Research Paper

APPLIED FOOD BIOTECHNOLOGY, 2026, 13 (1): e27 (1-19)
Journal homepage: www.journals.sbmu.ac.ir/afb

pISSN: 2345-5357
eISSN: 2423-4214

OPEN ACCESS

Effects of a Defined Functional Microbial Consortium on Cocoa Fermentation Dynamics and Bean Quality in Côte d'Ivoire

Ettien Y. Koffi, Hadja D. Ouattara, Lamine Samagaci, Wilfried K. Yao, Sebastien L. Niamke, Honore G. Ouattara*

Departement de Biotechnologies et Securite Alimentaire, UFR Biosciences, Universite Felix-Houphouet/ Boigny, Abidjan, Côte d'Ivoire.

Article History:
Received: 21 Aug 2026
Revised: 20 Oct 2026
Accepted: 22 Oct 2026
Published online: 31 Oct 2026

Keywords: Cocoa fermentation, Microbial starter, Quality, Improve, Cote d'Ivoire

How to cite this article: Koffi E, Ouattara H, Samagaci L, Yao W, Niamke S, Ouattara H. Effects of a Defined Functional Microbial Consortium on Cocoa Fermentation Dynamics and Bean Quality in Cote d'Ivoir. Appl Food Biotechnol. 2026; 13 (1): e27 (1-19).
http://doi.org/10.22037/afb.v13i1.53252

Corresponding Author:
Honore G. Ouattara

E-mail address:
kidou1212@gmail.com
Ouattarahonore@ufhb.edu.ci

© 2026 the authors. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0) License.

Abstract

Background and Aim: Use of starter cultures is considered as promising strategy for improving the cocoa fermentation process and ensuring consistent bean quality. Therefore, this study previously characterized valuable microbial strains isolated from fermenting cocoa, which showed potential for use as starter cultures. This study assessed the effects of these selected microbial starters on the cocoa fermentation at farm level as well as quality of the resulting beans.

Material and Methods: The methodology involved a comparative analysis of starter-inoculated and spontaneous cocoa fermentations. The starter consortium consisted of well-characterized functional microbial strains, including yeasts, lactic acid bacteria, acetic acid bacteria and *Bacillus* spp.

Results and Conclusion: Next-generation sequencing analysis revealed that in starter-assisted fermentation, the inoculated strains reached an amplicon sequence variant abundance of up to 69% of the total identified microbiota, compared with 24% in spontaneous fermentation. Fungal amplicon sequence variant abundance strongly decreased in starter-assisted fermentation. The inoculated starter stimulated the microbial activity, resulting in further rapid accumulation of acids in the cotyledons during starter-assisted fermentation, with total acid content reaching approximately 1.5-fold higher than that in spontaneous fermentation. Similarly, starter inoculation promoted a faster increase in reducing sugar content within the beans. Fermentation index reached one within 60 h in starter-assisted fermentation, whereas spontaneous fermentation needed 132 h to reach a similar index. The cut test further showed that 84 h were sufficient for starter-assisted fermentation to achieve 60% fully fermented cocoa beans, whereas spontaneous fermentation yielded only 34% fully fermented beans at a similar time point. At the end of fermentations, 92% of beans from starter-assisted fermentation were fully fermented, compared with 70% in spontaneous fermentation. Overall, use of the defined starter culture significantly accelerated the fermentation process and improved the quality of fermented cocoa beans.

Conflict of interest: The authors declare no conflict of interest.

What is "already known":	• Cocoa fermentation is driven by a complex microbial community. • Only a limited number of microbial species play key functional roles in cocoa fermentation. • The selection and application of functional microorganisms can significantly improve the efficiency and outcome of cocoa fermentation.
What this article adds:	• Validated microbial starters for farm-level cocoa fermentation in Côte d'Ivoire. • Selected starters accelerated fermentation and improved cocoa bean quality within 84 hours. • Microbial selection also reduced fungal ASV abundance and increased cotyledon acidity and reducing sugars. • This study provides first NGS-based characterization revealed high cocoa microbial diversity in Côte d'Ivoire.

1. INTRODUCTION

Fermentation of cocoa beans is an important and critical microbial process for production of high quality [1-3]. During this process, a spontaneous microbiota comprising yeasts, lactic acid bacteria (LAB), acetic acid bacteria (AAB) and *Bacillus* species use the cocoa pulp as substrate, driving diverse metabolic activities. Biochemical transformations include majorly oxidization of glucose into ethanol and lactic acid respectively by yeasts and LAB and further oxidization of ethanol by AAB. Microbial activities include production of pulp degrading enzymes by yeasts [4-6] and *Bacillus* [7, 8] as well as citrate lyase by LAB [9]. These processes trigger cascade reactions within the beans, decreasing bitterness, generating peptide precursors of chocolate aroma and promoting development of the brown color characteristic of well-fermented cocoa beans [10-12].

However, cocoa fermentation is an often-inconsistent process, difficult to control, leading to variable quality of fermented and cured beans and hence lesser crop values for farmers [13, 14]. This issue partly originates from the diversity and instability of the spontaneous microbial community involved in cocoa fermentation [15-17]. Only a limited group of microbial species with specific functional roles are needed to ensure successful fermentation and the production of high-quality chocolate [16, 18, 19]. Accordingly, the use of selected microbial strains with well-defined functional characteristics as starter cultures is regarded as an effective strategy to control and standardize the fermentation process. Numerous studies have demonstrated that microbial starter cultures can be used to fine-tune and modulate the quality of fermented cocoa and chocolate, highlighting the significant influence of specific microorganisms on the overall process [20-23].

However, various qualities of fermented cocoa bean were achieved using various compositions of microbial starters through various cocoa producing countries [24-26]. Moreover, several studies have shown that each cocoa-producing region possesses its own indigenous microbiota, well adapted to fermenting locally grown cocoa [17, 27]. Cote d'Ivoire, the world's leading cocoa producer, includes a limited experience with the use of starters culture for fermentation of cocoa. Through extensive studies, we have previously identified a microbial community, which plays a key role in Ivorian cocoa fermentation and screened restricted functional strains as potential microbial starter [17, 28-34]. The aim of this study was to assess the added value of these functional microbial strains when used as starter cultures in cocoa fermentation.

This study demonstrated that the starter strains were effective under natural farm-scale fermentation conditions, significantly accelerating the fermentation process and improving the quality of the resulting cocoa beans. The inoculation of the selected starters significantly modified the microbial dynamics, as reflected by a drastic reduction in the abundance of fungal amplicon sequence variants (ASVs). Inoculation induced a reported increase in the fermentation index (FI), as well as acid and reducing sugar contents in cotyledons. Collectively, these findings indicated that the selected microbial starters could effectively control and accelerate cocoa fermentation under real farm conditions, while improving key biochemical characteristics associated with cocoa quality. To authors' best knowledge, this study represented the first use of next-generation sequencing (NGS) to characterize the cocoa fermentation microbiota in Cote d'Ivoire and revealed an exceptionally high diversity of microbial species. Furthermore, *Bacillus A. bombysepticus, Lacticasei-bacillus porcinae* and *Talaromyces verruculosus* seem to be reported for the first time in cocoa fermentation. Overall, these results highlighted the potential of selected microbial starters as a practical strategy for shortening fermentation time, modulating microbial succession, enhancing biochemical fermentation processes and improving the quality and consistency of cocoa beans produced under farm-level conditions in Cote d'Ivoire.

2. MATERIALS AND METHODS

2.1. Composition of Microbial Consortium

Microbial strains used to compose the consortium in this study were isolated from fermenting cocoa in Cote d'Ivoire and then identified and characterized as potential starters regarding their interesting functional characteristics [17, 28, 31, 35]. These strains are listed in Table 1.

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0). To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

Table 1. Microbial strains included in the starter culture consortium

Microbial group	Microbial strains	Inoculum size (g)	Techno-functional properties	References
Yeasts	*Saccharomyces cerevisiae* (T7GB10)	0.918 (5.10^4 UFC)	High ethanol production Pectinolytic enzyme production	[5, 32]
	Candida tropicalis (D5P12)	0.820 (5.10^4 UFC)		
Lactic acid bacteria	*Lactobacillus casei* (T10G5)	0.299 ($3.33\ 10^4$ UFC)	Degradation of sugars and citric acid, production of lactic and acetic acids, aroma production, antifungal activity	[9, 17, 28, 29, 30, 46]
	Lactobacillus plantarum (T11G3)	0.248 ($3.33\ 10^4$ UFC)		
	Leuconostoc mesenteroides (T8AB6)	0.404 ($3.33\ 10^4$ UFC)		
Acetic acid bacteria	*Acetobacter pasteurianus* (T6HS14)	0.775 (10^5 UFC)	Resistance to ethanol and acetic acid production Thermotolerant up to 50°C	[34, 32, 35]
Bacillus	*Bacillus thuringiensis* (T11I10)	1.739 (10^5 UFC)	Production of pectinase, aroma compounds and organic acids	[30]

2.2. Preparation of Starter Cultures and Cells Freeze-Drying

Strains previously stored at -70 °C in 20 % glycerol were carefully thawed on ice, then transferred into liquid media and incubated at 30 °C for 24–72 h. The media used for microorganism isolation were yeast, peptone, glucose (YPG) (Difco, France) for yeasts, Mans de Rogosa Sharpe (MRS) (Difco, France) for LAB, yeast, ethanol, peptone, glucose (YEPG) (Difco, France) for AAB and nutrient broth (NB) for *Bacillus*. After incubation, the revitalized microbial cells were transferred from the liquid media to the equivalent agar solid media then set to grow at 30 °C for additional 24–72 h. Colonies on agar media were used to prepare the inoculum.

Two to three pure colonies of each strain were used to seed one liter of culture broth (YPG, MRS, YEPG and GN) depending on the strain and incubated in a similar condition as described previously. Then, cells were pelleted after centrifugation at 12,000× g for 10 min. Then, 10 ml of skimmed milk and 25 ml of 20% sucrose were added to the pellets as cryoprotectants. The highly cell-concentrated homogenized mixture containing approximately 2×10^9 (UFC).ml^{-1} was frozen at -60 °C for 1 h, followed by a drying process carried out at a similar temperature under a pressure of 1 Pa for 72 h [36].

2.3. Formulation of the Microbial Consortium

The resulting powder from freeze-drying was used for enumeration to estimate the number of viable cells par grams of powder, using standard decimal dilution method [37]. Viable cells from the lyophilisat were assessed as approximately 70 %. Then, formulation was realized with the aim of 10^5 microbial cells per gram of fermenting cocoa beans for each microbial group (yeasts, LAB, AAB and *Bacillus*). Thus, the formulated consortium for fermentation of 50 kg of cocoa bean consisted approximately of 5.3 g of powdered microorganisms comprising 1.75 g of yeasts, 1.00 g of LAB, 0.8 g of AAB and 1.74 g of *Bacillus*. The various inoculum sizes are detailed in Table 1.

2.4. Fermentation Conditions

Ripe cocoa pods were harvested from a farm located in Azaguie, geographic coordinate 5°37’ 60’’ North, 4°4’ 60’’West, at 40-km distance from Abidjan and transported to National Floristic Center in a farm environment, University Felix Houphouet-Boigny (Abidjan, Cote d’Ivoire). Pods were opened and beans were extracted manually and then placed on banana leaves. A hundred kilograms of beans were divided into two fermenting masses of 50 kg each. The first mass of cocoa beans was used in a spontaneous fermentation (SF), whereas the second mass was used in starter added fermentation (SAF) supplemented with the microbial consortium described

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0). To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

previously. The microbial consortium powder was evenly distributed over the cocoa bean heap, followed by thorough mixing to ensure uniform dispersion of the microorganisms. Cocoa beans were fermented in traditional conditions on banana leaves for 6 d. Whether it was SF or SAF, one fermentation was carried out per treatment. As technical replicates, three various samples from a similar treatment were collected for assays.

2.5. Analysis of Fermentation Dynamics

The monitoring of fermentation conditions, especially temperature and pH, was carried out directly on the fermenting heap. parameters were assessed at 15-cm depth in the heap with 12-h intervals, using portable pH-meter equipped with a temperature probe (HI7662 model, Hanna Instruments, USA). After the pH and temperature assessments, beans were withdrawn from the heap (top, middle, bottom and sides) to create a sample of approximately 500 g. These samples were used in further analyses at laboratory scale, including precisely fermentation index (FI), reducing sugar and acids assays. Prior to carrying out these analyses, beans were manually dehulled and the cotyledons (nibs) were collected.

2.6. Titratable Acids

For acid assay, 2 g of finely ground cotyledons were mixed with 18 ml of distilled water (DW) using 50-ml Erlenmeyer; the mixture was vortexed for 2 min and then filtered through Whatman membrane filter with a pore size of 0.45 µm (Cytiva, USA). To 5 ml of the resulting filtrate, two drops of phenolphthalein were added, allowing acids titration with a 0.1 N NaOH solution, until a persistent pink color was achieved (AOAC, 1990). Phenolphthalein and NaOH were purchased from Merck, Germany.

2.7. Reducing Sugars

To carry out reducing sugar assay, 5 g of ground cotyledon were mixed with 50 ml of hot (60 °C) DW using 200-ml flasks and the solution was allowed to cool at room temperature (RT). The cooled solution was filtered and the collected filtrate was made up to 100 ml using DW; this constituted the water-soluble sugar extract from the cotyledon [38]. Assessment of sugar content in this extract was carried out based on the method of Peter Bernfeld [39]. A volume of 200 µl of DNS (3,5-dinitrosalicylic acid) reagent (Sigma Aldrich, France) was added to 1 ml of water-soluble sugar extract and mixture was heated at 100 °C for 5 min using water bath. An additional volume of 2 ml of DW was added to the heated mixture upon cooling and the solution absorbance was read at 540 nm using spectrophotometer (Pioway, China) against a blank prepared under a similar conditions without water-soluble sugar extract.

2.8. Fermentation Index

The FI of cocoa beans was assessed based on changes in cocoa bean pigments associated with anthocyanin degradation and the formation of brown-colored phenolic compounds during fermentation. This was monitored using method described by Gourieva and Tserrevitinov [40] Fifty millimeters of a mixed solution composed of methanol and hydrochloric acid (Sigma Aldrich, France) in a ratio of 97:3 (V/V) were added to 0.5 g of ground cotyledon. The mixture was homogenized and stored at 4 °C for 17 h and then filtered and the absorbance of the filtrate was read at two wavelengths especially, 460 nm to measure the absorbance of degraded anthocyanins and 530 nm to measure the absorbance of non-degraded anthocyanins. The Equation 1 was used to assess the FI:

$$FI = \frac{A(460)}{A(530)} \quad \text{Eq.1}$$

where, FI was fermentation index, A(460) was absorbance at 460 nm and A(530) was absorbance at 530 nm.

2.9. Cut Test

The cut test is a method used to assess the physical quality of fermented and dried cocoa beans. This test was carried out using method described by Ching L Hii, *et al.* [41]. Three hundred fermented and dried cocoa beans were randomly selected and divided into three sets of 100 beans each. Each bean was cut lengthwise using cutter and the halves were investigated under daylight to assess the color, internal defects and the progress of fermentation. The beans were classified based on their internal coloration as brown beans (well fermented), brown purple beans (partially fermented), purple beans (unfermented), slate-colored beans, insect damaged beans, moldy beans and sprouted beans [14].

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0). To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

2.10. Genomic DNA Extraction and Sequencing of Ribosomal Genes from Cocoa Microbiota

Fermenting beans from Day 2, Day 4 and Day 6, representative of global microorganism diversity during the fermentation, were pooled into one sample of 20 g. This sample was vortex-homogenized into 70 ml of saline solution for 5 min. The mixture was then decanted and centrifuged at 170× g for 5 min at 4 °C using centrifuge (Eppendorf 5430 R, Eppendorf, Germany). The supernatant was filtered through cheesecloth with an approximate pore size of 20 µm. The resulting filtrate was centrifuged at 8,000× g for 20 min to pellet cells. Total DNA was extracted from the resulting cell pellet using phenol-chloroform method described by Camu et al. [42]. Extracted DNA was subjected to NGS targeting the bacterial 16S rRNA gene and the fungal internal transcribed spacer (ITS) region, including 5.8S rRNA gene. Amplification was carried out using universal bacterial primers of F27/R1492 and the fungal primers of ITS1/ITS2. The resulting amplicons were sequenced to characterize and identify the bacterial and fungal microbiota.

A total of two samples (one sample per fermentation) were used in NGS analysis. Each sample included the cocoa microbiota. After amplification and purification, full-length amplicons were converted into SMRTbell libraries through adapter ligation and subsequent cleanup. The resulting library was sequenced on PacBio Revio system using single molecule real-time (SMRT) sequencing technology. Raw sub-reads were processed for quality control using SMRT Link software v.25.1.0.257715; where, circular consensus sequencing (CCS) analysis was carried out to generate highly accurate consensus reads (> QV40). These highly accurate reads were then processed through DADA2 pipeline (https://benjjneb.github.io/dada2/index.html) for quality control assessment and inference of ASVs. Taxonomic assignments were then carried out using QIIME 2 v.2021.11 (https://docs.qiime2.org/2021.11/). Bacterial 16S rRNA gene sequences were classified using SILVA reference database v.138.1, whereas fungal ITS sequences were classified using UNITE database.

2.11. Statistical Analysis

One-way analysis of variance (ANOVA) was carried out using Stat-Plus software v.5.4 at a 95% confidence level to assess significant differences between the means of various treatments (SF and SAF). Each treatment was represented by three technical replicates, consisting of three distinct samples collected from a similar treatment. Results were expressed as means ±SD (standard deviation).

3. RESULTS AND DISCUSSION

3.1. Composition of the Microbiota in Spontaneous Fermentation and Starter Added Fermentation

Table 2 presents a comparative analysis of the microbiota identified in SF and SAF. Although the overall microbial community structure was roughly unchanged, several species detected in SF were absent in SAF, including *Malassezia globosa*, *M. restricta*, *Wickerhamomyces anomalus*, *Meyerozyma* sp., *T. verruculosus* and various species of the Genus *Enterococcus.* Fungal strains were commonly detected in fermenting cocoa as contaminants susceptible to damage the quality of fermented cocoa and its products. From the fungal species identified in this study, *W. anomalus* and *Meyerozyma* spp. have repeatedly been reported in cocoa fermentations from (Cote d'Ivoire, Ghana, Brazil and Ecuador) [16; 43; 44], while *M. globosa* and *M. restricta* were reported as dominant species in fine-flavor cocoa fermentations in Colombia [45]. Moreover, *T. verruculosus* was unusual as it was not reported from fermenting cocoa, to the best of the authors' knowledge.

More interestingly molds species were not detected in SAF, excepted *Fusarium* sp. A possible explanation for lesser abundance of fungal species in SAF was that starter inoculation might inhibit their growth. This hypothesis was supported by the authors' previous findings, which demonstrated the antifungal activity of *Lactiplantibacillus plantarum*, *Leuconostoc mesenteroides* and *L. casei* isolated from cocoa fermentations [46]. These strains, which were included in the starter consortium of the present study, demonstrated strong antagonistic activity against *Aspergillus* spp., *Fusarium* spp. and *Mucor* spp. [46]. Hence, their presence in the current starter culture might contribute to the inhibition of these fungal species during fermentation.

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0).
To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

Table 2. Microbial communities identified in various cocoa fermentation, spontaneous fermentation and starter added fermentation.

Microbial Group Identified	Spontaneous fermentation (SF)	Microbial mixture added as starters	Starter added fermentation (SAF)
Yeats	*Candida akabanensis, Malassezia globosa Malassezia restricta, Diutina rugosa Pichia manshurica,* ***Candida tropicalis*** *Hanseniaspora uvarum, Hanseniaspora opuntiae,* ***Saccharomyces cerevisiae****, Pichia kudriazevii, Pichia fermentans Debaromyces hansenii, Wickerhamomyces anomalus, Meyerozyma sp., Candida intermedia, Trichosporon asahii*	*Candida tropicalis, Saccharomyces cerevisiae,*	Candi*da akabanensis, Diutina rugosa Pichia manshurica,* ***Candida tropicalis*** *Hanseniaspora uvarum, Hanseniaspora opuntiae,* ***Saccharomyces cerevisiae****, Pichia kudriazevii, Pichia fermentans Debaromyces hansenii, Candida intermedia, Trichosporon sp.*
Molds	*Aspergillus subversicolor, Fusarium sp., Trichoderma sp., Talaromyces verruculosus,*		*Fusarium sp.,*
Lactic acid bacteria	*Limosilactobacillus fermentum,* ***Lactiplantibacillus plantarum,*** ***Leuconostoc mesenteroides****,* ***Lactocaseibacillus casei*** *Weissella confuse, Enterococcus italicus Weissella cibaria, Lacticaseibacillus rhamnosus, Enterococcus lactis, Enterococcus raffinosus, Ligilactobacillus agilis, Lacticaseibacillus porcinae, Liquorilactobacillus nagelii, Paucilactobacillus vaccinostercus, Lactobacillus amylovorus, Enterococcus faecalis, Pediococcus acidilactici, Levilactobacillus brevis*	*Lactiplantibacillus plantarum, Lacticaseibacillus casei, Leuconostoc mesenteroides*	*Limosilactobacillus fermentum,* ***Lactiplantibacillus plantarum,*** ***Leuconostoc mesenteroides****,* ***Lactocaseibacillus casei*** *Weissella confuse, Weissella cibaria, Lacticaseibacillus rhamnosus, Ligilactobacillus agilis, Lacticaseibacillus porcinae, Liquorilactobacillus nagelii, Lactobacillus amylovorus, Pediococcus acidilactici, Levilactobacillus brevis*
Acetic acid bacteria	*Acetobacter senegalensis, Acetobacter ghanensis, Acetobacter orientalis, Acetobacter fabarum,* ***Acetobacter pasteurianus****, Acetobacter syzygii, Acetobacter vaccinii, Acetobacter tropicalis Acetobacter pomorum, Acetobacter okinawensis, Acetobacter suratthaniensis*	*Acetobacter pasteurianus,*	*Acetobacter senegalensis, Acetobacter ghanensis, Acetobacter fabarum,* ***Acetobacter pasteurianus****, Acetobacter syzygii, Acetobacter vaccinii, Acetobacter tropicalis Acetobacter pomorum, Acetobacter okinawensis,*
Bacillus	*Bacillus subtilis, Bacillus pumilus,* ***Bacillus thuringiensis****, Bacillus A bombysepticus,*	*Bacillus thuringiensis*	*Bacillus subtilis, Bacillus pumilus****,*** ***Bacillus thuringiensis****,*
Other bacteria	*Pseudomonas asiatica, Acinetobacter baumannii, Corticimicrobacter populi Kerstersia gyiorum, Wolbachia pipientis*		*Pseudomonas asiatica, Acinetobacter baumannii,*

Fermenting beans from day-2, day-4 and day-6, representative of global microorganism diversity during the fermentation were pooled into one sample of 20 grams from which the total DNA was extracted [42]. Then, the microbiota was identified from the DNA extract, based on the NGS-sequencing of ribosomal genes (16S for bacteria and ITS 5,8S for fungi). Species corresponding to the starters are indicated in bold and underlined

Furthermore, all species constituting the starter culture especially *L. casei*, *L. plantarum*, *L. mesenteroides*, *Acetobacter pasteurianus*, *Bacillus thuringiensis*, *Saccharomyces cereviciea*, *Candida tropicalis* were detected in SF. This showed that these species were already present in SF prior to inoculation, indicating that the addition of the starter culture did not introduce new microbial species in the fermentation. However, the NGS analysis revealed clear differences in the microbial abundance between the two fermentation processes. The microbiota detected in SF

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0). To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

demonstrated a total absolute abundance of 37,184 reads, whereas starter-added fermentation (SAF) showed a higher absolute abundance, reaching 86,426 reads. Moreover, the starter species were detected at relatively small ASV abundance in SF, accounting for 24.63% of the total ASV abundance from microbial community (Figure 1). As expected, ASVs associated with these species were substantially further abundant in SAF where accounted for up to 69.08% of the total ASV abundance (Figure 1). From the microbial species composing the starter consortium, *A. pasteurianus*, *L. plantarum*, *L. mesenteroides*, *L. casei* and *B. thuringiensis* demonstrated the greatest increases in ASV abundance of SAF, compared with SF (Figure 1). The raw data of ASV abundance is provided in supplemental material.

Although NGS does not provide a direct estimate of viable microbial cells because DNA can originate from live and dead microorganisms, it offers valuable insights into the composition and diversity of the microbiota within the fermenting ecosystem [47]. Hence, the increased abundance of starter-associated ASVs in SAF could be consistent with enrichment of the inoculated strains, which might enhance the antifungal potential of the starter consortium and hence contribute to strongly decreases in the fungal growth in SAF. The NGS results revealed a high level of microbial diversity during cocoa fermentation in Cote d'Ivoire, including several microbial species that have not previously been reported in Ivorian cocoa fermentation, to the best of the authors' knowledge. Especially, species such as *B. A bombysepticus*, *L. porcinae* and *T. verruca-losus* were seemingly detected for the first time in cocoa fermentation. These findings significantly have expanded the currently known microbial diversity associated with cocoa fermentation and highlighted the potential richness of the microbial communities involved in Ivorian cocoa fermentation.

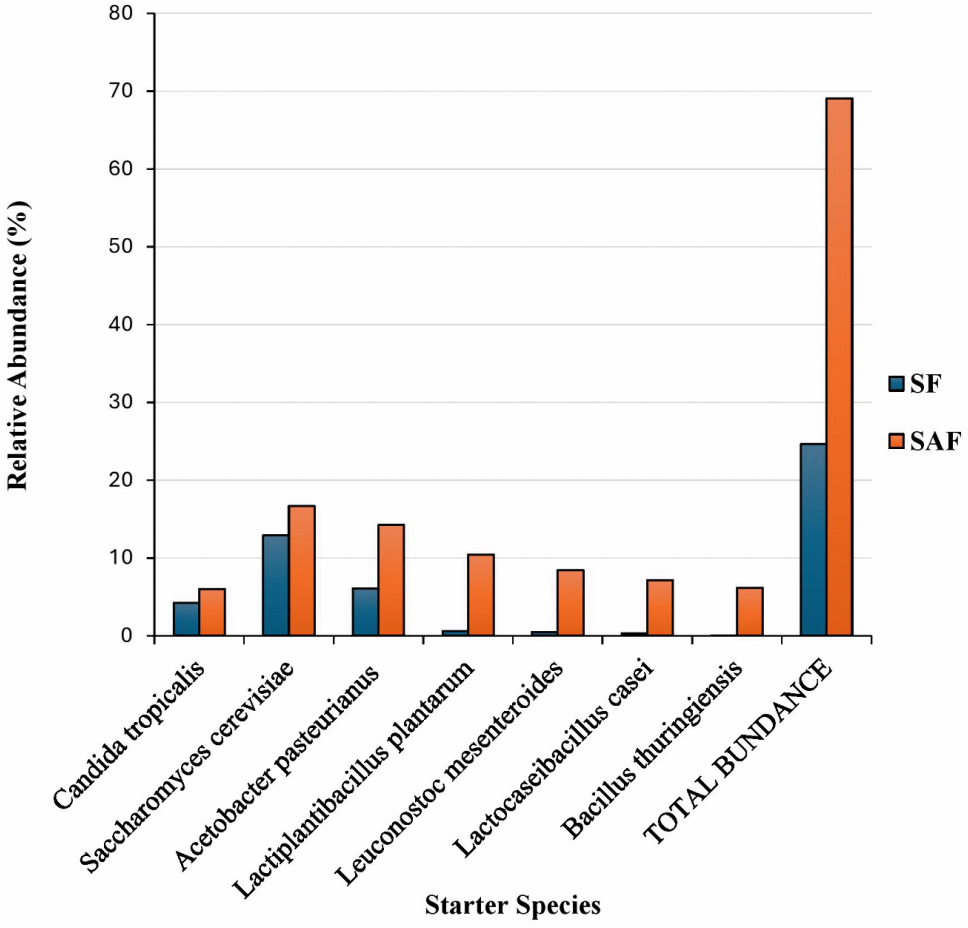


Figure 1. Relative abundance of starter species in spontaneous and starter-inoculated fermentations. The relative abundance of each starter species was calculated as the percentage abundance of that species relative to the total microbial abundance in each fermentation. Relative abundance values were calculated using raw abundance data provided in the Supplementary Material.

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0). To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

3.2. Variations of Temperatures During both Fermentations and Starter Added Fermentation

The increased ASV abundance of the starters strains in SAF seemed to include less effects of the fermentation dynamics. In the SF, a significant increase of temperature was observed during the first 84 h of the fermenting process. In this time, the temperature increased from 35 °C at 0 h, reaching the peak at 47.41 °C. A similar dynamic of temperature was observed in SAF with a reached peak of 47.80 °C. At the end of fermentation, the temperature was relatively higher in SAF (39.8 °C) than SF (32.23 °C) (Figure 2). Roughly, the dynamics of temperatures in SF and SAF processes were similar and no significant variation was observed. Temperature is closely associated to microbial activity during cocoa fermentation. Therefore, the limited variation observed in the temperature dynamics between the two fermentation treatments was unexpected.

Previously, Herrera-Rocha et al. [48] have described the temperature as a critical parameter during fine-flavor cocoa fermentation. The fermenting mass increased from nearly 27 to 49 °C and these researchers identified temperatures greater than 45 °C as critical control points for fermentation quality. This temperature increase contributes to the transition between microbial groups and promotes biochemical processes such as protein hydrolysis and polyphenol oxidation, which are essential for the formation of flavor precursors and the development of cocoa quality.

3.3. Acidification of Cocoa Beans During Fermentations

Acidification of cocoa beans during fermentation is important in the biochemical process leading to quality products, especially chocolate aroma production. In this study, bean acidification was monitored by assessing internal pH of the cotyledons and titratable acidity. Results showed that in SF, pH decreased from 6.98 at the beginning of the process to a value of 5.11 after 120 h of fermentation (Figure 3). Then, this pH increased slightly to reach 5.24 at the end of the process. In comparison, the pH in SAF changed with similar dynamics but the decrease was sharper leading to a lesser pH value of 4.61 after 120 h of fermentation and a pH value of 4.87 at the end of fermentation. Overall, decreasing of the pH was more rapid in SAF than SF.

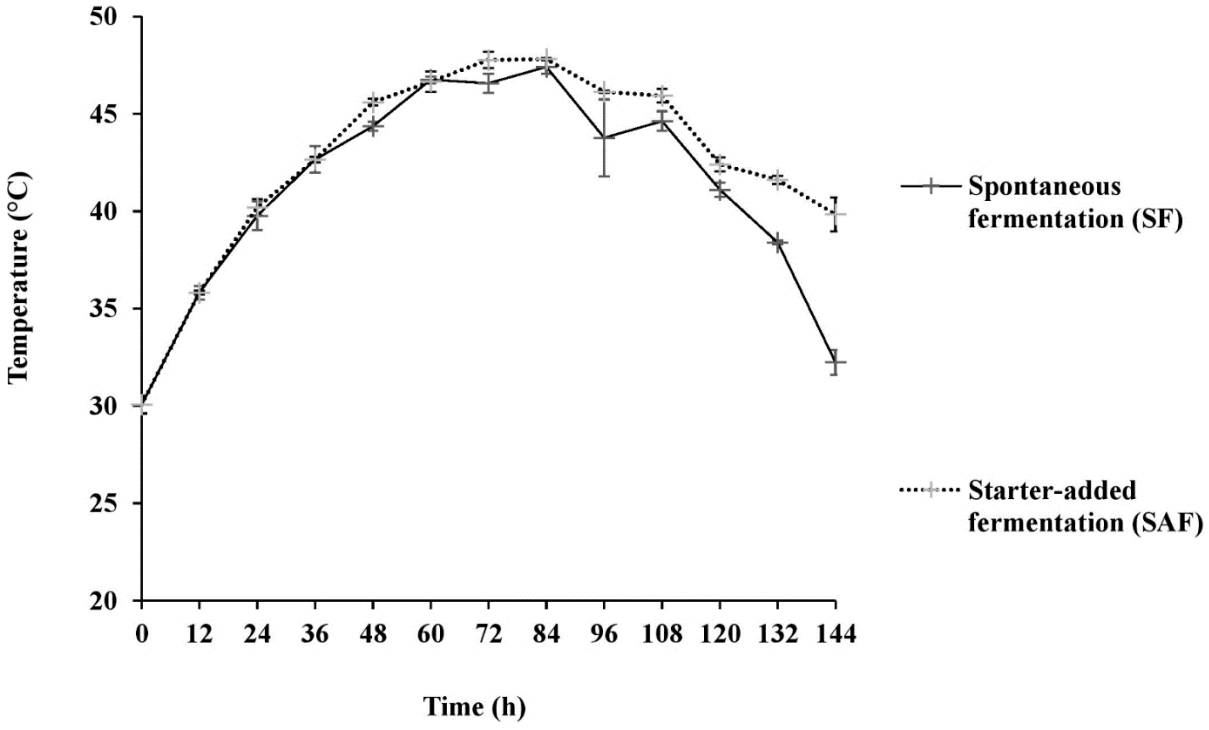


Figure 2. Development of the temperature of the fermenting heap during spontaneous fermentation and starter-added fermentation.

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0). To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

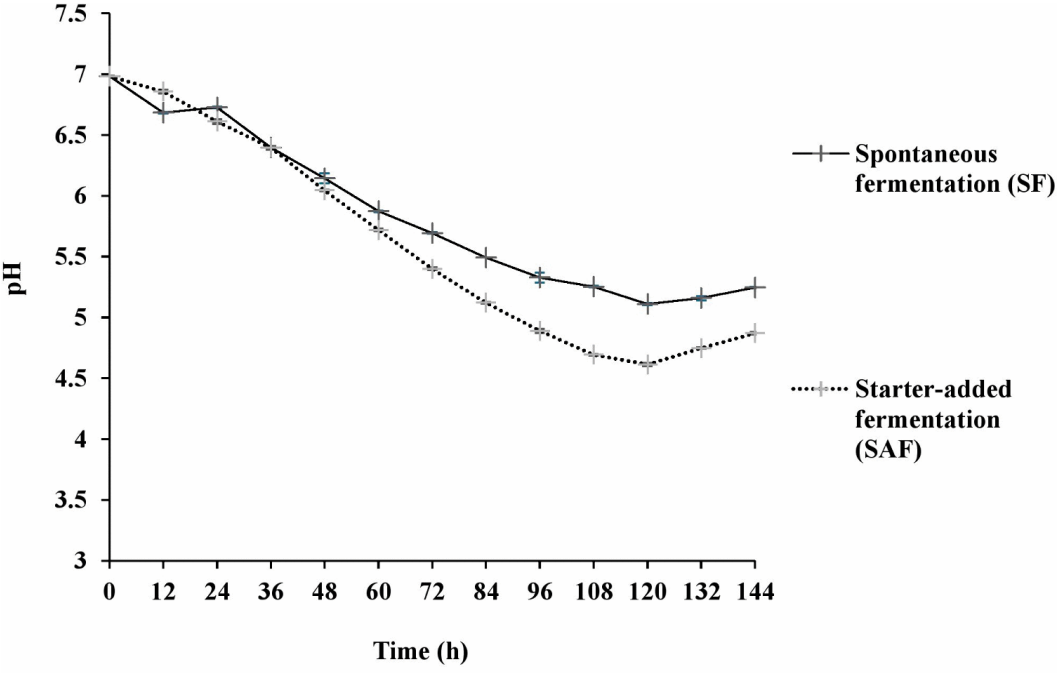


Figure 3. Comparative effects of spontaneous fermentation and starters-added fermentation on the pH in cotyledon.

Within approximately a similar period (0–108 h) in the two fermentations (SF and SAF), strong increase of the titratable acidity was observed within the cotyledons (Figure 4); the peak of acidity reached 0.86 (±0.06) mEq by gram of cotyledon in SAF and 0.6 (±0.06) mEq g^{-1} in SF (Figure 4). Then, this acidity decreased during the rest of the process. Significantly, the titratable acidity in the beans was further reported in SAF with a peak value of 1.5 folds, compared to the peak of acidity in SF.

Acidification is critical during cocoa fermentation, as it activates proteolytic enzymes, particularly proteases and therefore promotes pH-dependent biochemical reactions involved in the formation of chocolate aroma precursors. The enhanced acidification induced by the microbial consortium as observed in this study was likely to include strengthened and accelerated these biochemical processes; thereby, promoting the development of flavor precursors and ultimately improving the quality of the fermented cocoa beans [49, 50]. Since total titratable acidity was assessed, the individual organic acids responsible for the acidification could not be identified. Nevertheless, previous studies have consistently shown that acetic acid is the predominant organic acid responsible for the acidification of cocoa bean cotyledons and associated decreases in pH [16, 35, 51].

3.4. Production of Reducing Sugar During Fermentation Processing

Reducing sugars are well assessed as key compounds involved in the formation of chocolate aroma during roasting in industrial cocoa processing. In the current study, assays revealed a continuous increasing of reducing sugars content into beans during fermentations (Figure 5). Regarding the dynamics of productions of these compounds, three periods were distinguishable. The first period that could be considered as a lag phase occurred within the first 12–24 h of fermentation with no reducing sugars detected into beans. The second period spanning from 12–24 to 120 h showed a strong increase in reducing sugars content over the processes. At the end of this period (120 h), the yield of reducing sugar corresponded to 0.043 mg g^{-1} in SAF and 0.027 mg g^{-1} in SF. In the third period, the reducing sugars content were almost consistent; the yields at the end were 0.043 mg. g^{-1} and 0.029 mg. g^{-1} in SAF and SF, respectively. In summary, reducing sugars yield was higher in SAF than SF (Figure 5).

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0).
To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

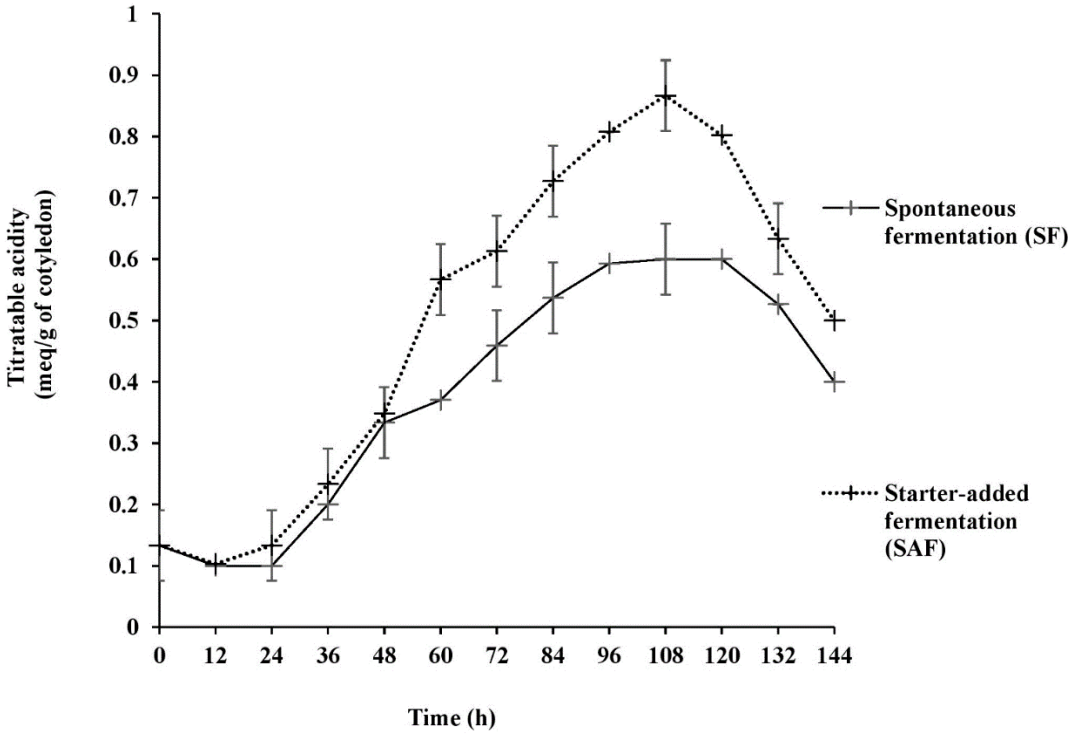


Figure 4. Comparison of acidity changes in cocoa cotyledons during spontaneous fermentation trials and starter-added fermentation. Error bars indicate standard deviation between three technical replicates.

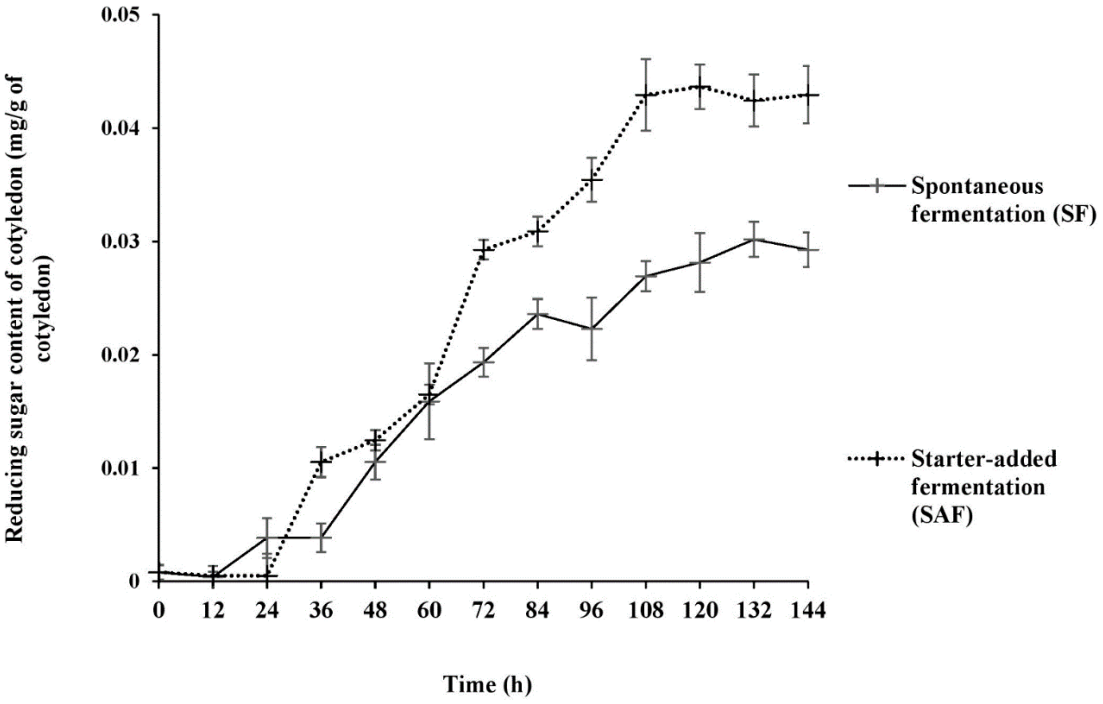


Figure 5. Comparative development of reducing sugars in cocoa cotyledons during spontaneous fermentation and starter-added fermentation. Error bars indicate standard deviation between three technical replicates.

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0). To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

Several reports have assessed that during roasting, reducing sugars participate in the Maillard reactions by reacting with free amino acids and short peptides generated during proteolysis, leading to the formation of volatile compounds responsible for the characteristic chocolate flavor [52]. The current study demonstrated that reducing sugars accumulated more rapidly and reached significantly higher concentrations in cocoa bean cotyledons during starter-inoculated fermentation than SF. Recently, Giulia Victoria Silva Lima, et al. [51] have observed an increase of reducing sugar under microbial starter-induced fermentation of Amazonian cocoa. Generally, the increase of reducing sugars, especially fructose and glucose, in fermenting cocoa beans results from sucrose hydrolysis by invertase [53]. The availability of reducing sugars enhances the likelihood of Maillard reaction during roasting [54]. The reported acidification of the cocoa bean cotyledons, coupled with the increased concentrations of reducing sugars during starter-inoculated fermentation, might possibly provide favorable biochemical conditions for the formation of chocolate aroma precursors.

3.5. Fermentation Index and Bean Quality

The FI assess the state of fermentation and indicate whether cocoa beans are under fermented (FI < 1), fully fermented (1 < FI < 1.59) or over fermented (FI > 1.59) [55]. Although FI is an indirect indicator of the degree of cocoa bean fermentation, this parameter is important to assess the quality of beans. The current results showed that in SF and starters added fermentation (SAF), FI increased progressively and continuously until the end of the process (Figure 6). In fact, fermentations began with 0.4 as FI and ended with FI values of 1.15 and 1.42 in SF and SAF, respectively. It is noteworthy that FI in SAF reached 1.11 after 60 h of fermentation (Figure 6), corresponding to 43% fully brown beans (Table 3). In contrast, the FI in SF was relatively small (0.85) at a similar time point with only 27% of brown beans, suggesting a less advanced fermentation process.

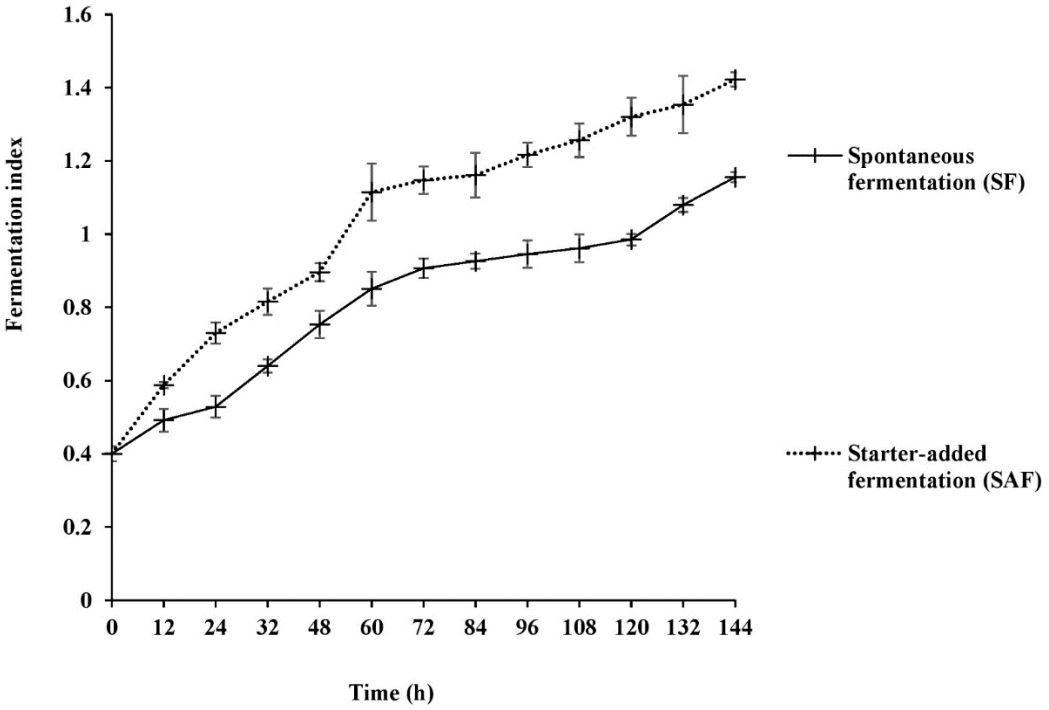


Figure 6. Development of fermentation index in spontaneous fermentation and starter-added fermentation. Error bars indicate standard deviation between three technical replicates.

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0). To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

Table 3. Summary of key fermentation indicators during fermentation with and without

		Treatment		
	Evaluation criteria	Spontaneous fermentation (SF)	Starter-added fermentation (SAF)	More advantageous treatment
Temperature	Temperature peak	47.4 °C	47.8 °C	Identical
	Time to reach peak	84 h	84 h	
Fermentation index (FI)	Increase in fermentation index	Moderate	Faster	SAF
	Time to reach FI = 1	132 h	60 h	SAF
Reducing sugars in the cotyledon	Amount of reducing sugars	Lower	Higher	SAF
	Maximum content and time to reach this maximum	0.030 mg/g after 132 h	0.043 mg/g after 108 h	
	Browning kinetics	Slow progressive	Fast and sustained	
Well-fermented beans (brown beans)	Percentage of fully-fermented (brown) beans at 60 h	27 %	43 %	SAF
	Percentage of fully-fermented (brown) beans at 84 h	34 %	60 %	
	Percentage of fully-fermented (brown) beans at the end, 144 h.	70 %	92 %	
Best times to stop fermentation (Fermentation with at least 60 % brown beans)		132 h	84 h	SAF
Time savings on fermentation			60 h	SAF

Brown color is the direct indicator of fully fermented beans, and 60 % of brown beans is the threshold of complete fermentation

In SF, 132 h of fermentation were needed to achieve a FI of 1.07 (Figure 6). The cut test revealed that SAF yielded 60% of fully fermented brown beans after 84 h of fermentation, whereas a similar rate of brown and cocoa beans was reached after 132 h in SF (Table 3). At the end of fermentation, SF showed a maximum of 70% brown beans, while 92% of brown beans was achieved in SAF (Table 3). These findings demonstrated that starter inoculation significantly enhanced the efficiency and progression and shortened the cocoa fermentation process.

Recently, Escobar et al. [56] reported that a decreased fermentation duration of 96 h was sufficient to achieve optimal development of global quality attributes and fine flavor characteristics of cocoa beans, whereas prolonged fermentation beyond this time resulted in a decreases in these desirable characteristics [56]. This highlighted the relevance of accelerating the fermentation process, as achieving optimal biochemical transformations within a shorter time may help preserve and enhance cocoa flavor potential while improving fermentation efficiency.

Based on the presence of *B. thuringiensis* and *C. tropicalis* in the microbial starter consortium, concerns may increase regarding their toxigenic potentials. However, the two species are naturally associated with cocoa fermentation ecosystem as reported in previous studies [30, 31, 57, 58, 59]. Furthermore, *C. tropicalis* has been investigated as a starter culture to improve cocoa bean quality and flavor [60], while *B. thuringiensis* has been used as a starter to suppress fungal growth during cocoa fermentation [59]. In the present study, use of these species was based primarily on their functional characteristics and potential technological benefits. Further characterizations, including whole genome sequencing (WGS), to screen toxin-associated genes and other relevant virulence determinants are necessary to better assess safety profiles before their full use at farm level.

4. CONCLUSION

In this study, the microbial consortium composed of selected starter strains positively affected cocoa fermentation by promoting higher microbial abundance, enhancing cotyledon acidification and increasing reducing sugar concentrations. This consortium induced higher FI and improved bean quality within a shorter fermentation time. Additionally, the starter consortium activated a strong decrease of fungal abundance in the fermenting cocoa. Collectively, these changes demonstrated the beneficial effects of starter culture inoculation on the fermentation process. Overall, the results evidenced the functional

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0). To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

potential of the selected starter strains and supported their use as an effective starter culture for improving the efficiency and quality of cocoa fermentation.

5. DECLARATION STATEMENTS

5.1. Funding statement

The authors received no financial supports for the research, authorship and/or publication of this study.

5.2. Conflict of interest

The authors declare no potential conflict of interest for the research, authorship and/or publication of this study.

5.3. Author contributions

Honore G.O., conceptualization, methodology, bioinformatics analysis, manuscript writing and review, supervision, validation, resources; Ettien Y.K., experiments, methodology, data curation and analysis, bioinformatics analysis, manuscript writing, review and editing; Lamine S., Wilfried Y. and Hadja O., experiments and manuscript reading and editing; Sebastien N., manuscript reading and editing.

5.4. Data availability

The experimental data supporting this article are provided in the main manuscript, including the additional data tables (Table 4) incorporated for production. Additional raw laboratory records and original figure-data files can be provided by the corresponding author on reasonable request.

ADDITIONAL SUPPLEMENTARY DATA TABLE

Table 4: Supplementary Material: Raw sequencing data showing the abundance of amplicon sequence variants (ASVs) detected in the different cocoa fermentation treatments.

	Spontanous fermentation		Starter-added fermentation	
Species identified from ASV	Absolute abundance	Relative abundance	Absolute abundance	Relative abundance
Aspergillus subversicolor	27	0.07	-	0.04
Fusarium sp.	19	0.05	21	0.02
Candida akabanensis	34	0.09	51	0.02
Malassezia globosa	49	0.13	0	0.00
Trichoderma sp.	163	0.42	0	0.11
Talaromyces verruculosus	177	0.45	-	0.00
Malassezia restricta	208	0.53	-	0.00
Diutina rugosa	1,041	2.66	708	0.84
Pichia manshurica	1,249	3.19	509	0.60
Candida tropicalis	**1,663**	**4.25**	**5,087**	**6.03**
Hanseniaspora uvarum	2,482	6.34	2,098	2.37
Hanseniaspora opuntiae	3,736	9.54	3,900	4.62
Saccharomyces cerevisiae	**5,046**	**12.88**	**14,075**	**16.67**
Pichia kudriazevii	11,184	28.55	9,813	11.62
Pichia fermentans	1,035	2.64	903	1.07
Debaromyces hansenii	103	0.26	56	0.07
Wickerhamomyces anomalus	77	0.20	89	0.11
Meyerozyma sp.	66	0.17	-	0.00
Candida intermedia	91	0.23	208	0.25
Trichosporon asahii	1,242	3.17	907	1.07
Acetobacter pasteurianus	2,382	6.08	12,030	14.25
Limosilactobacillus fermentum	1,392	3.55	6,071	7.19
Acetobacter senegalensis	658	1.68	542	0.54
Acetobacter ghanensis	507	1.29	613	0.73
Acetobacter orientalis	431	1.10	-	0.00
Acetobacter fabarum	285	0.73	133	0.16
Lactiplantibacillus plantarum	239	0.61	8,813	10.44
Leuconostoc Mesenteroides	179	0.46	7,095	8.40
Acetobacter syzygii	150	0.38	319	0.38
Bacillus subtilis	137	0.35	477	0.56
Lactocaseibacillus casei	**123**	**0.31**	**6,019**	**7.13**
Weissella confusa B	115	0.29	57	0.07
Acetobacter vaccinii	104	0.27	43	0.05
Wolbachia pipientis	83	0.21	-	0.00
Enterococcus G italicus	82	0.21	-	0.00
Weissella cibaria	71	0.18	89	0.11

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0). To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

Lacticaseibacillus rhamnosus	58	0.15	77	0.09
Acetobacter tropicalis	54	0.14	34	0.04
Enterococcus B lactis	40	0.10	-	0.00
Bacillus pumilus	40	0.10	56	0.07
Acetobacter pomorum	33	0.08	76	0.09
Pseudomonas E asiatica	30	0.08	56	0.07
Enterococcus A raffinosus	28	0.07	-	0.00
Acinetobacter baumannii	24	0.06	45	0.05
Acetobacter okinawensis	21	0.05	23	0.03
Bacillus A bombysepticus	19	0.05	-	0.00
Ligilactobacillus agilis	18	0.05	44	0.05
Lacticaseibacillus porcinae	18	0.05	72	0.09
Liquorilactobacillus nagelii	18	0.05	20	0.02
Bacillus thuringiensis	**17**	**0.04**	**5,204**	**6.16**
Paucilactobacillus vaccinostercus	14	0.04	-	0.00
Lactobacillus amylovorus	11	0.03	10	0.01
Enterococcus faecalis	11	0.03	-	0.00
Corticimicrobacter populi	10	0.03	-	0.00
Pediococcus acidilactici	10	0.03	10	0.01
Acetobacter suratthaniensis	50	0.13	52	0.06
Kerstersia gyiorumum	10	0.03	-	0.00
Levilactobacillus brevis	10	0.03	10	0.01
Total	37,174	100	86,426	100

Absolute abundance is expressed, as the number of sequencing reads obtained from the bioinformatics pipeline. Relative abundance is expressed as the percentage of each amplicon sequence variant (ASV) relative to the total ASV abundance in each sample. ASVs corresponding to the inoculated starter strains are indicated in bold.

6. REFERENCES

[1]. Ho VTT, Fleet GH, Zhao J. Unravelling the contribution of lactic acid bacteria and acetic acid bacteria to cocoa fermentation using inoculated organisms. Int J Food Microbiol. 2018; 279: 43-56. https://doi.org/10.1016/j.ijfoodmicro.2018.04.040

[2]. Ho VTT, Zhao J, Fleet G. Yeasts are essential for cocoa bean fermentation. Int J Food Microbiol. 2014; 174: 72-87. https://doi.org/10.1016/j.ijfoodmicro.2013.12.014

[3]. Lima LJR, Almeida MH, Nout MJR, Zwietering MH. Theobroma cacao L., "The Food of the Gods": quality determinants of commercial cocoa beans, with particular reference to the impact of fermentation. Crit Rev Food Sci Nutr. 2011; 51: 731-761. https://doi.org/10.1080/10408391003799913

[4]. Díaz-Muñoz C, De Vuyst L. Functional yeast starter cultures for cocoa fermentation. J Appl Microbiol. 2022; 133: 39-66. https://doi.org/10.1111/JAM.15312

[5]. Samagaci L, Ouattara HG, Goualie BG, Niamke SL. Polyphasic analysis of pectinolytic and stress-resistant yeast strains isolated from Ivorian cocoa fermentation. J Food Res. 2015; 4: 124-134. https://doi.org/10.5539/jfr.v4n1p124

[6]. Yao AK, Amien GFK, Assi-Clair BJ, Koné N, Koné MK, Bethune K, et al. Prospective yeast species with enzymatic, aromatic and antifungal applications isolated from cocoa fermentation in various producing areas in Côte d'Ivoire. Microbiol Res. 2025; 16: 256. https://doi.org/10.3390/microbiolres16120256

[7]. Ortiz Galeano LS, Sandoval Lozano A, Sandoval - Aldana A, Lozano - Tovar MD. Technological potential of Bacillus megaterium in Cacao bean fermentation. Int Microbiol. 2026; 29: 485-498. https://doi.org/10.1007/S10123-025-00775-Z

[8]. Ouattara HG, Reverchon S, Niamke SL, Nasser W. Regulation of the synthesis of pulp degrading enzymes in Bacillus isolated from cocoa fermentation. Food Microbiol. 2017; 63: 255-262. https://doi.org/10.1016/j.fm.2016.12.004

[9]. Ouattara D, Ouattara H, Adom J, Goualié B, Koua G, Doué G, et al. Screening of lactic acid bacteria capable to breakdown citric acid during Ivorian cocoa fermentation and response of bacterial strains to fermentative conditions. Br Biotechnol J. 2016; 10: 1-10. https://doi.org/10.9734/BBJ/2016/19279

[10]. Afoakwa EO, Quao J, Takrama J, Budu AS, Saalia FK. Chemical composition and physical quality characteristics of Ghanaian cocoa beans as affected

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0). To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

by pulp pre-conditioning and fermentation. J Food Sci Technol. 2013; 50: 1097-1105. https://doi.org/10.1007/S13197-011-0446

[11]. Castro-Alayo EM, Idrogo-Vásquez G, Siche R, Cardenas-Toro FP. Formation of aromatic compounds precursors during fermentation of Criollo and Forastero cocoa. Heliyon. 2019; 5. https://doi.org/10.1016/j.heliyon.2019.e01157

[12]. De Vuyst L, Leroy F. Functional role of yeasts, lactic acid bacteria and acetic acid bacteria in cocoa fermentation processes. FEMS Microbiol Rev. 2020; 44: 432-453. https://doi.org/10.1093/femsre/fuaa014

[13]. Koffi LB, Ouattara G, Karou T, Guehi ST, Nemlin J, Diopoh J. Impacts de la fermentation du cacao sur la croissance de la flore microbienne et la qualité des fèves marchandes. Agron Afr. 2013; 25: 159-170.

[14]. Schwan RF. Cocoa Fermentations Conducted with a Defined Microbial Cocktail Inoculum. Appl Environ Microbiol. 1998; 64: 1477-1483. https://doi.org/10.1017/CBO9781107415324.004

[15]. De Almeida SdFOd, Silva LRC, Junior GCAC, Oliveira G, Silva SHMd, Vasconcelos S, et al. Diversity of yeasts during fermentation of cocoa from two sites in the Brazilian Amazon. ACTA AMAZONICA. 2019; 49: 64-70. 64 VOL. 49(1) 2019: 64-70. https://dx.doi.org/10.1590/1809-4392201703712

[16]. De Vuyst L, Weckx S. The cocoa bean fermentation process: From ecosystem analysis to starter culture development. J Appl Microbiol. 2016; 121: 5-17. https://doi.org/10.1111/jam.13045

[17]. Ouattara HG, Niamké SL. Mapping the functional and strain diversity of the main microbiota involved in cocoa fermentation from Cote d'Ivoire. Food Microbiol. 2021; 98: 103767. https://doi.org/10.1016/j.fm.2021.103767

[18]. Hirko B, Mitiku H, Getu A. Role of fermentation and microbes in cacao fermentation and their impact on cacao quality. Syst Microbiol Biom. 2023; 3: 509-520. https://doi.org/10.1007/s43393-023-00160-9

[19]. Lefeber T, Papalexandratou Z, Gobert W, Camu N, De Vuyst L. On-farm implementation of a starter culture for improved cocoa bean fermentation and its influence on the flavour of chocolates produced thereof. Food Microbiol. 2012; 30: 379-392. https://doi.org/10.1016/j.fm.2011.12.021

[20]. Balcázar-Zumaeta CR, Pajuelo-Muñoz AJ, Trigoso-Rojas DF, Iliquin-Chavez AF, Fernández-Romero E, Yoplac I, et al. Reduction in the cocoa Spontaneous and starter culture fermentation time based on the antioxidant profile characterization. Foods. 2023; 12: 3291. https://doi.org/10.3390/foods12173291

[21]. Batista NN, Ramos CL, Ribeiro DD, Pinheiro ACM, Schwan RF. Dynamic behavior of saccharomyces cerevisiae, Pichia kluyveri and Hanseniaspora uvarum during spontaneous and inoculated cocoa fermentations and their effect on sensory characteristics of chocolate. LWT-Food Sci Technol. 2015; 63: 221-227. https://doi.org/10.1016/J.LWT.2015.03.051

[22]. Crafack M, Mikkelsen MB, Saerens S, Knudsen M, Blennow A, Lowor S, et al. Influencing cocoa flavour using Pichia kluyveri and Kluyveromyces marxianus in a defined mixed starter culture for cocoa fermentation. Int J Food Microbiol. 2013; 167: 103-116. https://doi.org/10.1016/j.ijfoodmicro.2013.06.024

[23]. Kresnowati M, Febriami H. Mapping the effects of starter culture addition on cocoa bean fermentation. ASEAN Eng J Part B. 2015; 5: 25-37. https://doi.org/10.11113/aej.v5.15465

[24]. Alvarez-Villagomez KG, Ledesma-Escobar CA, Priego-Capote F, Robles-Olvera VJ, García-Alamilla P. Influence of the starter culture on the volatile profile of processed cocoa beans by gas chromatography–mass spectrometry in high resolution mode. Food Bioscience. 2022; 47: 101669. https://doi.org/10.1016/j.fbio.2022.101669

[25]. Levai Lewis D, Fru-Cho J, Tatsinkou Fossi B, Nanganoa Lawrence T, Afoh Rauwitta O, Tah Y, et al. Comparison of cocoa bean quality produced with different starter cultures and fermentation methods.

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0). To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

Int J Microbiol Biotechnol. 2023; 8: 10-18. https://doi.org/10.11648/J.IJMB.20230801.12

[26]. Saunshi YB, Sandhya MVS, Rastogi NK, Murthy PS. Starter consortia for on-farm cocoa fermentation and their quality attributes. Prep Biochem Biotechnol. 2020; 50: 272-280. https://doi.org/10.1080/10826068.2019.1689508

[27]. Garcia-Armisen T, Papalexandratou Z, Hendryckx H, Camu N, Vrancken G, De Vuyst L, et al. Diversity of the total bacterial community associated with Ghanaian and Brazilian cocoa bean fermentation samples as revealed by a 16 S rRNA gene clone library. Appl Microbiol Biotechnol. 2010; 87: 2281-2292. https://doi.org/10.1007/s00253-010-2698-9

[28]. Ouattara HD, Ouattara HG, Droux M, Reverchon S, Nasser W, Niamke SL. Lactic acid bacteria involved in cocoa beans fermentation from Ivory Coast: Species diversity and citrate lyase production. Int J Food Microbiol. 2017; 256: 11-19. https://doi.org/10.1016/j.ijfoodmicro.2017.05.008

[29]. Ouattara HG, Ouattara H, Yao W, Williams N, Reverchon S, Niamke S, et al. Lactic Acid Bacteria (LAB) strains are actively involved in the occurrence of aroma compounds during cocoa fermentation. Microbiol Nat. 2019; 1: 63-72. https://doi.org/https://doi.org/10.26167/7mz4-r345

[30]. Ouattara HG, Reverchon S, Niamke SL, Nasser W. Molecular identification and pectate lyase production by Bacillus strains involved in cocoa fermentation. Food microbiol. 2011; 28: 1-8. https://doi.org/10.1016/j.fm.2010.07.020

[31]. Samagaci L, Ouattara H, Niamké S, Lemaire M. Pichia kudrazevii and Candida nitrativorans are the most well-adapted and relevant yeast species fermenting cocoa in Agneby-Tiassa, a local Ivorian cocoa producing region. Food Res Int. 2016; 89: 773-780. https://doi.org/10.1016/j.foodres.2016.10.007

[32]. Soumahoro S, Ouattara HG, Goualié BG, Koua G, Doue G, Niamke SL. Occurrence of high acetic acid-producing bacteria in Ivorian cocoa fermentation and analysis of their response to fermentative stress. Amer J BioSci. 2015; 3: 70-79. https://doi.org/10.11648/j.ajbio.20150303.12

[33]. Yao W, Doue G, Ouattara H, Goualie B, Koua G, Niamke S. Selection of potential Bacillus starters for cocoa beans fermentation improvement. Food Technol. 2017; 41.

[34]. Yao W, Ouattara HG, Goualie B, Soumahoro S, Niamke S. Analysis of some functional properties of acetic acid bacteria involved in Ivorian cocoa fermentation. J Appl Biosci. 2014; 75: 6282-6290. https://doi.org/10.4314/jab.v75i1.13

[35]. Soumahoro S, Ouattara HG, Droux M, Nasser W, Niamke SL, Reverchon S. Acetic acid bacteria (AAB) involved in cocoa fermentation from Ivory Coast: species diversity and performance in acetic acid production. J Food Sci Technol. 2020; 57: 1904-1916. https://doi.org/10.1007/s13197-019-04226-2

[36]. Kadet V, Samagaci L, Ouattara HD, Ahoussi JM, Koffi EY, Ouattara HG, Lemaire M, Niamké SL. Cocoa residues as alternative support for freeze drying of Candida tropicalis for controlled fermentation of cocoa. J Food Nutr Sci. 2024; 12: 239-245. https://doi.org/10.11648/J.JFNS.20241205.15

[37]. Ben-David A, Davidson CE. Estimation method for serial dilution experiments. J Microbiol Methods. 2014; 107: 214-221. https://doi.org/10.1016/j.mimet.2014.08.023

[38]. Agbo NG, Uebersax M, Hosfield G. An efficient extraction technique of sugars from dry edible beans (Phaseolus vulgaris) and estimation by HPLC. 1985.

[39]. Bernfeld P. Amylases, α and β. Methods in Enzymology. 1955; 1: 149-158. https://doi.org/10.1002/9780470122570.CH7

[40]. Gourieva KB, Tserrevitinov OB. Method of evaluating the degree of fermentation of cocoa beans. 1979. Patent. SU646254A1 1979-02-05.

[41]. Hii CL, Law CL, Cloke M, Sharif S. Improving Malaysian cocoa quality through the use of dehumidified air under mild drying conditions. J Sci Food Agric. 2011; 91: 239-246. https://doi.org/10.1002/jsfa.4176

[42]. Camu N, De Winter T, Verbrugghe K, Cleenwerck I, Vandamme P, Takrama JS, et al. Dynamics and biodiversity of populations of lactic acid bacteria and

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0). To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

acetic acid bacteria involved in spontaneous heap fermentation of cocoa beans in Ghana. Appli Environ Microbiol. 2007; 73: 1809-1824. https://doi:10.1128/AEM.02189-06

[43]. Daniel HM, Vrancken G, Takrama JF, Camu N, De Vos P, De Vuyst L. Yeast diversity of Ghanaian cocoa bean heap fermentations. FEMS Yeast Res. 2009; 9: 774-783. https://doi.org/10.1111/J.1567-1364.2009.00520.X

[44]. Meersman E, Steensels J, Mathawan M, Wittocx PJ, Saels V, Struyf N, Verstrepen KJ. Detailed Analysis of the Microbial Population in Malaysian Spontaneous Cocoa Pulp Fermentations Reveals a Core and Variable Microbiota. PLoS ONE. 2013; 8: 0081559. https://doi.org/10.1371/journal.pone.0081559

[45]. Fernández-Niño M, Rodríguez-Cubillos MJ, Herrera-Rocha F, Anzola JM, Cepeda-Hernández ML, Aguirre Mejía JL, González Barrios AF. Dissecting industrial fermentations of fine flavour cocoa through metagenomic analysis. Sci Rep. 2021; 11(1): 8638. https://doi.org/10.1038/s41598-021-88048-3

[46]. Ouattara H, Yao W, Ouattara H, Germain K, Sebastien N. Fungal inhibition by lactic acid bacteria (LAB) is modulated in vitro by cocoa fermentation-related conditions: towards a biocontrol of fungi in processing cocoa. Emir J Food Agric. 2023; 35: 853-861.

[47]. Mayo B, TCC Rachid C, Alegría Á, MO Leite A, S Peixoto R, Delgado S. Impact of next generation sequencing techniques in food microbiology. Curr Genom. 2014; 15: 293-309. https://doi.org/10.2174/1389202915666140616233211

[48]. Herrera-Rocha F, Cala MP, Aguirre-Mejía JL, Rodríguez-López CM, Chica MJ, Olarte HH, Gonzalez Barrios AF. Dissecting fine-flavor cocoa bean fermentation through metabolomics analysis to break down the current metabolic paradigm. Sci Rep. 2021; 11: 21904-21908. https://doi.org/10.1038/s41598-021-01427-8

[49]. Janek K, Niewienda A, Wöstemeyer J, Voigt J. The cleavage specificity of the aspartic protease of cocoa beans involved in the generation of the cocoa-specific aroma precursors. Food Chem. 2016; 211: 320-328. https://doi.org/10.1016/j.foodchem.2016.05.033

[50]. Voigt J, Textoris-Taube K, Wöstemeyer J. pH-Dependency of the proteolytic formation of cocoa- and nutty-specific aroma precursors. Food Chem. 2018; 255: 209-215. https://doi.org/10.1016/j.foodchem.2018.02.045

[51]. Lima GVS, Pinto ASO, Correia MGdS, Filho MPM, Freitas AdLd, Santos PO, et al. Starter Culture-Induced Fermentation Reveals Genotype-Driven Variability in the Composition and Quality of Brazilian Amazon Forastero Cocoa Beans. J Food Sci. 2026; 91: e70908. https://doi.org/10.1111/1750-3841.70908

[52]. Konar N, Palabiyik I, Karimidastjerd A, Toker OS, Gorgulu A. Recent advances in cocoa chemistry and roasting. Trends Food Sci Technol. 2025; 162: 105036. https://doi.org/10.1016/J.TIFS.2025.105036

[53]. Akoa SP, Boulanger R, Effa Onomo P, Lebrun M, Ondobo ML, Lahon M-C, et al. Sugar profile and volatile aroma composition in fermented dried beans and roasted nibs from six controlled pollinated Cameroonian fine-flavor cocoa (Theobroma cacao L.) hybrids. Food Biosci. 2023; 53: 102603. https://doi.org/10.1016/J.FBIO.2023.102603

[54]. Moretti LK, Ramos KK, Ávila PF, Goldbeck R, Vieira JB, Efraim P. Influence of cocoa varieties on carbohydrate composition and enzymatic activity of cocoa pulp. Food Res Int. 2023; 173: 113393. https://doi.org/10.1016/J.FOODRES.2023.113393

[55]. Melo TS, Pires TC, Engelmann JVP, Monteiro ALO, Maciel LF, Bispo EDS. Evaluation of the content of bioactive compounds in cocoa beans during the fermentation process. J Food Sci Technol. 2020; 58: 1947-1957. https://doi.org/10.1007/S13197-020-04706-W

[56]. Escobar S, Santander M, Zuluaga M, Chacón I, Rodríguez J, Vaillant F. Fine cocoa beans production: Tracking aroma precursors through a comprehensive analysis of flavor attributes formation. Food Chem.

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0). To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

2021; 365: 130627.
https://doi.org/10.1016/j.foodchem.2021.130627

[57]. Schwan RF, Wheals AE. The microbiology of cocoa fermentation and its role in chocolate quality. Crit Rev Food Sci Nutr. 2004; 44: 205–221.
https://doi.org/10.1080/10408690490464104

[58]. Koffi O, Samagaci L, Goualie B, Niamke S. (2017). Diversity of Yeasts Involved in Cocoa Fermentation of Six Major Cocoa-Producing Regions in Ivory Coast. Eur Sci J. 2017; 13: 496-516.
http://dx.doi.org/10.19044/esj.2017.v13n30p496

[59]. Kadjo AC, Beugre GC, Sess Tchotch DA, Kedjebo KBD, Mounjouenpou P, Durand N, Fontana A, Tagro GS. Screening of anti-fungal *Bacillus* strains and influence of their application on cocoa beans fermentation and final bean quality. J Adv Microbiol. 2023; 23: 8-17.
https://doi.org/10.9734/JAMB/2023/V23I1700

[60]. Kamarisima FAM, Aditiawati P, Yusantri SD, Kumalasari DP, Mutsla D, Rachmawati N, Dewi M, Pratama F. Enhancing fermented cocoa bean quality and flavor through short secondary fermentation using Candida tropicalis as starter and Citrus limon peel. J Sci Food Agric. 2025; 105: 8616-8624.
https://doi.org/10.1002/JSFA.70158

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0). To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

Research Paper

pISSN: 2345-5357
eISSN: 2423-4214

APPLIED FOOD BIOTECHNOLOGY, 2026, 13 (1): e27 (1-19)
Journal homepage: www.journals.sbmu.ac.ir/afb

OPEN ACCESS

تأثیر کنسرسیوم میکروبی عملکردی تعریف‌شده بر پویایی تخمیر کاکائو و کیفیت دانه‌ها در ساحل عاج

اتین کوفی، حاجا واتارا، لامین ساماگاسی، ویلفرید یائو، سباستین نیامکه، اونوره واتارا*

گروه زیست‌فناوری و ایمنی مواد غذایی، دانشکده علوم زیستی، دانشگاه فلیکس اوفویه-بوانی، آبیجان، ساحل عاج

چکیده

سابقه و هدف: استفاده از کشت‌های آغازگر به‌عنوان راهبردی امیدبخش برای بهبود فرایند تخمیر کاکائو و تضمین یکنواختی کیفیت دانه‌ها در نظر گرفته می‌شود. ازاین‌رو، در مطالعه پیشین، سویه‌های میکروبی ارزشمند جداسازی‌شده از کاکائوی در حال تخمیر، که ظرفیت بالقوه‌ای برای استفاده به‌عنوان کشت آغازگر داشتند، مشخصه‌یابی شدند. در مطالعه حاضر، اثر کشت‌های آغازگر میکروبی منتخب بر تخمیر کاکائو در مقیاس مزرعه و نیز بر کیفیت دانه‌های حاصل ارزیابی شد.

مواد و روش ها: روش کار شامل مقایسه تخمیر کاکائو با تلقیح کشت آغازگر و تخمیر خودبه‌خودی بود. کنسرسیوم آغازگر از سویه‌های میکروبی عملکردی و به‌خوبی مشخصه‌یابی‌شده تشکیل شده بود که شامل مخمرها، باکتری‌های اسید لاکتیک، باکتری‌های اسید استیک و گونه‌های جنس *باسیلوس* می‌شد.

یافته‌ها و نتیجه‌گیری: نتایج توالی‌یابی نسل جدید نشان داد که در تخمیر با کمک کشت آغازگر، فراوانی واریانت‌های توالی آمپلیکونی مربوط به سویه‌های تلقیح‌شده به حداکثر ۶۹ درصد از کل میکروبیوتای شناسایی‌شده رسید، درحالی‌که این مقدار در تخمیر خودبه‌خودی ۲۴ درصد بود. فراوانی واریانت‌های توالی آمپلیکونی قارچی در تخمیر با کمک کشت آغازگر به‌طور چشمگیری کاهش یافت. کشت آغازگر تلقیح‌شده موجب تحریک فعالیت میکروبی شد و در نتیجه، اسیدها در طول تخمیر با کمک کشت آغازگر با سرعت بیشتری در لپه‌های دانه تجمع یافتند، به‌طوری‌که محتوای کل اسیدها تقریباً به ۱/۵ برابر مقدار مشاهده‌شده در تخمیر خودبه‌خودی رسید. به‌طور مشابه، تلقیح کشت آغازگر افزایش سریع‌تر محتوای قندهای احیاکننده را درون دانه‌ها تسهیل کرد. شاخص تخمیر در تخمیر با کمک کشت آغازگر طی ۶۰ ساعت به یک رسید، درحالی‌که در تخمیر خودبه‌خودی، دستیابی به شاخص مشابه به ۱۳۲ ساعت زمان نیاز داشت. همچنین، آزمون برش دانه‌ها نشان داد که در تخمیر با کمک کشت آغازگر، ۸۴ ساعت برای دستیابی به ۶۰ درصد دانه‌های کاکائوی کاملاً تخمیرشده کافی بود، درحالی‌که در همین بازه زمانی، تنها ۳۴ درصد دانه‌ها در تخمیر خودبه‌خودی به‌طور کامل تخمیر شده بودند. در پایان فرایند تخمیر، ۹۲ درصد دانه‌های حاصل از تخمیر با کمک کشت آغازگر کاملاً تخمیر شده بودند، درحالی‌که این میزان در تخمیر خودبه‌خودی ۷۰ درصد بود. در مجموع، استفاده از کشت آغازگر تعریف‌شده، فرایند تخمیر را به‌طور معنی‌داری تسریع کرد و کیفیت دانه‌های تخمیرشده کاکائو را بهبود بخشید.

واژگان کلیدی: تخمیر کاکائو، کشت آغازگر میکروبی، کیفیت، بهبود، ساحل عاج

تاریخچه مقاله

دریافت: ۲۱ اوت ۲۰۲۶
داوری: ۲۰ اکتبر ۲۰۲۶
پذیرش: ۲۲ اکتبر ۲۰۲۶
چاپ: ۳۱ اکتبر ۲۰۲۶

نویسنده مسئول

اونوره واتارا

پست الکترونیک:
kidou1212@gmail.com
Ouattarahonore@ufhb.edu.ci

This open-access article distributed under the terms of the Creative Commons Attribution Non Commercial 4.0 License (CC BY-NC 4.0).
To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.