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RESEARCH ARTICLE

Optimization of Cocoa Nibs Fermentation Using *Saccharomyces cerevisiae* Starter Cultures: Impact on Physicochemical Properties and Aroma Profile

Natwalinkhol Settapramote[1], Nittaya Phungam[2], Kanrawee Hunsakul[3], Threethip Chuensun[4], Tanongsak Yatale[1] and Arthitaya Kawee-ai[5,*]

[1]*Division of Food Business and Nutrition, Faculty of Science and Agricultural Technology, Rajamangala University of Technology Lanna, Tak 63000, Thailand*
[2]*Department of Agro-Industry, Rajamangala University of Technology Isan Surin, Nogmuang Surin 32000, Thailand*
[3]*Department of Culinary Technology and Service, Faculty of Science and Technology, Rajamangala University of Technology Tawan-Ok, Chonburi 20110, Thailand*
[4]*Faculty of Engineering and Agro-Industry, Maejo University, Chiang Mai 50290, Thailand*
[5]*Division of Cannabis and Medicinal Plants for Local Development, Graduate School, Payap University, Chiang Mai 50000, Thailand*

([*]Corresponding author's e-mail: kaweeai@gmail.com)

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Abstract

Cocoa fermentation is a crucial post-harvest process that significantly influences cocoa beans' flavor, aroma, and quality. This study investigates the impact of controlled fermentation using *Saccharomyces cerevisiae* strains 71B and K1V-1116 on cocoa nibs' physicochemical and aromatic properties. The fermentation process was analyzed through total soluble solids (°Brix), pH, titratable acidity, cut test scores, sugar and acid composition, and volatile aroma compounds. Results indicate that total soluble solids decreased progressively as fermentation advanced, with natural fermentation retaining higher sweetness levels (0.66 °Brix on day 9) than K1V-1116 (0.43 °Brix) and 71B (0.46 °Brix). pH levels fluctuated, initially decreasing before rising in later stages, with natural fermentation reaching the highest final pH (6.28). Titratable acidity exhibited an inverse trend, where 71B fermentation maintained higher lactic acid levels (75.80 mg/100 mL), while acetic acid levels were lowest in K1V-1116 (45.87 mg/100 mL), suggesting a milder fermentation profile. After nine days, the cut test score was highest in K1V-1116 (86.66 %), indicating superior fermentation efficiency. Sugar analysis revealed a significant reduction in sucrose for inoculated fermentations, while 71B produced the highest fructose levels (31.47 mg/100 mL). Aroma analysis confirmed that controlled fermentation enhanced floral and sweet notes while reducing undesirable fatty and amine compounds. Pearson's correlation analysis revealed strong positive correlations between sugar content and aroma compound concentrations, and negative correlations between organic acid levels and aroma compound. These findings demonstrate that targeted yeast fermentation enhances cocoa quality and may support consistent flavor production. This approach presents a promising strategy for improving post-harvest processing and achieving high-quality cocoa suitable for cocoa nib' production.

Keywords: Cocoa fermentation, Physicochemical properties, Aroma compounds,microbial metabolism, Controlled fermentation, Starter cultures, *Saccharomyces cerevisiae*

Introduction

Cocoa nibs result from processing cocoa beans through fermentation, drying, roasting, and winnowing [1]. These nibs, representing the purest form of cocoa solids, have gained significant attention due to their health benefits and versatility in culinary and industrial

applications [2]. The global demand for cocoa products has increased due to the growing awareness of their nutritional and therapeutic properties, particularly in functional foods and nutraceuticals [2,3]. Today, cocoa is cultivated in tropical regions worldwide, with key production centers in West Africa, South America, and Southeast Asia. The post-harvest processing of cocoa beans, including fermentation, drying, and roasting, is crucial in determining cocoa nibs' flavor profile, nutrient retention, and bioactive compound content [3].

Fermentation is critical in cocoa processing, initiating biochemical reactions that develop characteristic cocoa flavors [4]. The two primary fermentation methods are commercial and natural fermentation. Commercial fermentation involves controlled conditions with selected microbial cultures to ensure uniformity. In contrast, natural fermentation relies on indigenous microorganism [5]. Enhancing cocoa and nib quality is essential for the food and beverage industry, as fermentation significantly influences the taste and aromas of cocoa products [1-3]. Natural fermentation, or traditional or spontaneous fermentation, is the predominant method used by small-scale farmers in wooden boxes, baskets, or directly on banana leaves, allowing a diverse microbial community to drive the fermentation [6]. This method is influenced by environmental factors such as temperature, humidity, and the native microbial population [1,5,6]. While natural fermentation often results in more significant variability in flavor profiles, it also retains a broader spectrum of bioactive

compounds, particularly polyphenols and flavonoids [1,6]. Conversely, commercial fermentation employs controlled conditions to standardize cocoa bean quality and flavor. This method involves inoculating the beans with specific strains of yeast and bacteria to ensure predictable biochemical transformations [1,3,6].

Commercial fermentation can enhance the efficiency of the process, reduce fermentation time, and improve consistency in the final product [3,7]. However, some studies suggest that controlled fermentation may reduce certain beneficial polyphenols, potentially impacting the antioxidant properties of cocoa nibs [8]. Hurana *et al.* [1,9] and Hirko *et al.* [10] concluded that the quality of cocoa nibs is affected by factors including the cacao bean's origin and variety, fermentation and roasting processes, farming practices, processing techniques, storage conditions, and overall freshness. Similarly, Camargo *et al.* [11] and Oliveira *et al.* [12] found that a pH lower than 5.0 can lead to reduced flavor quality.

Several commercial microbial strains have been identified as beneficial for cocoa fermentation [7,10,13,14]. For example, *S. cerevisiae* strains used in wine and bread production have been adapted for cocoa fermentation, promoting efficient sugar metabolism and flavor development [6,7,13]. Díaz-Muñoz *et al.* [15] highlighted that the use of *S. cerevisiae* IMDO 050523 as a starter culture leads to a higher degree of fermentation and positively influences the aroma profiles of cocoa liquor and chocolate. *S. cerevisiae* enhances cocoa nib quality during fermentation by developing complex flavors, lowering pH to increase acidity, reducing bitterness, outcompeting undesirable microorganisms, and producing volatile aroma compounds that contribute to the overall richness of cocoa's flavor profile [7]. Additionally, *Lactobacillus plantarum* and *Lactobacillus fermentum* have been shown to enhance lactic acid production, improving the balance between acidity and sweetness in cocoa beans [13,16,17]. *Acetobacter* species optimized for cocoa fermentation can efficiently oxidize ethanol, preventing excessive acidity while enhancing fruity and floral aroma compounds [10,14].

In Thailand, cocoa farmers traditionally produce cocoa nibs using conventional fermentation methods. They typically ferment cocoa in baskets covered with cocoa or banana leaves, allowing natural microorganisms to facilitate the process [18]. However, this traditional approach often results in inconsistent and suboptimal cocoa nib quality. This research aims to enhance cocoa nib fermentation by employing commercial yeast as a starter culture. Specifically, it examines how using *S. cerevisiae* starter cultures optimizes the physicochemical properties and aroma profile of the final product. In this study, two commercial *S. cerevisiae* strains were used to ferment cocoa, and their effects were compared to those of natural fermentation. The evaluation focused on the appearance as well as the chemical and physical properties of the cocoa nib. Understanding the influence of microorganisms on cocoa nib quality can contribute to developing more efficient fermentation techniques,

potentially increasing the market value of cocoa products. Additionally, this knowledge may assist small-scale producers in improving their production processes, thereby enhancing product quality and market differentiation.

Materials and methods

Raw material preparation

In December 2023, fresh cocoa pods with at least 70 % ripening were collected from Mae Ramat distinct (16° 52.0′ N, 99° 08.0′ E), Tak province, Thailand. The pods were washed with a 100 ppm chlorine solution, and the spoiled pods were discarded [19]. The pods were then manually cut horizontally with a shape knife, and the fresh cocoa beans were gathered in a plastic box with good sanitation in a plastic box for further research.

Starter culture preparation

In this study, three treatments were employed: Two commercial strains of *Saccharomyces cerevisiae*—K1V-1116 and 71B, obtained from Lallemand's Lalvin® (USA)—at a concentration of 0.2 % of the total cocoa weight, as well as a natural yeast treatment. The commercial yeast strains were mixed with water at a 1:2 ratio and sealed in an airtight container to prevent air entry [20]. The mixture was then left to activate at room temperature (28 ± 5 °C) for 20 min before use.

Cocoa nibs' fermentation

The activated yeasts were introduced into fresh cocoa beans within a 5-L cylindrical plastic container and subjected to fermentation for nine days. For the natural yeast fermentation process, banana leaves were thoroughly cleaned and slit to facilitate drainage. Fresh cocoa beans and banana leaves were alternately layered in a 5-L cylindrical plastic fermentation vessel, ensuring that the underside of the banana leaves faced the cocoa beans. The sequencing of the fermentation process is presented in **Figure 1**. Four alternating layers were arranged before sealing the vessel for fermentation [18].

Samples were collected on the first day of fermentation, followed by the third day, and each subsequent day until the completion of the nine-day fermentation period. The collected samples were analyzed for physical quality using the cut test method, followed by chemical properties, including total soluble solids, pH, and acidity content.

After fermentation, the cocoa beans were dried at 50 °C for 18 h and then roasted at 140 °C for 45 min to produce cocoa nibs [21]. The cocoa nibs were collected and analyzed for their chemical and physical properties.

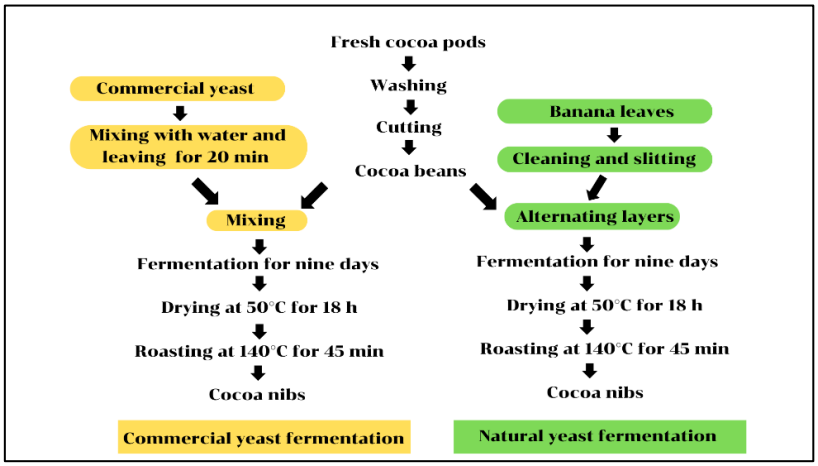

Figure 1 Schematic representation of the fermentation process, detailing the sequential stages of raw material preparation, starter culture preparation, and fermentation.

Determination of total soluble solids, pH, and titratable acidity

The physicochemical parameters of the separated sample, including total soluble solids, pH, and acidity, were determined. Before analysis, ten whole cocoa beans with attached pulp were vortexed in 20 mL of Milli-Q water for 2 min [22] and then filtered using Whatman filter paper No.4 (England). Total soluble

solids were evaluated using a digital refractometer (PAL-1, ATAGO, Japan). The pH was measured using a digital pH meter (F20 FiveEasy™, Mettler Toledo, Switzerland).

The acidity, expressed as lactic acid, was determined by titration [23]. Briefly, a 5 mL aliquot of the sample was accurately pipetted into a clean conical flask or beaker. To this, 25 mL of distilled water was added to dilute the sample, ensuring proper mixing of components. Following dilution, 3 - 4 drops of phenolphthalein indicator were introduced into the flask and stirred to ensure even distribution. Titration was carried out using a 0.1 M sodium hydroxide (NaOH) solution, which was added dropwise while continuously stirring. The endpoint of the titration was identified by the first persistent color change to faint pink, indicating acid neutralization. The volume of NaOH used to reach the endpoint was recorded from the burette reading. The acidity, expressed as lactic acid, was calculated using the following Eq. (1):

$$\text{Lactic acid (\%)} = \frac{(0.1\text{M NaOH} \times \text{vol.of NaOH (L)} \times 90.08) \times 100}{\text{Weight of sample}} \quad (1)$$

Cut test analysis

A randomized sample of 100 g of fermented beans was longitudinally sliced at the midpoint using a sharp knife to expose the largest cotyledon surface. The exposed surfaces were inspected under artificial light and categorized into five groups: entirely brown, partially brown, partially purple, entirely purple, slaty, by cacao quality, and flavor assessment guidelines [24]. To assess the extent of fermentation, the percentage was calculated based on the cut test score (CTS), as presented in Eq. (2) [25].

$$\text{CTS (\%)} = (10 \times \%\text{brown}) + (5 \times \%\text{partly purple/brown}) + (0 \times \%\text{purple and slaty}) \quad (2)$$

Determination of sugar content, acid content, and aroma compound

Before analysis, 25 g of coarsely ground cocoa nibs was placed in a 250-mL round-bottom flask with 100 mL of distilled water. The mixture then underwent steam distillation at 90 °C for 60 min, following the method of Mohamadi Alasti *et al.* [26]. The extracted oil was separated using hexane (1:1 ratio), evaporated, re-disolved in 1 mL of hexane, and filtered through a 0.45-µm nylon filter (Agilent Technologies, USA) for further analysis.

For sugar content analysis, the sample was diluted with distilled water at a 1:2 (v/v) ratio and mixed using a vortex mixer (Vortex Mixer Genie 2, Scientific Industries, USA) for 10 s. The mixture was left to settle at 4 °C for 14 - 16 h. The supernatant was filtered through a 0.2 -µL syringe filter membrane, stored in vials, and analyzed for sugar type and content using HPLC with an Agilent Hi-Plex H column (7.7×300 mm^2, 8 µm). A refractive index detector was employed at 50 °C, with a 5-µL injection volume. The mobile phase consisted of 5 mM sulfuric acid in methanol at a 0.6 mL/min [27] flow rate. Quantification was performed by comparing the results to the standard glucose, fructose, and sucrose solutions.

To determine acidity, the sample was diluted with distilled water at a 1:2 (v/v) ratio and mixed using a vortex mixer for 10 s. After settling at 4 °C for 14 - 16 h, the supernatant was filtered through a 0.2 µm syringe filter membrane, stored in vials, and analyzed for acid composition using high-performance liquid chromatography (HPLC). The analysis used an Agilent Zorbax Eclipse Plus C8 column (4.6×250 mm^2, 5 µm) with a diode array detector equipped with a UV lamp at 203 nm. The column temperature was maintained at 40 °C with a 5 µL injection volume. The mobile phase consisted of 5 mM sulfuric acid in methanol, delivered at a flow rate of 1 mL/min [27]. Acid quantification was performed by comparing the sample results with the standard lactic acid, acetic acid, and citric acid solutions.

The analysis of aroma compounds was performed using Gas Chromatography-Mass Spectrometry (GC-MS), following the method of Mohamadi Alasti *et al.* [26]. An Agilent 6890N system equipped with an HP-5MS column (30 m × 0.25 mm × 0.25 µm) was utilized. A 5 µL sample was injected in split mode with a 15:1 ratio. The chromatographic conditions included a helium flow rate of 1.0 mL/min and a temperature program starting at 70 °C (held for 1 min), increasing to 300 °C at a rate of 20 °C/min, and maintained for 8 min, resulting in a total runtime of 25 min. Mass spectrometry (MS) analysis was conducted using an Agilent 5973 inert Quadrupole system with Electron Impact

Ionization (EI) and a 30 - 500 AMU scan range. Component identification was performed by comparing mass spectra with the NIST02.L library.

Statistical analysis

All experiments were conducted in triplicate, except for the cut test analysis. The results are presented as mean values ± standard deviation (SD) based on these triplicate measurements. Statistical comparisons were performed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test to assess significance at a *p*-value of ≤ 0.05. Pearson's correlation coefficients are used to analyze the relationships among the sugar content, organic acid content, and aroma compounds in cocoa nibs' products. All statistical analyses were conducted using SPSS software (Version 17, SPSS, Inc., USA).

Results and discussion

Effect of starter on the total soluble solids

During fermentation, continuous physicochemical changes occur in cocoa beans, leading to alterations in the composition and concentration of pulp nutrients [7,28]. The analysis of the total soluble solids (°Brix) of cocoa beans fermented with two types of commercial yeast, K1V-1116 and 71B, compared to cocoa beans fermented with natural yeast, revealed that as the fermentation time increased, the sweetness of cocoa beans tended to decrease for all three types of yeast (**Figure 2**).

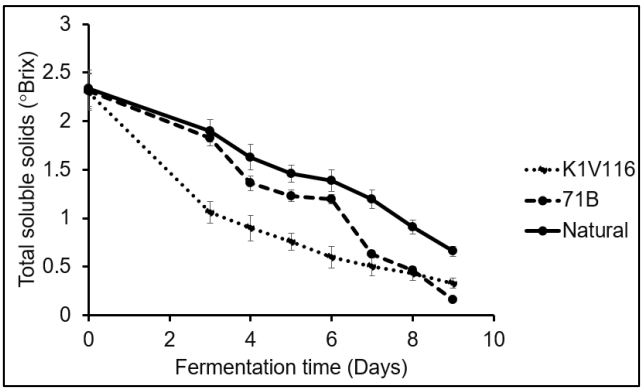

Figure 2 Changes in total soluble solids of cocoa beans fermented with three different starter cultures. Data are presented as mean ± SD from triplicate measurements.

Fermentation using the commercial yeast 71B resulted in sweetness levels comparable to those achieved with natural yeast during the first three days of fermentation, ranging from 2.34 to 1.83 °Brix. During the fermentation period of 4 - 9 days, cocoa beans fermented with natural yeast exhibited sweetness levels ranging from 1.63 to 0.66 °Brix, significantly higher than those fermented with both types of commercial yeast ($p \leq 0.05$). When comparing the commercial yeast 71B, the sweetness of cocoa beans fermented for 4 - 8 days ranged from 1.36 to 0.46 °Brix, which was higher than the beans fermented with the commercial yeast K1V-1116, whose sweetness levels ranged from 0.90 to 0.43 °Brix. Since commercial yeast has a higher fermentation efficiency than natural yeast, the sweetness of cocoa beans fermented with commercial yeast was lower than that of beans fermented with natural yeast [28]. This is because yeast utilizes sugars from the cocoa pulp for growth, and the better the yeast grows, the more complete the fermentation process becomes [7]. Consequently, commercial yeast tends to lower sweetness levels than natural yeast fermentation. Moreover, fermentation with natural yeast cannot regulate the yeast population [29].

Total soluble solids represent the concentration of dissolved sugars, organic acids, and other soluble compounds within the cocoa pulp. At the onset of fermentation, total soluble solid levels are high due to fermentable sugars, such as glucose and fructosel, which serve as substrates for yeast and bacterial metabolism [6,13,28]. As fermentation progresses, yeasts and lactic acid bacteria metabolize these sugars, converting them into ethanol, organic acids (e.g., lactic and acetic acid), and carbon dioxide [28,29]. This metabolic activity

leads to a gradual decline in total soluble solids, accompanied by increased acidity. Consequently, total soluble solids and acidity exhibit a negative correlation, wherein a reduction in total soluble solids corresponds to an increase in acid concentration (**Figure 4**).

Effect of starter on pH

Compared to those fermented with natural yeast, the analysis of pH levels in cocoa beans fermented with two commercial yeast strains, K1V-1116 and 71B, revealed distinct trends throughout the fermentation process (**Figure 3**). During the first five days, the pH of cocoa beans fermented with 71B remained relatively high, ranging from 4.89 to 5.52, exceeding the levels observed in beans fermented with K1V-1116 and natural yeast. As fermentation progressed to days 6 - 8, the pH of beans fermented with natural yeast increased, ranging from 6.38 to 6.28, surpassing the levels recorded for commercial yeast fermentation. Specifically, the pH of beans fermented with K1V-1116 increased from 4.89 to 5.24, while those fermented with 71B rose from 4.89 to 5.81. In contrast, the pH of beans fermented with natural yeast increased from 4.90 to 5.15.

These findings align with Calvo, Botina [30] those who reported that pH levels fluctuate throughout cocoa fermentation, corresponding to different stages. Initially, pH levels drop rapidly, followed by a gradual rise during the middle stage and a significant increase in the final stage. However, when fermentation exceeds seven days, pH levels may decline due to inadequate aeration or insufficient turning of the beans, conditions that promote the growth of acetic-acid-producing bacteria, leading to increased acid production [7,28]. Furthermore, excessively high temperatures and humidity can stimulate microbial activity, further contributing to acid accumulation and a continued decline in pH [7,28,30].

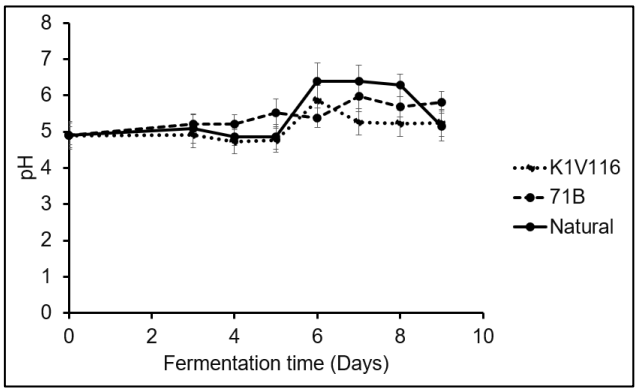

Figure 3 Changes in pH of cocoa beans fermented with three different starter cultures. Data are presented as mean ± SD from triplicate measurements.

The pH of cocoa beans during fermentation is primarily influenced by the accumulation of organic acids, mainly lactic acid, acetic acid, and citric acid, which lower the cocoa pulp and beans [6,7,28,29]. At the initial fermentation stage, pH values are relatively high (approximately 4.8 - 5.5). However, as microbial activity intensifies, acid production increases, leading to a decrease in pH. In the later stages of fermentation, some acids volatilize or are neutralized by microbial metabolism, resulting in a gradual rise in pH [7,28,31]. This pattern demonstrates an inverse relationship between pH and acidity, wherein an increase in acidity leads to a decrease in pH and vice versa.

Effect of starter on the titratable acidity

The total lactic acid content in cocoa beans fermented with two commercial yeast strains, K1V-1116 and 71B, and natural yeast exhibited a decreasing trend throughout fermentation (**Figure 4**). During the first five days, cocoa beans fermented with 71B had the highest lactic acid levels, ranging from 3.10 % to 1.50 % acidity, compared to those fermented with K1V-1116 and natural yeast.

Between days 6 and 9, the total lactic acid content stabilized across all fermentation conditions, with no significant differences observed ($p > 0.05$) and an average acidity of approximately 0.90 %. This pattern can be attributed to the malolactic fermentation

capability of 71B, which converts malic acid in the cocoa pulp into lactic acid, enhancing overall acidity. In contrast, natural yeasts generally exhibit lower efficiency in malic acid metabolism, resulting in reduced lactic acid production in naturally fermented cocoa beans [28,31].

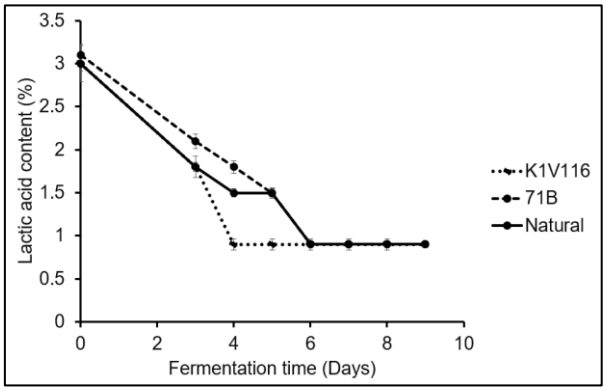

Figure 4. Changes in titratable acidity of cocoa beans fermented with three different starter cultures. Data are presented as mean ± SD from triplicate measurements.

The interplay between total soluble solids, pH, and acidity is a critical indicator of fermentation efficiency and microbial activity [6,7,13,28,31]. As fermentation progresses, lactic acid bacteria (LAB) convert sugars and ethanol into lactic acid, reducing the pH of the cocoa mass [10,19]. This acidification inhibits undesirable microbial growth while promoting enzymatic reactions that contribute to flavor development [1,21,28]. Acetic acid bacteria (AAB) further oxidize ethanol into acetic acid, generating heat that raises the temperature of the fermenting mass [28]. This exothermic reaction is critical for disrupting seed viability and triggering enzymatic reactions inside the cocoa beans [3,17,24].

Cut test analysis

The fermentation process significantly influences cocoa beans' visual characteristics. The images in **Table 1** illustrate the progressive color and structural changes in cocoa beans subjected to different fermentation treatments: Natural, K1V-1116, and 71B starter cultures.

Table 1 The change of appearance and cut test score of cocoa beans fermented with different starter cultures.

Fermentation time (days)	**Starter types**		
	Natural	**K1V-1116**	**71B**
0			
CTS	0.00 (%)	0.00 (%)	0.00 (%)
3			
CTS	10.00 (%)	13.33 (%)	13.33 (%)

Fermentation time (days)	Starter types		
	Natural	**K1V-1116**	**71B**
6			
CTS	30.00 (%)	36.66 (%)	40.00 (%)
9			
CTS	66.66 (%)	86.66 (%)	83.33 (%)

On day 0, all samples appear dark and uniform, indicating unfermented beans with no visible internal modifications. However, as fermentation progresses, noticeable differences emerge among the treatments. By day 3, some beans exhibit a lighter hue, particularly in the K1V-1116 and 71B treatments, suggesting the initial stages of microbial activity and enzymatic reactions affecting pigmentation. By day 6, the transformation becomes more pronounced. The beans under all treatments exhibit a more evident brown coloration, with a more significant number of well-fermented beans observed in the K1V-1116 and 71B groups compared to the Natural fermentation. This trend continues to day 9, where all samples show a significant increase in brown coloration, indicating a more advanced fermentation stage, with K1V-1116 displaying the most uniform and extensive transformation.

The cut test score (CTS), expressed as a percentage, represents the proportion of properly fermented beans within each treatment group. The initial CTS at day 0 is 0 % across all treatments, confirming no fermentation [6,22]. By day 3, the CTS increases slightly, with natural (10.00 %), K1V-1116 (13.33 %), and 71B (13.33 %). This suggests that fermentation has begun but is still in the early stages [22].

On day 6, the CTS continues to increase with natural fermentation of 30.00 %, K1V-1116 of 36.66 %, and 71B of 40.00 %. This indicates that starter cultures, particularly 71B, enhance fermentation efficiency compared to natural fermentation. The microbial activity associated with starter cultures accelerates the breakdown of bean pulp and internal enzymatic reactions, contributing to faster bean transformation [7,28,29,31]. By day 9, a substantial improvement was observed, with the highest CTS values recorded for natural fermentation at 66.66 %, K1V-1116 at 86.66 %, and 71B at 83.33 %. These findings confirm that starter cultures K1V-1116 and 71B significantly enhance the fermentation process compared to natural fermentation. K1V-1116 exhibits the highest fermentation efficiency, achieving 86.66 % CTS, indicating a superior ability to promote biochemical transformations within the cocoa beans.

The results suggest that fermentation is more effective when starter cultures accelerate microbial activity and biochemical changes within the beans. The increased CTS values in K1V-1116 and 71B treatments suggest that these starter cultures enhance fermentation homogeneity and effectiveness. The natural fermentation method shows slower progression, possibly due to reliance on spontaneous microbial activity, leading to variability in fermentation efficiency [29]. According to the International Standards for the Assessment of Cocoa Quality and Flavour (ISCQF), a CTS of more than 60 % is considered an indicator of good quality [24]. A CTS > 60 % was observed on day 8 (data not shown). Therefore, to achieve high-quality cocoa nib production, the optimal fermentation time for

the commercial yeasts K1V-1116 and 71B was 8 days. This finding was consistent with the work of Koné *et al.* [32], who found that cocoa stored for 8 days exhibited excellent quality.

The superior performance of K1V-1116 may be attributed to its metabolic activity, which enhances acetic acid production, pulp degradation, and enzymatic reactions that contribute to the breakdown of polyphenols. This leads to improvements in aroma precursors [7,31]. Similarly, 71B exhibits a high fermentation rate, further confirming the effectiveness of selected microbial inoculants in cocoa fermentation.

Sugar content in cocoa nibs

Table 2 presents the sucrose, glucose, and fructose concentrations in cocoa nibs subjected to different fermentation starters, including natural, K1-V116, and 71B yeast inoculation. The data indicate significant differences ($p \leq 0.05$) in sugar content among the treatments. Sucrose concentration was highest in naturally fermented cocoa nibs (97.37 ± 6.67 mg/100 mL), followed by K1-V116 (70.73 ± 9.42 mg/100 mL) and 71B (54.00 ± 2.35 mg/100 mL). The significant reduction in sucrose levels in inoculated fermentations (K1-V116 and 71B) suggests enhanced sucrose hydrolysis due to yeast metabolism.

Table 2 Types and quantities of sugar in cocoa nibs.

Yeasts	Concentration (mg/100 mL)		
	Sucrose	**Glucose**	**Fructose**
Natural	97.37 ± 6.67^{a}	13.13 ± 0.95^{a}	20.20 ± 2.48^{b}
K1-V116	70.73 ± 9.42^{b}	11.09 ± 2.52^{ab}	20.53 ± 4.57^{b}
71B	54.00 ± 2.35^{c}	10.27 ± 0.23^{bc}	31.47 ± 1.63^{a}

Note: a, b, c, mean values within each column with different superscript letters were significantly different ($p \leq 0.05$).

Yeasts produce invertase, an enzyme that breaks down sucrose into glucose and fructose, which are more readily utilized during fermentation [1,27]. The significantly lower sucrose content in 71B-treated nibs implies a more active enzymatic breakdown compared to the other starters. On the other hand, the higher residual sucrose content in naturally fermented cocoa nibs suggests a slower fermentation process, likely due to the lack of specific yeast strains that accelerate the enzymatic hydrolysis [2]. This may result in less uniform sugar degradation, affecting the consistency of flavor precursors in the final product.

Glucose concentrations exhibited a downward trend similar to sucrose, with the highest value recorded in natural fermentation (13.13 ± 0.95 mg/100 mL). The glucose levels in K1-V116 (11.09 ± 2.52 mg/100 mL) and 71B (10.27 ± 0.23 mg/100 mL) were slightly lower, although the differences were not as pronounced as those for sucrose. The variation in glucose content may be attributed to microbial consumption during fermentation, as glucose serves as a primary substrate for yeast and bacterial metabolism [6,10,14]. The lower glucose concentration in the 71B treatment, accompanied by a significant increase in fructose levels, suggests preferential utilization of glucose over fructose in this fermentation system.

Unlike sucrose and glucose, fructose levels increased in the 71B fermentation (31.47 ± 1.63 mg/100 mL), which was significantly higher than both K1-V116 (20.53 ± 4.57 mg/100 mL) and natural fermentation (20.20 ± 2.48 mg/100 mL). The higher fructose accumulation in the 71B fermentation could be linked to yeast strain-specific metabolic activity, where glucose is preferentially consumed, leaving behind more fructose. This phenomenon is commonly observed in yeast fermentation, where certain strains exhibit glucose repression, a mechanism that favors glucose utilization over other sugars [13,28,29,31].

The lower glucose content in 71B-treated nibs and higher fructose retention highlight the distinct metabolic pathways involved. This trend suggests that 71B yeast exhibits a stronger glucose metabolism, leading to the preferential breakdown of glucose and accumulation of fructose [7,31]. This could have implications for the flavor profile of cocoa nibs, as fructose contributes to sweetness and caramelization reactions during roasting, potentially enhancing the sensory attributes of the final cocoa product.

Acid content in cocoa nibs

Table 3 presents the concentrations of lactic acid, acetic acid, and citric acid in cocoa nibs subjected to different fermentation starters, including natural, K1-V116, and 71B yeast inoculation.

Table 3 Types and quantities of acid in cocoa nibs.

Yeasts	Concentration (mg/100 mL)		
	Lactic acid	**Acetic acid**	**Citric acid**
Natural	56.13 ± 3.07^{c}	58.40 ± 4.42^{ab}	64.87 ± 3.20^{b}
K1-V116	64.07 ± 7.90^{b}	45.87 ± 4.80^{c}	62.00 ± 2.03^{b}
71B	75.80 ± 4.46^{a}	54.50 ± 1.97^{b}	74.43 ± 2.50^{a}

Note: a, b, c, mean values within each column with different superscript letters were significantly different ($p \leq 0.05$).

Lactic acid was highest in the 71B fermentation (75.80 ± 4.46 mg/100 mL), followed by K1-V116 (64.07 ± 7.90 mg/100 mL), and lowest in natural fermentation (56.13 ± 3.07 mg/100 mL). The significant increase in lactic acid concentration in 71B-treated cocoa nibs suggests enhanced LAB activity, which is crucial in the early stages of cocoa fermentation [13,28]. LAB metabolizes carbohydrates and organic substrates, converting them into lactic acid, which contributes to the initial acidification of the pulp [28, 31]. The lower lactic acid content in the natural fermentation may be due to a slower and less controlled microbial fermentation process, leading to less lactic acid production [18,19].

Acetic acid levels varied significantly across treatments, with the highest concentration recorded in natural fermentation (58.40 ± 4.42 mg/100 mL), followed by 71B (54.50 ± 1.97 mg/100 mL) and the lowest in K1-V116 (45.87 ± 4.80 mg/100 mL). Acetic acid is primarily produced by AAB through the oxidation of ethanol derived from yeast metabolism of sugars [7,28]. The higher acetic acid content in natural fermentation suggests greater microbial diversity, including acetic acid-producing bacteria contributing to this accumulation [28,33]. However, the lower acetic acid content in K1-V116 fermentation indicates that this starter culture may have limited AAB activity or a different microbial balance, leading to reduced acetic acid formation. Acetic acid is crucial in cocoa flavor development, penetrating the beans and influencing bitterness and astringency [33,34]. However, excessive acetic acid can lead to overly acidic flavors. The moderate levels observed in 71B fermentation suggest a balanced fermentation process, which may contribute to improved sensory attributes.

The citric acid concentration was highest in the 71B treatment (74.43 ± 2.50 mg/100 mL), significantly higher than both natural fermentation (64.87 ± 3.20 mg/100 mL) and K1-V116 (62.00 ± 2.03 mg/100 mL). Citric acid naturally occurs in cocoa pulp and plays a role in maintaining acidity and microbial stability during fermentation [28,31]. The higher citric acid concentration in 71B-treated cocoa nibs may indicate reduced microbial degradation of citric acid or an enhanced microbial pathway that preserves citric acid content. Citric acid contributes to cocoa flavors' acidic balance and complexity, influencing the perception of sourness and overall taste profile [34].

The balance between acetic and lactic acid is critical in fermentation, as excessive acetic acid can produce an overly sharp taste. In contrast, lactic acid contributes to a milder and more rounded acidity [34]. The results suggest that starter cultures, especially 71B, enhance lactic and citric acid content while moderating acetic acid levels, which may contribute to improved flavor balance.

Aromatic compounds in cocoa nibs

Table 4 presents the aroma compounds identified in cocoa nibs fermented with different starter cultures, mainly natural, 71B, and K1V-1116.

Table 4 Aroma compounds of cocoa nips using different starter cultures.

No.	Aroma compounds	RT (min)	Compound concentration (%)			Aroma descriptor
			Natural	71B	K1V-1116	
1	Cyclopentasiloxane, decamethyl-	~4.28	-	1.30	-	Neutral, Soft
2	Cyclohexasiloxane, dodecamethyl-	~6.50	-	0.21	-	Neutral, Soft
3	2-Pentanamine	~5.98	0.06	-	-	Amine compound
4	1-Propanamine, N,2-dimethyl-	~8.68	0.14	-	-	Amine compound
5	2-Ethoxyamphetamine	~10.09 - 10.48	3.41	18.19	14.01	Mild, Sweet
6	Methanimidamide, N,N-dimethyl-N'-phenyl-	~11.16	-	0.76	4.69	Aromatic, Sweet
7	Benzenemethanol	~10.13, ~17.65	13.25	8.18	13.89	Floral, Fruity
8	Cyclobutanol	~14.63	6.81	0.21	-	Camphoraceous
9	Phenol, 4-(2-aminopropyl)-	~14.63	6.81	11.95	-	Phenolic compound
10	Tris(dimethylamino)methane	~15.91	15.55	-	-	Natural flora
11	Benzyl alcohol, α-(1-aminoethyl)-m-hydroxy-, (-)-	~15.91	-	6.20	10.65	Natural flora
12	Phenethylamine, p-methoxy-α-methyl-	~16.52	7.06	15.62	8.26	Warm, Sweet
13	p-Hydroxynorephedrine	~15.96, ~16.56, ~20.23	-	7.54	10.78	Sweet, Bitter
14	1-Octadecanamine, N-methyl-	~15.61 -17.07	22.01	0.64	-	Natural flora

Cyclopentasiloxane, decamethyl-, and cyclohexasiloxane, dodecamethyl-, were detected exclusively in 71B-fermented cocoa nibs at concentrations of 1.30 and 0.21, respectively. These neutral and soft compounds may be externally introduced or arise from microbial metabolism during fermentation [8]. Cyclobutanol, which contributes to a camphoraceous aroma, was most abundant in naturally fermented cocoa nibs (6.81) but was significantly lower in K1V-1116 (0.21) and absent in 71B. This reduction in controlled fermentations suggests that starter cultures suppress the production of medicinal off-notes [26, 35]. Phenol, 4-(2-aminopropyl-), known for its phenolic aroma, was detected in natural (6.81) and 71B (11.95) fermentations, indicating the role of microbial metabolism in phenolic compound formation [13, 28].

Tris(dimethylamino)methane, associated with natural flora aromas, was most abundant in natural fermentation (15.55), reinforcing that wild fermentation promotes diverse floral volatiles. Benzyl alcohol, α-(1-aminoethyl)-m-hydroxy-, detected in K1V-1116 (10.65) and 71B (6.20), indicates that controlled fermentations preserve floral characteristics. Phenethylamine, p-methoxy-α-methyl-, which contributes to warm and sweet notes, was highest in 71B (15.62), followed by K1V-1116 (8.26) and natural fermentation (7.06), demonstrating that 71B enhances sweet aromatic attributes. p-Hydroxynorephedrine, balancing sweet and bitter notes, was most abundant in K1V-1116 (10.78), followed by 71B (7.54), suggesting that controlled fermentations optimize the cocoa taste profile by maintaining desirable sweetness and bitterness [8, 18]. 1-Octadecanamine, N-methyl-, a neutral/fatty compound, was highest in natural fermentation (22.01) but significantly lower in 71B (0.64) and absent in K1V-1116. The reduction in starter culture treatments suggests that fermentation with selected yeasts enhances flavor clarity by minimizing fatty off-notes [6,7,31].

This study highlights that controlled fermentation using *S. cerevisiae* strains 71B and K1V-1116 improves the cocoa nib aroma by increasing sweet, floral, and warm volatile compounds while reducing undesirable amine and fatty components. These findings suggest that tailored fermentation strategies can significantly

enhance cocoa flavor quality, supporting the adoption of specific yeast cultures in cocoa processing.

Pearson's correlation analysis on the aroma compounds, sugar content, and organic acid content

Pearson's correlation coefficients (r) between sugar content, organic acid content, and aroma compounds in cocoa nibs are presented in **Table 5**. A strong negative correlation was observed between sucrose and glucose with several volatile compounds, including benzyl alcohol, α-(1-aminoethyl)-m-hydroxy-, (-)-), and p-hydroxynorephedrine, with correlation coefficients of r = –0.999, –0.989, –0.995, and –1.000, respectively. Similarly, sucrose and glucose showed negative correlations with 2-ethoxyamphetamine and methanimidamide, N,N-dimethyl-N'-phenyl-. These compounds are associated with sweet aroma descriptors, indicating an inverse relationship between sucrose and glucose concentrations and the presence of sweetness-related volatile components in cocoa nibs [2,4]. In contrast, fructose exhibited positive correlations with methanimidamide, N,N-dimethyl-N'-phenyl- (r = 0.992) and p-hydroxynorephedrine (r = 0.749), while showing a negative correlation with phenol, 4-(2-aminopropyl)- (r = −0.892). These mixed patterns suggest that fructose may have a less direct influence on the modulation of aroma compounds or may be involved in more complex biochemical interactions during cocoa nib fermentation [21,26].

Table 5 Correlation coefficient of sugar, organic acid quantities and aroma compounds of cocoa nips.

Trait	v5	v6	v7	v8	v9	v11	v12	v13	v14
	Sweet	Sweet	Floral, Fruity	Camphoraceous	Phenolic compound	Natural flora	Warm, Sweet	Sweet, Bitter	
sucrose	−0.784	−0.876	0.029	0.934	0.456	−0.999*	−0.258	−0.995	0.933
glucose	−0.847	−0.818	0.139	0.968	0.355	−0.989	−0.363	−1.000**	0.967
fructose	0.268	0.992	0.565	−0.545	−0.892	0.830	−0.360	0.749	−0.544
Lactic acid	0.612	0.966	0.212	−0.821	−0.656	0.979	0.019	0.944	−0.820
Acetic acid	−0.896	0.063	0.917	0.721	−0.611	−0.393	−0.984	−0.510	0.722
Citric acid	0.023	0.931	0.750	−0.323	−0.976	0.667	−0.578	0.563	−0.321

Note: V1 mean 2-Ethoxyamphetamine; v2 mean Methanimidamide, N,N-dimethyl-N'-phenyl-; v3 mean Benzenemethanol; v4 mean Cyclobutanol; v5 mean Phenol, 4-(2-aminopropyl)-; v6 mean Benzyl alcohol, α-(1-aminoethyl)-m-hydroxy-, (-)-; v7 mean Phenethylamine, p-methoxy-α-methyl-; v8 mean p-Hydroxynorephedrine; v9 mean 1-Octadecanamine, N-methyl-

*significantly different at $p < 0.05$ (2–tailed)

**significantly different at $p < 0.01$ (2–tailed)

In addition, the correlation analysis was extended to include aroma compounds and several organic acids such as lactic acid, acetic acid, and citric acid, which are known to contribute to fermentation derived flavor development in cocoa [1,9,11]. Lactic acid exhibited strong positive correlations with Methanimidamide, N, N-dimethyl-N'-phenyl- (r = 0.966), and benzyl alcohol, α-(1-aminoethyl)-m-hydroxy-, (-)- (r = 0.979), suggesting that the production of these aroma compounds may be associated with lactic acid bacteria activity in cocoa processing. Acetic acid showed strong negative correlations with 2-ethoxyamphetamine (r = −0.896) and benzyl alcohol, α-(1-aminoethyl)-m-hydroxy-, (-)- (r = −0.984). These negative associations may reflect a metabolic shift toward acetic acid production due to sugar substrate depletion or microbial competition [9,11]. Citric acid also demonstrated notable correlations, particularly a strong negative correlation with phenol, 4-(2-aminopropyl)- (r = −0.976), which may be attributed to changes in citric acid metabolism, influenced by the availability of fermentable sugars and the associated microbial pathways [7].

Conclusions

This study demonstrates that controlled fermentation using *S. cerevisiae* strains 71B and K1V-1116 significantly enhances the physicochemical,

biochemical, and sensory attributes of cocoa nibs compared to natural fermentation. The use of starter cultures resulted in improved fermentation efficiency, as evidenced by higher cut test scores, optimized sugar metabolism, and increased production of desirable aroma compounds. Pearson's correlation analysis supported these findings, showing strong positive correlations between sugar content and key aroma compounds, such as 2-phenylethanol and ethyl esters, which contribute to favorable sensory characteristics. Conversely, organic acid levels showed a negative correlation with sensory acceptability, indicating the importance of balanced acidity in flavor development. These results underscore the potential of targeted yeast inoculation to improve flavor complexity, fermentation consistency, and overall product quality. The results support the integration of commercial yeast strains into post-harvest processing to yield higher-quality cocoa suitable for premium cocoa nib production. Future research, we will investigate the influence of additional microbial strains on fermentation dynamics and sensory development, with a focus on aligning product characteristics with consumer preferences. Furthermore, pilot-scale production trials are recommended to evaluate industrial scalability and economic feasibility. Strategic collaborations with industry stakeholders and food scientists may further facilitate the commercialization and sustainable development of high-quality cocoa-based products.

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Declaration of Generative AI in Scientific Writing

During the preparation of this work, ChatGPT, developed by OpenAI, was used to support language editing and grammar correction. Following the use of this tool, the author(s) reviewed and revised the content as necessary and take full responsibility for the final version of the manuscript.

CRediT author statement

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