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Research Article

Impact of New Fermentation Supports on the Quality of Cocoa Beans (*Theobroma cacao* L.) From Côte d'Ivoire

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This study investigated the influence of various fermentation supports on cocoa bean quality, aiming to identify alternatives to banana leaves, which are facing scarcity issues. Supports tested included banana leaves (support used as a reference), palm leaves, cocoa pods, jute bags, polypropylene tarpaulins and polypropylene bags. Key parameters monitored during fermentation included temperature, pH, microbial dynamics and concentrations of reducing sugars and organic acids. The beans underwent physicochemical characterisation and market value evaluation after fermentation and drying. The results revealed that fermentation in cocoa pods and polypropylene bags was suboptimal. Cocoa pods exhibited low fermentation temperatures, delayed microbial activity, high pH and increased mould proliferation, leading to poor marketability with low cut-test scores. Polypropylene bags retained higher humidity levels, posing a risk for mould contamination, although they showed higher phenolic compound content. By contrast, beans fermented on jute bags, palm leaves and reusable polypropylene tarpaulins displayed fermentation profiles and final product characteristics similar to banana leaves, making them viable alternatives for local producers. Further analysis of the aromatic characteristics of beans fermented on these supports is needed to provide a holistic evaluation. This study offers valuable insights into identifying sustainable alternatives for cocoa fermentation and serves as a reference for resource optimisation in other food production systems.

Keywords: chemical characteristics; cocoa; fermentation; fermenting microorganisms; market value; physicochemical properties

1. Introduction

Cocoa (*Theobroma cacao* L.) ranks third among the world's primary commodities, following sugar and coffee [1]. With an annual production of 2.23 million tons, representing 42.22% of global production in the 2022–2023 season, Côte d'Ivoire remains the world's leading cocoa producer [2]. Cocoa is a crucial ingredient in products like chocolate, biscuits, confectionery, cocoa drinks and ice creams [1, 3]. Cocoa beans and their derivatives are high in antioxidants, such as catechins, epicatechin and procyanidins, which are polyphenols also found in wine, vegetables and tea [4, 5]. The process of transforming cocoa fruit into marketable beans involves postharvest treatments: pod breaking, fermentation and drying [6], with fermentation being the most critical. Bankoff et al. [7] highlight that fermentation promotes physicochemical changes in the cocoa beans, causing colour modification, pH and organic acid variations and temperature increase.

In rural areas, farmers often subject raw seeds to spontaneous fermentation after harvesting and breaking cocoa pods, which is crucial for developing the typical cocoa flavour during roasting [4]. Fermentation removes the pulp surrounding the cocoa beans, develops chocolate aroma

precursors, reduces astringency and brings bitterness to a pleasant level [8]. Poor management of fermentation can result in low market quality and loss of desired characteristics in cocoa beans.

Fermentation involves two stages: microbial fermentation of the sweet pulp into citric acid through alcoholic processes initiated by yeasts and lactic acid bacteria (LAB), followed by a more exothermic stage characterised by acetic fermentation from internal biochemical changes in the cotyledon involving acetic bacteria and *Bacillus* genus bacteria [9].

Ivorian producers often face issues like slaty and mouldy beans due to poor fermentation and drying [10]. The most common local fermentation support, banana leaves, yields beans with the best market, microbiological, and organoleptic qualities [11]. However, the use of banana leaves has declined due to climatic variations, soil aging and shading of cocoa trees.

Producers have turned to plastic tarpaulins and wooden crates as alternatives, but these do not offer uniform treatment, leading to defective beans and a degradation of the market and organoleptic quality of the final product [12]. Recently, new supports like palm leaves, cocoa pods, polypropylene tarpaulins, polypropylene bags and jute bags have been used [13]. However, there is limited data on the quality of beans from these supports in Côte d'Ivoire. The lack of knowledge about the impact of new fermentation supports on good postharvest treatment practices could explain the heterogeneity of cocoa bean batches produced and their variable quality.

This study aims to evaluate these new fermentation supports to find viable alternatives to banana leaves and determine which supports yield beans of the highest commercial quality. The impact of five new fermentation supports on the fermentation process and the quality of dried beans was evaluated to provide producers with advice on obtaining consistently high-quality cocoa beans with high commercial value.

2. Materials and Methods

2.1. Cocoa Bean Fermentation and Drying. Cocoa beans from the forastero variety originated from a single producer. Cultivation, fermentation steps and drying were performed in 2022 in San-Pedro in the Bas-Sassandra region of Côte d'Ivoire. The entire fermentation process (for each of the fermentation support) was carried out over 6 days on a single production site in a single shaded area, protected from extreme weather conditions. Six fermentation supports (Figure 1) were used: banana leaves (Reference), palm leaves, cocoa pods, polypropylene tarpaulin, polypropylene bags and jute bags. These supports underwent three stirrings (at 48, 96 and 120 h) during the process. A mass of 250 kg of fresh beans was fermented for each support except cocoa pods. In this case, 4000 pods (approximating 250 kg of beans) were distributed into eight polypropylene bags and fermented together. These bags were stored in a clean and shaded area on wooden shelves to allow the juice to drain out.

Humidity and temperature, varying from one support to another, was monitored throughout the fermentation process. After 6 days of fermentation, all cocoa beans obtained for each fermentation support were sun-dried on a polypropylene tarpaulin measuring $3 \times 2\ m^2$, at an ambient temperature (between 28°C and 30°C) for 7 days. The beans fermented in the cocoa pods were removed and separated from their placenta after the 6 days of fermentation and before drying. This was performed to ensure that the beans are in the same form as for other supports. The beans were sun-dried between 8 a.m. and 4 p.m. The masses being dried were stirred once every hour (8 stirring per day in total). After 4 p.m., the beans were covered with polypropylene tarpaulins until 8 a.m. the next day. The fermentation and drying protocol described in this section is the most frequently used by local producers in this region. As described earlier, the latter usually use banana leaves as fermentation support. The protocol has therefore been extrapolated to other fermentation supports, ensuring therefore that a single protocol is used and in which the only varying parameter is the fermentation support.

Following these two steps, fermented and dried cocoa beans were obtained. These are the beans on which the market value was studied, as described in Section 2.5.

2.2. Sampling. During fermentation, samples ($n = 4$) were collected aseptically between 20- and 90-cm deep in each fermenting batch and placed in sterile pots. 50 g were sampled every 24 h from different supports over six days of fermentation. After fermentation, samples ($n = 3$, 1 kg each) of dried beans from different supports were collected. All samples were transported to the laboratory for various analyses. Upon arrival, dried beans were shelled, finely ground into powder (IKA A11 basic; Germany) under liquid nitrogen. These powders were kept at −80°C until further analyses.

2.3. Fermentation Parameter Monitoring

2.3.1. Determination of pH and Fermentation Temperature. Temperature measurement during fermentation was performed using an electronic probe thermometer. The pH was measured using a pH metre (Hanna Instrument, Romania) with a combined glass electrode, according to Tsighe et al. [14] with slight modifications. The probe was immersed to a depth of 5 cm in the fermenting mass, and values were read after maintaining the probe for 2 min in the mass. These parameters are the only which were directly monitored in the fermenting mass. pH and temperature ($n = 6$) were recorded every morning at 7 a.m. during 6 days.

2.3.2. Enumeration of Microbial Flora of Cocoa Beans From Different Fermentation Supports. Microbial flora, including yeasts, LAB, acetic acid bacteria (AAB) and *Bacillus* genus bacteria, were enumerated using standard microbiological spread plate methods. LAB were incubated on MRS agar at 30°C for 48 h, *Bacillus* on Mossel agar supplemented with emulsified egg yolk [15] at 30°C for 24 h, AAB on Acetobacter

Figure 1: Different fermentation supports: (a) palm leaves; (b) cocoa pods; (c) polypropylene tarpaulin; (d) polypropylene bags; (e) jute bags; (f) banana leaves (reference).

formulated medium at 30°C for 24 h [16] and yeasts on Sabouraud medium with chloramphenicol at 25°C for 5 days. The LAB, *Acetobacter*, and AAB media were supplemented with oxytetracycline to inhibit yeast growth. The observed colonies were expressed in colony-forming units (CFU/g). Analyses were performed on four biological replicates ($n = 4$).

2.3.3. Determination of Organic Acids and Soluble Sugars. To analyse the evolution of induced metabolites (organic acids and reducing sugars) during fermentation, 100 g of beans from each fermentation medium were collected in stomachers. In the laboratory, the beans were dehulled and ground in liquid nitrogen using a grinder (IKA A11 basic; Germany) before being divided into 20-mL jars. Extraction was performed using 10 g of ground cocoa beans for each fermentation medium. The resulting powder was transferred to a 100-mL volumetric flask containing 50 mL of distilled water, heated to 75°C and filtered through 0.45-μm filters prior to injection.

Organic acids (oxalic, malic, lactic, citric, acetic and succinic acids) and reducing sugars (glucose and fructose) were analysed by high-performance liquid chromatography (HPLC; Agilent 1200, Hewlett-Packard-Strasse 8, 76,337 Waldbronn, Germany) coupled with a refractive index detector (RID) according to the method described by Ho et al. [17]. Elution was performed with sulphuric acid (5 mM) at a flow rate of 0.6 mL/min for 25 min in an ion exclusion column (Bio-Rad Aminex HPX-87H, Hercules, USA) at a temperature of 50°C. Identification was based on the retention times of corresponding standards, and quantification was carried out using calibration curves. Analyses were performed on four biological replicates ($n = 4$).

2.4. Physicochemical Characteristics of the Final Product

2.4.1. Proximate Composition. Humidity content was measured on 500 g of beans using a mini GAC (Dickey John multigrain) according to the method of Akmel et al. [18]. Water activity was measured with an Aw meter (AQUA LAB), and ash content was determined using AOAC 2000 [19] methods. The fat (cocoa butter) of the cocoa powder, extracted with hexane in Soxhlet for 8 h, as well as the pH, were analysed in accordance with the method of Nogbou et al. [20].The fat analysis allowed us to determine the total lipid content found in the sample. The total protein content (i.e., percentage of crude protein) is determined using the Kjeldahl method, and the obtained nitrogen percentage (% N) is used to calculate the crude protein percentage (% P) using the relation: % P = % N × 6.25 [21]. Carbohydrates and energy value are calculated using the following formulae [19, 22]: Carbohydrates = 100 − (% moisture + % protein + % fat + % ash) and energy value (EV, kcal/100 g) = [(9 × (fat (%)) + (4 × (protein (%)) + (4 × carbohydrate (%))]. Analyses were performed on three biological replicates ($n = 3$). Fibre content (in % dry matter) was estimated using the method described in [23, 24].

2.4.2. Acidity Determination. The acid value represents the number of milligrams of potash required to neutralise 1 g of fat. It can be converted into acidity (oleic), expressed as a percentage of oleic acid to indicate the level of free fatty acids (FFA). The acid value is calculated as follows: Acid value = (VKOH × NKOH × 56.1)/PMG, where VKOH represents the volume of alcoholic potash used in ml, NKOH is the normality of the potash, PMG is the mass of fat extracted in milligrams, and 56.1 is the molecular weight of KOH [25].

2.4.3. Total Polyphenol Content, Total Flavonoid Content and Total Tannin Content. Total polyphenol content was determined by the Folin–Ciocalteu colorimetric method. Absorbance was measured at 760 nm, and concentration was expressed in mg of gallic acid equivalents (standard) (mg GAE) per 100 g of fresh extract [26]. Total flavonoid content

was quantified by spectrophotometry as described by Dehpour et al. [27]. Absorbance was measured at 415 nm with total concentration expressed in mg of quercetin equivalents (standard) (mg QE) per 100 g of fresh extract. Tannin content was measured by spectrophotometry according to Luczaj et al. [28].

2.4.4. Phenolic Compounds and Alkaloids Analysis. Alkaloids and phenolic compounds in defatted cocoa powder were analysed by HPLC-DAD according to Bachir Bey et al. [29]. The quantification of each identified compound (theobromine, caffeine, catechin and epicatechin) was performed on each obtained sample using an external standard calibration curve for each compound [30]. A volume of 10 µL of each standard and samples were injected into the HPLC (Agilent 1100, Hewlett Packard-Strasse 8, 76,337 Waldbronn, Germany) using the following characteristics: C18 column (Poroshell 120 EC-C18 (100 × 4.6 mm). The column temperature was maintained at 40°C, and phenolic compounds were eluted using two separate mobile phase gradients: phase A (H_2O/MeOH, 9:1, with 0.1% H_3PO_4) and phase B (MeOH with 0.1% H_3PO_4), at a flow rate of 1 mL/min.

2.4.5. Fatty Acid Analysis. The total fatty acid profile of cocoa butter was analysed through an esterification process to convert them into methyl esters of FFA. Methyl esters were obtained from metabolised fatty acids with a BF3/methanol mixture and analysed by gas chromatography (GC 6890A system) coupled with an FID detector [31]. For this, 10 mg of cocoa butter sample was added to 0.2 mL of hexane and 0.5 mL of BF3 reagent (methanol/BF3 14% hexane (55:25:20)) and heated at 70°C in a water bath for 1.5 h. After transesterification, methyl esters were extracted by adding 0.5 mL of saturated NaOH solution, 0.2 mL of 10% H_2SO_4 and 8 mL of hexane. After vigorous shaking, 0.5 µL of the upper layer of the solution was withdrawn and injected. The GC system (Agilent Technologies, Inc.) equipped with a CP9205 VF-WAX capillary column (30 m × 250 µm × 0.25 µm, Agilent Technologies, Inc.) was used. Helium was used as the carrier gas at a flow rate of 1.234 mL/min. Methyl esters of fatty acids were identified based on retention times and compared to FAME Mix C4–C24 analytical standards (Supelo37 component in dichloromethane, Sigma-Aldrich). The areas under the peaks corresponding to the fatty acids were summed, and the relative concentration of a fatty acid was calculated by dividing its area by the sum of the areas of all the peaks, using the following equation: FA concentration = FA area/total FA area. Analyses were performed on three biological replicates ($n = 3$).

2.4.6. Mineral Composition. Minerals such as macroelements: sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) were determined by a flame atomic absorption spectrometer (Varian 220FS Spectra AA, Varian, Les Ulis, France). The P concentrations in the filtrate were determined using the vanado-molybdate colorimetric method at 420 nm [32].

2.5. Market Value of Cocoa Beans. Bean count (i.e., graining) was determined by counting healthy beans in 100 g according to ISO 1114 (1977). Shell percentage was determined using the AOAC methods 968.10 and 970.23 [33]. The yield obtained from 250 kg of raw beans put into fermentation was determined after drying using the following equation: yield = [(fermented beans − dried beans)/250] × 100. Analyses were performed on three biological replicates ($n = 3$). The Cut test was performed according to the method of Hii et al. [34], and the cut test score was calculated using the equation: Cut test score = (10 × % brown) + (5 x % violet/brown) + (0 × % violet and slaty).

2.6. Statistical Analyses. Firstly, statistical analyses were performed on parameters monitored during the fermentation process. Differences in terms of temperature, pH, yeast, LAB, AAB, and *Bacillus* between the banana leaves, set as reference group and the other fermentation supports were analysed using a robust two-way mixed ANOVA. The within-subject factor was time and between-subject factor was fermentation support. Pearson correlation coefficients were also calculated between lactic acid production and LAB growth and between temperature and *Bacillus* growth. A heatmap representing the consumed sugars and produced organic acids during fermentation was also elaborated.

Statistical analyses were also performed on cocoa final product obtained after fermentation and drying of this one. One-way ANOVA followed by Dunnett's multiple comparison test (in the case of significant differences) were performed on the physicochemical, chemical and market quality parameters. Lastly, a principal component analysis (PCA) was also elaborated with all the data obtained for the final product. Physicochemical, chemical and market characteristics were used here and auto-scaled prior to analysis.

With the exception of the heatmap elaborated with the Morpheus software (https://software.broadinstitute.org/morpheus/), all other analyses were performed using R software (Version 4.3.2, R Development Core Team, Boston, USA).

3. Results and Discussion

3.1. Temperature and pH Evolution During Fermentation. Temperature and pH are important factors to assess the progress of fermentation [35]. Throughout the fermentation process, all the fermentation support showed a temperature (23°C–48°C) and pH (2–6) profile close to that frequently obtained when using banana leaves, with the exception of the cocoa pods fermentation support (See Figures 2(a) and 2(b)), which showed a lower temperature due to the low number of beans in a pod compared with the other support. According to De Vuyst and Weckx [36], beans considered to be well fermented are those whose maximum fermentation temperature is between 45°C and 50°C. Thus, the maximum temperature and pH values observed on these supports are linked to the emergence of AAB that oxidise ethanol produced by the yeasts into acetic acid [37].

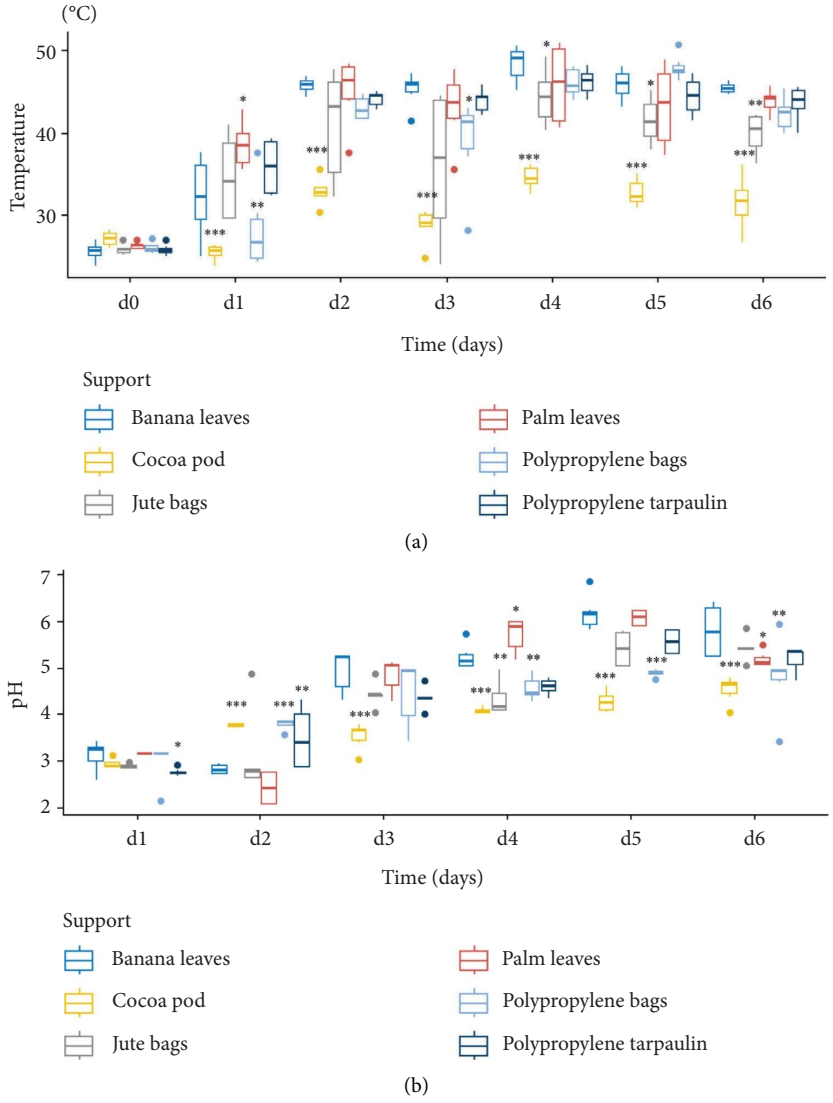

FIGURE 2: Variation in (a) temperature and (b) pH during fermentation of cocoa beans on different supports. *Significant difference, **highly significant difference, and ***very highly significant difference in comparison to the reference support (banana leaves).

3.2. Microbial Fermentation Flora. Cocoa bean fermentation involves various groups of microorganisms which play a decisive role in the final quality of chocolate. This study isolated a fermentation microflora which includes yeasts, LAB, AAB and *Bacillus*. These have been studied on numerous previous occasions [7, 38].

Yeast population increased from 0.5×10^6 CFU/g to a maximum of 3×10^6 CFU/g, with significant differences observed on Day 6 when comparing cocoa pods to banana leaves (Figure 3). Cocoa pods showed slower yeast growth, reaching a maximum on Day 6, while the yeast population on the other supports reached a maximum earlier in the fermentation process. As opposed to when other fermentation supports are used, cocoa beans fermented in cocoa pods are not confronted to an exogenous microflora. This means that the initial yeast population is exclusively constituted of those found in the cocoa pods, which therefore require more time to reach a maximum. Reducing sugars reserve being less depleted in cocoa pods (detailed later in Figure 4) can also explain the increase in yeast population in this case when compared to other supports (decreasing yeast populations linked to reducing sugars reserves depletion).

LAB varied from 1×10^7 to 20×10^7 CFU/g (Figure 5). Cocoa pods showed the lowest population, significantly different from banana leaves on days 2 and 3. The maximum population was reached in beans fermented on polypropylene bags on the last day of fermentation (d6) with $20.61 \pm 5.46 \times 10^7$ CFU/g, significantly higher than LAB on banana leaves. This disparity can be explained by the characteristics of these supports (shape, size, impermeability, depth), which offer a better impermeability than banana leaves, thus limiting the entry of air but also the flow of juice during fermentation and favouring anaerobic conditions preferred by yeasts and lactic bacteria.

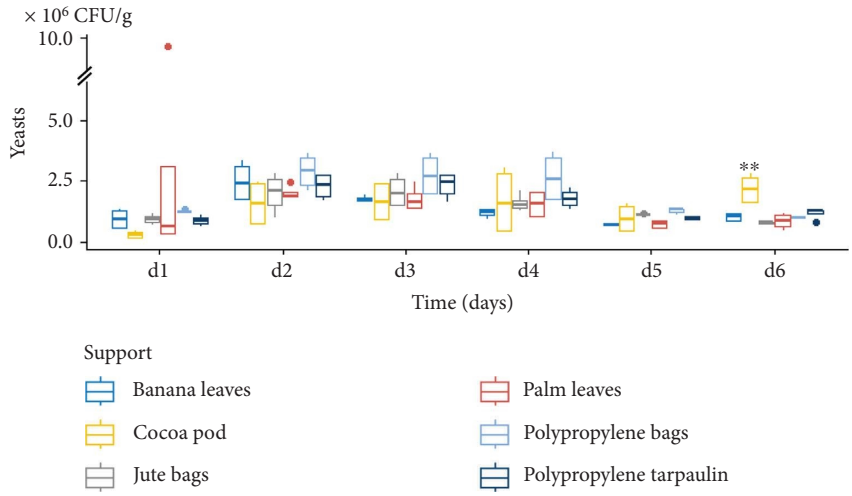

FIGURE 3: Yeast growth during the fermentation of cocoa beans on different support. **Highly significant difference in comparison to the reference support (banana leaves).

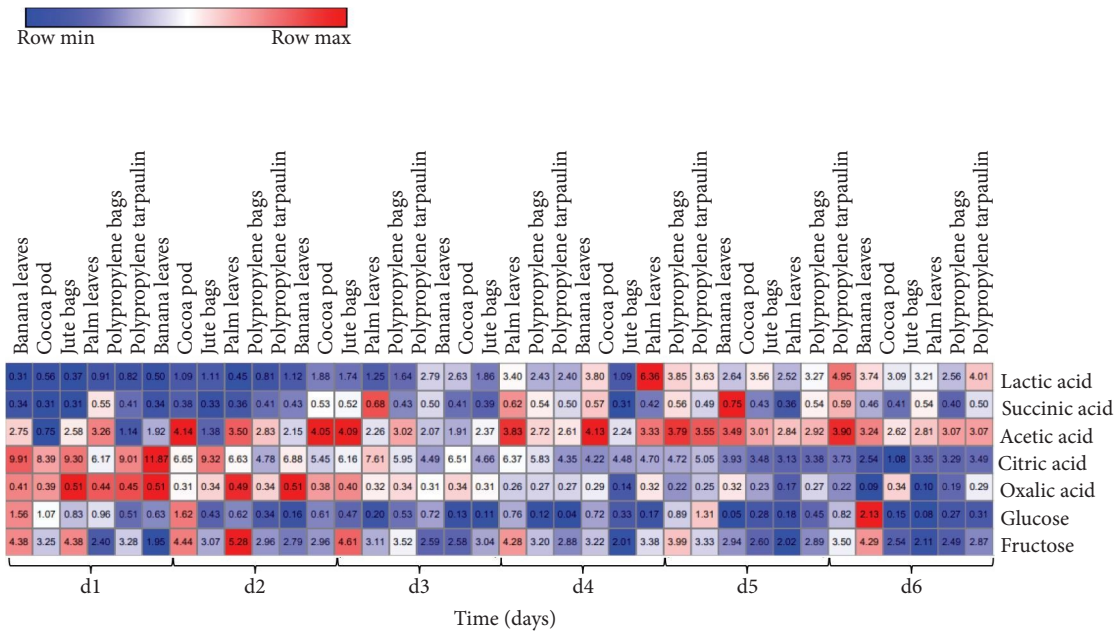

FIGURE 4: Heatmap showing the evolution of reducing sugars and organic acids during fermentation. Each column represents a fermentation support at a specific time interval. Values are in mg/g of cocoa beans.

The increase in the population of yeast and LAB and their activities led to aeration of the fermenting mass, allowing AAB to continue fermenting. The growth of AAB was accompanied by a rapid increase in temperature, from 40 to a maximum of 48°C (Figure 2(a)). To the exception of the population growing on the cocoa pods support, AAB population increased up to Day 5 on all support, with maximum values for the palm leaves support (34×10^7 CFU/g). These populations then decreased towards the end of fermentation, right after the second mechanical stirring (Figure 6). AAB in cocoa pods support showed a significantly lower growth than those on banana leaves throughout fermentation (from Day 2 to Day 6), which could be explained by less yeast and LAB activity in the past.

The results obtained for the AAB population differ both in terms of maximum growth and the time at which this maximum growth is reached, compared with the work of Lagunes Gálvez et al. [39] who reported maximum growth up to 1.5×10^8 CFU/g after 48 h. The discrepancy could be explained by the fact that wooden boxes were used in the latter study, which have not been used in the present study.

When looking at the *Bacillus* (Figure 7), it can be seen that its population tends to increase over the last days of fermentation (Day 4 to Day 6), as reported in the literature [38, 40]. Their population varied between 1×10^7 and 11×10^7 CFU/g on all support. The maximum population ($11.77 \pm 6.68 \times 10^7$ CFU/g) was recorded on Day 6 for banana leaves, significantly higher compared to all other

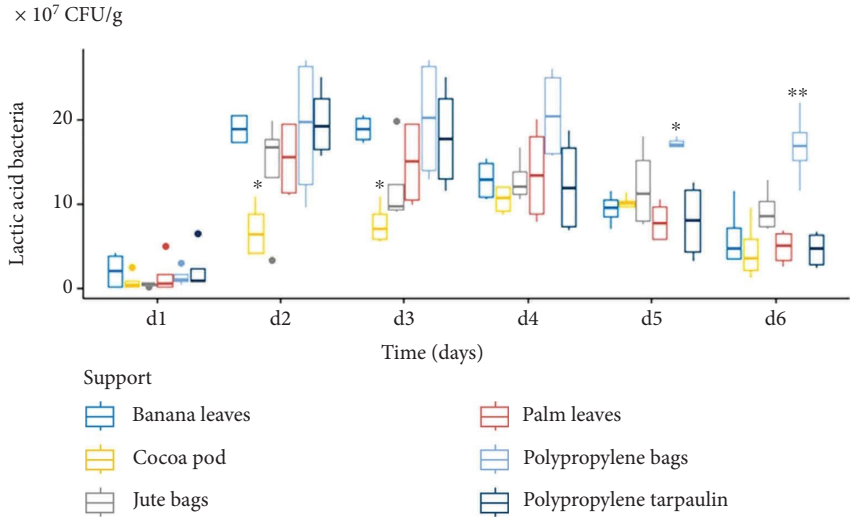

FIGURE 5: LAB growth during the fermentation of cocoa beans on different fermentation supports. *Significant difference and **highly significant difference in comparison to the reference support (banana leaves).

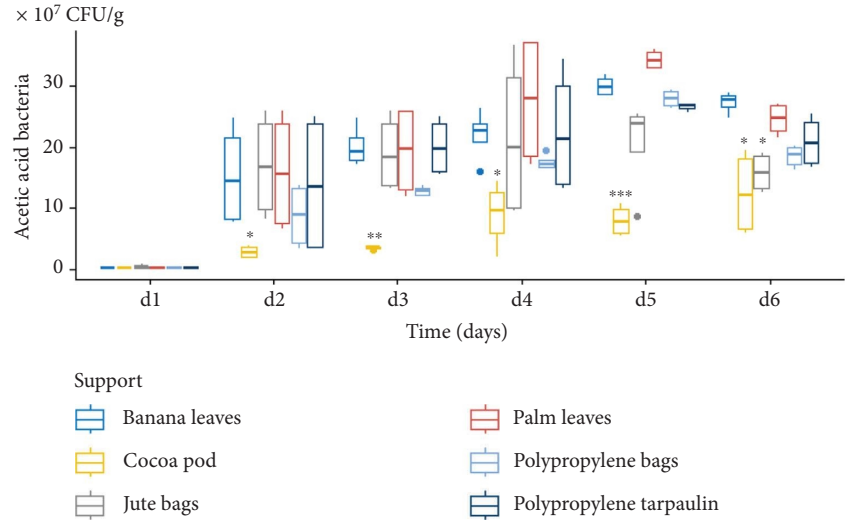

FIGURE 6: Growth kinetics of AAB during the fermentation of cocoa beans on different supports. *Significant difference, **highly significant difference, and ***very highly significant difference in comparison to the reference support (banana leaves).

fermentation support. Overall, the growth of *Bacillus* was positively correlated with the rise in temperature and could be explained by the ability of *Bacillus* bacteria to resist to high temperatures during fermentation [41].

3.3. Organic Acids and Reducing Sugars Profiles. To the exception of cocoa pods, the concentration in fructose and glucose is high at the beginning of the fermentation process (Figure 4) and decreases towards the end, as indicated by Hirko et al. [42].

The concentration of glucose increased from 2 to 4 mg/g in 24 h of fermentation, while that of fructose varied between 1 and 5 mg/g from 24 to 96 h. Maximum glucose and fructose concentrations were observed in cocoa beans fermented on jute bags (4.45 ± 0.61 mg/g and 5.27 ± 0.70 mg/g, respectively) followed by banana leaves (4.32 ± 1.56 mg/g and 4.43 ± 1.65 mg/g, respectively). However, an increase in glucose and fructose content on cocoa pods was observed on the last days of fermentation, with respective maximum concentrations of 3.07 ± 2.13 mg/g and 4.29 ± 3.10 mg/g (d6 in Figure 4). The concentrations observed for cocoa pods could be due to the initial sucrose disparity in the bean mucilage and the pods' ability to accelerate yeast and lactic bacteria activity, converting reducing sugars until exhausted, as reported by De Vuyst and Leroy [43]. The peak concentration on Day 6 aligns with the late yeast activity previously observed in Figure 3.

Organic acids play a key role in the development of chocolate character [36]. The analysis of organic acids revealed that citric acids (from 1.08 to 11.87 mg/g), lactic acids (from 0.30 to 6.36 mg/g) and acetic acids (from 0.74 to 4.14 mg/g) were the main organic acids, while the concentrations of succinic acids (from 0.31 to 0.75 mg/g) and oxalic acids (from 0.40 to 0.49 mg/g) were lower.

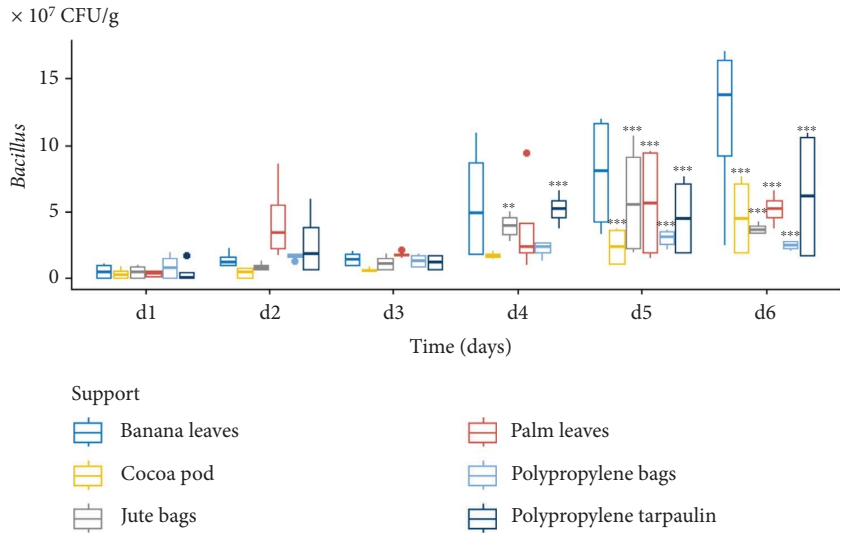

FIGURE 7: *Bacillus* growth during fermentation of cocoa beans on different supports. **Highly significant difference and ***very highly significant difference in comparison to the reference support (banana leaves).

The concentration of citric acid was higher at the start of fermentation from Day 1 to Day 3, between 8.39 and 11.87 mg/g. This high concentration of citric acid on all supports at the start of fermentation may be explained by the initial composition of the bean mucilage, rich in citric acid before the cocoa pods are opened [44, 45]. These results are close to those of Ho et al. [46] in unfermented cocoa beans with 13–14 mg/g citric acid.

The concentration of citric acid drastically decreases over the fermentation period. In fact, as explained by Sarbu and Csutak [47]; the decrease in citric acid concentration is associated with the activity of microorganisms such as LAB and yeasts. The latter use citric acid as a source of carbon and energy, metabolising it to produce lactic acid and other compounds. The greatest decrease is observed for cocoa beans fermented on jute bags, with a decrease from 9.30 mg/g on Day 1 to 1.08 mg/g on Day 6. Those fermented on banana leaves on the other hand decreased from 9.91 mg/g on Day 1 to 3.73 mg/g on Day 6. This difference can be attributed to the configuration and layout of the supports during fermentation, that is, jute bags are more perforated than banana leaves. In addition, before fermentation, these bags are placed on wooden pallets, at a certain height above the ground, allowing the juice containing these acids to run off more quickly.

Lactic acid possesses an opposite behaviour to that of citric acid. Its initial concentration was lower and increased after 4 days of fermentation on all support. The maximum concentration was observed on the polypropylene tarpaulin (6.36 ± 0.64 mg/g) on Day 4 and was equivalent to approximately twice that obtained on the banana leaves (3.85 ± 0.16 mg/g). This greater increase in concentration on polypropylene tarpaulins is attributed to their watertightness, which favours anaerobic conditions and in turn leads to optimal growth of the LAB responsible for converting ethanol into lactic acid. In fact, the activity of lactic bacteria on polypropylene tarpaulins was positively correlated ($r = 0.26$; $p = 0.012$) with the production of lactic acid, in contrast to other fermentation support. This correlation indicates that greater lactic acid production is associated with increased LAB activity.

3.4. Analysis of the Obtained Final Product Following Fermentation and Drying. A comprehensive evaluation of the physicochemical characteristics and market value of the final product from each fermentation support was conducted. A PCA was performed and summarised in two biplots: one displaying physicochemical characteristics (Figure 8(a)), and the other showing market value parameters (Figure 8(b)).

The first two axes (PC1 and PC2) account for 52.8% of the total variability. Results showed three distinct groupings: cocoa beans from cocoa pods (strongly negatively correlated with PC1), beans fermented on palm leaves and jute bags (negatively correlated with PC2) and beans fermented on banana leaves and polypropylene tarpaulins (positively correlated with PC1). A fourth, less distinct group comprised beans from polypropylene bags, dispersed along PC2 but positively correlated with PC1. This PCA individual plot has been elaborated with the data presented in Table 1.

The trends observed in Figure 8 can be interpreted based on these results. Concerning the first group observed in Figure 8, composed of banana leaves (reference) and polypropylene tarpaulin, it can be seen that these had lower ash and graining compared to cocoa pods but higher than beans fermented on jute bags and palm leaves (second group). Although cocoa pods had higher graining, all values met international standards according to which cocoa beans are considered constant when their graining is less than 100 beans/100 g [12]. Additionally, beans fermented on polypropylene tarpaulins and banana leaves had the highest concentrations of C18:2 (3.40 ± 0.18 and 3.25 ± 0.14%), an unsaturated fatty acid known for its cardiovascular benefits [48]. These beans also had similar alkaloid content, with theobromine at 13.71 ± 1.46 and 13.28 ± 1.20 mg/g, and caffeine at 2.89 ± 1.32 and 2.45 ± 0.71 mg/g.

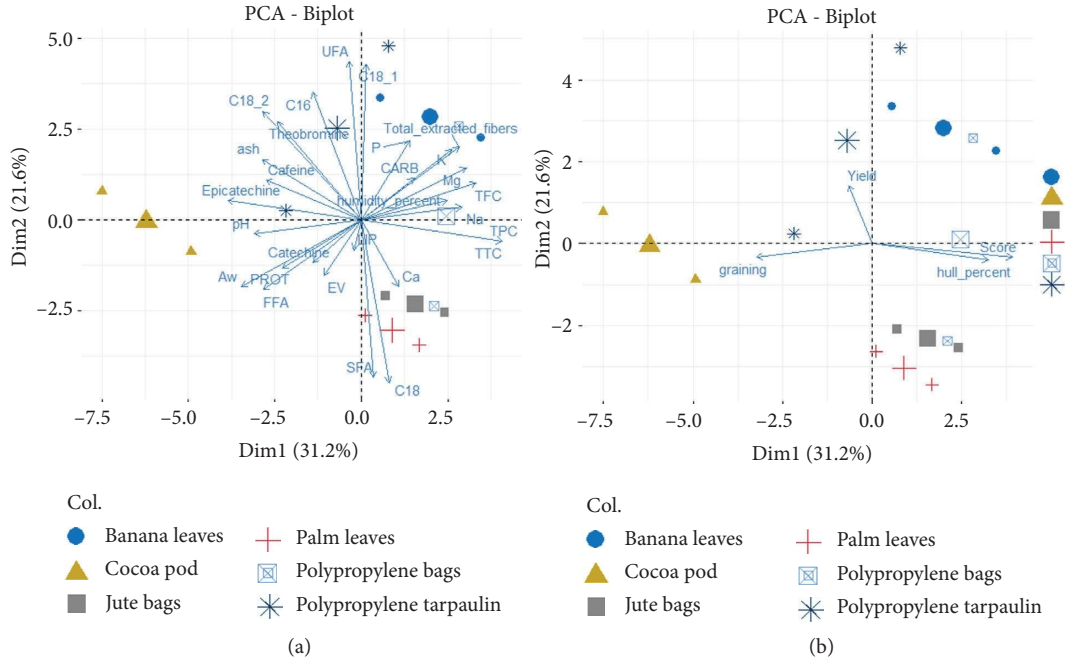

Figure 8: Principal component analysis biplots individual plot representing the cocoa beans final products based on their (a) physicochemical and (b) market characteristics. EV = energetic value, CARB = total carbohydrates, LIP = total lipids, PROT = total proteins, FFA = free fatty acids, UFA = unsaturated fatty acids, SFA = saturated fatty acids, TFC = total flavonoid content, TPC = total polyphenolic content, TTC = total tannin content.

From the PCA in Figure 8(a), polypropylene bags resemble the characteristics of banana leaves, particularly in total fibre content (i.e., total extracted fibres of, respectively, 78.49 ± 3.96% and 74.96 ± 0.18%) and carbohydrates (29.17 ± 2.81% and 31.53 ± 11.5%, respectively). However, polypropylene bags have higher levels of total flavonoids (156.29 ± 5.84 mgEQu/g), polyphenols (72.29 ± 0.37 mgEAG/g) and tannins (72.22 ± 0.38 mgEAcTa/g). These higher levels of phenolic compounds, likely due to better sealing (retained over 8% humidity during drying), are beneficial for the prevention of neurodegenerative and cardiovascular diseases, metabolic disorders and cancers [49]. Although some beneficial properties are observed, this greater content in phenolics compounds could lead to a more astringent and bitter final product.

Beans fermented in polypropylene bags as well as those fermented on polypropylene tarpaulins had a humidity content of, respectively, 8.90 ± 0.18% and 8.35 ± 0.69%. To avoid mould contamination, moisture content should not exceed 8% [7]. The higher humidity in polypropylene bags and polypropylene tarpaulin is likely due to their inert nature, reducing juice flow during fermentation and slowing drying.

Beans fermented on jute bags and palm leaves showed similar total saturated fatty acids (60.21 ± 0.52% and 60.20 ± 0.17%) and C18:0 levels (32.77 ± 0.56% and 33.16 ± 0.01%). The higher saturated fatty acids are attributed to lower fermentation temperatures (below 50°C), which limit lipid hydrolysis into unsaturated fatty acids. Indeed, according to Kagambèga et al. [50], lipid hydrolysis reactions are accelerated by ultraviolet radiation and high temperatures, which could release unsaturated fatty acids.

The last group concerns cocoa pods, negatively correlated with PC2, characterised by beans with a higher ash concentration (3.148 ± 0.11%), water activity (Aw) of 0.44 ± 0.01, an energetic value of 653 ± 17.7 kcal/100 g and a pH of 6.31 ± 0.07. According to the Codex International Cocoa Standard [51], the pH maximum threshold for fermented and dried beans is 5.5. The higher pH in cocoa pods results from lower fermentation temperatures (below 40°C), which delay microbial activity and can lead to mould proliferation [52].

Beans fermented in cocoa pods had a lower Cut test score of 835.83 ± 3.54, the lowest observed in the experiments, due to higher rates of sprouted beans (5.00 ± 1.80%), purple beans (8.17 ± 2.08%) and mouldy beans (3.00 ± 1.00%). According to international standards (ISO 2451), beans exceeding 3% for sprouted, slate and defective beans, and 8% for purple beans, are classified as Grade II. The high mould content, despite low water activity (0.48 ± 0.01%), suggests pre-drying mould contamination linked to high germination rates during fermentation in the pod after the fourth day. All other fermentation supports had Cut test scores above 950, comparable to values obtained by Hii et al. [53], indicating good fermentation practices. Beans from cocoa pods had higher concentrations of phenolic compounds like epicatechin (4.02 ± 2.2 mg/g) and catechin (0.47 ± 0.67 mg/g), as well as increased protein (15.79 ± 1.49%) and FFA (1.58 ± 0.13%). The increased concentration of epicatechin and catechin seems to be linked to the high proportion of purple beans (8.83 ± 3.19%) observed after the cut test for fermented beans in the cocoa pod. Previous studies by Davrieux et al. [54] have shown that the colour of the

Table 1: Physicochemical characteristics and market value of cocoa beans final product.

	Banana leaves	Cocoa pod	Jute bags	Palm leaves	Polypropylene bags	Polypropylene tarpaulin
Physicochemical characteristics						
Humidity (% FM)	7.65 ± 0.30[a]	6.65 ± 0.21	7.917 ± 0.44[a]	7.50 ± 0.47[a]	8.90 ± 0.18	8.35 ± 0.69
Aw	0.44 ± 0.01[a]	0.47 ± 0.02[a]	0.45 ± 0.01[a]	0.45 ± 0.03[a]	0.45 ± 0.00[a]	0.45 ± 0.02[a]
pH	5.29 ± 0.30[a]	6.31 ± 0.07	5.42 ± 0.20[a]	5.42 ± 0.37[a]	5.46 ± 0.03[a]	5.16 ± 0.36[a]
Ash (% DM)	2.89 ± 0.36[a]	3.15 ± 0.11[a]	2.47 ± 0.35	2.53 ± 0.18	2.75 ± 0.16[a]	2.60 ± 0.04[a]
Total lipid (% DM)	50.82 ± 11.05[a]	50.28 ± 7.38[a]	47.75 ± 0.77[a]	49.75 ± 2.90[a]	53.18 ± 1.96[a]	47.10 ± 6.65[a]
Total protein (% DM)	14.65 ± 0.04[a]	15.79 ± 1.49	14.38 ± 0.18[a]	14.78 ± 0.48[a]	14.50 ± 0.17[a]	14.25 ± 0.69[a]
Total extracted fibres (% DM)	74.96 ± 0.18[a]	67.11 ± 7.09	72.73 ± 5.25[a]	65.82 ± 0.24	78.49 ± 3.96[a]	70.30 ± 11.06[a]
Total carbohydrates (% DM)	31.53 ± 11.5[a]	27.91 ± 4.60[a]	32.41 ± 3.88[a]	32.91 ± 3.29[a]	29.17 ± 2.81[a]	35.99 ± 7.27[a]
Energetic value (kcal/100 g)	640.2 ± 50.6[a]	653 ± 17.7[a]	643.5 ± 15.80[a]	638.5 ± 14.90[a]	656.67 ± 11.7[a]	624.9 ± 33.5[a]
FFA (mg KOH/g)	1.14 ± 0.17[a]	1.58 ± 0.13[a]	1.44 ± 0.46[a]	1.35 ± 0.33[a]	1.30 ± 0.26[a]	1.30 ± 0.39[a]
TPC (mgEAG/g)	69.69 ± 7.2[a]	57.59 ± 4.12	67.14 ± 2.46[a]	70.28 ± 0.48[a]	72.288 ± 0.37[a]	62.89 ± 3.54[a]
TFC (mgEQu/g)	101.39 ± 9.43[a]	40.79 ± 3.28	63.34 ± 2.69[a]	100.41 ± 0.33[a]	156.29 ± 5.84	89 ± 34.5[a]
TTC (mgEAcTa/g)	69.62 ± 7.22[a]	57.51 ± 4.11[a]	67.06 ± 2.48[a]	70.20 ± 0.49[a]	72.22 ± 0.38[a]	62.82 ± 3.53[a]
Theobromine (mg/g)	13.28 ± 1.20[a]	14.09 ± 1.67[a]	9.87 ± 4.28	9.48 ± 1.48	12.42 ± 0.64[a]	13.71 ± 1.46[a]
Caffeine (mg/g)	2.45 ± 0.71[a]	2.72 ± 0.65[a]	1.86 ± 0.78[a]	2.05 ± 1.12[a]	1.94 ± 0.07[a]	2.89 ± 1.32[a]
Catechin (mg/g)	0.41 ± 0.58[a]	0.47 ± 0.67[a]	0.00 ± 0.00[a]	1.38 ± 0.16	0.00 ± 0.00[a]	0.49 ± 0.69[a]
Epicatechin (mg/g)	1.59 ± 0.06[a]	4.02 ± 2.20	1.78 ± 1.83[a]	1.12 ± 0.81[a]	1.05 ± 0.07[a]	2.49 ± 1.40[a]
C16:0 (% TF)	28.35 ± 0.33[a]	28.44 ± 0.41[a]	27.43 ± 0.04[a]	27.03 ± 0.17[a]	27.08 ± 0.43[a]	27.90 ± 1.61[a]
C18:0 (% TF)	30.29 ± 1.27[a]	29.08 ± 1.25[a]	32.77 ± 0.56[a]	33.16 ± 0.01	31.40 ± 0.04[a]	29.00 ± 2.77[a]
SFA (% TF)	58.64 ± 0.94[a]	57.53 ± 0.84[a]	60.21 ± 0.52[a]	60.20 ± 0.17[a]	58.48 ± 0.48[a]	56.90 ± 1.16[a]
C18:1 (% TF)	38.1 ± 0.80[a]	39.47 ± 0.54[a]	36.97 ± 0.50[a]	36.97 ± 0.16[a]	38.71 ± 0.68[a]	39.69 ± 0.97[a]
C18:2 (% TF)	3.25 ± 0.14[a]	2.99 ± 0.29[a]	2.80 ± 0.0	2.82 ± 0.01[a]	2.79 ± 0.20	3.40 ± 0.18[a]
UFA (% TF)	41.35 ± 0.94[a]	42.46 ± 0.84[a]	39.78 ± 0.52[a]	39.79 ± 0.17[a]	41.51 ± 0.48[a]	43.09 ± 1.16[a]
P (% MB)	0.11 ± 0.00[a]	0.15 ± 0.00	0.13 ± 0.00	0.10 ± 0.00	0.11 ± 0.00[a]	0.12 ± 0.00
Ca (% MB)	0.12 ± 0.00[a]	0.12 ± 0.00[a]	0.86 ± 0.00	0.13 ± 0.00	0.14 ± 0.00	0.13 ± 0.00
Mg (% MB)	0.24 ± 0.00[a]	0.36 ± 0.00	0.29 ± 0.00	0.27 ± 0.00	0.3 ± 0.00	0.26 ± 0.00
K (% MB)	0.68 ± 0.00[a]	1.02 ± 0.00	0.76 ± 0.00	0.72 ± 0.00	0.86 ± 0.00	0.72 ± 0.00
Na (% MB)	0.04 ± 0.00[a]	0.05 ± 0.00	0.05 ± 0.00	0.04 ± 0.00[a]	0.05 ± 0.00	0.04 ± 0.00[a]
Market value						
Graining (beans per 100 g)	87.83 ± 10.14[a]	94.17 ± 6.36[a]	86.67 ± 5.66[a]	87 ± 14.60[a]	88 ± 10.84[a]	88 ± 7.07[a]
Shell (% DM)	11.71 ± 0.19[a]	10.27 ± 0.45	11.79 ± 0.47[a]	12.05 ± 1.29[a]	12.06 ± 0.45[a]	12.10 ± 0.99[a]
Yield (%)	54.1 ± 2.97[a]	58 ± 2.83	52 ± 0.00[a]	58 ± 2.83	59.6 ± 0.56	62.00 ± 2.83
Cut test results						
Purple beans (% DM)	4.83 ± 0.28[a]	8.17 ± 2.08	1.66 ± 1.52[a]	2.50 ± 1.00[a]	2.83 ± 0.28[a]	3.67 ± 2.36[a]
Light-coloured beans (% DM)	0.33 ± 0.57[a]	0.25 ± 0.25[a]	0.16 ± 0.28[a]	0.00 ± 0.00[a]	0.50 ± 0.00[a]	0.16 ± 0.28[a]
Slate (%)	0.00 ± 0.00[a]	0.83 ± 0.28	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
Sprouted (% DM)	0.33 ± 0.57[a]	5.00 ± 1.80	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.16 ± 0.28[a]
Mite (% DM)	0.00 ± 0.00[a]	1.58 ± 0.87[a]	0.33 ± 0.57[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.33 ± 0.28[a]
Mouldy (% DM)	0.00 ± 0.00[a]	3.00 ± 1.00[a]	0.83 ± 0.57[a]	0.16 ± 0.28[a]	0.50 ± 0.50[a]	0.00 ± 0.00[a]
Cut test score (/1000)	976.67 ± 2.36[a]	835.83 ± 3.54	980 ± 11.79[a]	985.83 ± 5.89[a]	974.17 ± 10.61[a]	973.33 ± 7.07[a]

Note: Means which do not possess any superscript are significantly different from the mean obtained for the reference support (i.e., banana leaves) when performing Dunnet's test. For each component, the means ± standard deviations are presented.

Abbreviations: DM = dry matter, FFA = free fatty acids, FM = fresh matter, TF = total fat, TFC = total flavonoid content, TPC = total polyphenol content, TTC = total tannin content, SFA = saturated fatty acids, UFA = unsaturated fatty acids.

cotyledon changes from purple to brown during fermentation, due to the high oxidation and polymerisation of polyphenols within the molecular structures. In addition, they showed a higher concentration of major minerals such as phosphorus (0.15 ± 0.00% MB), potassium (1.02 ± 0.00% MB) and magnesium (0.36 ± 0.00% MB) compared with the other groups, attributable to the higher ash content observed previously.

4. Conclusion

This study highlights the significant impact of fermentation supports on the process and final product characteristics of cocoa beans. Different supports lead to variations in the physicochemical and microbiological properties of the beans, ultimately influencing their metabolite composition postdrying. Beans fermented in cocoa pods exhibited a lower pH and fermentation temperature (< 40°C) and delayed microbial growth, particularly for yeasts and AAB. In terms of final bean quality, cocoa pod fermentation resulted in higher ash and mineral content but also higher proportions of mouldy and sprouted beans, leading to a low cut-test score. Additionally, the higher final product pH from cocoa pod fermentation poses risks for conservation due to increased susceptibility to mould proliferation.

Mould proliferation is also a concern with beans fermented in polypropylene bags, which showed the highest humidity content. Furthermore, these beans had elevated phenolic compound concentrations, potentially increasing bitterness and astringency. Fermentation in cocoa pods and polypropylene bags is therefore suboptimal. Conversely, fermentation on polypropylene tarpaulins, palm leaves and jute bags produced beans with characteristics similar to those fermented on banana leaves, the current quality benchmark. This was evident both during the fermentation process and in the physicochemical and market characteristics of the final product.

These findings suggest that polypropylene tarpaulins, palm leaves and jute bags are viable alternatives to banana leaves for ensuring optimal cocoa bean quality in Côte d'Ivoire. Additionally, the reusability of polypropylene tarpaulins and jute bags, along with the cost-effectiveness of palm leaves, offers practical solutions to address the scarcity of banana leaves. However, further research is needed to evaluate the impact of these fermentation supports on the aroma and flavour of the final product. A deeper understanding of these relationships could enable optimisation of the fermentation process to produce high-quality cocoa beans with distinct aromatic profiles. These results suggest that, despite the shortage in banana leaves as fermentation support, cocoa producers could benefit from support to optimise the fermentation process, thereby guaranteeing better quality beans and a more competitive price on the market.

On a larger scale, these findings provide valuable insights for cocoa producers worldwide and set a precedent for identifying local alternatives to scarce resources commonly used in food production in Southern countries.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

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