



Research Article

On-farm Controlled Cocoa Fermentation with Lyophilized *Candida tropicalis* Improves Efficiency and Sensory Attributes

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Abstract

The fermentation of cocoa beans is one of the most critical steps in processing the beans and producing high-quality chocolate and related products. To control this step and ensure the quality of the cocoa beans, the main microorganisms responsible for this process have been isolated, characterized, and used in cocoa bean processing. The objective of this study was to evaluate the effect of using the yeast *Candida tropicalis*, either in monoculture or in coculture with *Saccharomyces cerevisiae*, on the controlled fermentation of cocoa beans at the pilot scale, in order to improve the sensory, physicochemical, and nutritional quality of the beans and their derivative products. To this end, the harvested cocoa pods were shelled, and the beans were collected, homogenized, and divided into three 50-kg batches, including one control and two experimental batches. The control batch was not inoculated; as for the other two batches, one received primarily freeze-dried *Candida tropicalis* at a concentration of 10^5 cells/g of beans, and the other received a mixture of *Candida tropicalis* and *Saccharomyces cerevisiae* at a concentration of 10^5 cells/g of beans. Fermentation was carried out for 144 hours (6 days); samples were taken every 12 hours for analyses of fermentation index, cut test and temperature. After fermentation of the cocoa beans, the 72-hour and 144-hour beans were characterized biochemically and organoleptically. The freeze-dried microbial starters of *Candida tropicalis* promoted a more rapid increase in temperature, fermentation index, and the proportion of well-fermented brown beans compared to the trial combining the two strains and the control. Furthermore, biochemical analyses indicate that the fermentation trial using *Candida tropicalis* alone for 72 hours showed the highest levels of bioactive compounds and a low concentration of antinutritional compounds. Organoleptic analyses, meanwhile, reveal a more pronounced preference for the sample fermented with *Candida tropicalis* alone for 72 hours. In summary, freeze-dried microbial starters of *Candida tropicalis* alone reduces fermentation time from 144 hours to 72 hours—a reduction of 3 days—while preserving the bioactive properties.

Keywords

Fermentation, Cocoa, Starters, Freeze Drying, Yeast, *Candida tropicalis* and *Saccharomyces cerevisiae*

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1. Introduction

Cocoa (*Theobroma cacao* L.) is a globally important agricultural commodity with significant economic value. Its beans serve as essential raw materials to produce chocolate and a wide range of food products [1]. The global cocoa and chocolate market has shown steady growth, increasing from USD 46.61 billion in 2021 to USD 48.29 billion in 2022, and is projected to reach USD 67.88 billion by 2029 [2]. This expansion is largely driven by the high content of bioactive compounds in cocoa beans and their associated health benefits [3, 4]. In 2025, global cocoa production was estimated at 4.8 million tonnes, according to the ICCO, with Cote d'Ivoire and Ghana remaining the leading producers, jointly accounting for more than 50% of global output [5].

Over the past decade, cocoa production has increased significantly, rising from approximately 1.22 million tonnes in 2008/2009 season to 1.80 million tonnes in the 2023/2024 season, representing an increase of about 47.54% [5-7]. However, despite this growth, quality-related constraints continue to limit the sector's full economic potential. These challenges are mainly linked to post-harvest practices, particularly fermentation, which is widely recognized as the most critical step influencing cocoa quality [8, 9].

Indeed, cocoa fermentation is a complex biochemical process driven by a succession of microbial activities, involving yeasts, lactic acid bacteria, acetic acid bacteria, and *Bacillus* species [1, 10]. These microorganisms play a key role in initiating and regulating a series of physical and chemical transformations that are essential for flavor development and overall bean quality. Nevertheless, despite decades of research, cocoa fermentation is still predominantly carried out spontaneously and remains largely uncontrolled at the farm level. As a result, the lack of standardization leads to considerable variability in the quality of fermented and dried cocoa beans [11, 12].

To address these limitations, various methods and processing techniques have been proposed [13]. Among them, the use of microbial starter cultures has emerged as the most promising strategy, as it directly targets the key microorganisms responsible for cocoa fermentation [14-16].

In this context, numerous studies have been conducted in Côte d'Ivoire and other cocoa-producing countries to isolate, characterize, and select microbial starters based on their ability to accelerate fermentation and enhance the quality of fermented cocoa beans. These starters have been evaluated at both laboratory and industrial scales, either as single yeast cultures or in combination with other dominant cocoa microorganisms [17, 18].

Among these microorganisms, yeasts play a central role, as they are capable of initiating cocoa fermentation. They contribute through the production of pectinolytic enzymes and ethanol, which facilitate pulp degradation, reduce pectin content, soften the beans, and promote the growth of acetic acid bacteria [19, 20]. Among yeast species, *Saccharomyces cerevisiae* is the most widely used in starter cultures due to its positive impact

on the quality of fermented cocoa beans [20]. Its application as a starter culture allows better control of the fermentation process, leading to faster fermentation and improved sensory properties of the resulting chocolate. Furthermore, some of these research efforts have resulted in patented technologies that are currently utilized by major chocolate producers, particularly in Latin America [21, 22].

However, despite producing more than 60% of the world's cocoa, Africa has achieved limited success in implementing microbial starter cultures for controlled fermentation. This highlights the need for continued research to identify yeast starter cultures capable of ensuring efficient and consistent fermentation under local conditions. Côte d'Ivoire, as the world's leading cocoa producer, stands to benefit significantly from such advancements. In this regard, increasing attention has been given to alternative yeast genera involved in cocoa fermentation, such as *Pichia*, *Kluyveromyces*, *Candida*, *Sporilla*, *Hanseniaspora*, and *Torulaspora*. Among these, *Candida tropicalis* has been identified as one of the dominant yeast species during cocoa fermentation in Côte d'Ivoire [23-25].

Despite its prevalence, the use of *Candida tropicalis* as a starter culture remains largely underexplored. A laboratory-scale study conducted in Indonesia using a *C. tropicalis* strain (350 g of cocoa beans) reported a significant improvement in bean quality (greater than 60%) compared to the control [26]. However, no pilot-scale studies (e.g., 50 kg fermentations) have yet been conducted, which limits its practical application under real production conditions. Therefore, investigating the use of *Candida tropicalis*, either alone or in combination with *Saccharomyces cerevisiae*, represents a promising strategy to improve cocoa bean quality. For large-scale application, the preservation of these microbial strains through freeze-drying is essential to ensure their stability, viability, and ease of use over time.

The objective of this study is to evaluate the effect of *Candida tropicalis*, used in monoculture or in co-culture with *Saccharomyces cerevisiae*, on the controlled fermentation of cocoa beans at pilot scale. The goal is to improve the sensory, physicochemical, and nutritional quality of cocoa beans and their derived products.

2. Materials and Methods

2.1. Harvesting of Cocoa Pods

As part of this research, the plant material consists of all-purpose cocoa pods (mixed varieties of cocoa grown in Cote d'Ivoire). A total of 2,500 cocoa pods were harvested in Ivory Coast, in the Agnɔby-Tiassa region (Latitude: 6° 09' 00" North; Longitude: 4° 00' 00" West) located 80 kilometers or 1h30 min from the city of Abidjan.

2.2. Microbial Strains

The two (2) yeast strains (*Candida tropicalis* and *Saccharomyces cerevisiae*) used in this study were provided by the Laboratory of Biotechnologies Agriculture and valorization of Biological Resources of the Felix Houphouet Boigny University of Abidjan, Cote d'Ivoire. These strains were previously isolated from Ivorian cocoa and characterized and identified before being preserved in Yeast Peptone Glucose (YPG) broths supplemented with glycerol. The *Candida tropicalis* strain was selected for its ability to strongly produce pectinolytic enzymes while the *Saccharomyces cerevisiae* strain was selected for its ability to strongly produce ethanol. These two metabolites, ethanol and pectinolytic enzymes, are essential for the fermentation of cocoa beans.

2.3. Lyophilization of Microorganisms

The yeast starter cultures (*Candida tropicalis* and *Saccharomyces cerevisiae*) were cultivated separately in 2 L of Yeast Peptone Glucose (YPG) broth at 30 °C for 24 h. After incubation, 10 mL of each culture were aseptically transferred into sterile 15 mL tubes and centrifuged at 12,000 rpm for 10 minutes (Labao, China). The resulting cell pellets were collected into Erlenmeyer flasks [27]. Subsequently, cryoprotectants were added by incorporating skimmed milk and a 25% sucrose solution at final concentrations of 10% and 20%, respectively. The mixtures were homogenized for 1 minute, then frozen at -60°C for 2 hours (Haier, China). The frozen samples were thereafter subjected to freeze-drying for 60 hours using a lyophilizer (Biobase, China) set at -50°C and 1 Pa. Following lyophilization, both the viability and functional capacity of the yeast strains were re-evaluated. For this purpose, 1 mL of each culture before freeze-drying and 1 g of each lyophilized sample were used to inoculate 4 mL of YPG broth. The inoculated media were incubated at 30 °C for 2 hours. Serial decimal dilutions (10^{-1} to 10^{-5} cells/mL) were then prepared, and the last three dilutions were plated on YPG agar to determine the survival rate of the yeast cells.

2.4. Enzyme Production

A specific culture medium was prepared and poured into Petri dishes. After solidification, wells of 5 mm diameter were made in the agar. The *Candida tropicalis* strain was then inoculated into the wells at a concentration of 10^5 cells/mL. The plates were incubated at 30 °C for 48 hours. After incubation, a potassium iodide/iodine (KI/I₂) solution was applied to reveal enzyme activity. The diameters of the clear zones formed around the wells were measured to assess pectinolytic activity. These measurements were used to evaluate enzyme production after freeze-drying [28, 29].

2.5. Ethanol Production

The method described by [30], based on CO₂ production,

was used. Briefly, 10 mL of YPG broth were dispensed into test tubes, each containing a Durham tube to capture gas. The media were sterilized by autoclaving at 121 °C for 15 minutes, cooled to 30 °C, and then inoculated with the yeast starters to reach a final concentration of 10^5 cells/mL. The cultures were incubated at 30 °C for 48 hours. After incubation, the volume of CO₂ produced was measured as an indicator of ethanol production. This allowed the evaluation of fermentative activity after lyophilization.

2.6. Fermentation of Cocoa Beans Using Freeze-Dried Microbial Starters

Cocoa pods were opened two (2) days after harvest using a wooden club. The beans were manually extracted and placed on banana leaves until a total mass of 150 kg was obtained. The entire batch was then thoroughly mixed to ensure a uniform distribution of the natural microbiota.

The homogenized beans were subsequently divided into three (3) equal batches of 50 kg each and arranged on banana leaves. The first batch was left uninoculated and served as the control. The second batch was inoculated with *Candida tropicalis* at a concentration of 10^5 cells/g of beans. The third batch was inoculated with a mixed culture of *Candida tropicalis* and *Saccharomyces cerevisiae*, also at a final concentration of 10^5 cells/g of beans.

Fermentation was carried out for 6 days using the heap method on banana leaves. During the process, the temperature of each fermentation batch was recorded every 12 hours. At the same intervals, samples were collected for laboratory analyses to monitor key fermentation parameters.

2.6.1. Temperature

The temperature of the cocoa fermentation mass was measured following the method described by [31], using a previously calibrated thermometer. To ensure accurate readings, the thermometer probes were inserted at different locations within each fermentation batch. The temperatures were then recorded directly from the device at regular intervals.

2.6.2. Fermentation Index of Cocoa Beans

The fermentation index (FI) of cocoa beans was determined according to the method of [32]. An amount of 10 g of cocoa beans was taken and manually pulped. The cotyledons were then separated and crushed using a porcelain mortar. From this paste, 0.5 g was weighed and mixed with 50 mL of a solution containing methanol and hydrochloric acid (97:3, v/v). The mixture was stored in a refrigerator at 8 ± 2 °C for 17 hours to allow proper extraction and then filtered using Whatman filter paper. The absorbance of the filtrate was measured using a spectrophotometer at two wavelengths 460 nm and 530 nm. The fermentation index was calculated as the ratio of absorbance at 460 nm to that at 530 nm.

2.6.3. Cut Test of Dried Cocoa Beans

The cut test of cocoa beans was performed according to the method described by [33]. A total of 100 fermented and dried cocoa beans were randomly selected from each sample. Each bean was cut lengthwise into two equal halves. The internal appearance of the beans was then examined visually to detect any defects. Based on their internal color, the beans were classified into four categories [34]: brown (well-fermented), purple (partially fermented), brown purple (intermediate fermentation) and slaty (unfermented or poorly fermented).

2.6.4. Biochemical Analysis of High-Quality Fermented Cocoa Beans

At the end of the fermentation process, cocoa beans that exhibited the best overall quality were selected for biochemical characterization after drying. The selection was based on both the reduction of fermentation time, indicated by a fermentation index (FI) greater than 1, and the preservation of the physical quality of the beans, as shown by a cut test result with more than 60% well-fermented (brown) beans. Only the samples that met these criteria were retained for further biochemical analyses.

2.7. Nutritional and Antinutritional Compounds Determination in Fermented Dried Cocoa Beans

Methanolic extraction of nutritional and antioxidant compounds was performed following the method described by [35], with slight modifications. An amount of 1 g of finely crushed cocoa cotyledon from each sample was mixed with 10 mL of 70% methanol. The mixture was vortexed vigorously for 1 minute to ensure proper homogenization, then centrifuged at 3000 rpm for 10 minutes. The supernatant obtained was carefully collected and transferred into a 50 mL volumetric flask. The remaining pellet was subjected to a second extraction using 70% methanol under the same conditions (vortexing and centrifugation). The supernatant from this second extraction was combined with the first. Finally, the total volume was adjusted to 50 mL with distilled water. The resulting solution constituted the methanolic extract, which was subsequently used for the determination of nutritional compounds and antioxidant activity.

2.7.1. Phenolic Compounds in Fermented and Dried Cocoa Beans

The total phenolic content was determined using the Folin-

Ciocalteu method as described by [35]. For each sample, 1 mL of the methanolic extract was transferred into a test tube. Then, 1 mL of Folin-Ciocalteu reagent was added, followed by 1 mL of 20% (w/v) sodium carbonate solution. The mixture was thoroughly homogenized and incubated in the dark for 30 minutes at room temperature. After incubation, distilled water was added to each tube to bring the final volume to 10 mL. The absorbance of the resulting solution was measured at 725 nm using a spectrophotometer, with a reagent blank used as control. Quantification of phenolic compounds was performed using a calibration curve prepared under the same conditions with gallic acid as standard (1 mg/mL). Results were expressed as gallic acid equivalents (GAE).

2.7.2. Flavonoids Content of Fermented and Dried Cocoa Beans

The total flavonoid content was determined following the method described by [36]. For each sample, 0.5 mL of the methanolic extract was placed into a test tube. Then, 0.5 mL of aluminum chloride solution, 0.5 mL of sodium acetate, and 2 mL of distilled water were added successively. The mixture was thoroughly homogenized and allowed to stand at room temperature.

for 30 minutes. After incubation, the absorbance of the solution was measured at 415 nm using a spectrophotometer, with a reagent blank used as control. Flavonoid concentration was quantified using a calibration curve prepared under the same conditions with quercetin as standard (1 mg/mL). Results were expressed as quercetin equivalents (QE).

2.7.3. Anthocyanins Content of Fermented and Dried Cocoa Beans

The anthocyanin content was determined using the pH differential method described by [37]. This method is based on the structural change of anthocyanins at different pH values.

A volume of 1 g of finely crushed cocoa cotyledon from each sample was mixed with 9 mL of 0.025 M sodium chloride buffer (pH 1.0). In addition, another 1 g of the same sample was mixed with 9 mL of 0.4 M sodium acetate buffer (pH 4.5). Both mixtures were centrifuged at 2000 rpm for 10 minutes, and the supernatants were carefully collected. The absorbance of each extract was then measured at 520 nm and 700 nm using a spectrophotometer. The anthocyanin concentration was calculated using the difference in absorbance between the two pH conditions, according to the formula below:

$$\text{Anthocyanin pigment (cyanidin-3-glucoside equivalents, mg/L)} = \frac{A \cdot M \cdot df \cdot 103}{\epsilon \cdot 1} \quad (1)$$

A: (A_{520 nm} - A_{700 nm}) pH 1,0 - (A_{520 nm} - A_{700 nm}) pH 4,5 M (molar mass): 449,2 g/mol cyanidin-3-glucoside df: dilution factor

2.7.4. Antioxidant Activity of Fermented and dried Cocoa Beans (DPPH Assay)

The antioxidant (antiradical) activity of the samples was

evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method, as described by [38]. For the assay, 2.5 mL of each methanolic extract was mixed with 1 mL of a 3 mM DPPH solution. The reaction mixtures were incubated in the dark at room temperature for 30 minutes to allow the reaction to occur. After incubation, the absorbance was measured at 415 nm using a spectrophotometer. A control sample was prepared under the same conditions, replacing the extract with methanol. In addition, a blank consisting of 1 mL of DPPH solution mixed with 2.5 mL of methanol was also measured. The antiradical activity (AA) was expressed as the percentage of DPPH radical scavenging (decolorization), calculated using the formula below:

$$AA (\%) = \frac{(DOc - (DOe - DOb)) \times 100}{DOc} \quad (2)$$

DOc: optical density of control (1 mL DPPH + 2,5 mL methanol) DOe: optical density of sample (1 mL DPPH + 2,5 mL extract) DOb: optical density of control (1 mL de methanol + 2,5 mL extract)

2.7.5. Determination of Phytate Content in Fermented and Dried Cocoa Beans

Phytate content was determined using the method described by [39]. For each analysis, one (1) gram of finely crushed cocoa cotyledon was placed into an Erlenmeyer flask and mixed with 20 mL of 0.65 N hydrochloric acid (HCl). The mixture was homogenized and left to extract for 12 hours at room temperature (27 °C). After extraction, the mixture was centrifuged at 12,000 rpm for 40 minutes. Then, 0.5 mL of the supernatant was collected and mixed with 3 mL of Wade reagent. The resulting solution was incubated in the dark at room temperature (27 °C) for 15 minutes. The absorbance was measured at 490 nm using a spectrophotometer, with a blank used as reference. Phytate content was then calculated using a calibration curve prepared with a standard sodium phytate solution at 10 µg/mL.

$$\text{Phytate content (mg/100 g)} = \frac{DO \times 4}{0,033 \times me} \quad (3)$$

DO: optical density 490 nm me: sample mass (g) 4: dilution factor 0,033: slope of calibration line

2.7.6. Determination of Oxalate Content in Fermented and Dried Cocoa Beans

The oxalate content of cocoa beans was determined following the method described by [40]. For this analysis, one (1) gram of finely crushed cocoa cotyledon was homogenized in 75 mL of 3 M sulfuric acid (H₂SO₄). The mixture was then stirred continuously for one hour using a magnetic stirrer to ensure complete extraction. After extraction, the mixture was filtered using Whatman filter paper. A volume of 25 mL of the filtrate was collected and heated, then titrated with a 0.05 M potassium permanganate (KMnO₄) solution until a persistent

light pink color appeared, indicating the endpoint of the reaction. The oxalate content in the samples was subsequently calculated using the formula below:

$$\text{Oxalate content (mg/100 g)} = \frac{2,2 \times Veq \times 100}{me} \quad (4)$$

Veq: equivalent volume (mL) of KMnO₄ poured me: sample mass (g)

2.7.7. Determination of Tannin Content in Fermented and Dried Cocoa Beans

The tannin content of the samples was determined according to the method described by [41]. For this analysis, one (1) mL of the methanolic extract from each sample was transferred into test tubes, followed by the addition of 5 mL of vanillin reagent. The mixtures were incubated in the dark for 30 minutes at room temperature to allow color development. After incubation, the absorbance was measured at 500 nm using a spectrophotometer, with a control sample prepared under the same conditions used as reference. Tannin quantification was performed using a calibration curve established from a standard solution of catechin (2 mg/mL), prepared and analyzed under the same experimental conditions.

2.8. Sensory Evaluation of Chocolate, Cocoa Beans, and Cocoa Herbal Tea

Cocoa beans obtained from the best fermentation treatments (72 and 144 hours), as well as control samples fermented for the same durations (72 and 144 hours), were subjected to sensory evaluation by tasters.

2.8.1. Roasting of Fermented and Dried Cocoa Beans

Fermented and dried cocoa beans obtained from the pilot-scale test (50 kg), inoculated with yeast starters at the optimal concentration, as well as control samples collected at the same fermentation times, were subjected to roasting. For this purpose, one (1) kilogram of cocoa beans was spread evenly on baking paper and placed in an oven preheated to 120 °C. The beans were roasted for 20 minutes following the protocols described by [42, 43]. After roasting, the beans were removed from the oven and allowed to cool at room temperature prior to further processing.

2.8.2. Preparation of Herbal Teas from Roasted Cocoa Beans

After roasting, the cocoa beans were shelled to obtain the cotyledons, which were then finely ground using a blender for one minute until a homogeneous powder was obtained. A quantity of two (2) g of the ground cotyledons was weighed and placed into porous tea bags. Each tea bag was then immersed in a glass container containing 250 mL of distilled water previously

brought to boiling (100 °C). The infusion was allowed to proceed for five (5) minutes. At the end of the infusion period, the tea bag was removed from the container, following the method described by [11]. A commercial tea sample, purchased from local supermarkets, was prepared under the same conditions and used as a reference.

2.8.3. Panel Composition and Sensory Evaluation

The organoleptic evaluation of the different products was conducted using an untrained panel composed of 45 voluntary participants, including both men and women, selected based on their availability. The panel consisted predominantly of Ivorian participants, along with individuals from other nationalities, including Togolese, Central African, Senegalese, Burkinabe, and Ghanaian. The age of the panelists ranged from 18 to 60 years. For the evaluation, samples of each product were coded using alphanumeric identifiers and presented simultaneously to each panelist in a randomized order to minimize bias. Sensory responses were collected using a 10-point hedonic scale, where 1 corresponded to “very unpleasant” and 10 to “very pleasant.”

2.9. Statistical Analysis

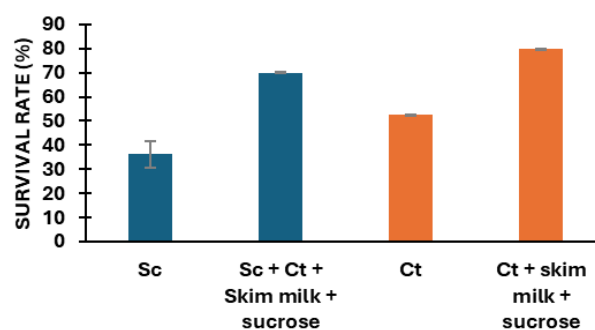
All quantitative analyses were performed in triplicate, and results were expressed as mean $\pm$ standard deviation. Differences among treatments were assessed using analysis of variance (ANOVA). When significant differences were observed, Duncan’s multiple range test was applied for mean comparison at a significance level of 5% ($p < 0.05$). To further explore consumer preferences, preference mapping (PREFMAP) was conducted to better visualize and interpret the relationships between products and panelist scores. All statistical analyses were performed using XLSTAT (version 2019.2.2) and RStudio (2024).

3. Results

3.1. Survival Rate and Metabolite Production of Microbial Starters After Freeze-Drying

3.1.1. Survival Rate of Microbial Starters After Freeze-Drying

The survival rate of microbial starters after freeze-drying is shown in Figure 1. Overall, survival rates ranged from 36.19% to 70.73%. The use of the cryoprotectant (skim milk and sucrose) significantly improved cell survival. Under these conditions, *Saccharomyces cerevisiae* showed a survival rate of 70.03%, while *Candida tropicalis* reached a higher survival rate of 79.73%. In general, *Candida tropicalis* demonstrated better resistance to freeze-drying than *Saccharomyces cerevisiae*, both in the presence and absence of cryoprotectant.

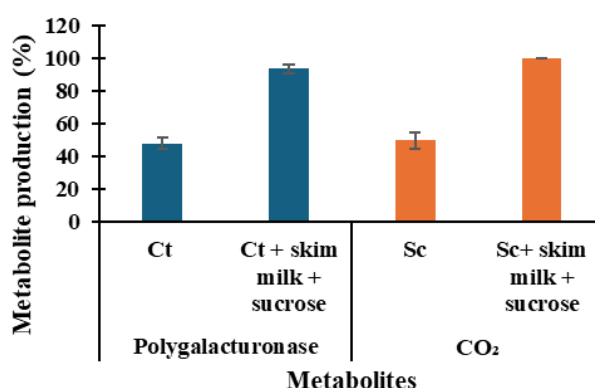


Candida tropicalis (Ct); *Saccharomyces cerevisiae* (Sc).

Figure 1. Survival rate of microbial yeast starters after freeze-drying.

3.1.2. Production of Metabolites of Interest by Microbial Starters After Freeze-Drying

Figure 2 illustrates the ability of yeast microbial starters to produce key metabolites (polygalacturonases and CO₂) after freeze-drying. Overall, metabolite production capacity ranged from 48.14% to 100%. The addition of cryoprotectants (skim milk and sucrose) significantly improved metabolite production. Under these conditions, *Candida tropicalis* retained 93.70% of its production capacity, while *Saccharomyces cerevisiae* maintained full activity (100%).



Candida tropicalis (Ct); *Saccharomyces cerevisiae* (Sc).

Figure 2. Metabolite production by microbial yeast starters after freeze-drying.

3.2. Dynamics of Cocoa Bean Fermentation at Pilot Scale Using *Candida tropicalis* Alone or in Co-Culture with *Saccharomyces cerevisiae*

3.2.1. Temperature Profile of Fermentation Heap

The evolution of temperature during cocoa fermentation using *Candida tropicalis* alone and in co-culture with *Saccharomyces cerevisiae* is shown in Figure 3. Overall, all fermentation treatments exhibited a similar temperature pattern characterized by two main phases: an initial increase followed by a decrease, with a peak temperature separating these phases.

The traditional fermentation (without added microbial starters) recorded the lowest temperature values, ranging from 35 to 49.16 ± 0.15 °C, with the maximum temperature reached at the 108th hour.

Fermentation trials inoculated with *Candida tropicalis* alone and in co-culture with *Saccharomyces cerevisiae* followed comparable trends. However, higher temperatures were observed in the fermentation heap inoculated with *Candida tropicalis* alone, with values ranging from 35 to 50.2 ± 0.03 °C. In contrast, the co-culture system showed slightly lower temperatures, ranging from 35 to 49.46 ± 0.03 °C.

Notably, the highest temperature recorded (50.2 ± 0.03 °C) was reached at the 84th hour in the fermentation inoculated with *Candida tropicalis* alone. In comparison, the co-culture system reached its peak temperature of 49.46 ± 0.03 °C later, at the 108th hour.

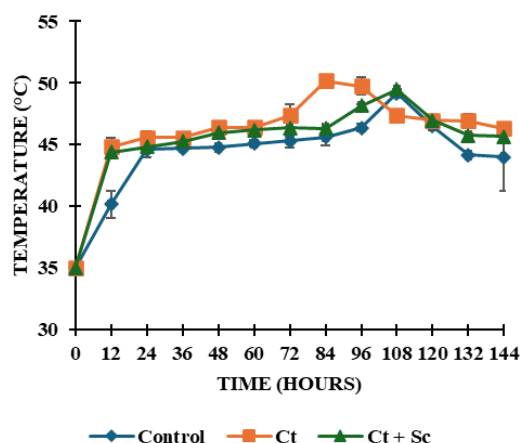


Figure 3. Temperature profile of cocoa fermentation using *Candida tropicalis* alone or in co-culture with *Saccharomyces cerevisiae*.

Traditional fermentation (control); Fermentation with added inoculum of *Candida tropicalis* (Ct); Fermentation with added inoculum of *Saccharomyces cerevisiae* (Sc).

3.2.2. Fermentation Index During Cocoa Bean Fermentation

The evolution of the fermentation index during cocoa bean fermentation is presented in Figure 4. An overall increase in the fermentation index was observed in all treatments, with values ranging from 0.45 ± 0 to 1.69 ± 0.07 . During the first 48 hours, the fermentation index increased more rapidly in the fermentation inoculated with *Candida tropicalis* alone (from 0.45 to 1.12) compared to both the traditional fermentation (control) (from 0.45 to 0.75) and the co-culture with *Candida tropicalis* and *Saccharomyces cerevisiae* (from 0.45 to 0.84). Furthermore, fermentation with *Candida tropicalis* alone reached a fermentation index ≥ 1 as early as 24 hours. In contrast, this threshold was reached later in the co-culture system (60 hours) and in the traditional fermentation (96 hours).

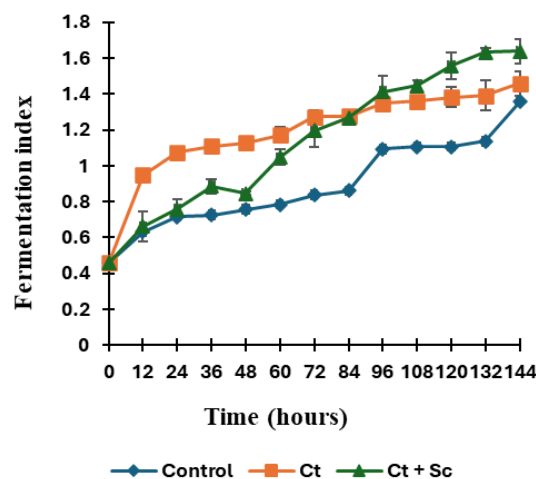


Figure 4. Fermentation index evolution in cocoa beans fermented with *Candida tropicalis* and *Saccharomyces cerevisiae*.

Traditional fermentation (control); Fermentation with added inoculum of *Candida tropicalis* (Ct); Fermentation with added inoculum of *Saccharomyces cerevisiae* (Sc).

3.2.3. Percentage of Well-Fermented Brown Beans After Drying

The evolution of the proportion of well-fermented brown beans during fermentation is presented in Figure 5. Overall, the percentage increased progressively in all treatments, rising from 0 to 99% over time. Fermentation inoculated with *Candida tropicalis* alone showed a faster increase in the proportion of well-fermented brown beans, exceeding 60% after 72 hours. In contrast, this threshold was reached later in the co-culture with *Candida tropicalis* and *Saccharomyces cerevisiae* (96 hours) and in the traditional fermentation (control) (108 hours).

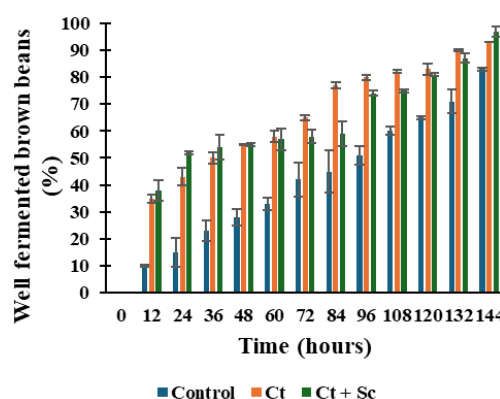


Figure 5. Evolution of the proportion of well-fermented brown cocoa beans during fermentation with *Candida tropicalis* alone or in co-culture with *Saccharomyces cerevisiae*.

Traditional fermentation (control); Fermentation with added inoculum of *Candida tropicalis* (Ct); Fermentation with added inoculum of *Saccharomyces cerevisiae* (Sc).

3.3. Biochemical and Sensory Analysis of Fermented and Dried Cocoa Beans from the Best Fermentation Treatment

3.3.1. Nutritional Compounds

The concentrations of nutritional compounds in cocoa beans obtained from the best fermentation treatment (*Candida tropicalis* alone) at 72 h and 144 h, as well as from traditional fermentation (control), are presented in Table 1. Cocoa beans

fermented with *Candida tropicalis* at 72 h exhibited the highest total phenolic content ($69,068.58 \pm 666.66$) and antioxidant activity (89.11 ± 0.73) compared to beans from traditional fermentation at both 72 h and 144 h. The flavonoid and anthocyanin contents of beans fermented with *Candida tropicalis* at 72 h were higher than those of the control at 72 h, but lower than those observed at 144 h. Regarding reducing sugars, cocoa beans fermented with *Candida tropicalis* at 72 h showed higher values than those at 144 h, but lower values than those obtained in traditional fermentation at 72 h.

Table 1. Nutritional profile of cocoa beans fermented with freeze-dried microbial starters.

Sample	Phenolic compounds	Flavonoids	Anthocyanins	Reducing sugars	Anti-oxydant power
T+ / 72 h	66186.28 ± 756.73 a	405.51 ± 13.29 c	0.69 ± 0.02 d	0.158 ± 0.01 a	88.56 ± 0.29 ab
F.Ct / 72 h	69068.58 ± 666.66 b	591.32 ± 6.10 b	1.36 ± 0.14 c	0.112 ± 0.00 b	89.11 ± 0.73 a
T+ / 144 h	50787.29 ± 611.50 c	606.20 ± 5.80 ab	2.27 ± 0.06 a	0.069 ± 0.00 c	88.67 ± 0.53 ab
F.Ct / 144 h	47611.42 ± 323.57 d	620.45 ± 12.09 a	2.19 ± 0.19 b	0.070 ± 0.01 c	87.65 ± 0.53 b

T+ / 72 h: beans from traditional fermentation (control) at 72h; F.Ct / 72 h: beans from fermentation with *Candida tropicalis* at 72h; T+ / 144 h: beans from traditional fermentation (control) at 144h; F.Ct / 144 h: beans from fermentation with *Candida tropicalis* at 144h. values are expressed as mean $\pm$ standard deviation of three independent replicates. Within the same column, values followed by different letters are significantly different ($p < 0.05$).

3.3.2. Antinutritional Compounds in Fermented and Dried Cocoa Beans

The contents of antinutritional compounds in fermented and dried cocoa beans obtained from the best fermentation treatment (*Candida tropicalis* alone) at 72 h and 144 h, as well as from traditional fermentation (control), are presented in Table 2. Phytate content was lowest in cocoa beans fermented with

Candida tropicalis at 72 h, followed by those at 144 h. Higher phytate levels were observed in the control samples at both 72 h and 144 h. For oxalates, cocoa beans fermented with *Candida tropicalis* at 72 h showed higher contents than the control samples (72 h and 144 h), but lower levels than those recorded at 144 h with *Candida tropicalis*. Regarding tannins, cocoa beans fermented with *Candida tropicalis* at 72 h exhibited higher contents than those at 144 h, but lower values than those observed in traditional fermentation at 72 h.

Table 2. Antinutritional profile of cocoa beans fermented with freeze-dried microbial starters.

Samples	Oxalates	Phytates	Tanins
T+ / 72 h	623.33 ± 45.80 d	33.61 ± 0.37 b	1862.24 ± 51.02 a
F.Ct / 72 h	1085.33 ± 45.79 b	24.24 ± 0.67 d	1335.45 ± 6.37 b
T+ / 144 h	931.33 ± 55.36 c	43.07 ± 1.22 a	693.87 ± 5.10 c
F.Ct / 144 h	1246.66 ± 67.21 a	26.78 ± 0.43 c	565.05 ± 3.83 d

T+ / 72 h: beans from traditional fermentation (control) at 72h; F.Ct / 72 h: beans from fermentation with *Candida tropicalis* at 72h; T+ / 144 h: beans from traditional fermentation (control) at 144h; F.Ct / 144 h: beans from fermentation with *Candida tropicalis* at 144h. Values are expressed as mean $\pm$ standard deviation of three independent replicates. Within the same column, values followed by different letters are significantly different ($p < 0.05$), depending on the parameter analyzed.

3.3.3. Sensory Analysis of Fermented, Dried and Roasted Cocoa Beans

The principal component analysis (PCA) of the sensory attributes of cocoa beans after fermentation, drying, and roasting is presented in Figure 6. The first two principal components (F1 and F2) explain 95.69% of the total variance, with 61.04% and 34.65%, respectively, indicating a strong representation of the sensory data. The projection of variables on the factorial plane highlights a clear structuring of sensory attributes into two main groups. The first group, consisting of acidity, astringency, and bitterness, is positively correlated with the F1 axis. The second group, comprising cocoa aroma and taste, is negatively correlated with the F1 axis. In addition, these latter attributes show positive coordinates on the F2 axis, indicating their strong contribution to the second dimension. Figure 6 shows a clear differentiation between samples. Samples KA047 and KA072 are positioned on the positive side of the F1 axis and in the lower part of the F2 axis, indicating an association with acidity, astringency, and bitterness. Sample KA025 is located on the negative side of the F1 axis and in the lower part of F2. In contrast, sample KA059 is positioned on the negative side of F1 and the positive side of F2, close to the variables associated with cocoa aroma and taste.

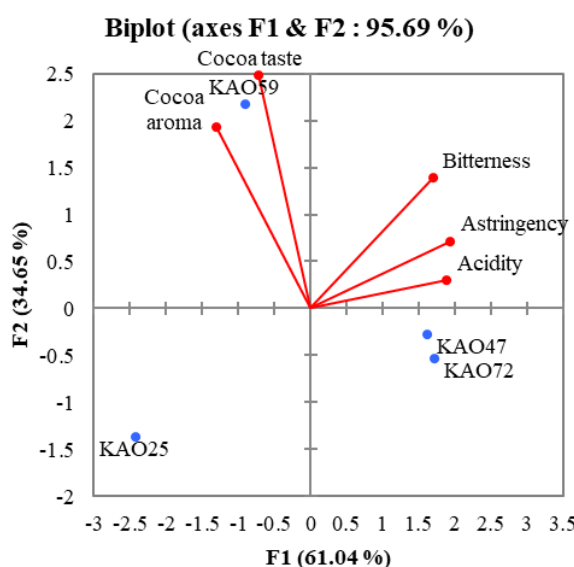


Figure 6. Principal component analysis of the key sensory attributes of fermented, dried, and roasted cocoa beans.

Beans obtained from traditional fermentation after 72 hours (KA047); Beans obtained after 72 hours of fermentation with added *C. tropicalis* inoculum (KA059); Beans obtained from traditional fermentation after 144 hours (KA025); Beans obtained after 144 hours of fermentation with added *C. tropicalis* inoculum (KA072).

3.3.4. Sensory Analysis of Herbal Teas Made from Fermented, Dried and Roasted Cocoa Beans

The principal component analysis (PCA) performed on the sensory and physical attributes of the cocoa samples revealed

that the first two principal components accounted for 91.82% of the total variance, with 51.87% explained by the first axis (F1) and 39.96% by the second axis (F2) (Figure 7). This high percentage indicates that the F1-F2 plane provides a reliable representation of the data structure. The projection of variables on the factorial plane highlights distinct groupings of attributes. On axis F1, color intensity and appearance are located on the positive side, indicating a strong positive correlation with this axis. In contrast, bitterness, acidity, and astringency are positioned on the negative side of F1, suggesting an opposition between visual attributes and undesirable taste characteristics. Regarding axis F2, cocoa aroma, cocoa taste, and fluidity are projected on the positive side, showing that these attributes strongly contribute to this dimension. Conversely, appearance and acidity exhibit negative coordinates on F2, indicating an inverse relationship with these sensory qualities. The distribution of samples across the factorial plane reveals clear differentiation among them. Sample KA072 is located in the positive region of both F1 and F2, closely associated with cocoa taste and fluidity, suggesting a more desirable sensory profile. Sample KA007 is positioned in the positive region of F1 but negative on F2, aligning more with appearance and color intensity attributes. Samples KA025 and KA047 are grouped in the negative region of F1, in association with bitterness and acidity, indicating less favorable sensory characteristics. Finally, sample KA059 is situated in the negative region of F1 but positive on F2, and is relatively close to the cocoa aroma attribute.

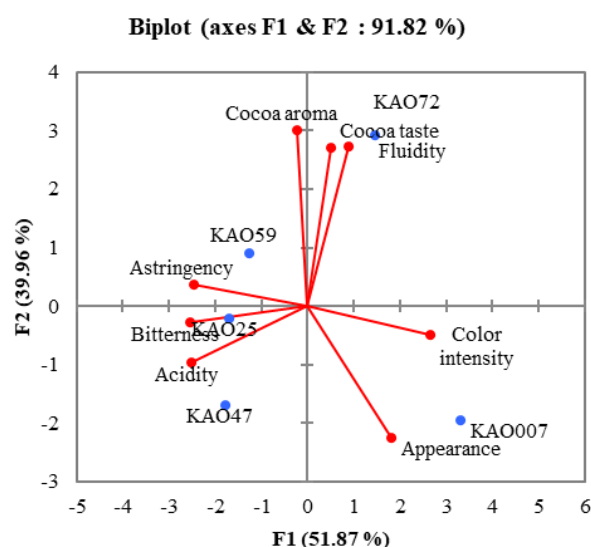


Figure 7. Multivariate analysis of the sensory quality of herbal teas derived from fermented, dried, and roasted cocoa beans.

Cocoa tea produced from beans obtained after 72 hours of traditional fermentation (KA047); Cocoa tea produced from beans obtained after 72 hours of fermentation with added *C. tropicalis* inoculum (KA059); Cocoa tea produced from beans obtained after 144 hours of traditional fermentation (KA025); Cocoa tea produced from beans obtained after 144 hours of fermentation with added *C. tropicalis* inoculum (KA072); Commercial tea (KA007).

4. Discussion

The present study demonstrates that freeze-dried *Candida tropicalis* and *Saccharomyces cerevisiae* starters supplemented with skimmed milk and sucrose exhibited significantly higher viability and retained a greater capacity to produce metabolites of interest compared to non-protected strains. These findings are consistent with previous reports, notably those of [44] who used skimmed milk and glucose, [45], who employed cocoa pulp and pod, and [46], who used trehalose and sucrose, all of which highlighted the beneficial role of cryoprotectants in preserving microbial functionality during freeze-drying. The enhanced preservation of both cell viability and metabolic activity can be attributed to the protective mechanisms of cryoprotectants during dehydration and freezing. Cryoprotectants rich in mono- and disaccharides are known to promote the formation of a glassy (vitreous) matrix at the cell membrane. This matrix stabilizes cellular structures by forming a protective layer around the cell wall and membrane, thereby reducing mechanical and osmotic stresses induced by ice crystal formation and water loss. As a result, cellular integrity is maintained, which explains the higher survival rates and the sustained production of metabolites observed in treated starter cultures.

In the context of cocoa processing, the availability of stable, viable, and metabolically active starter cultures is essential for ensuring consistent fermentation, improving bean quality, and enhancing the sensory properties of derived products.

During the fermentation trials, key parameters including temperature, pH of both pulp and cotyledon, reducing sugar content in the pulp and cotyledon, fermentation index, and bean quality assessed by the cut test exhibited trends consistent with those reported for cocoa fermentations in various regions worldwide [20, 47-50].

In terms of temperature evolution, fermentation trials conducted with *Candida tropicalis* in monoculture and in co-culture with *Saccharomyces cerevisiae* exhibited similar overall trends. However, the highest temperatures were recorded in the fermentation pile inoculated with *C. tropicalis* alone, ranging from 35 to 50.2 ± 0.03 °C, with a maximum peak of 50.2 ± 0.03 °C reached at 84 hours. In comparison, the co-culture system showed a slightly lower maximum temperature of 49.46 ± 0.03 °C, reached later at 108 hours. These temperature peaks, approaching 50 °C, are consistent with previous studies indicating that cocoa fermentation is characterized by significant heat generation (>40 °C) due to intense microbial activity and exothermic reactions [51, 52]. Although ethanol production contributes to heat generation, the main source of this temperature increase is the oxidation of ethanol into acetic acid by acetic acid bacteria, a highly exothermic process. This rise in temperature plays a crucial role in cocoa fermentation, as it leads to the death of the seed embryo, induces structural changes in the cotyledons, increases bean permeability, and promotes the formation of flavor precursors. The higher and

earlier temperature peak observed with *C. tropicalis* in monoculture suggests a strong metabolic activity of this yeast, particularly in pulp degradation and substrate transformation. Therefore, freeze-dried *C. tropicalis* used in monoculture appears to be a promising candidate for controlled cocoa fermentation, as its ability to induce high temperature peaks reflects its significant metabolic potential and its key role in initiating biochemical processes essential for the development of desirable sensory attributes.

The intense metabolic activity observed with *Candida tropicalis* can be attributed, on the one hand, to the intrinsic capabilities of certain strains, which exhibit high enzymatic activity toward sugars and strong tolerance to fermentation conditions such as temperature and acidity. These characteristics enable the yeast to rapidly consume pulp sugars and facilitate their diffusion into the cotyledon [26]. In addition, *C. tropicalis* is known to produce key hydrolytic enzymes, including polygalacturonases, pectinases, and invertases. These enzymes promote the degradation of pulp polysaccharides, leading to the release of simple sugars and enhancing their migration into the cotyledon. This enzymatic activity contributes to the breakdown of the pulp matrix, thereby improving substrate availability for subsequent microbial and biochemical processes.

Regarding the fermentation index, the trial using *Candida tropicalis* in monoculture showed a faster progression, increasing from 0.45 to 1.12, compared to traditional (control) fermentation (0.45 to 0.75) and the co-culture system with *C. tropicalis* and *Saccharomyces cerevisiae* (0.45 to 0.84). Notably, the threshold value of 1 commonly associated with well-fermented beans, was reached more rapidly in the monoculture system. These observations indicate that *C. tropicalis* alone significantly accelerates the fermentation process. This effect is likely due to its strong metabolic potential, which promotes rapid pulp degradation, efficient production of ethanol and organic acids, and early activation of endogenous enzymes within the cotyledon. As a result, key biochemical transformations occur earlier, reducing the time required to achieve optimal fermentation. This acceleration may have direct implications for bean quality, as it enhances the formation of flavor precursors while shortening the fermentation duration. The findings are consistent with previous studies showing that the use of selected yeast starters, particularly *C. tropicalis*, can improve the consistency and efficiency of cocoa fermentation compared to spontaneous processes or less active co-culture systems [51, 53]. Overall, the use of *C. tropicalis* in monoculture appears to be a promising strategy for controlled cocoa fermentation, enabling faster attainment of key fermentation indicators and potentially improving the sensory quality of the final product.

Concerning the proportion of well-fermented brown beans after drying, the fermentation trial using *Candida tropicalis* in monoculture showed a more rapid progression, exceeding 60% of well-fermented beans after only 72 hours. In contrast, this

threshold was reached later in the traditional (control) fermentation and in the co-culture system with *C. tropicalis* and *Saccharomyces cerevisiae*, after 108 hours and 96 hours, respectively. This difference can be attributed to the high metabolic activity of *C. tropicalis*, which promotes rapid pulp degradation, efficient production of ethanol and organic acids, and early activation of endogenous enzymes within the cotyledon. These processes accelerate tissue breakdown and metabolite diffusion, leading to the characteristic brown coloration of well-fermented beans. These findings are consistent with previous studies [15, 50], which demonstrated that inoculation with selected yeasts, including *C. tropicalis*, enhances fermentation efficiency and increases the proportion of well-fermented beans.

Overall, the use of *C. tropicalis* in monoculture appears to significantly shorten fermentation time while maintaining or even improving bean quality, making it a promising strategy for controlled cocoa fermentation. Beyond these technological and sensory advantages, it is also important to consider how such fermentation conditions influence the nutritional composition of cocoa beans.

The analysis of nutritional compounds revealed that cocoa beans fermented with *Candida tropicalis* for 72 hours and subsequently dried exhibited the highest levels of total phenolic compounds, as well as the greatest antioxidant activity, compared to beans from traditional fermentation at 72 and 144 hours and those from the 144-hour fermentation test. This observation can be attributed to the role of yeasts in driving the early biochemical transformations of cocoa fermentation. At the initial stages, yeasts particularly *C. tropicalis* are the dominant microorganisms and actively degrade the mucilaginous pulp rich in sugars. Through their metabolic activity, they produce ethanol and a range of metabolites that alter the biochemical environment surrounding the beans. These changes may facilitate the release, preservation, or reduced degradation of phenolic compounds, which are responsible for the antioxidant properties of cocoa. Moreover, a shorter and more controlled fermentation period, as observed with *C. tropicalis* at 72 hours, may limit excessive oxidation and loss of phenolic compounds, thereby maintaining higher antioxidant potential. This suggests that accelerated fermentation can help preserve bioactive compounds before they are significantly degraded during prolonged fermentation. These findings are consistent with previous studies [54, 55], which reported that the use of *Candida tropicalis* as a starter culture not only improves the technological quality of cocoa fermentation but also enhances the retention of total phenolic compounds and the production of amino acids involved in flavor development.

Furthermore, the higher levels of total phenolic compounds and antioxidant activity observed at 72 hours can be explained by the progressive decline of these compounds during extended fermentation. Several studies have shown that cocoa polyphenols undergo oxidation and polymerization reactions under the influence of enzymatic activity and fermentation conditions, leading to a gradual decrease in their concentration

over time [56]. This reduction is also associated with the diffusion of phenolic compounds from the cotyledonary cells and their involvement in enzymatic reactions that contribute to the formation of aroma precursors. Regarding flavonoids and anthocyanins, the results indicate that beans fermented with *Candida tropicalis* at 72 hours contained higher levels than those from traditional fermentation at the same time point, but lower levels than those observed in samples fermented for 144 hours. Anthocyanins, which are responsible for the purple coloration of fresh cocoa beans, undergo significant transformations during fermentation [57, 58]. Under the combined effects of enzymatic oxidation and acidic conditions, these compounds are partially degraded or converted into other phenolic derivatives [56]. The observed trends therefore reflect the dynamic and progressive transformation of these bioactive compounds throughout the fermentation process. Beyond these changes in phenolic composition, it is also important to consider the fate of antinutritional compounds during fermentation.

The results obtained indicate that cocoa beans fermented with *Candida tropicalis* at 72 hours exhibited the lowest phytate contents compared to the other treatments. This reduction in phytates during fermentation has been widely reported in the literature, notably by [8], who highlighted the role of microbial activity in the degradation of antinutritional compounds. The decrease in phytate content can be attributed to the enzymatic activity of microorganisms involved in fermentation. Certain yeasts and bacteria possess phytase enzymes capable of hydrolyzing phytic acid into simpler, less complex molecules, thereby reducing its concentration in the fermented matrix. In addition to microbial enzymes, endogenous phytases present in cocoa beans may also contribute to this process. Furthermore, several studies [59, 60] have demonstrated that fermentation enhances phytate degradation through the combined action of microbial and endogenous enzymes. This reduction is of nutritional importance, as it improves the bioavailability of essential minerals such as iron, zinc, and calcium by limiting their chelation by phytic acid. Overall, the use of *Candida tropicalis* in controlled fermentation appears to promote a more efficient degradation of phytates, thereby contributing to the improvement of the nutritional quality of cocoa beans.

Regarding oxalate content, the results indicate that cocoa beans fermented with *Candida tropicalis* for 72 hours exhibited higher levels than those observed in traditional fermentations at 72 and 144 hours, but lower levels than those recorded in samples fermented with the starter for 144 hours. This pattern suggests a dynamic evolution of oxalates throughout the fermentation process. These variations can be linked to the metabolic transformations occurring during fermentation. Oxalate levels are influenced by several factors, including the degradation of organic acids and the metabolic activities of the microorganisms present in the fermentation environment. During fermentation, biochemical reactions involving organic

acids and microbial metabolites may lead to either the formation or degradation of oxalates [58]. Furthermore, the evolution of oxalate content appears to depend on both fermentation duration and the composition of the microbial consortium. As fermentation progresses, shifts in microbial populations and metabolic pathways can alter oxalate dynamics [52]. The intermediate levels observed at 72 hours with *C. tropicalis* may therefore reflect a transitional stage, where oxalate formation and degradation processes are still in balance. Overall, these findings highlight the importance of controlling fermentation conditions particularly duration and microbial composition to better manage antinutritional compounds such as oxalates and optimize the nutritional quality of cocoa beans.

Regarding tannin content, cocoa beans fermented with *Candida tropicalis* for 72 hours exhibited higher levels than those observed in samples fermented for 144 hours, but lower levels than those recorded in traditional fermentation at 72 hours. This trend can be explained by the progressive transformation of phenolic compounds during cocoa fermentation. Tannins, which belong to the polyphenol family, are gradually oxidized and polymerized under the action of oxidative enzymes such as polyphenol oxidases. These reactions lead to a progressive decline in tannin concentration as fermentation time increases. In addition, these transformations contribute to the reduction of astringency and bitterness, thereby improving the sensory quality of cocoa beans [57, 58]. The lower tannin levels observed after 144 hours can thus be attributed to the intensification of oxidation and condensation reactions during prolonged fermentation. Importantly, controlled fermentation using *C. tropicalis* for 72 hours appears to provide a favorable biochemical balance. At this stage, beans retain relatively high levels of bioactive compounds with antioxidant activity while showing a reduction in certain antinutritional factors, such as phytates and tannins. This balance suggests a simultaneous improvement in both nutritional value and sensory potential. Overall, the use of *Candida tropicalis* as a starter culture emerges as a promising strategy to optimize cocoa fermentation, enabling the production of beans with enhanced technological, nutritional, and sensory quality suitable for chocolate processing. Given these biochemical and functional changes, it becomes essential to further assess the overall quality of the fermented beans, as well as that of derived products such as cocoa-based herbal teas, in order to better understand the impact of controlled fermentation on final product acceptability and value.

The principal component analysis (PCA) performed on the sensory attributes, as well as on the combined sensory and physical attributes of cocoa beans after fermentation, drying, and roasting, revealed that the first two principal components explained a large proportion of the total variance (95.69% for sensory attributes and 91.82% for combined sensory and physical attributes). This indicates that the F1-F2 planes provide a reliable representation of the data structure. The high variance captured by these two axes suggests that most of the variability among samples can be effectively described by

these principal dimensions. This finding highlights the strong influence of key sensory and physical parameters in differentiating the cocoa samples. It is consistent with previous studies [8, 58], which emphasize the critical role of fermentation and post-harvest processing in shaping the sensory profile and overall quality of cocoa beans.

The projection of variables onto the factorial plane reveals two distinct groups of attributes. The first group comprises acidity, astringency, and bitterness, which are positively correlated with axis F1. The second group includes cocoa aroma and cocoa taste, which are negatively associated with F1 and positively correlated with F2. This distribution indicates a clear opposition between undesirable sensory attributes (bitterness, astringency, acidity) and desirable attributes (cocoa aroma and taste).

These results suggest that astringency and bitterness are closely linked to the biochemical transformations occurring during fermentation and drying, particularly the oxidation and polymerization of polyphenols and tannins, as reported by [57]. As these compounds are progressively modified, their contribution to sensory perception evolves, influencing the overall quality of the beans. With regard to cocoa aroma and taste, previous studies [50, 61] have shown that the use of selected microbial starters, such as *Candida tropicalis*, in controlled fermentation can enhance the development of desirable sensory attributes. These improvements are associated with the formation of flavor precursors and their subsequent transformation during drying and roasting, leading to a richer and more balanced cocoa profile.

The distribution of samples on the factorial plane shows a clear differentiation, reflecting the combined effects of fermentation treatment and fermentation duration on the sensory quality of cocoa beans. This separation highlights the strong influence of process conditions on the development of sensory and physical attributes. For instance, samples KA072 and KA059 are positioned close to the cocoa aroma and taste vectors, indicating high aromatic intensity and good fluidity during herbal tea preparation. This suggests that the use of *Candida tropicalis* as a starter, together with appropriate control of fermentation time, promotes the development of richer and more balanced sensory profiles.

In contrast, samples KA025 and KA047 are associated with acidity and bitterness, indicating that less controlled or prolonged fermentations may enhance astringency and undesirable taste attributes [52, 62]. These results underline the importance of controlling both fermentation duration and microbial composition to avoid excessive development of negative sensory characteristics. Overall, these findings confirm that controlled fermentation, particularly with selected starters such as *Candida tropicalis*, plays a key role in shaping the sensory and physical structure of cocoa-derived products. By modulating the balance between desirable attributes (aroma and taste) and undesirable ones (bitterness and astringency), it offers an effective strategy for producing cocoa beans and derived products such as cocoa-based herbal teas with premium

sensory quality. This is consistent with previous studies highlighting the impact of starter cultures on phenolic transformations and the formation of flavor precursors.

5. Conclusions

The objective of this study was to develop and evaluate the effect of *Candida tropicalis*, used in monoculture or in co-culture with *Saccharomyces cerevisiae*, on the controlled fermentation of cocoa beans at scale, with the aim of improving their sensory, physicochemical, and nutritional quality, as well as that of derived products.

The results demonstrated that inoculation with *C. tropicalis* in monoculture for 72 hours enabled a faster attainment of optimal fermentation compared to traditional methods. This approach significantly increased the proportion of well-fermented beans and improved overall sensory quality. Biochemical analyses further revealed that beans fermented under these conditions exhibited higher levels of total phenolic and flavonoid compounds, along with enhanced antioxidant activity, indicating better preservation of health-promoting bioactive compounds. In parallel, antinutritional factors such as phytates and tannins were reduced, while oxalate levels were modulated by fermentation duration, suggesting an overall improvement in nutritional quality and mineral bioavailability.

Sensory evaluation and principal component analysis confirmed that controlled fermentation favored desirable attributes such as cocoa aroma and taste, while effectively modulating undesirable characteristics including astringency, acidity, and bitterness. These improvements were also observed in cocoa-based herbal teas, highlighting the positive impact of controlled fermentation on the quality of derived products.

From a scientific and technological perspective, this study demonstrates that *Candida tropicalis* is an effective microbial starter for optimizing cocoa fermentation. Its use enhances the formation of aromatic and bioactive compounds while reducing antinutritional factors, thereby contributing to both improved product quality and functional value. These findings have important implications for several industrial sectors, including the chocolate industry, which can benefit from more consistent and higher-quality beans; the nutraceutical sector, through the valorization of antioxidant-rich cocoa products; and the beverage industry, particularly for cocoa-based herbal teas with improved sensory profiles.

Finally, this work opens promising perspectives for the development of tailored microbial starters, the optimization of fermentation conditions at industrial scale, and the integration of controlled fermentation strategies into local cocoa production systems to enhance both product quality and economic value.

Abbreviations

ICCO International Cocoa Organization

YPG Yeast Peptone Glucose
KI/I2 Potassium Iodide/Iodine
FI Fermentation Index

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Conflicts of Interest

The authors declare no conflicts of interest.

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