br> [42] <br> [43] <br> [44] <br> [45] <br> [46] |

### 3.2.3. Addition of enzymes during fermentation

During spontaneous fermentation, the degradation of cocoa bean pulp is assisted by microbial activity that can release pectinase enzymes into the environment. Pectinase enzyme from *Kluyveromyces fragilis* degraded pulp tissue and caused liquid to leak out of the seed pile into the surrounding environment [47]. According to Ganda-Putra et al. [48], the enzymes pectinase (poligalacturonase) were added to a pulp and the temperature was set to 47.5°C with an initial pH of 4.6. After four days of treatment, it was determined that the fermentation index has increased above one. The increase in the value of the fermentation index was attributed to the change in the purple color in the cocoa chip beans, which turned brown. In the cotyledons of freshly harvested cocoa beans, a tiny number of purple cells spread between the colorless cells. As a result of acid entering the seeds, cells containing anthocyanin (purple pigment) and the enzyme polyphenol oxidase burst. The seeds turn purple due to the bursting of cells carrying anthocyanin. They then turn brown due to the anthocyanin being oxidized by the polyphenol oxidase enzyme.

The abundance of proteolytic enzymes and invertase in fermenting cocoa beans have been determined. Semi-fermented beans have a lower concentration of internal enzymes such as aspartic endoprotease, carboxypeptidase, and invertase than raw beans [43]. Table 3 showed articles which studied the effect of intracellular enzymes in cocoa beans that are optimized during fermentation.
Table 3. Effect of enzymes addition during fermentation.

| No | Research Result | Variable - Result | Accelerated | Author |
|----|-----------------|-------------------|-------------|--------|
|    |                 | Subst. Var. Mikroorg. Method Enzyme Aroma | Yes | No | |
| 1  | Fermentation time was reduced by two days under ideal circumstances when endogenous pectolytic enzymes depolymerized pectin (PG, temperature 47.5°C, starting pH 4.6), | 1 | 1 | [48] |
| 2  | Saccharomyces cerevisiae strain isolated from Malaysian cocoa fermentation with desired technological qualities (high temperature tolerance and at least moderate polygalacturonase activity). | 1 | 1 | [49] |
| 3  | DegU promotes the formation of pectate lyases (Pel) in Bacillus sp during the late stages of cocoa fermentation, when simple sugars are depleted, to supply carbon to microbial cells via polymeric pectic compounds. | 1 | 1 | [50] |

3.2.4. Pulp reduction. Pulp is a suitable medium for the growth of microbes during fermentation, which produces alcohol, acid, and heat. The amount of sugar contained in the pulp depends on the level of ripeness of the fruit and when stored will increase more reducing sugars such as glucose and fructose components. The amount of pulp that can be removed is at least 10% by weight by pressing the cocoa beans so that the pulp will be released from the cocoa beans without any risk of a non-optimal fermentation process [24]. It also reported a reduction in the amount of pulp up to 20% will accelerate fermentation due to faster development in the microbial dominance process, a faster temperature rise and a pH value start point which was previously around 3.5-4.8 to 4.8-5.5 [24]. Reduced acidity can be achieved by reducing the amount of pulp present before fermentation, and this could be a potential solution to the acidity problem.

4. Conclusion
Small farmers require the specific fermentation technology that is faster, easier, and low cost. The result of a literature review on fermentation techniques that result in shorter fermentation times through (1) adding inoculum, (2) determining the pH and temperature required to activate intracellular enzyme, (3) Addition of external enzymes during fermentation, and (4) pulp reduction. Integrating these technologies can increase farmers cocoa bean quality while reducing processing time.

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