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TEMPORAL MICROBIAL FLORA OF FERMENTED CACAO BEANS: ITS EFFECT ON THE SENSORY QUALITIES OF COCOA

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ABSTRACT

Cocoa bean fermentation is a microbially driven process that determines much of the flavor, texture, and quality of cocoa, yet the microbial succession that accompanies different fermentation durations has received little attention in Philippine cacao production. This study investigated the effects of 3-, 5-, and 7-day fermentation periods on the microbial composition and sensory properties of Forastero cacao beans fermented in wooden sweatboxes, using a Completely Randomized Design with three treatments and three replications. Microorganisms were isolated, cultured, and documented through photomicrography, and the resulting cocoa products were evaluated by a sensory panel of 30 participants (10 processors, 10 teachers, and 10 students) for aroma, flavor, acidity, bitterness, astringency, mouthfeel, and overall acceptability. Key microorganisms identified included *Lactobacillus fermentum*, *Acetobacter* spp., *Bacillus subtilis*, and *Saccharomyces cerevisiae*, each playing a role in the organic transformations vital for flavor development. Results indicated that the 7-day fermentation significantly enhanced sensory qualities, with the highest scores for aroma (8.50), flavor (9.00), and overall acceptability (8.80). Statistical analysis (analysis of variance, $p < 0.05$) confirmed reductions in bitterness and astringency, along with improved mouthfeel, as fermentation was prolonged. These findings are consistent with earlier reports that longer fermentation moderates the bitterness and astringency of bulk cocoa. Because the microorganisms were identified morphologically, species names should be regarded as presumptive pending molecular confirmation. Well-fermented beans could offer economic benefits because of their superior taste and reduced defects, which support farmers and processors. Within the conditions of this study, 7 days was the optimal fermentation duration for maximizing cocoa flavor and quality. Further research may focus on recording physicochemical changes, confirming species identities, and exploring microbial interactions to improve consistency and marketability.

Keywords: *Cacao Fermentation, Microbial Succession, Sensory Evaluation, Cocoa Quality, Forastero Cacao, Lactic Acid Bacteria, Acetic Acid Bacteria*

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INTRODUCTION

Background of the Study

Cocoa (*Theobroma cacao* L.) and its derivatives are valued for their aroma, color, and health benefits, making them essential commodities worldwide. Various conventional methods have been used for years to ensure cocoa quality. However, the global demand for cocoa and the criteria for sensory, functional, and safety attributes have recently undergone significant evolution. On the one hand, society and health authorities are increasingly requiring more precise quality control tests, not only analyzing physicochemical and sensory parameters but also assessing functional compounds and contaminants (Thompson et al., 2013; Schwan & Fleet, 2014; Pereira et al., 2016).

On the other hand, increased production prompts industries to adopt quality control techniques that are fast and nondestructive. Finally, a rise in global cocoa demand and prices could lead to future cases of fraud. To address this, new investigations, technologies, and data analysis methods are being researched, developed, and implemented in research or quality laboratories to monitor cocoa quality and authenticity. The main advances in critical techniques focus on creating new, more sensitive methods such as chromatographic analysis to detect metabolites and contaminants in trace amounts.

These methods are used to assess cocoa quality, study new functional properties, control cocoa authenticity, or detect frequent emerging frauds. Regarding non-destructive methods, spectroscopy is the most extensively explored technique, which is conducted within the near-infrared range and, to a lesser extent, within the mid-infrared range. It is primarily applied in the post-harvest stage of cocoa beans to analyze various biochemical parameters or assess the authenticity of cocoa and its derivatives.

Substantial research studies have examined cacao fermentation and its sensory evaluation. However, no studies have been conducted to identify the microbial flora inhabiting the cacao bean during the fermentation process in relation to the sensory qualities of cocoa products. Farmers needed a low-cost, profitable production method. Similarly, buyers and producers preferred fewer days, as demand increases rapidly. The Department of Agriculture in South Cotabato reported low productivity, particularly in cacao processing technology, in various areas of the Philippines, such as Lake Sebu and Sto. Nino and Surallah in South Cotabato province of Mindanao (Muyrong & Dizon, 2020).

Ecological studies to date have shown that a variety of yeast species can be isolated from cocoa bean fermentations. However, only a few species consistently grow and become dominant. These include *Hanseniaspora* (*Kloeckera*) species, which generally initiate the fermentation and then die off, followed by *S. cerevisiae*, *P. kudriazevii*, and *P. menbranifaciens*, with *Pichia* species tending to be more prevalent toward the end of fermentation. A recent comparative survey of yeasts isolated from fermentations conducted in Brazil, Ecuador, Ivory Coast, and Malaysia used both traditional culture methods and culture-independent molecular techniques (Papalexandratou & De Vuyst, 2011; Papalexandratou et al., 2013).

Fermentation is the stage at which the potential for chocolate flavor is created. Microbial activity in the pulp produces ethanol and organic acids that diffuse into the cotyledons, kill the embryo, and trigger enzymatic reactions that generate flavor, aroma, and color precursors, which are fully expressed only after drying and roasting (Schwan & Wheals, 2004; De Vuyst & Weckx, 2016). The process is generally described as a succession in which yeasts, lactic acid bacteria (LAB), acetic acid bacteria (AAB), and, at later stages, aerobic spore-forming *Bacillus* species dominate in turn (De Vuyst & Leroy, 2020). Because spontaneous fermentation depends on the microorganisms that colonize the beans from the surrounding environment, its outcome may differ among batches, farms, and fermentation practices. The duration of fermentation is therefore one of the main levers available to producers for controlling bean quality.

Duration matters in practice. Forastero beans, which make up the bulk of world cocoa production, are commonly fermented for about five to six days, whereas Criollo beans typically require far shorter periods (Afoakwa et al., 2008). A direct comparison of 3- and 7-day fermentations showed that the 3-day beans retained more phenolic

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compounds and that the drink prepared from 7-day liquor had the best sensory notes (Efraim et al., 2010). In Davao del Norte, Philippines, basket fermentation of cacao for five and seven days produced Grade 1A beans, and fermentation duration and quantity significantly affected the proportion of defective beans, acidity, and bitterness (Remitar & Magbalot-Fernandez, 2023). These studies focused on bean quality and sensory outcomes rather than on the microbial communities present at each fermentation stage.

In the Philippines, cacao is grown mostly by smallholders in Mindanao, and the 2017–2022 Philippine Cacao Industry Roadmap set a target of 100,000 metric tons of quality beans by 2022 (Department of Agriculture & Department of Trade & Industry, 2017). Fragmented smallholder production and poor post-harvest practices have been identified as constraints to the competitiveness of Philippine cocoa in the global value chain (Duke Center on Globalization, Governance & Competitiveness, 2017). Improving and standardizing fermentation is therefore a practical entry point for raising bean quality at the farm and cooperative levels.

Microbiological studies of cocoa fermentation come mainly from West Africa, Latin America, Malaysia, and Indonesia (Ardhana & Fleet, 2003; Papalexandratou et al., 2013). To the authors' knowledge, no peer-reviewed study has linked the microbial succession of spontaneous fermentation to the sensory qualities of cocoa produced from cacao grown in South Cotabato. The present study addresses this gap by documenting the microorganisms found in Forastero cacao beans fermented for 3, 5, and 7 days and by relating the fermentation period to the sensory qualities of the resulting cocoa. The findings are intended to give farmers, cooperatives, and extension workers evidence-based guidance on how long cacao beans should be fermented under local conditions.

The study primarily investigates the biochemical changes cocoa undergoes during fermentation, which may indicate cocoa quality. The complex flavor profile of cocoa beans depends on factors such as bean genotype, postharvest treatments like pulp pre-conditioning, fermentation, and drying, as well as industrial processing steps like roasting, along with soil type and the age of the cocoa tree. Many bacteria and fungi inhabit the beans at the start of fermentation until they are ready for drying. These microorganisms present on or acting upon the beans influence flavor development, affecting both the type and quality of the final product. Therefore, this study sought to identify the microbial flora that inhabit cacao beans during fermentation and to relate these to the sensory qualities of the resulting cocoa products.

Conceptual Framework of the Study

The conceptual framework of the study is shown in Figure 1. This study investigates the progression of microbial flora during cacao bean fermentation and its impact on the sensory quality of cocoa. The framework follows a chronological sequence, starting with the types of microorganisms that emerge over time during fermentation.

Initially, yeasts such as *Saccharomyces cerevisiae* dominate the early phase, fermenting sugars into ethanol and carbon dioxide. As fermentation progresses, lactic acid bacteria (LAB) like *Lactobacillus fermentum* and *Lactiplantibacillus plantarum* become prominent, producing lactic acid and further lowering the pH. In the middle to late stages, acetic acid bacteria (AAB) such as *Acetobacter pasteurianus* and *Gluconobacter spp.* oxidize ethanol into acetic acid, raising the temperature and increasing the acidity. *Bacillus* species, including *B. subtilis*, may emerge in the later stages, contributing to the breakdown of proteins and flavor development.

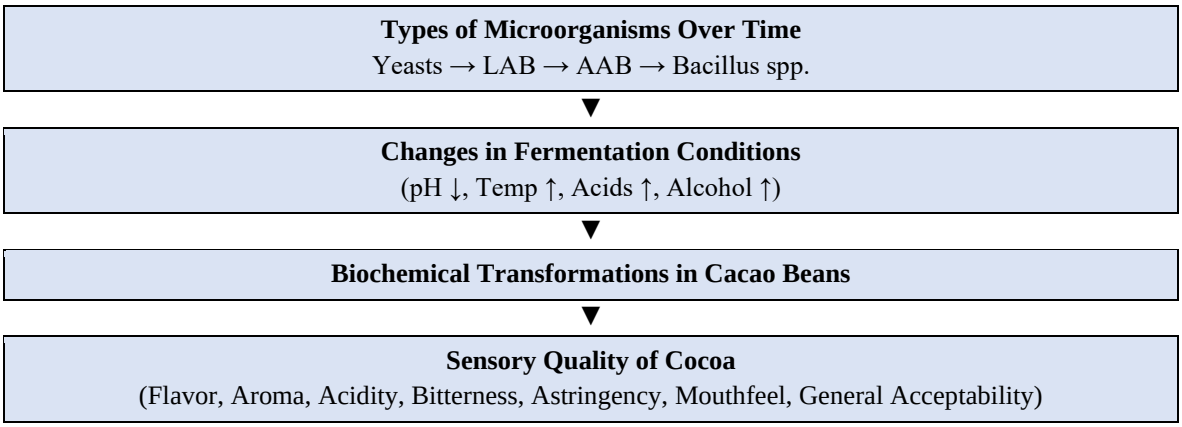
These microbial activities cause notable changes in fermentation conditions, including a decrease in pH, a rise in temperature, and the production of organic acids and alcohols. These changes lead to biochemical transformations within the cacao beans, such as the death of the seed embryo and the formation of precursors for cocoa flavor and aroma.

Ultimately, the interaction between microbial succession and the fermentation environment directly affects the sensory quality of cocoa, shaping key attributes like flavor, aroma, bitterness, and acidity. Understanding this relationship is essential for optimizing fermentation practices to improve cocoa quality.

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Figure 1. Conceptual Framework of the Study



Review of Related Literature

This section reviews the literature and studies relevant to the present research, drawing on books, journal articles, published and unpublished theses and dissertations, and online sources by international and local authors.

Bean Fermentation

Bean fermentation is a critical stage in cocoa processing, as it determines much of the chocolate's final flavor, aroma, and color. According to Thompson et al. (2001), microbial activity during fermentation solubilizes the pulp material surrounding the beans. It produces a variety of metabolic end-products, such as alcohols and organic acids, which diffuse into the beans and cause their death. These internal changes trigger a cascade of biochemical reactions that create the chemical precursors responsible for cocoa's distinctive sensory characteristics.

The significance of fermentation for cocoa quality has long been recognized. Schwan et al. (1995) emphasized that research on cocoa fermentation has been ongoing for more than a century, with numerous studies identifying the microbial species involved in the process. Thompson et al. (2001) summarized that the bean pulp is rich in fermentable sugars, including glucose, fructose, and sucrose, and has a naturally low pH of 3.0–3.5 due to the presence of citric acid. These conditions favor the initial growth of yeasts and lactic acid bacteria. Yeasts such as *Kloeckera* and its teleomorphic form *Hanseniaspora*, as well as *Saccharomyces*, *Candida*, *Pichia*, and *Kluyveromyces* species, initiate alcoholic fermentation. Meanwhile, lactic acid bacteria—including *Lactobacillus*, *Leuconostoc*, and *Lactococcus*—ferment pulp sugars and metabolize citric acid. As fermentation progresses, acetic acid bacteria (*Acetobacter* and *Gluconobacter*) oxidize the ethanol produced by yeasts into acetic acid. Finally, species of *Bacillus* develop as the bean mass becomes less acidic and the temperature rises to 40–50°C due to the heat generated by microbial activity.

Although extensive progress has been made in identifying the microbial diversity associated with fermentation, Schwan (1998) noted that quantitative data on the growth dynamics of individual species remain limited. Such data, when paired with chemical and sensory analyses, are vital to understanding which microorganisms play the most significant roles in flavor and quality development. With this knowledge, it becomes possible to optimize fermentation conditions and even select appropriate microbial starter cultures for consistent, high-quality cocoa production.

Amoa-Awua (2014) described the cocoa fermentation process as largely traditional and spontaneous. After harvesting, pods are manually opened, and the beans—ranging from 50 to 500 kilograms—are heaped on the ground or placed in boxes, trays, or baskets. Fermentation then occurs naturally, driven by indigenous microorganisms that colonize the beans from the surrounding environment. Following fermentation, the beans are sun-dried or mechanically dried until their moisture content drops below 11%. A small amount of microbial activity may persist in the early stages of drying. Biologically, the fermentation process involves both microbial

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reactions in the pulp and the biochemical transformations they induce inside the beans. Factors such as the cocoa cultivar, pod maturity, pod health, postharvest handling, and fermentation methods influence the specific reactions and, consequently, the quality of the resulting cocoa beans. Among these, the chemical and physical composition of the pulp plays a particularly decisive role in determining which microbial species dominate and how they interact.

Lima et al. (2011) further explained that mature cocoa fruits develop directly from the tree's stem as pods containing 30–40 seeds. Each bean comprises two cotyledons with an embryo, enclosed by a seed coat (testa) and surrounded by a sweet, white, mucilaginous pulp that accounts for approximately 40% of the bean's fresh weight. This pulp serves as a rich medium for microbial growth, consisting of 82–87% water, 10–15% fermentable sugars, 2–3% pentosans, 1–3% citric acid, 1–1.5% pectin, and small amounts of other polysaccharides, proteins, amino acids, vitamins (mainly vitamin C), and minerals. Its relatively high pectin and polysaccharide content make the pulp viscous, restricting air diffusion into the bean mass. The pulp's pH ranges from 3.0 to 4.0, depending on citric acid concentration, and this acidic environment strongly influences microbial activity and succession during fermentation.

These studies show that cocoa bean fermentation is a complex biochemical and microbiological process involving a sequence of microbial communities. The interactions among these microorganisms, coupled with the intrinsic properties of the pulp and environmental conditions, ultimately define the flavor potential and quality of the finished cocoa product.

Dynamics of Biological Reactions within the Pulp and Seeds

Early investigations into the chemical changes occurring during cocoa fermentation date back nearly a century. Preyer (1913) was among the first to observe that microorganisms in the cocoa pulp produce ethanol and organic acids, initiating complex biochemical transformations. During fermentation, the temperature of the cocoa bean mass increases from approximately 25–30°C to about 50°C by the end of the process. This temperature rise plays a crucial role in developing the characteristic flavor of chocolate. Researchers later concluded that metabolites generated by microbial activity in the pulp could diffuse through the testa into the seed, while compounds normally present in the seed—such as polyphenols, peptides, alkaloids, sugars, and proteins—could also move outward into the pulp (Knapp, 1937; Roelofsen, 1958; Quesnel, 1966).

Wood and Lass (1985) further explained that the permeability and integrity of the testa determine the movement of microbial metabolites during fermentation. As the process advances, the testa becomes increasingly permeable, allowing key compounds such as ethanol, acetic acid, and lactic acid to penetrate the seeds. These acids, together with the heat generated by microbial metabolism, cause the death of the embryo and a significant reduction in the pH of the cotyledons—from around 7.0 to approximately 5.0–5.5. This acidification is a critical step that initiates major biochemical changes inside the seed.

According to Thompson et al. (2013), the death of the embryo is accompanied by the disintegration of the seed's cellular organization, leading to the activation of two important pH-dependent enzymes in the cotyledons: an aspartic endoprotease and a serine carboxypeptidase. These enzymes, which were previously compartmentalized within the cells, begin breaking down storage proteins into amino acids and peptides. These breakdown products are essential precursors for flavor development during the subsequent roasting stage, when the characteristic chocolate aroma and taste fully emerge.

Leal et al. (2008) added that the combined actions of proteolytic enzymes, along with residual lactic and acetic acids, known respectively as fixed and volatile acidity, serve as useful indicators of fermentation success and overall bean quality. Other enzymes, such as glycosidases and polyphenol oxidases, are likewise activated by fermentation-induced changes. Furthermore, microbial metabolites beyond ethanol and organic acids, including higher alcohols, esters, aldehydes, and ketones, may penetrate the seed and influence its chemical composition and eventual chocolate profile.

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Overall, Thompson et al. (2013) emphasized that microbial metabolism in the pulp creates both substances and conditions that activate a cascade of endogenous biochemical reactions within the seeds. These processes result in the formation of color, flavor, and aroma precursors that define the distinct chocolate character later enhanced during drying and roasting. Thus, the dynamic interaction between microbial activity in the pulp and enzymatic reactions within the seeds is fundamental to the successful fermentation and quality development of cocoa beans.

Source of Microorganisms That Conduct the Fermentation

Ostovar and Keeney (1973) noted that the microorganisms responsible for fermentation are indigenous species that originate as natural contaminants of the process. As long as the cocoa pod remains healthy, undamaged, and intact, it is considered sterile. However, some researchers have isolated yeasts such as *Candida krusei*, a species frequently associated with fermentation, from the inner tissues of apparently healthy, undamaged pods (Maravalhas, 1966; Faparusi, 1974; Jespersen et al., 2005).

During the stages of pod-breaking and bean removal for fermentation, the pulp and beans become contaminated with a variety of microorganisms, many of which contribute to the subsequent fermentation. The contaminating organisms are thought to originate from the outer surface of the pods, the workers' hands, knives, unwashed baskets used for seed transport, dried mucilage left on the walls of boxes from previous fermentations, and various plant materials and vegetation (e.g., banana leaves) that come into contact with the beans (Rombouts, 1952; Ostovar & Keeney, 1973). Jespersen et al. (2005) demonstrated that certain yeast species associated with cocoa bean fermentations in Ghana came from the surface of the pods and from the trays used for fermentation. Similar findings were reported by Ostovar and Keeney (1973) and Camu et al. (2007, 2008) regarding the origins of LAB and AAB. Fermenting cocoa beans can attract masses of flying insects such as fruit flies (*Drosophila melanogaster*), beetles, bees, and wasps, and various authors have suggested these to be potential vectors of microorganisms (Wood & Lass, 1985).

Therefore, based on recent research about the microbiota involved in wine fermentation, Barata et al. (2012) suggested mechanisms by which microorganisms might enter and establish themselves in cocoa bean fermentations. These insights offer a comparative view on how microbial communities colonize and affect fermentative ecosystems across different substrates.

Contribution of Various Microbial Groups to Cocoa Fermentation

There were different studies that profiled the microbial groups in the fermentation of cacao. This section will discuss the three (3) major microbial groups in the fermentation of cacao, which include the yeasts, lactic acid bacteria, and acetic acid bacteria.

Yeasts

Yeasts are among the most commonly isolated microorganisms from fermenting cocoa beans, especially in the early stages of fermentation. They are considered essential to the fermentation process and the development of chocolate flavor. As a result, their role in cocoa fermentation has been studied for over 100 years. Early research on cocoa yeasts has been reviewed by Knapp (1937), Rombouts (1952), Roelofsen (1958), and Forsyth and Quesnel (1963). These studies reported the flora associated with fermentations carried out in the West Indies (Trinidad, Jamaica, Puerto Rico), Southeast Asia (Java, Ceylon), and Africa (Gold Coast). A variety of yeast species was found, primarily in the genera now known as *Saccharomyces*, *Hanseniaspora* (anamorph *Kloeckera*), and *Pichia*. These early studies also showed that yeasts grew successively during fermentation, with *Hanseniaspora* species developing first, followed by *Saccharomyces* species, and then by less fermentative species in *Pichia*, *Candida*, and other genera.

According to Fleet (2003), the unique chemical composition of the cocoa pulp, especially its acidity, is likely to encourage the initial growth of the more acid-tolerant species such as those of *Hanseniaspora*. The increasing concentrations of ethanol and acetic acid, and the increase in temperature to about 50°C as fermentation proceeds, will be the determining factors because some species are not tolerant to such extreme stresses. The early death of

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Hanseniaspora species is probably due to their sensitivity to the increasing ethanol concentrations, but other factors may also be involved. Because of the yeast and bacterial complexity of the fermentation, various forms of interactions between species are also likely to occur and affect the overall yeast ecology, as has been found for wine fermentations.

Lactic Acid Bacteria

Nielsen et al. (2007) and Camu et al. (2008) studied the lactic acid bacteria (LAB) associated with the fermentation of cocoa beans and used molecular methods for species and strain identification; similar profiles were obtained. Both *L. plantarum* and *L. fermentum* dominated the fermentations, with *L. plantarum* more prevalent during the early stages and *L. fermentum* becoming dominant later. Several other species, including newly identified ones, were also isolated from these fermentations, but their presence was generally random and inconsistent. These species included *Enterococcus casseli-flavus, Fructobacillus pseudoficulneus, Leuconostoc pseudomesenteroides, Pediococcus* species, *Lactobacillus cacaonum, Lactobacillus fabifermentans, Lactobacillus ghanaensis, Weissella fabaria, and Weissella ghanaensis.*

Furthermore, Papalexandratou et al. (2011) and Pereira et al. (2013) examined the diversity of LAB species present during cocoa fermentations. The results confirmed the dominance of L. *fermentum* in all fermentations. The other LAB species reported in these studies were generally in accordance with previous results obtained through culture-dependent analysis (Passos et al., 1984a,b; Schwan et al., 1995). These species included *L. plantarum, L. cacaonum, L. mali, L. nagelii, L. brevis, L. vaginalis, L. durianis, Vagococcus carniphilus, Leuconostoc pseudomesenteroides, Weissella sp.,* and *Pediococcus acidilactici; L. plantarum, L. durianis, L. fermentum, L. mali, L. nagelii, L. pseudomesenteroides,* and *Pediococcus acidilactici*; and *L. plantarum, L. vaccinostercus, and Weissella sp.*

Acetic Acid Bacteria

The characteristic vinegar-like aroma of cocoa bean fermentations that occurs after the “alcoholic” phase of fermentation led early investigators to conclude that acetic acid bacteria (AAB) were important contributors to the process (Bainbridge & Davies, 1912; Loew, 1913; Nicholls 913; Knapp, 1937).

Additionally, Roelofsen and Giesberger (1947) conducted the first significant study on the species involved in these fermentations. They tracked the growth of these bacteria during bean fermentation and noted a dominance of *Acetobacter rancens (now Acetobacter pasteurianus)* and *Acetobacter melanogenum* (now *Gluconobacter oxydans*). Since then, many researchers have reported isolating and identifying AAB from cocoa bean fermentations in various countries.

Moreover, in different locations where various studies were conducted, diverse species now classified as A. *pasteurianus, Acetobacter aceti, Gluconoacetobacter xylinus*, and *G. oxydans* were isolated from beans fermented in Trinidad (Ostovar & Keeney, 1973), Ghana, Malaysia (Carr et al., 1979; Carr & Davies, 1980), and Indonesia (Ardhana & Fleet, 2003). Populations of 10^6–10^8 cfu/g of these species have been recorded at various stages of fermentation, with *A. pasteurianus* being the most prevalent and dominant organism.

More recent studies using both cultural and molecular methods have confirmed the prevalence of *A. pasteurianus* in heap and tray fermentations conducted in Ghana, along with *Acetobacter tropicalis* and *Acetobacter syzgii* (Camu et al. 2007, 2008; Nielsen et al., 2007a). As determined by 16S rRNA gene clone library sequencing, Garcia-Armisen et al. (2010) confirmed the dominance of *A. pasteurianus* in Ghanaian cocoa bean fermentations and, for the first time, revealed the presence of *Gluconoacetobacter* species in these ferments. Other newly described species that were less frequently isolated from these fermentations were *Acetobacter fabarum* (Cleenwerck et al., 2008), *Acetobacter ghanensis* (Cleenwerck et al., 2007), *Acetobacter senegalensis* (Camu et al., 2007), and *Acetobacter lovaniensis*- like species (Camu et al., 2008).

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Fermentations conducted in Ecuador, Malaysia (Papalexandratou et al., 2011a, b, 2013), and Australia (Dircks, 2009) also showed a predominance of *A. pasteurianus*. However, *Acetobacter lovaniensis* was the only species recovered from cocoa fermentations in the Dominican Republic (Lagunes-Galvez et al., 2007).

Role of Yeasts in Fermentation

The functional roles of yeasts in contributing to the fermentation process and development of quality of cocoa have been discussed in several reviews.

Ugliano and Henschke (2009) noted that the yeasts initiate an alcoholic fermentation of pulp sugars to produce mainly ethanol and carbon dioxide, and a vast array of secondary metabolites, such as higher alcohols, organic acids, esters, aldehydes, ketones, sulfur, and nitrogen volatiles, as has been well established for other fermented commodities.

Additionally, Pereira et al. (2012) explained that the qualitative and quantitative profile of these metabolites will vary with the species and strain of yeasts that predominate. The ethanol concentration will determine the ecology of the microbiota that subsequently grows in the fermenting bean mass, and it becomes a substrate for the growth of AAB. However, it also passes into the inner tissues of the bean, thereby contributing to its intracellular disorganization and death, along with acetic acid later produced by the AAB. The carbon dioxide that is produced will contribute to the microaerobic conditions of the bean mass, thereby encouraging the growth of LAB.

Fleet (2008) also discussed that the death and autolysis of the yeast cells are likely to provide micronutrients, such as vitamins and amino acids, that also support the growth of these bacteria. Rodriguez-Campos et al. (2012) reported that the secondary metabolites generally have high flavor impact and will also diffuse into the bean to affect cocoa quality.

Schwan et al. (1997) further discussed that several yeast species isolated from cocoa bean fermentations are pectinolytic and are considered to play an important role in the degradation and solubilization of the pulp, especially during the first 24 hours. Silva et al. (2005) supported this by stating that hydrolysis of pectin requires several enzymes, including polygalacturonase, pectin methylesterase, and pectin lyase. This activity decreases the viscous nature of the bean mass and facilitates its mixing and the penetration of air (oxygen), which encourages the growth of AAB.

According to Daniel (2009), the metabolism of organic acids is another mechanism by which yeasts are believed to influence cocoa bean composition and quality. The utilization of citric acid in the pulp and lactic acid produced by the LAB would reduce the acidity of the beans. Among the main species associated with cocoa fermentation, *P. kudriazevii* (*C. krusei*) has the ability to assimilate citric acid and lactic acid.

Ho et al. (2014) likewise noted that, as a group, yeasts play a key role in cocoa bean fermentation. Yeast growth during the fermentation was specifically inhibited by Natamycin, but normal growth of LAB and AAB occurred. Beans from this fermentation did not meet acceptable criteria and gave chocolate of inferior flavor. While several species contribute to the fermentation, it is not known if all of them are essential to the process and whether or not individual species contribute positively or negatively to cocoa bean quality and chocolate character. Controlled fermentations with starter cultures using particular yeast species show that chocolate flavor can vary according to the presence and dominance of particular species.

Role of Lactic Acid Bacteria

The functional roles of LAB during cocoa fermentation have likewise been discussed by several researchers. Pereira (2013) discussed that the sugary, acidic, microaerobic environment of the pulp is conducive to the growth of LAB. Moreover, some species within this group are tolerant of the higher ethanol and temperature conditions that develop during fermentation (e.g., *L. plantarum and L. fermentum*). Metabolically, LAB conduct three major reactions during bean fermentation; they ferment pulp sugars, they utilize citric acid, and some may reduce

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fructose to mannitol. These reactions contribute to changes in the composition of the pulp that impact cocoa bean and chocolate quality and, possibly, the microbial ecology of the fermentation.

Lefeber (2011) also noted that *Homofermentative* species (*L. plantarum, Pediococcus acidilactici, Lactococcus lactis*) ferment pulp sugars via the *Embden–Meyerhof* pathway to produce mainly lactic acid. Heterofermentative species (*L. fermentum. Leuconostoc spp., and Weissella spp.*) ferment the sugars via the hexose monophosphate pathway, which produces a mixture of mainly lactic acid, ethanol, acetic acid, and CO2. Lactic acid can diffuse into the cotyledon and, since it is nonvolatile, it will remain in the bean after drying and roasting. In this way, it will impact the acidity and quality of the beans and chocolate. Thus, a predominance of heterofermentative species is likely to give less acidic beans. The metabolism of fructose into mannitol varies with the strain, with a stronger tendency being found in some strains of L. *fermentum*. The more fructose used by this reaction, then, the less will be available for fermentation into lactic acid, thereby giving less acid beans.

Moreover, De Vuyst et al. (2010) reported that citric acid is utilized by many species and strains of LAB, including those associated with cocoa fermentation. It is largely metabolized into a mixture of lactic acid, acetic acid, ethanol, and diacetyl. Its utilization by LAB is considered the main factor in decreasing the acidity of the pulp and in equilibrating the bean pH to values around 5.0–5.5, which are considered optimal for endogenous proteolytic and other enzymatic activities. Both *L. plantarum* and *L. fermentum* are strong utilizers of citric acid.

Fleet (2003) also discussed that apart from organic acids, LAB are known to produce various other antimicrobial substances such as bacteriocins that could impact the yeast and bacterial ecology of the fermentation, as well as later growth of spoilage or toxigenic fungi in beans during drying and subsequent storage.

Adler et al. (2013) provide a summary of the role of LAB in fermentation, as they noted that LAB are universally found as predominant organisms in cocoa bean fermentations. While a diversity of species has been isolated from these ecosystems, *L. plantarum and L. fermentum* have consistently emerged as the main species contributing to the fermentation. Their production and metabolism of organic acids impact the pH of the bean, and this will modulate the activity of endogenous enzymes that contribute to the generation of chocolate flavor. However, their production of lactic acid is not always seen as a desirable property, as it may lead to excessive bean and chocolate acidity. Their other major property, utilization of citric acid, is a reaction that could be done by yeasts. As mentioned previously, acetic acid is a key player in the overall process of bean death and its consequences in generating the precursors of chocolate flavor.

Role of Acetic Acid Bacteria in Fermentation

Lima et al. (2011) present the functional roles of AAB in the fermentation of cocoa beans. Metabolically, these bacteria conduct two primary reactions: the oxidation of pulp sugars and acids, and the oxidation of ethanol. Although AAB may be present in pulp at the commencement of fermentation, its potential to grow and utilize the pulp sugars would be limited by the absence of oxygen at this stage. Their main growth and activity generally occur after yeast growth and bean mixing, when oxygen has had a greater opportunity to permeate the bean mass.

It is also reported by de Vuyst et al. (2010) that at this time, AAB rapidly utilizes the ethanol produced by the yeasts, oxidizing it to acetic acid and producing concentrations as great as 20 mg/g pulp. This oxidation reaction is quite exothermic, and is considered to be a main factor in causing the temperature of the bean mass to increase to 50°C or more.

Additionally, Copetti et al. (2012) presented several implications of acetic acid for cocoa bean and chocolate quality. First, it diffuses into the bean, where, in combination with ethanol and elevated temperature, it causes cellular disorganization and bean death. These changes, along with the decreased pH, trigger endogenous bean reactions responsible for producing chocolate flavor and color precursors. Acetic acid contributes to the volatile acidity of the bean and, if excessive and not properly removed during subsequent processing (e.g., drying and roasting), it may reduce bean and chocolate quality. Second, acetic acid is antimicrobial, and its presence in the pulp may selectively influence the growth of yeasts, bacteria, and filamentous fungi, which can, in turn, have

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specific impacts on bean and chocolate quality. Indeed, residual acetic acid may limit the growth of spoilage and mycotoxigenic fungi during the drying stage.

Sengun and Karabiyikli (2011) noted that AAB may also oxidize sugars and acids (e.g., lactic acid) to give various metabolites, such as gluconic acid, aldehydes, and ketones, that may affect bean quality and flavor.

In summary, AAB emerges as essential microorganisms of cocoa bean fermentation. *Acetobacter pasteurianus* is the species most frequently recovered from these fermentations, but others, such as *G. oxydans, A. tropicalis, A. lovaniensis, and A. syzgii*, may also be prevalent.

Temporal Microbial Flora

Steinkraus (2004) used the technology of temporal microbial flora (starter cultures) from a spontaneous fermentation to a controlled industrialized process based on the use of well-defined microbial strains as starter cultures to conduct the fermentation. The concept of using starter cultures to conduct cocoa bean fermentations is not new, and the early literature contains several anecdotal discussions of experiments where cocoa bean fermentations were deliberately inoculated with specific microorganisms, mostly yeasts (Knapp, 1937; Roelofsen & Giesberger, 1947; Roelofsen, 1958). At that time, the aim was to induce a faster, more consistent fermentation, and this could be achieved by adding preparations of baker's or brewer's yeasts, without adverse impact on chocolate quality. More specific investigations on the use of starter cultures have now been conducted, where the main goals have been to develop a faster, more consistent fermentation process, which yields cocoa beans with predictable qualities. An additional goal is to have some controlled influence on the sensory character of the final chocolate product through fermentation with particular microbial species or strains.

Following earlier studies of pectin-degrading yeasts isolated from traditional cocoa bean fermentations, Sanchez et al. (1984) and Sanchez et al. (1985) inoculated fermentations with the yeasts *Saccharomyces chevalieri* (now classified as *Saccharomyces cerevisiae), Candida zeylanoides*, and *Kluyveromyces fragilis* (now classified as *K. marxianus*). Freshly extracted beans (70 kg in boxes) were inoculated at 5×10^6 cfu/g with either species. *Saccharomyces chevalieri* and *Candida zeylanoides* were chosen because they produced pectinolytic enzymes and formed part of the natural flora of these local fermentations, and *Kluyveromyces fragilis* (obtained from a culture collection) was selected because of its pectinolytic activity. Although indigenous yeast species also grew throughout these fermentations, the authors observed clear impacts of the inoculated yeasts. Fermentations with *S. chevalieri* were fast, with good pulp breakdown, and yielded chocolate with sensory characteristics similar to those of the control, uninoculated fermentation. Fermentations with *C. zeylanoides* were also successful but gave chocolate of a different flavor; it was still acceptable. Fermentations with K. *fragilis were slow, with weak degradation of the pulp, and these beans yielded* unacceptable chocolate. The authors noted that the inoculated yeasts could impact the species of indigenous yeasts that developed during the fermentations. It was concluded that pectinolytic strains of S. *chevalieri (S. Cerevisiae)* would be the best yeasts for development as a starter culture.

There was a preliminary investigation in Malaysia that reported cocoa fermentations inoculated with *Saccharomyces cerevisiae*, but the experimental design of this study and lack of microbiological analyses precluded any firm conclusion (Samah et al., 1992). With the goal of maximizing the production of cocoa sweatings, Buamah et al. (1997) and Dzogbefia et al. (1999) conducted aseptic laboratory-scale fermentations of cocoa beans using inoculated yeasts. The beans were inoculated singly or with mixtures of *Kluyveromyces fragilis* (*K. marxianus*), *Candida norvegensis* (*Pichia norvegensis*), *Torulopsis* candida (*Candida saitoana*), and *Saccharomyces chevalieri* (*S. cerevisiae*), all of which were selected for their production of pectinolytic enzymes. In addition to presenting data on the yield of cocoa sweatings, the authors noted that the fermented beans had potential for producing good quality chocolate, with the exception of those produced with *K. fragilis*.

Schwan (1998) conducted inoculated cocoa fermentations in Brazil using a mixture of *Saccharomyces cerevisiae* var. chevalieri as the yeast, L. Lactis and *L. plantarum* as the LAB, and *Acetobacter aceti* and *Gluconobacter*

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oxydans as the AAB. All the species had been isolated and characterized from previous spontaneous cocoa bean fermentations conducted at that locality, and the strain of *S. cerevisiae* used was pectinolytic. Fermentations were conducted in boxes containing about 200 kg of beans that had been aseptically extracted from surface-sterilized pods. In one trial, all the species were inoculated simultaneously at time zero, whereas in another trial a phased inoculation was conducted, with the yeast inoculated at time zero, LAB after 24 h, and AAB at 48 h.

The microbial succession, speed of process, and quality of beans (and quality of chocolate) for the inoculated fermentations were generally similar to those of a spontaneous control (uninoculated) fermentation, thereby demonstrating that a controlled inoculated process could mimic a traditional process. The phased inoculation trial was not as effective as simultaneous inoculation. Some indigenous species also developed in the inoculated fermentations, highlighting the practicalities of conducting controlled fermentations on an industrial scale where it is difficult to manage the hygiene of the bean extraction and fermentation facilities.

In contrast to the reports of Sanchez et al. (1985) and Buamah et al. (1997), Leal et al. (2008) reported successful cocoa bean fermentations inoculated with a pectinolytic strain of *K. marxianus*. Box-like fermentations with commercially extracted beans (45 kg) in Brazil were conducted after inoculation with approximately 2 × 106 cfu/g of *K. marxianus*. Although no cultural and population data for yeasts and bacteria were reported for the fermentations, the authors used PCR-DGGE analyses to demonstrate that the inoculated *K. marxianus* had colonized the fermentation. The final dried beans showed a higher degree of fermentation and less acidity than those obtained from parallel, spontaneous fermentations and gave chocolate with a higher sensory acceptability. It was suggested that increased pulp degradation during fermentation by the inoculated yeast enhanced the overall process and contributed to beans of higher quality.

Also, Lefeber et al. (2010, 2011) investigated the growth kinetics and metabolic behavior of cocoa fermentation isolates of *L. fermentum*, *L. plantarum*, and *Acetobacter pasteurianus* in a simulated cocoa pulp medium. They concluded that these species had properties suitable for use as cocoa starter cultures. Selected strains of *L. fermentum* and *A. pasteurianus* were then used in combination with or without a selected strain of *Saccharomyces cerevisiae* to inoculate (105 cfu/g each) commercial-scale heap or box fermentations in Ivory Coast, Ghana, and Malaysia (Lefeber et al., 2012). Although no culture or population analyses were conducted to monitor the microbiology of the fermentations, PCR-DGGE analyses demonstrated the presence of the inoculated species. The study involved many variables and analyses, making it difficult to draw firm conclusions. However, sensory evaluation of the chocolates made from the fermented beans suggested that yeast needed to be inoculated along with the mixture of LAB and AAB to produce acceptable chocolate.

Moreover, the use of the Glucono acetobacter xylinus subsp. Xylinus as the starter strain for fermentation of cocoa beans, without an intermediate LAB fermentation phase, resulted in cocoa beans with a lower pH and higher levels of acetic acid but less cocoa flavor than naturally fermented cocoa beans (Samah et al., 1993).

Dircks (2009) reported the use of specific yeast species as starter cultures for laboratory-scale (5 kg boxes) and pilot-scale (50 kg) fermentations of cocoa beans grown in Queensland, Australia. The yeasts, strains of *H. guilliermondii*, *P. kudriazevii* (*Issatchenkia orientalis*/*Candida krusei*), and *S. cerevisiae*, were selected because they consistently occur in spontaneous fermentations of these beans. A laboratory strain of *K. marxianus* was also used because of its pectinolytic properties and previous association with cocoa fermentations (Schwan, 1998). The laboratory-scale studies were carried out under aseptic conditions—pods surface sterilized, beans aseptically extracted, and fermented. The beans were also inoculated with a mixture of *L. plantarum*, *Pediococcus acidilactici*, and *A. pasteurianus* to introduce the bacterial flora. These bacterial strains had been previously isolated and purified from spontaneous fermentations of the beans. Growth profiles of the individual species were monitored throughout each fermentation, along with chemical changes in the pulp and cocoa nibs. Based on these criteria, all the fermentations were successfully completed.

Beans from fermentations inoculated with a mixture of *H. guilliermondii* and *P. kudriazevii* gave chocolate with a flavor similar to that of an uninoculated, spontaneous control and with high liking scores. Beans from

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fermentations inoculated with a mixture of *S. cerevisiae* and *K. marxianus* or a mixture of *H. guilliermondii*, *P. kudriazevii*, *S. cerevisiae*, and K. marxianusgave chocolates with a different flavor and lower liking scores. These studies were followed up with pilot-scale fermentations in boxes and barrels. In these experiments, the beans were extracted from the pods and fermented under commercial conditions, with no inoculation of the bacterial species, but inoculation with the same mixtures of yeast species (each at approximately 106 cfu/g) as for the laboratory experiments.

Microbiological and chemical analyses indicated that all fermentations were successfully completed and that the inoculated species had implanted the fermentations. Chocolates prepared from beans fermented with a mixture of either (i) *H. guilliermondii* and *P. kudriazevii*; (ii) S. Cerevisiae and *K. marxianus*; or (iii) *H. guilliermondii*, *P. kudriazevii*, *S. cerevisiae*, and *K. marxianus* were all considered acceptable according to sensory criteria, and had higher liking scores than a standard chocolate prepared in parallel from Ghanaian beans. The three yeast combinations gave chocolates with clearly different flavors, which were also different from chocolates prepared from spontaneous fermentation controls. Beans fermented with a mixture of *H. guilliermondii* and *P. kudriazevii* in barrels gave chocolate with the highest liking score. Dircks (2009) concluded that the use of starter cultures for cocoa fermentation could make the process more consistent and faster and that different mixtures of yeasts produced cocoa beans that gave chocolate with distinctively different flavor profiles.

Crafack et al. (2013) conducted tray cocoa bean fermentations in Ghana, inoculated with either a cocktail of selected strains of *Pichia kluyveri*, *L. fermentum*, and *A. pasteurianus* or *K. marxianus*, *L. fermentum*, and *A. pasteurianus*. Molecular identification was used to demonstrate the presence of the inoculated yeasts. Sensory evaluation of the chocolates prepared from beans from the different fermentations showed distinctive differences. Beans fermented with *Pichia kluyveri* gave chocolate with the highest liking score and desirable attributes of sweetness, fruitiness, and chocolate aroma. Chocolate prepared from the *K. marxianus*-fermented beans was considered to be more bitter, sour, and astringent.

Most of the starter culture research on cocoa bean fermentation has focused on inoculation with yeasts. It seems from the data so far that yeasts are essential to the fermentation and development of chocolate character, although this may be modulated by the species or strain of yeast that is used. Consequently, more research is needed to determine how different yeast species/strains impact the finer properties of chocolate flavor, as has been well established for other fermented products such as beer, wine, and bread (Fleet, 2007). However, very little is known about the flavor impacts or, indeed, essentiality of the LAB and AAB. Controlled studies with inoculated species and strains of these bacteria are required. While the functionality of AAB in producing the acetic acid that has a major role in killing the beans seems essential, the functionality of the LAB is less evident. In contrast, their presence and contribution to bean acidity through the production of lactic acid could be detrimental to the process. Similarly, controlled fermentations with inoculated *Bacillus* species are also needed to better define their role, possibly in contributing to pectin degradation in the pulp and production of desirable pyrazines. Also, the lipolytic enzymes produced by these bacteria could affect the fat structure and composition of the bean and resultant chocolate quality.

Many of the controlled, inoculated studies of cocoa bean fermentations have also reported the growth of indigenous yeasts and bacteria during these fermentations (Sanchez et al., 1985; Schwan, 1998; Lefeber et al., 2012; Crafack et al., 2013). If an industrialized starter culture process is to be successful, it will be necessary to minimize and manage such uncontrolled impacts and to provide conditions that will favor the growth and predominance of the inoculated species. Two issues are important in this context. One is the selection of appropriate strains of yeasts, LAB, and AAB that are capable of growing and exhibiting the appropriate metabolic activities under the stressful environmental conditions of the cocoa fermentation ecosystem (e.g., tolerances to acidity, increasing ethanol concentration, increasing temperature to 50°C, and changing redox potential) as discussed by Lefeber et al. (2010, 2011) and Pereira et al. (2012a).

The second issue is the capacity to control the microbiological hygiene of the bean processing and fermentation environments in order to minimize indigenous contamination. Bean extraction processes that minimize bean

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contact with the outer surfaces of the pods will be important in giving a bean mass with a low initial microbiota. The design and construction material of the fermentation vessel would also be expected to have a major influence. Significant criteria, here, are cleaning and sanitation, mixing and aeration of the bean mass, and temperature control—bearing in mind the importance of achieving a phased increase of temperature up to 45–50°C during the process. Well-designed vessels constructed of stainless steel or plastic, that could be easily cleaned, would be an advance on wooden boxes or baskets.

Dircks (2009) conducted inoculated fermentations with 50–75 kg of beans in food-grade, plastic compost barrels that were mounted on a frame to enable their manual turning for mixing and aeration. When housed in a room controlled at 30°C, the bean mass readily increased to 45–50°C. As mentioned previously, beans processed in these barrels gave good-quality chocolate. Pereira et al. (2013) have conducted spontaneous cocoa fermentations in a mechanized, rotatable stainless-steel fermenter (50 kg bean capacity) that enabled controlled mixing and aeration of the beans. The microbial ecology of the fermentation was comparable to that in the traditional box process. The extent of mixing and aeration in the new fermenter needed to be controlled, as these processes caused dissipation of heat in the bean mass, thereby preventing the fermentation from increasing to 45–50°C. It was concluded that this type of design could be further developed for controlled inoculated fermentations.

The design of fermentation vessels is not only important for future starter culture technologies, but is also equally important for better controlled, spontaneous fermentations. In this context, it is relevant that the beans are extracted and prepared for fermentation in a manner that maximizes their contact with the pod surface and environmental surroundings in order to provide a load of indigenous microbiota that is essential to conduct the fermentation (Schwan, 1998; Schwan & Wheals, 2004).

Fermentation Duration and Cocoa Bean Quality

The length of fermentation that yields good cocoa depends on the cacao type. Afoakwa et al. (2008), in a critical review of flavor formation, reported typical fermentation periods of five to six days for Forastero and one to three days for Criollo, and noted that the aroma compounds formed during roasting increased with fermentation time. Regional practice varies as well; Malaysian fermentations, for example, last about six days.

The study that is closest in design to the present work was conducted by Efraim et al. (2010), who compared 3- and 7-day fermentations combined with sun or artificial drying. Most phenolic compounds, including total phenolics, flavan-3-ols, and procyanidins, were lost during fermentation, and the 3-day beans retained higher phenolic levels. A drink prepared from the 7-day liquor had the best sensory notes. Because phenolic compounds contribute to bitterness and astringency, these results explain why short fermentations tend to yield harsher cocoa.

Calvo et al. (2021) followed fermentations of five to six days at three locations in Colombia. Epicatechin fell by 51%, 78%, and 71% by day 5, theobromine declined from 9.5–11 g kg^{-1} in fresh beans to about 8.7 g kg^{-1}, and bean acidity peaked on day 5. The authors concluded that temperature and pH profiles were the key quality-control variables, with a pH of 5.0–5.5 being optimal for the enzymes that release aroma precursors.

Fermentation time has also been shown to be the most influential variable shaping both microbial community structure and the volatile profile of cocoa beans; turning came second, and pod storage had a minor effect (Hamdouche et al., 2019). A systematic review and meta-analysis found that most spontaneous fermentations lasted 96–144 hours, or four to six days (Taylor et al., 2022).

The optimal duration is not universal, however. In Caquetá, in the Colombian Amazon, wooden-box fermentations sampled on days 4 and 7 showed declines in total phenolics, flavonoids, and antioxidant activity with time, and some sites displayed over-fermentation notes at 7 days while others gave favorable profiles (González et al., 2024). In the Philippines, the 5-day, 50 kg basket treatment was the most profitable in Davao del Norte, although 7 days also produced Grade 1A beans (Remitar & Magbalot-Fernandez, 2023). These findings suggest that the best duration should be established for each bean type, fermentation vessel, and production environment.

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Microbial Succession in Box Fermentations

Box fermentation in Southeast Asia has been characterized in some detail. In Malaysian box fermentations, well-performed batches were dominated by *Hanseniaspora opuntiae*, *Saccharomyces cerevisiae*, *Lactobacillus fermentum*, and *Acetobacter pasteurianus* (Papalexandratou et al., 2013). Another Malaysian study described a conserved core microbiota in which apiculate yeasts dominated early, and *S. cerevisiae* dominated late, with *A. pasteurianus* the only AAB isolated (Meersman et al., 2013). In Indonesia, the first two to three days of fermentation consisted of a succession of filamentous fungi, yeasts, LAB, and AAB, while later stages were dominated by *Bacillus*, mostly *B. pumilus* and *B. licheniformis* (Ardhana & Fleet, 2003).

In wooden boxes holding about 100 kg of beans in Ecuador, microbial diversity peaked at 72 hours and fell sharply by 96 hours, and *Pichia kudriavzevii* was among the most abundant fungi at the start of fermentation (Tigrero-Vaca et al., 2022). The indispensable role of yeasts was demonstrated by Ho et al. (2014), who showed that when yeast growth was suppressed with natamycin, the beans were under-fermented and the chocolate lacked the higher alcohols and esters derived from yeast metabolism. It should be noted that recent taxonomic revisions have renamed several species, so that *Lactobacillus fermentum* and *Lactobacillus plantarum* now appear in the literature as *Limosilactobacillus fermentum* and *Lactiplantibacillus plantarum* (De Vuyst & Leroy, 2020).

Bacillus Species and Food Safety Considerations

Bacillus species appear mainly in the late, less acidic stage of fermentation and are not uniformly beneficial. In Côte d'Ivoire, 90 of 98 *Bacillus* isolates from cocoa fermentation were pectinolytic, suggesting a role in pulp degradation (Ouattara et al., 2008). A later study identified six species, namely *B. subtilis*, *B. pumilus*, *B. sphaericus*, *B. cereus*, *B. thuringiensis*, and *B. fusiformis*, and found that the best pectate lyase producers were *B. fusiformis*, *B. subtilis*, and *B. pumilus*, whereas *B. cereus* and *B. thuringiensis* generally showed low activity (Ouattara et al., 2011). In box fermentation in Nigeria, *Bacillus* species persisted until 168 hours, or seven days (Obinze et al., 2022).

Bacillus cereus is a toxigenic pathogen, and its spores can survive processing. In simulated cocoa fermentations, *B. cereus* spore counts increased by about 2–3 log units per gram (Pereira et al., 2020). Spore heat resistance in cocoa beans was high, with decimal reduction times (δ-values) of 148.5, 55.9, and 23.3 minutes at 110, 125, and 140 °C, respectively, which exceed values reported in aqueous media (Pereira et al., 2019). Roasting therefore cannot be relied upon to sterilize cocoa beans, and good hygiene during fermentation and drying remains essential.

Starter Cultures and Controlled Fermentation

Proof-of-concept studies show that the choice of starter culture influences the speed and community dynamics of fermentation and the flavor of the chocolate (De Vuyst & Weckx, 2016). A cocktail of *S. cerevisiae*, *Lactiplantibacillus plantarum*, and *A. pasteurianus* was proposed as a starter for hybrid cocoa (Moreira et al., 2017), and starter mixtures were able to steer fermentations in Costa Rica (Díaz-Muñoz et al., 2021). A comparison of inoculated and spontaneous fermentations in Brazil, with identification by MALDI-TOF mass spectrometry of 873 isolates, found that chocolate from spontaneous fermentation was dominated by bitterness, whereas chocolate from inoculated fermentation was bitter, astringent, and acidic (Miguel et al., 2017). These results indicate that knowledge of the natural microbial succession is a prerequisite for designing local starter cultures.

Sensory Evaluation and Hedonic Scaling

Consumer acceptance of cocoa products is commonly measured with hedonic scales. The 9-point hedonic scale was introduced by Peryam and Pilgrim (1957), and a review of hedonic scaling concluded that it works simply and effectively when the goal is to measure hedonic differences and predict acceptance (Lim, 2011). Parametric analysis of variance of such data is generally justified by large samples, around 100 or more consumers per product (Lim, 2011), and the routine treatment of hedonic data as interval data has been criticized, with alternatives such

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as ranking proposed (Wichchukit & O'Mahony, 2015). ISO 11136:2014 provides guidance for conducting hedonic tests with consumers in a controlled area.

Affective tests, which measure liking among consumers, are distinguished from descriptive tests, in which trained panelists rate the intensity of attributes (Lawless & Heymann, 2010). Many cocoa studies use trained panels; for example, Calvo et al. (2021) used seven trained panelists to score cocoa, astringency, bitterness, acidity, and fruity, floral, and nutty notes. Studies that use untrained panels, such as the present one, are best interpreted as measures of consumer perception.

Identification of Cocoa Fermentation Microbiota

Culture-dependent methods remain widely used, but they have known limits. The identification of 1,126 isolates from Bolivia and Brazil by MALDI-TOF mass spectrometry recovered LAB such as *L. plantarum* and *L. fermentum*, yeasts such as *H. opuntiae* and *S. cerevisiae*, and AAB such as *A. pasteurianus*, *A. fabarum*, *A. ghanensis*, and *A. senegalensis*, and the authors noted that cocoa microbiota had often been identified by time-consuming biochemical phenotyping (Miescher Schwenninger et al., 2016). *Bacillus* species have been resolved with amplified ribosomal DNA restriction analysis and 16S rRNA gene sequencing (Ouattara et al., 2011), and combining culture with amplicon sequencing detects taxa that plating misses (Tigrero-Vaca et al., 2022). Identification based on colony and cell morphology alone can therefore assign isolates reliably only to broad groups such as yeasts, LAB, AAB, and spore formers.

Synthesis and Research Gap

Taken together, the literature shows that cocoa fermentation follows a recognizable microbial succession; that longer fermentation reduces phenolic compounds and, in many studies, improves sensory quality; and that the best duration depends on cacao type and local conditions. It also shows that *Bacillus* species, which become prominent late in fermentation, deserve attention for both their enzymatic benefits and their safety risks. What is lacking is evidence that links the microbial flora at specific fermentation stages to the sensory qualities of cocoa produced from Philippine Forastero cacao. The present study contributes such evidence for South Cotabato.

Objectives and Hypothesis of the Study

The general objective of the study is to identify the different microbial flora that inhabit the cocoa bean during the entire duration of cacao bean fermentation in relation to the sensory qualities of the cocoa product.

Specifically, this study aimed to:

1. Identify the different microbial flora that inhabit the cacao beans during the fermentation period; and

2. Evaluate the sensory qualities of cocoa products as influenced by the action of the microbial flora across the fermentation period, particularly in terms of:

    2.1 aroma;
    2.2 flavor;
    2.3 acidity;
    2.4 bitterness;
    2.5 astringency;
    2.6 mouthfeel; and
    2.7 overall acceptability.

The following null hypothesis was tested:

H_0: The temporal changes in microbial flora during cacao fermentation have no significant effect on the sensory quality of cocoa, indicating that microbial activity does not play a critical role in flavor, aroma, or texture development.

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Significance of the Study

This study is significant as it provides valuable insights into the role of microbial succession during the fermentation of Forastero cacao beans and its impact on the sensory quality of cocoa. Understanding this relationship is crucial for cacao farmers, chocolatiers, and food scientists, as it could lead to improvements in fermentation practices, quality control, and flavor development in cocoa production.

Specifically, this study is essential in terms of the following:

Enhancement of Cocoa Quality. By analyzing the microbial flora at different fermentation phases, this study can help identify beneficial microorganisms that contribute to desirable sensory attributes such as rich flavor, balanced acidity, and optimal texture.

Improved Fermentation Techniques. Understanding microbial succession can help farmers and processors refine fermentation methods to enhance flavor consistency and reduce off-flavors, leading to higher-quality cocoa beans.

Scientific and Industrial Applications. This study provides scientific data on the role of microorganisms in cocoa fermentation, which can be useful for food scientists, microbiologists, and the chocolate industry in optimizing fermentation conditions for better chocolate production.

Support for Sustainable Agriculture. By improving fermentation efficiency through microbiological insights, this research can help small-scale cacao farmers produce premium-quality cocoa, increasing their market value and economic sustainability.

Contribution to Sensory Science. This study expands knowledge on how microbial activity influences the biochemical pathways that develop cocoa's sensory characteristics, contributing to food sensory research and the chocolate industry's flavor profiling.

Philippine Cacao Industry. The findings provide locally generated evidence that supports the post-harvest strategies of the Philippine cacao industry, particularly the clustering of smallholders around shared fermentation facilities and the standardization of fermentation practice.

Extension Workers and Cooperatives. Extension workers and farmer cooperatives may use the results to advise growers on the fermentation duration that gives the best sensory outcome and to emphasize hygiene during fermentation and drying.

Future Researchers. The microbial inventory and sensory data may serve as a baseline for studies that apply molecular identification, record physicochemical changes, or develop starter cultures suited to Philippine cacao.

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METHODS

This section presents the research design, the locale and scope of the study, the materials, the experimental layout and procedures, the flow of gathering data and specimens, and the statistical analysis employed.

Research Design

The study consisted of two sub-studies: (1) the identification of the temporal microbial flora of fermented cacao beans and (2) the sensory evaluation of cocoa products derived from beans fermented for varying periods. The study was laid out in a Completely Randomized Design (CRD) with three treatments replicated three times.

The treatments were as follows:

T_1 –Cacao bean fermented for three (3) days

T_2 –Cacao bean fermented for five (5) days

T_3 –Cacao bean fermented for seven (7) days (standard practice).

A Completely Randomized Design (CRD) assigns treatments entirely at random to experimental units and is appropriate when the units are relatively uniform, as were the nine sweatboxes used in this study (Gomez & Gomez, 1984). A CRD with three replications has also been used in cacao fermentation research in the Philippines (Remitar & Magbalot-Fernandez, 2023). The three fermentation periods bracket the five to seven days commonly reported for Forastero beans (Afoakwa et al., 2008) and add a shorter, 3-day treatment.

The experimental layout of the nine fermentation boxes (three treatments × three replications; T = treatment, R = replication) is shown in Figure 2.

Figure 2. Experimental Layout of the Study

T3R1	T3R2	T2R1	T3R3	T1R2	T1R3	T2R2	T2R3	T1R1

Time and Place of the Study

This study was conducted in two research locations. The microbiological analysis for the identification of microorganisms inhabiting the cacao beans during fermentation was carried out at the Biological Science Building, Rooms BS B-225 and BS D-113, University of the Philippines – Los Baños (UPLB), Laguna. Meanwhile, the evaluation of the sensory qualities of the fermented cacao beans was conducted in Barangay San Felipe, Tantangan, South Cotabato, from January to February 2025.

Scope and Limitation of the Study

This study was limited only to the microbial flora present in the fermentation of cacao beans. It focused solely on documenting and identifying the microbial flora that inhabits the cocoa beans throughout the fermentation period and evaluating the cocoa product based on its sensory qualities**.**

Materials

For sub-study 1, the materials used included cacao beans, baskets, a fermentation box, banana leaves, a camera, record books, a pen for data and specimen recording, and a microscope to examine the microflora on a larger scale.

On the other hand, sub-study 2 used cacao beans, roasting machines, packaging materials, knives, face masks, hair nets, dash trays, tasting cups, and soup spoons.

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Sub-Study 1: Temporal Microbial Flora of Fermented Cacao Beans

Harvesting. The Forastero cacao pods were carefully harvested from Kablon Farm in Tupi, South Cotabato. They were inspected for ripeness before being clipped from the trees with sharp knives or machetes to avoid damaging the branches. The harvested pods were then gathered in baskets and transported to a central processing area.

Breaking of Pods and Removal of Placental Tissue. Once the pods were opened, the wet beans and pulp were carefully extracted by hand. The placental tissue, which connects the beans inside the pod, was gently separated and discarded. This step ensured that only clean, high-quality beans were collected for fermentation. After removal, the extracted beans were placed in baskets and immediately transferred to fermentation containers to maintain freshness and optimize flavor development.

Assignment of Cacao Beans in Sweat Wooden Boxes. The extracted Forastero cacao beans were carefully collected and assigned to sweat wooden boxes for fermentation. These wooden boxes, traditionally used for their ability to retain heat and allow proper aeration, played a crucial role in the fermentation process. After the beans were placed inside, they were evenly distributed to ensure uniform fermentation.

Fermentation. Cacao beans were placed in wooden sweatboxes for fermentation. A total of nine sweatboxes, each measuring 30 cm by 30 cm by 15 cm, were used across three treatments, with three replications per treatment. The wooden slats allowed for air circulation and proper drainage.

To ensure uniform fermentation, beans were covered with banana leaves and turned every two days until the designated fermentation period was completed. The only variation among treatments was the fermentation duration.

Preparation of Sample Beans for the Isolation of Microbial Flora

Samples of cocoa beans were collected at different fermentation stages—three, five, and seven days. Sufficient quantities were carefully gathered and transported to the laboratory for microscopic examination and microbial isolation. A small scrape from each sample was used to isolate and identify the microorganisms present.

Preparation of Culture Media

a. Potato Dextrose Peptone Agar (PDPA). Potato Dextrose Peptone Agar (PDPA) was prepared by boiling peeled potatoes to extract nutrients, then filtering the infusion and enriching it with dextrose for energy, peptone for nitrogen, and agar for solidification. The mixture was heated until dissolved and sterilized in an autoclave to eliminate contaminants. After cooling, it was poured into sterile Petri dishes under aseptic conditions. Once solidified, the PDPA was ready for isolating and identifying microorganisms in fermented cacao beans.

b. Potato Dextrose Agar (PDA). Potato Dextrose Agar (PDA) was prepared by boiling peeled potatoes to extract nutrients, then filtering the infusion and combining it with dextrose as a carbohydrate source and agar for solidification. The mixture was heated until fully dissolved, sterilized in an autoclave to remove contaminants, and cooled before being poured into sterile Petri dishes under aseptic conditions. Once solidified, PDA was ready for isolating and identifying fungi and yeasts in fermented cacao beans.

Isolation of Microorganisms

The isolation of microorganisms that inhabit the cacao fermentation process was done at the National Institute for Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños (UPLB), Los Baños, Laguna, Philippines.

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Preparation of Pure Culture

The pure culture media were obtained by spreading bacteria on a solid medium, ensuring that a single cell occupied an isolated area of the agar surface. Through repeated multiplication, the cell formed a visible colony of identical clones, allowing for further study and identification.

Identification and Photo Documentation

Photomicrography was used to document the microorganisms living in the cacao beans during fermentation at the University of the Philippines Los Baños (UPLB). The identification of specific bacterial and fungal species was validated using illustrated taxonomic guides, ensuring accurate classification of the microbial communities involved throughout the fermentation process.

Sub-Study 2: Sensory Evaluation of Fermented Cacao Beans Fermented in Varying Periods

Drying cocoa beans. Cocoa beans were sun-dried on a wooden floor. The drying area, measuring 2 m × 2 m, could hold 5–8 kg of beans. Typically, 50 kg of beans require about 2.8 square meters of space. The beans were spread evenly to a depth of about 5 cm and raked with a wooden palette into long ridges, exposing the drying floor between them. This setup ensured even sun exposure for optimal drying.

Roasting. The fermented cocoa beans were evenly spread on a tray and placed on the middle rack of the oven. They were roasted for 10 minutes, stirring halfway through. Roasting was considered complete when a rich, brownie-like aroma filled the kitchen. The beans were then immediately removed from the oven and left to cool for 10 minutes. Once cooled, the shells were gently cracked and removed by twisting them between the fingers.

Breaking and Winnowing. Breaking and winnowing of fermented cocoa beans were done to produce nibs. The beans were either poured into a breaking device or manually cracked using a rolling pin or hammer. The resulting nibs were then winnowed to separate them from the shells. The roasted fermented cocoa beans were stored and made ready for grinding.

Grinding. The roasted nibs were processed using a grinding machine. The nibs were cooled first before grinding, which was done six times to obtain consistent quality.

Packaging. After the cacao beans were ground into cocoa liquor, the chocolate-making process continued with several key steps. First, the cocoa liquor was refined to reduce particle size, resulting in a smoother texture. This refined cocoa was then conched, a process involving continuous mixing and aeration at controlled temperatures to develop flavor and enhance smoothness. Next, the conched cocoa was tempered by carefully cooling and reheating to stabilize cocoa butter crystals, ensuring a glossy finish and proper snap. Finally, the tempered cocoa was poured into molds and cooled to solidify into the final product.

These steps were essential in transforming ground cacao into high-quality chocolate with desirable texture and flavor.

Sensory Evaluation

The sensory evaluation was conducted at Barangay San Felipe, Tantangan, South Cotabato. The sensory quality of cocoa fermented in different time-based processes was evaluated using the 10-Point Sensory Evaluation Scale for Fermented Cocoa Beans (Table 1). During the evaluation, the samples were properly coded to avoid switching of treatment identities. The samples were placed on top of the table for evaluation. Instructions and directions were provided on the evaluation sheet on how to rate the cocoa samples. Descriptors of the samples of cocoa were evaluated using the 10-Point Sensory Evaluation Scale for Fermented Cocoa Beans.

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Respondents of the Study

Thirty respondents evaluated the sensory qualities of cocoa products fermented over three different days. The evaluators, who were randomly selected, comprised ten Grade 12 TVE – Food Processing students, ten faculty and staff members from San Felipe Elementary School, and ten cocoa processors or food technologists. Score sheets were used to assess the cocoa products.

Because the panel combined food-processing professionals and lay consumers, the evaluation represents a mixed affective panel rather than a trained descriptive panel (Lawless & Heymann, 2010). The three groups were intended to capture the perceptions of those who process cocoa, those who teach in the community, and those who consume cocoa products.

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Research Instrument

Sensory quality was rated on the 10-Point Sensory Evaluation Scale for Fermented Cocoa Beans presented in Table 1. The scale describes, for each sensory attribute, the characteristics corresponding to each score range.

The rating format is related to the hedonic scaling tradition (Peryam & Pilgrim, 1957; Lim, 2011) but uses ten points and provides descriptors for each range of scores, so that panelists can anchor their ratings to a defined quality description. The scale is applied to the seven attributes listed in Table 1.

Table 1. 10-Point Sensory Evaluation Scale for Fermented Cocoa Beans

Sensory Attribute	1.0 - 2.4 (Poor)	2.5 - 4.4 (Below Average)	4.5 - 6.4 (Acceptable)	6.5 - 8.4 (Good)	8.5 - 9.4 (Very Good)	9.5 - 10.0 (Excellent)
Aroma *(Intensity & pleasantness of cocoa scent)*	Very weak, unpleasant, strong unwanted odors	Slight cocoa aroma, but weak or slightly off	Moderate cocoa aroma, acceptable but not rich	Strong cocoa aroma, pleasant & well-developed	Rich, deep cocoa aroma with slight room for improvement	Exceptionally rich, premium cocoa aroma
Flavor *(Balance of cocoa taste & desirable flavors)*	Unpleasant, harsh, overly acidic or bitter	Weak cocoa flavor, slightly sour or off-tasting	Moderate cocoa taste with minor imperfections	Well-balanced cocoa flavor with minimal bitterness	Strong cocoa taste with well-developed notes	Premium cocoa taste, full-bodied & smooth
Acidity *(Sourness level in cocoa beans)*	Extremely sour, overpowering acidity	Strong acidity, slightly unpleasant	Moderate acidity, noticeable but balanced	Mild acidity, contributing positively to flavor	Smooth acidity with only minor variations	No excess acidity, perfectly smooth & well-rounded
Bitterness *(Presence of bitter compounds in cocoa)*	Extremely bitter, harsh, & unpleasant	Strong bitterness, overwhelming the cocoa taste	Noticeable bitterness, but tolerable	Mild bitterness, blending well with cocoa flavor	Balanced bitterness with slightly intense notes	Perfectly balanced bitterness, enhancing richness
Astringency *(Drying/puckering sensation in the mouth)*	Very high astringency, rough, & unpleasant	Strong astringency, interfering with flavor	Moderate astringency, noticeable but tolerable	Mild astringency, blending well with other attributes	Well-controlled astringency, slightly present	Minimal or no astringency, smooth & pleasant
Mouthfeel *(Texture & smoothness of cocoa)*	Very rough, grainy, & unpleasant	Slightly rough, lacking smoothness	Moderate smoothness, slightly dry	Smooth & velvety texture	Near creamy texture with minor dryness	Exceptionally creamy & well-balanced
Overall Acceptability *(General preference based on all attributes)*	Very poor, undesirable	Below average, not recommended	Acceptable but needs improvement	Good, enjoyable, & well-balanced	High-quality cocoa with slight refinements needed	Excellent, premium-quality cocoa

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References for the scale:

ISO 11136:2014 - Sensory Analysis — Methodology — General Guidance for Conducting Hedonic Tests.

Afoakwa, E. O. (2016). *Chocolate Science and Technology*. Wiley Blackwell.

Beckett, S. T. (2008). *The Science of Chocolate*. Royal Society of Chemistry.

Prawira, M. A., & Barringer, S. A. (2009). Cocoa Bean Fermentation and Its Effect on Sensory Quality. *Journal of Food Science*.

Explanation of the scale:

- **1.0 - 2.4 (Poor):** Unpleasant characteristics, strong defects
- **2.5 - 4.4 (Below Average):** Noticeable imperfections, not desirable
- **4.5 - 6.4 (Acceptable):** Moderate quality, some minor defects
- **6.5 - 8.4 (Good):** Well-developed qualities, minimal defects
- **8.5 - 9.4 (Very Good):** High-quality attributes with minor enhancements needed
- **9.5 - 10.0 (Excellent):** Premium quality, exceptional characteristics

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Data Gathered

Sub-Study 1: Temporal Microbial Flora of Fermented Cacao Beans

Cacao fermentation is a dynamic process characterized by a succession of microbial populations that drive essential biochemical changes. This study monitored bacterial communities at three key fermentation stages—Day 3, Day 5, and Day 7—to understand their role in flavor development and overall fermentation progress.

Day 3 (Early Fermentation Stage): The fermentation process begins with the dominance of *lactic acid bacteria* (LAB), which initiate acidification and create favorable conditions for microbial succession.

Day 5 (Peak Fermentation Stage): *Acetic acid bacteria* (AAB) become predominant, oxidizing ethanol into acetic acid, generating heat, and contributing to essential flavor precursor formation.

Day 7 (Late Fermentation Stage): Residual AAB gradually declines, while spore-forming bacteria (e.g., *Bacillus* spp.) become more dominant, aiding in organic matter decomposition and stabilization of the fermentation process.

These findings highlight the development of microbial succession, emphasizing its significance in optimizing fermentation practices for enhanced cocoa quality.

Sub-Study 2: Sensory Evaluation of Fermented Beans

This sub-study evaluates the sensory quality of fermented cacao beans using a 10-Point Hedonic Scale. A panel of 30 evaluators (processors, teachers, and students) assessed key attributes, including aroma, flavor, acidity, bitterness, astringency, and mouthfeel. The data collected were:

Aroma: 8.2 (High intensity and pleasant cocoa scent)
Flavor: 8.0 (Well-balanced cocoa taste with desirable notes)
Acidity: 5.0 (Moderate sourness contributing to flavor complexity)
Bitterness: 4.5 (Within acceptable limits, not overpowering)
Astringency: 4.8 (Mild drying/puckering sensation)
Mouthfeel: 8.3 (Rich texture and smoothness, enhancing the experience)
Overall Acceptability: 8.6 (Indicating improved sensory qualities through controlled fermentation)

Operational Definition of Terms

In this study, key terms are defined operationally to ensure clarity and precision in interpretation. These definitions specify how terms are applied within the research context, particularly in examining the fermentation phases of cocoa beans and their impact on the sensory qualities of the Forastero cacao hybrid. By establishing clear definitions, this section helps prevent ambiguity and enhances the consistency of the study's findings. Moreover, the following terms are defined based on their relevance to the study's objectives and methodologies.

Cacao pods are the fruit of the cacao plant and contain the seeds, known as beans, which are used to produce chocolate. Each pod holds 30-50 seeds.

Cocoa is a tropical tree native to the Amazon jungles. It has a dense canopy, with fully green adult leaves. Its flowers, which grow on the stem or branches, are white or rosy. The fruit is a pod. Usually, the cocoa tree grows from 6 to 20 meters tall.

Cocoa bean is the almond inside the cocoa pod, healthy, clean, already fermented or not, dried, husked, and without mucilage (COVENIN 50: 1995, 2nd revision).

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Dry cocoa beans are the grains that have been evenly dried and have a moisture content between 7 and 8% (COVENIN 50: 1995, 2nd revision).

Fermentation is the process of removing the slime or mucilage from cocoa and producing precursor substances of chocolate flavor and aroma within the almond.

Fermented bean is the almond that, when cut lengthwise by the cross section, shows on both sides of the almond well-defined and deep grooves, with a very fragile dark brown husk (forestero and trinitarian cocoas) and light brown (Criollo cocoas).

Forastero is one of the three main bean types commonly known alongside Criollo and Trinitario. Strains of Forastero make up 90% of the world's chocolate production. Although known as a "bulk" bean, Forastero strains have their own unique characteristics and are by no means inferior.

Good Agricultural Practices (GAP) are a set of principles, standards, and technical recommendations applicable to the production, processing, and transportation of food. They aim to ensure hygiene, protect human health and the environment, and use methods that are ecologically safe, hygienically acceptable, and economically feasible.

Grinding is a stage in the chocolate production process. Roasted cocoa nibs are ground for different durations to release cocoa butter and create cocoa liquor or mass.

Hedonic Scale is a scale that measures how much respondents like or dislike something.

Nibs are roasted, de-shelled cocoa bean pieces.

Roasting is an essential step in chocolate production. It adds additional character to the flavor and can have a major impact on the chocolate's profile. Roasting helps separate the outer husk from the inner bean, making cracking and winnowing much easier. It also virtually sterilizes the cocoa bean. This is rather important as the conditions in which cocoa beans are fermented are naturally full of bacteria, fungi, and molds.

Sensory evaluation is a science that measures, analyzes, and interprets people's reactions to products as perceived through their senses. It helps determine whether product differences are noticeable, the reasons for these differences, and if one product is more liked than another.

Temporal relates to a term involving measured time or time in laboratory studies.

Varietal is the type of cocoa bean used in making chocolates. Some chocolate bars use a single origin bean, while others blend different varietals together. Though Criollo, Trinitario, and Forastero are the most common names seen, there are actually many other strains of cacao cultivated worldwide.

Winnowing is the process of removing the roasted cocoa nibs from the shell.

Statistical Treatment of Data

Data gathered were analyzed using Analysis of Variance (ANOVA) for two factorial experiments in Completely Random Design (CRD). The Least Significant Difference (LSD) was employed to compare the significant differences among the treatment mean values.

Treatment means were separated using the Least Significant Difference (LSD). With only three treatments, Fisher's protected LSD, applied when the F-test is significant, is a defensible procedure for pairwise comparisons (Gomez & Gomez, 1984). In the results, means that share a common superscript letter are not significantly different at $p < 0.05$, and the coefficient of variation (CV%) is reported as a measure of the variability of the data.

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RESULTS AND DISCUSSION

This section presents the results of the experiment based on the actual observations made during the conduct of the study, together with the analysis and interpretation of the data gathered. Tables are presented to reveal the significant findings.

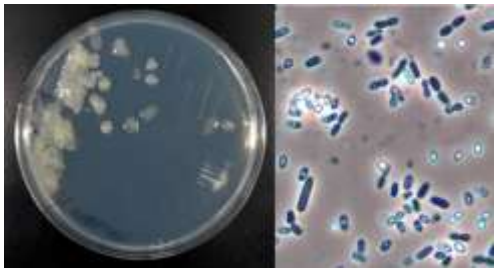
Temporal Microbial Flora of Fermented Cacao Beans at Day 3, 5, and 7

Microbial succession drives essential biochemical changes during cacao fermentation, influencing cocoa quality. At Day 3, *Acetobacter pasteurianus* and *Acetobacter fabarum* (Figures 3 and 4) dominated, oxidizing ethanol into acetic acid and generating heat (50–55°C), which halts embryo growth and triggers key flavor precursor formation (Schwan & Wheals, 2004). The rising acidity accelerates pulp degradation, improving aeration and fermentation uniformity.

Concurrently, *Lactobacillus fermentum* and *Lactiplantibacillus plantarum* (Figures 5 and 6) contributed to acidification by breaking down residual sugars, fostering optimal conditions for microbial succession (Ardhana & Fleet, 2003).

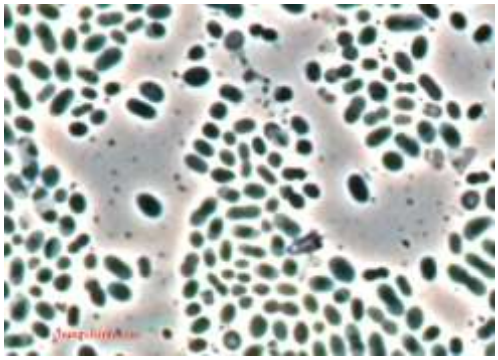
The yeast *Saccharomyces cerevisiae* and the acetic acid bacterium *Gluconobacter* spp. (Figures 7 and 8) further aided fermentation. *S. cerevisiae* converted pulp sugars into ethanol, fueling acetic acid production, while *Gluconobacter* spp. assisted in sugar metabolism and early acetic acid formation (Pereira et al., 2012).

Figure 3. ***Acetobacter pasteurianus***



Type: Bacteria
Traits: Needs oxygen, turns alcohol into vinegar (acetic acid)
Role: Raises temperature and acidity; helps develop chocolate flavor

Figure 4. ***Acetobacter fabarum***

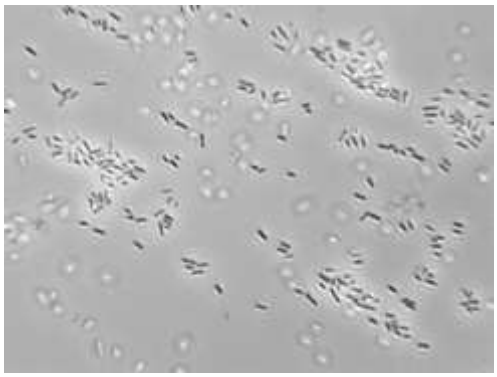


Type: Bacteria
Traits: Acid- and heat-tolerant, needs oxygen
Role: Converts alcohol to acid; adds heat and sour taste

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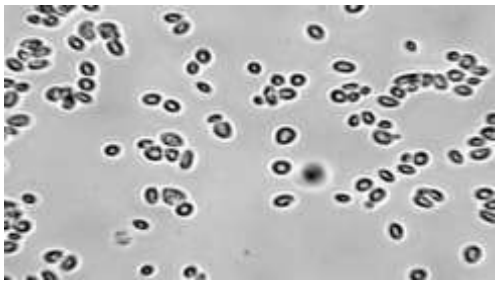
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Figure 5. ***Lactobacillus fermentum***



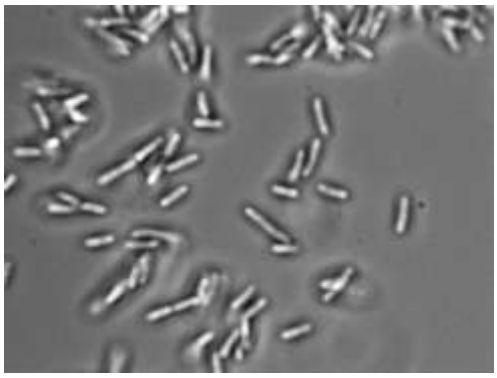
Type: Bacteria
Traits: Works without oxygen, loves low pH
Role: Makes lactic acid, lowers pH, helps flavor and food safety

Figure 6. ***Lactiplantibacillus plantarum***



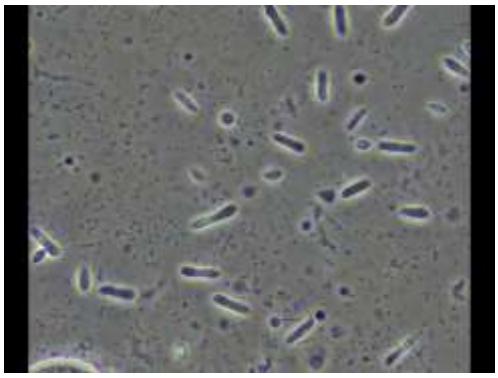
Type: Bacteria
Traits: Very adaptable, grows in low oxygen
Role: Starts fermentation early; makes acid and controls spoilage

Figure 7. ***Saccharomyces cerevisiae***



Type: Yeast
Traits: Ferments sugar into alcohol and gas
Role: Begins fermentation; provides alcohol for bacteria to make acid

Figure 8. ***Gluconobacter spp.***



Type: Bacteria
Traits: Needs oxygen; produces gluconic acid
Role: Adds acidity and unique flavor

By Day 5, fermentation was at its most intense phase. *Acetobacter spp.* (Figure 9) inhabited the surface layers in the fermentation of cacao beans, where oxygen was more available. These bacteria continued converting ethanol into acetic acid, producing heat that broke down polyphenols, lightened the beans, and activated key enzymatic reactions (Schwan & Wheals, 2004). These changes were essential for developing cocoa's characteristic flavors.

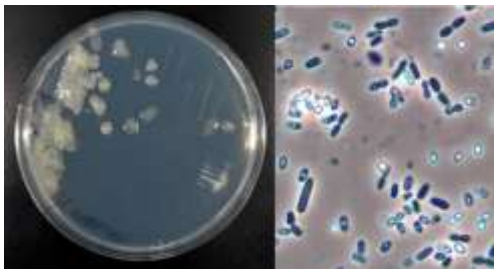
At the same time, *Bacillus* species—including *Bacillus subtilis, Bacillus cereus,* and *Bacillus coagulans* (Figures 14, 15, and 16) were also found. Their role in breaking down organic material further supported fermentation and improved aeration (Ho et al., 2014). Increased airflow during this stage also helped stabilize pH, reduce sourness, and prevent unwanted off-flavors, making the process more controlled. However, if fermentation lasted too long, acidity levels could become too high, or mold could develop, affecting cocoa quality (Lefeber et al., 2010).

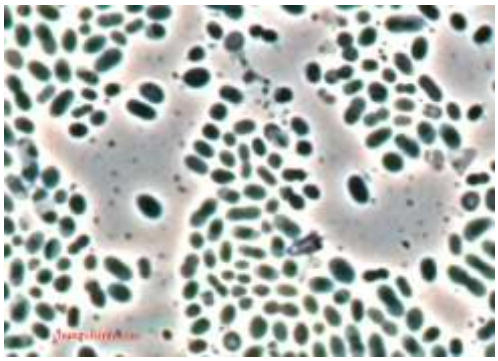
Other microorganisms were also present at this stage. There were *Lactobacillus plantarum* (Figure 13), which remained active in the pulp-rich areas, contributing to acid production and maintaining fermentation balance, and *Pichia kudriavzevii* (Figure 12) and *Gluconobacter spp.* (Figure 11) which were still present in the sugary pulp layers, helping to break down sugars and support the chemical changes needed for flavor formation.

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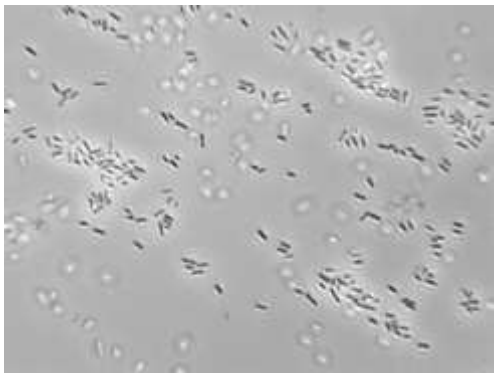
Figure 9. ***Acetobacter spp.***





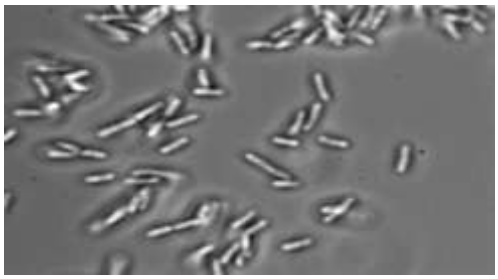
Type: Bacteria
Traits: Needs oxygen; turns alcohol to acetic acid
Role: Increases acidity and heat; essential for flavor development

Figure 10. ***Lactobacillus fermentum***



Type: Bacteria
Traits: Works without oxygen, loves low pH
Role: Makes lactic acid, lowers pH, helps flavor and food safety

Figure 11. ***Gluconobacter spp.***

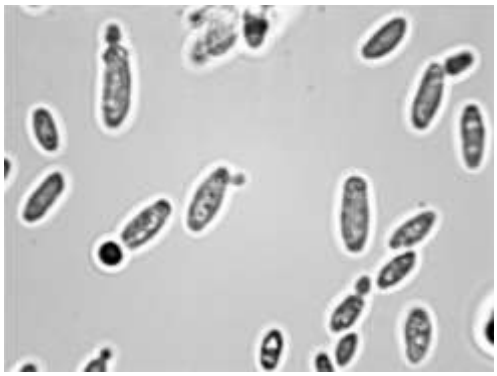


Type: Bacteria
Traits: Needs oxygen; produces gluconic acid
Role: Adds acidity and unique flavor

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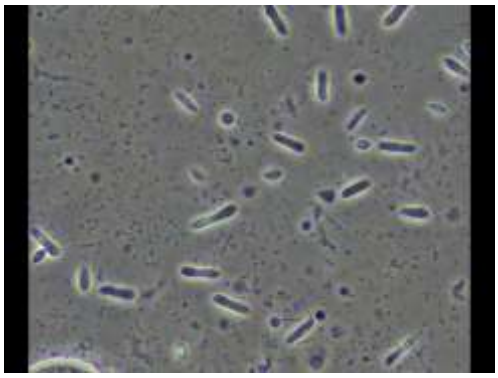
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Figure 12. ***Pichia kudriavzevii***



Type: Yeast
Traits: Grows in heat and acid; adds aroma
Role: Helps break down pulp and boosts fruity flavor

Figure 13. ***Lactiplantibacillus spp.***



Type: Bacteria
Traits: Acid-tolerant; produces lactic acid
Role: Lowers pH, improves taste and safety

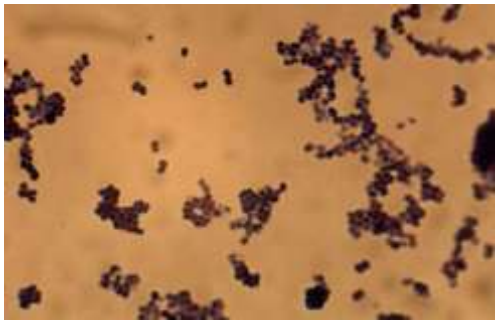
On Day 7, microbial activity gradually declined as fermentable compounds became fully depleted. As a result, acetic acid bacteria began to subside, while *Bacillus subtilis*, *Bacillus cereus*, and *Bacillus coagulans* (Figures 14, 15, and 16) inhabited the fermenting cacao mass, actively facilitating the breakdown of residual organic material (Ho et al., 2014).

Meanwhile, a slight increase in pH occurred due to acetic acid evaporation, which in turn reduced sourness and refined the final flavor. Additionally, the beans gradually developed a deep brown color, indicating the completion of biochemical transformations. At the same time, the reduction in *lactic acid bacteria* and *yeast* populations confirmed the transition toward stabilization.

Furthermore, among the *Bacillus* species (Figures 14, 15, and 16), *Bacillus subtilis* enhanced enzymatic reactions, thereby improving the overall quality of the beans. Likewise, *Bacillus cereus* and *Bacillus coagulans* played a crucial role in organic matter decomposition and flavor enhancement (Camu et al., 2008).

Figure 14. ***Bacillus subtilis***





Type: Bacteria
Traits: Forms spores; produces enzymes
Role: Breaks down proteins and starch; adds flavor

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Figure 15. ***Bacillus cereus***



Type: Bacteria
Traits: Spore-forming; can be harmful
Role: Unwanted; may spoil food or cause illness

Figure 16. ***Bacillus coagulans***



Type: Bacteria
Traits: Heat-tolerant, makes lactic acid
Role: Supports fermentation; adds acid and stability

Comparison with Previous Studies on Microbial Succession

The succession reported here follows the general pattern described in the literature, in which the yeasts, LAB, AAB, and *Bacillus* appear in turn (Schwan & Wheals, 2004; De Vuyst & Leroy, 2020). Most of the species recovered, including *A. pasteurianus*, *L. fermentum*, *L. plantarum*, *S. cerevisiae*, and *P. kudriavzevii*, are regarded as core members of the cocoa fermentation microbiota. The recovery of *A. fabarum* is also consistent with earlier work in which this species was identified among AAB isolates from Bolivian and Brazilian fermentations (Miescher Schwenninger et al., 2016).

One difference deserves comment. In Malaysian box fermentations, apiculate yeasts such as *Hanseniaspora opuntiae* dominate the first hours, and *S. cerevisiae* becomes prominent later (Papalexandratou et al., 2013; Meersman et al., 2013). In the present study, the earliest sample was taken at Day 3, by which time AAB and LAB were already prominent. In Ecuadorian box fermentations, microbial diversity peaked at 72 hours and then declined (Tigrero-Vaca et al., 2022), which suggests that samples taken before 72 hours would be needed to document the initial yeast-dominated phase in South Cotabato fermentations.

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The temperature of 50–55 °C attributed to AAB activity at Day 3 is slightly higher than the roughly 50 °C commonly cited for the bean mass in the literature reviewed above. Because mass temperature, pH, and acidity were not monitored in this study, the figure should be interpreted as indicative. Recording these variables at regular intervals would allow the microbial succession to be tied directly to the physical and chemical conditions of the fermentation.

The appearance of *Bacillus subtilis*, *B. cereus*, and *B. coagulans* at Days 5 and 7 agrees with reports that aerobic spore formers become prominent late in fermentation, when the bean mass is less acidic and aeration has increased (Ardhana & Fleet, 2003; Obinze et al., 2022). *Bacillus* species may contribute to pectin degradation, as shown by pectinolytic isolates (Ouattara et al., 2008, 2011), but *B. cereus* is also a toxigenic species whose spores resist roasting (Pereira et al., 2019, 2020). The longer fermentation that gave the best sensory scores should therefore be accompanied by hygienic handling, proper drying, and monitoring for spore-forming contaminants.

Finally, the microorganisms were identified through photomicrography and illustrated taxonomic guides. Such methods can assign isolates reliably to broad groups, but they cannot separate closely related species, for example *A. pasteurianus* from *A. fabarum*, which has been achieved with MALDI-TOF mass spectrometry and gene sequencing (Miescher Schwenninger et al., 2016; Ouattara et al., 2011). The species names in this section are therefore presumptive and should be confirmed by molecular methods in future work.

Sensory Evaluation of Fermented Cacao Beans at 3, 5, and 7 Days

The sensory attributes of cacao beans fermented for 3, 5, and 7 days are summarized in Table 2. Statistical analysis showed significant differences among the treatments in terms of aroma, flavor, acidity, bitterness, astringency, mouthfeel, and overall acceptability. These variations highlight the impact of fermentation duration on the final sensory profile of cacao beans.

Table 2. Means of the Sensory Attributes of Fermented Cocoa Beans at Day 3, 5, and 7 Fermentation Period

Treatments	Aroma	Flavor	Acidity	Bitterness	Astringency	Mouthfeel	Overall Acceptability
3-Day	5.70^{c}	5.33^{c}	6.47^{b}	7.80^{a}	8.03^{a}	6.20^{c}	5.43^{c}
5-Day	7.30^{b}	7.53^{b}	5.97^{b}	6.33^{b}	6.20^{b}	7.40^{b}	7.50^{b}
7-Day	8.50^{a}	9.00^{a}	5.00^{c}	5.00^{c}	4.80^{c}	8.50^{a}	8.80^{a}
CV%	4.82	5.10	6.75	5.80	5.92	6.03	5.50

Legends:

Means with common letter superscripts are not significantly different at $p < 0.05$.

Significant = There is a significant difference between treatments.

Not significant (ns) = No significant difference.

CV% (Coefficient of Variation) = Measures the variability of the data.

Overview of the Treatment Means

Table 2 shows that fermentation duration shifted every sensory attribute in a favorable direction. From the 3-day to the 7-day treatment, aroma increased by 2.80 points, flavor by 3.67, mouthfeel by 2.30, and overall acceptability by 3.37, while acidity decreased by 1.47 points, bitterness by 2.80, and astringency by 3.23. Judged against the scale in Table 1, the 3-day beans were rated Acceptable for aroma (5.70), flavor (5.33), and overall acceptability (5.43); the 5-day beans were rated Good (aroma 7.30, flavor 7.53, overall acceptability 7.50); and the 7-day beans were rated Very Good (aroma 8.50, flavor 9.00, overall acceptability 8.80).

The gain from extending fermentation from five to seven days was smaller than the gain from three to five days. Aroma rose by 1.60 points between Days 3 and 5 but by 1.20 between Days 5 and 7, and flavor rose by 2.20 and

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1.47, respectively. Acidity was the only attribute for which the 3-day and 5-day means did not differ significantly, as both share the letter b. These patterns suggest diminishing returns as fermentation proceeds, which is consistent with reports that fermentation of Forastero beans is largely complete after about five to six days (Afoakwa et al., 2008).

Aroma

The results revealed that the aroma intensity of fermented cacao beans increased with fermentation time. Specifically, the 3-day fermentation had the lowest aroma score (5.70), whereas the 5-day fermentation exhibited a moderate score (7.30). The highest aroma score (8.50), considered excellent, was recorded during the 7-day fermentation. This enhancement in aroma is attributed to the extended fermentation period, which promotes the production of volatile compounds, thereby contributing to a richer aroma profile. These findings suggest that fermenting cacao beans for 5 to 7 days is ideal for maximizing aroma quality (Afoakwa et al., 2008).

The increase in aroma with fermentation time agrees with the finding that the aroma compounds formed during roasting increase with fermentation time (Afoakwa et al., 2008) and that fermentation time is the main factor shaping the volatile profile of cocoa beans (Hamdouche et al., 2019). The weak aroma of the 3-day beans is consistent with incomplete formation of aroma precursors, in which proteolysis in the cotyledons is limited by insufficient acidification and bean death.

Flavor

Similarly, the flavor of fermented cacao beans significantly improved with longer fermentation. The 3-day fermentation yielded the lowest score (5.33), while the 5-day and 7-day fermentations demonstrated substantial improvements, with scores of 7.53 and 9.00, respectively. This increase in flavor complexity is primarily due to the prolonged breakdown of sugars and amino acids, which enhances the development of desirable chocolate notes. Based on these results, fermenting cacao beans for at least 5 to 7 days is recommended to achieve optimal flavor quality (De Brito et al., 2000).

The highest flavor score at 7 days parallels the finding that 7-day liquor gave the best sensory notes compared with 3-day liquor (Efraim et al., 2010). Flavor precursors arise when the cotyledon pH falls to about 5.0–5.5, the range in which the endogenous enzymes that release aroma precursors are most active (Calvo et al., 2021), which is a condition that is more completely reached in longer fermentations.

Acidity

In contrast, acidity in fermented cacao beans exhibited a decreasing trend over time. The highest acidity score (6.47) was observed in the 3-day fermentation, whereas the 5-day and 7-day fermentations resulted in lower scores of 5.97 and 5.00, respectively. This decline in acidity is attributed to the extended fermentation duration, which facilitates the breakdown of organic acids, thereby reducing the sourness of the beans. These findings indicate that longer fermentation is beneficial in moderating acidity levels, ultimately improving the balance of fermented cacao beans (Schwan & Wheals, 2004).

Acidity scores decreased as fermentation was prolonged, in line with the observation in this study that acetic acid evaporates during the later stage, which reduces sourness. It should be noted that instrumental measures of acidity do not always track sensory ratings. In Colombian fermentations, bean acidity peaked on day 5 (Calvo et al., 2021), and in Davao del Norte, pH and titratable acidity differed significantly with duration, whereas the sourness of the liquor did not (Remitar & Magbalot-Fernandez, 2023). Measuring pH and titratable acidity alongside sensory ratings would clarify how chemical acidity relates to perceived sourness in South Cotabato cacao.

Bitterness

A similar decreasing trend was observed in the bitterness of fermented cacao beans, which significantly declined with longer fermentation. The 3-day fermentation exhibited the highest bitterness score (7.80), while the 5-day and 7-day fermentations recorded lower scores of 6.33 and 5.00, respectively. This reduction in bitterness is

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largely attributed to the degradation of polyphenols and alkaloids, such as theobromine, which occurs more effectively with extended fermentation.

Consequently, fermenting cacao beans for 5 to 7 days results in a more palatable and less bitter profile (Afoakwa et al., 2008).

The lower bitterness of the 7-day beans is consistent with the loss of phenolic compounds during fermentation. Epicatechin declined by 51% to 78% by day 5, and theobromine also declined during fermentation (Calvo et al., 2021), and 3-day beans retained more phenolic compounds than 7-day beans (Efraim et al., 2010). The 3-day bitterness score of 7.80 is therefore what would be expected from beans in which phenolic degradation was still incomplete.

Astringency

Astringency in fermented cacao beans also decreased with increasing fermentation time. The highest astringency score (8.03) was noted in the 3-day fermentation, whereas the 5-day and 7-day fermentations exhibited progressively lower scores of 6.20 and 4.80, respectively. The significant reduction in astringency, particularly in the 7-day fermentation, can be attributed to the breakdown of tannins and other polyphenolic compounds.

These findings suggest that fermenting cacao beans for at least 5 days effectively reduces astringency, resulting in a smoother sensory experience (De Brito et al., 2000).

The reduction in astringency, from 8.03 at 3 days to 4.80 at 7 days, is likewise consistent with the degradation and complexation of polyphenols during fermentation (Efraim et al., 2010; Afoakwa et al., 2008). The result differs, however, from the Davao del Norte basket study, in which the astringency of the liquor was not significantly affected by fermentation duration and quantity (Remitar & Magbalot-Fernandez, 2023), indicating that the effect of duration on astringency may depend on the fermentation vessel, bean quantity, and panel.

Mouthfeel

Regarding mouthfeel, the fermentation duration had a positive impact on the texture of fermented cacao beans. The 3-day fermentation had the lowest mouthfeel score (6.20), whereas the 5-day fermentation showed a noticeable improvement (7.40). The highest score (8.50) was observed in the 7-day fermentation, which provided the smoothest and most refined texture.

This enhancement is due to better bean breakdown and softening, which occurs more efficiently with prolonged fermentation (Schwan & Wheals, 2004).

Mouthfeel is also affected by grinding, refining, conching, and tempering. In this study, these steps followed a single procedure described under Sub-Study 2, so differences in mouthfeel among treatments are more likely to reflect the fermentation period than the processing method. The improvement in texture with longer fermentation may be linked to the better bean breakdown described above, although texture was not measured instrumentally.

Overall Acceptability

Taking all sensory attributes into account, the study revealed that the overall acceptability of fermented cacao beans increased with fermentation time. The 3-day fermentation had the lowest acceptability score (5.43), while the 5-day fermentation exhibited a moderate score (7.50). Notably, the highest acceptability (8.80) was recorded for the 7-day fermentation.

These results indicate that extending the fermentation period from 5 to 7 days enhances the overall sensory profile, making fermented cacao beans more desirable for chocolate production (Afoakwa et al., 2008).

The highest acceptability was recorded for the 7-day treatment, in agreement with the sensory preference for 7-day over 3-day fermentation reported by Efraim et al. (2010). The result does not mean that longer fermentation

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is always better. Some wooden-box fermentations in the Colombian Amazon showed over-fermentation notes at 7 days (González et al., 2024), and the most profitable treatment in Davao del Norte was a 5-day fermentation (Remitar & Magbalot-Fernandez, 2023). Because the present study did not extend beyond 7 days, it cannot determine whether quality would decline at longer durations.

Integrated Discussion

The results can be read as a coherent sequence. Yeast metabolism of pulp sugars supplies ethanol; LAB acidify the pulp and use citric acid; AAB oxidize ethanol to acetic acid, which, together with heat, kills the embryo and lowers the cotyledon pH; and the late *Bacillus* flora contributes to the breakdown of residual organic material (Schwan & Wheals, 2004; De Vuyst & Leroy, 2020). The enzymatic and chemical changes that follow reduce phenolic compounds and generate flavor precursors, which explains why the 7-day beans, which passed through the entire succession, received the highest aroma, flavor, mouthfeel, and acceptability scores and the lowest bitterness and astringency scores.

An important qualification is that fermentation duration and microbial flora were not independent in this study. Each longer treatment contained both a later stage of the microbial succession and a longer period of chemical change, so the design cannot separate the effect of the microorganisms from the effect of time. The null hypothesis, that temporal changes in microbial flora have no significant effect on sensory quality, is therefore addressed only indirectly: the sensory attributes differed significantly among fermentation periods, and the microbial composition differed among periods, but no analysis relating specific microorganisms to specific attributes was performed. Experiments that include controlled inoculation, as in the starter culture studies cited above, would be needed to establish a causal role for individual species.

Implications for Practice

For producers and cooperatives, the results support a 7-day fermentation of Forastero beans in wooden sweatboxes as the practice that gave the best sensory scores under the conditions of this study. The 5-day treatment gave Good ratings and may be a reasonable alternative where time or space is limiting, as the gain from Day 5 to Day 7 was smaller than the gain from Day 3 to Day 5. A 3-day fermentation produced bitter, astringent cocoa and should be discouraged for bulk Forastero beans.

Hygiene should accompany any extension of fermentation, given the late appearance of *Bacillus* species, including *B. cereus*. Clean boxes, baskets, and drying surfaces, together with proper drying, reduce the risk of spore-forming contaminants. Producers who intend to sell beans through formal channels should also be aware of the national standard for cacao beans, PNS/BAFS 58:2019, which specifies grading requirements (Bureau of Agriculture & Fisheries Standards, 2019). The economic consequences of a longer fermentation, including labor, box occupancy, and price premiums for higher quality, were not analyzed here and should be examined before the 7-day practice is recommended as the most profitable option.

Limitations of the Study

Several limitations should be considered when interpreting the findings. First, microorganisms were identified through photomicrography and illustrated taxonomic guides, so species names are presumptive. Second, mass temperature, pH, titratable acidity, and phenolic content were not reported, which limits the ability to explain the sensory differences chemically. Third, the microbial sampling began at Day 3, so the early yeast-dominated phase was not documented.

Fourth, the sensory panel consisted of 30 untrained evaluators, 10 from each of three groups, and the sample is small by the standards recommended for hedonic tests (Lim, 2011). The rating sheet (Appendix D) defines bitterness and astringency as intensities (for example, 1 = too weak and 10 = too strong for bitterness) and acidity as 1 = unbalanced to 10 = balanced, whereas Table 1 describes higher scores as better quality for every attribute. The direction of the acidity, bitterness, and astringency scales is therefore ambiguous, and the interpretation used

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here (lower scores indicating milder sensations) should be confirmed by the author. Future studies should use separate liking and intensity scales and, ideally, a trained descriptive panel.

Fifth, the study used a single cacao type (Forastero) from one farm, with the sensory evaluation conducted at one location in January to February 2025 and three replications per treatment. Treating panelists as blocks in the analysis could be considered in future work. The findings should therefore be generalized with caution.

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CONCLUSIONS AND RECOMMENDATIONS

Summary of Findings

This study used a completely randomized design (CRD) to investigate the microbial succession and sensory characteristics of cocoa beans fermented for 3, 5, and 7 days. The primary objective was to identify key microorganisms present at different fermentation stages and assess their impact on sensory attributes.

The microbial flora found to inhabit the cacao beans at 3, 5, and 7 days of fermentation were as follows.

Early fermentation (Days 1–3). *Acetobacter pasteurianus*, *Acetobacter fabarum*, *Lactobacillus fermentum*, *Lactiplantibacillus plantarum*, and *Gluconobacter* spp. were responsible for breaking down sugars and producing ethanol and organic acids.

Middle fermentation (Days 4–5). *Acetobacter* spp. became dominant, converting ethanol into acetic acid, which contributed to flavor development. Meanwhile, *Lactobacillus fermentum* and *Gluconobacter* spp. declined, while *Pichia kudriavzevii* and *Lactiplantibacillus* spp. emerged, helping to break down sugars and support the chemical changes needed for flavor formation (Rohan, 1963; Schwan & Wheals, 2004; Afoakwa, 2010, 2016).

Late fermentation (Days 6–7). *Bacillus subtilis*, *Bacillus cereus*, and *Bacillus coagulans* were prevalent, breaking down complex compounds and further enhancing the sensory quality of the beans.

Sensory panel. Sensory evaluation was conducted by 10 processors, 10 teachers, and 10 students, who assessed aroma, flavor, acidity, bitterness, astringency, mouthfeel, and overall acceptability of the cocoa beans.

Statistical analysis. Statistical analysis (ANOVA, $p < 0.05$) showed significant differences in sensory attributes across different fermentation durations.

Optimal fermentation period. The 7-day fermentation period yielded the best sensory results, with the highest scores in flavor (9.00), aroma (8.50), and overall acceptability (8.80).

Three-day fermentation. The 3-day fermentation led to increased bitterness (7.80) and astringency (8.03), making it the least preferred.

Five-day fermentation. The 5-day fermentation resulted in moderate sensory quality, balancing acidity and flavor development.

These findings confirm that a longer fermentation period significantly enhances sensory qualities, making the beans more suitable for high-quality chocolate production.

Taken together, the microbial and sensory results indicate that Forastero cacao beans fermented in wooden sweatboxes pass through a succession of microorganisms that is broadly consistent with the literature, and that sensory quality improved progressively as fermentation was extended from 3 to 7 days. The gain was greatest between Days 3 and 5 and smaller between Days 5 and 7.

Conclusions

Based on the findings of the study, the following conclusions are made:

1. The fermentation process follows a structured microbial succession, beginning with yeasts and lactic acid bacteria, followed by acetic acid bacteria, and ending with *Bacillus spp.*

2. Fermentation duration has a significant effect on sensory attributes, with longer fermentation (7 days) improving aroma, flavor, and overall acceptability while reducing bitterness and astringency.

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3. A 7-day fermentation period is optimal for enhancing cocoa bean quality, making it more suitable for premium chocolate production.

4. The microbial succession observed is consistent with the general pattern reported for cocoa fermentation, although species identities were established morphologically and should be considered presumptive until confirmed by molecular methods.

5. Because fermentation duration and microbial succession were confounded, the study demonstrates an association between fermentation stage and sensory quality but does not establish that individual microorganisms caused the observed sensory differences.

Recommendations

Based on the findings and conclusions of this study, it is recommended that cocoa bean fermentation be carried out for seven (7) days to achieve optimal sensory quality, including enhanced aroma, flavor, and overall acceptability, while reducing undesirable attributes such as bitterness and astringency. This duration allows key microorganisms to effectively contribute to the development of desirable flavor compounds.

Furthermore, more research is recommended to:

1. **Optimize fermentation conditions**, including temperature, aeration, and turning frequency, to support consistent microbial activity.

2. **Isolate and study dominant microbial strains** involved in each fermentation stage to better understand their specific roles and interactions.

3. **Assess the impact of fermentation on different cacao varieties** to evaluate if similar results are observed beyond the Forastero hybrid.

4. **Explore scalable fermentation techniques** that can be adopted by local farmers and cooperatives to improve cocoa quality and increase economic returns.

5. To producers and cooperatives: adopt a 7-day fermentation of Forastero cacao in wooden sweatboxes, maintain clean boxes and drying surfaces to limit spore-forming contaminants, and keep records of fermentation dates, turning, and bean quantity.

6. To extension workers and agricultural agencies: incorporate the findings into training on post-harvest handling, and advise against 3-day fermentation of bulk Forastero beans.

7. To researchers: record mass, temperature, pH, and titratable acidity during fermentation; sample earlier than Day 3 to capture the yeast phase; confirm species identities by MALDI-TOF mass spectrometry or gene sequencing; and test intermediate and longer durations, such as 4, 6, and 8 days, to locate the optimum.

8. To researchers: evaluate sensory quality with a trained descriptive panel in addition to a consumer panel, consider analyzing the data with panelists as blocks, and quantify *B. cereus* and other spore formers in the fermented, dried, and roasted beans.

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