

Impact of new fermentation supports on the physico-chemical quality of Mercedes cocoa beans (*Theobroma cacao* L.1753) grown at Soubré in the NAWA region.

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ABSTRACT

Objective: The cocoa bean fermentation process is a crucial step for obtaining quality beans. This study aims to evaluate the impact of six fermentation supports: banana leaves (control), palm leaves, cocoa pods, tarpaulins, polypropylene bags, and jute bags on the quality of Mercedes variety cocoa beans in Côte d'Ivoire.

Methodology and Results: Fermentation lasted six days on six different supports at the same producer's site. The analyses monitored temperature and pH variations. The results show that, except for beans fermented in cocoa pods, those on banana leaves, palm leaves, tarpaulins, polypropylene bags, and jute bags maintained a fermentation temperature above 45°C and an acidic pH between 2 and 6. After drying, beans fermented in cocoa pods exhibited higher proportions of mouldy and germinated beans, exceeding 3%, with a high pH of 6.23±0.04.

Conclusion and Application of Results: Monitoring the fermentation allowed the evaluation of temperature and pH conditions for each support. After drying, principal component analysis (PCA) and hierarchical clustering analysis (HCA) performed with R and Minitab 18 software, integrating all physicochemical parameters, reveal that beans fermented on palm leaves and jute bags have similar compositions to those fermented on banana leaves. Additionally, these supports are available, accessible, economical, and less labour-intensive. It follows that palm leaves and jute bags could be the best alternatives to banana leaves for maintaining cocoa bean quality in Côte d'Ivoire.

Keywords: Cocoa, Fermentation, Temperature, Support, pH, Quality.

INTRODUCTION

The cocoa tree (*Theobroma cacao* L.) was introduced to Côte d'Ivoire in the late 19th century, specifically in its Eastern region (Bastide, 2007; Kouakou *et al.*, 2013). Despite challenging beginnings, cocoa cultivation, which has become emblematic of

Côte d'Ivoire, quickly thrived, benefiting from the availability of fertile land in the forested area as well as a significant influx of labour from both within and outside the country (UN-REDD, 2016; Koua *et al.*, 2018). With an annual production of 2,230

million tonnes, representing 42.22% of global production during the 2022-2023 campaign, Côte d'Ivoire remains the world's leading cocoa producer (ICCO, 2023). Three main varieties, namely Criollo, Forastrero, and Trinitario, are traditionally cultivated globally (Bartley, 2005). Recently, scientific advancements have allowed for the creation of new clones and hybrids through genetic crosses, including hybrids such as Catongo, SNK.10, UPA 134, LCS95, as well as a recently developed variety named "Mercedes." This latter variety was introduced in response to the observed aging phenomenon in older cocoa varieties in Côte d'Ivoire (Djocgoue *et al.*, 2007; Karim *et al.*, 2020). However, the technological transformation of cocoa into commercial beans is lengthy and requires a primary post-harvest handling process (Thompson *et al.*, 2001); with fermentation being, the most critical stage (Beckett, 2009). One of the problems often faced by Ivorian producers is the significant presence of slaty beans and mouldy beans, attributable to improper fermentation and drying processes (Kouakou *et al.*, 2013). Banana trees, known for their leaves offering the best commercial,

microbiological, and organoleptic qualities of cocoa beans, are commonly grown in association with cocoa trees (Barel, 2013). However, in recent decades, banana trees have encountered limitations. Climate variations, soil degradation, and the shading progression of cocoa trees after about five years of cultivation have led to a gradual decline in banana trees (*Musa* sp). As an alternative, producers are turning to the use of plastic tarps and wooden boxes. Unfortunately, research has shown that beans from these fermentation substrates were categorized as utilizing methods with low uniformity, with the presence of defective beans and a deterioration in commercial, microbiological, and organoleptic quality (Guehi *et al.*, 2010). Thus, producers, always seeking substrates capable of ensuring better cocoa bean quality, are now turning to fermentation substrates without evaluating the resulting bean quality. This research work was designed to evaluate the commercial quality of Mercedes variety cocoa beans in the Soubré region for each fermentation substrate, in order to select the best ones in comparison to banana leaves.

MATERIAL AND METHODS

Material: The biological material consists of cocoa beans during fermentation (Figure 1a) and fermented and dried beans (Figure 1b)

from different fermentation processes of cocoa beans of the hybrid variety proposed by CNRA/Divo "Mercedes" for the Soubré area.



Figure 1: Types of samples collected from each fermentation substrate
a: beans in fermentation b: fermented and dried beans

Post-harvest procedures: The post-harvest handling process leading to the production of fermented and dried cocoa beans was carried out according to the sequential steps: pod breaking-fermentation-drying.

Husking and Fermentation: Pod breaking was carried out using knives three days after harvest with 4000 pods, mainly under rural conditions using knives and clubs. Fermentation trials of 250 kg of fresh beans

or 1000 cocoa pods without separating the beans from the placenta were conducted at the same producer's site using six substrates: banana leaves (Control); palm leaves; cocoa pods; polypropylene tarpaulin; polypropylene bags; and jute bags. The industrial protocol was followed (144 hours or 6 days of fermentation with stirring intervals of 48 hours, 96 hours, and 120 hours), except for cocoa pods.



Figure 2: Different fermentation supports

a: Banana leaves (control); b: Palm leaves; c: Cocoa pods; d: Polypropylene tarp; e: Polypropylene bags; f: Jute bags.

Drying: The drying of all beans from various fermentation substrates was carried out in a thin layer on polypropylene tarps, commonly used by buyers or cooperative agents to store dried beans before transporting them to the factory. The daily drying duration was 7 hours, taking place from 9:00 a.m. to 4:00 p.m.

Sampling: After drying, a quantity of 2 kg of dried beans was collected from each fermentation substrate for the various analyses. The samples were then transported to the agro-valorization laboratory at Jean Lorougnon Guédé University (Côte d'Ivoire) for further analysis. These dried beans were shelled, finely ground (using an IKA A11 basic mill; Germany), and placed in 100 g

containers, then stored at a temperature of -80°C for further analysis.

Determination of fermentation pH and temperature: Temperature and pH were monitored during the six days of fermentation using a thermometer and a pH meter, early in the morning (between 6 a.m. and 8 a.m.). The thermometer was powered on for 15 minutes beforehand. Then, the probe was immersed into the mass to a depth of 20 cm, and the value was recorded after a 2-minute stabilization period. pH was measured according to ICCO Method No. 9 (1963) using a pH meter equipped with a combined glass electrode (Hanna Instrument, Romania) (Hii *et al.*, 2009).

Physical and Chemical Characteristics of the Final Product: The moisture content was assessed by measuring 500 grams of beans from each fermentation substrate using a mini GAC (Dickey John multigrain), following the method of Lainé (2001). The water activity of the samples was measured using an aw-meter (AQUA LAB). The pH was determined according to the AOAC method (2005). The ash content (total mineral salts) was evaluated according to the AOAC method (1995). The grading of cocoa beans after drying was determined by counting the number of healthy and normal beans per 100 grams weighed, following ISO 1114 (1977) standard. The percentage of husk was determined according to AOAC methods 968.10 and 970.23 (AOAC, 1972). The level and degree of fermentation (cut test) were assessed according to the method of Efraim *et al.* (2010). Additionally, the cut test score was calculated using the equation by Hii *et al.*

(2011): Cut test score = (10 x % brown) + (5 x % purple/brown) + (0 x % purple and slaty). 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] x 100. Three trials were conducted per sample. The evaluation of the fermentation index was carried out according to the method of Gourieva K.B & Tserévitinov OB (1979), revised by Misnawi (2008).

Statistical Analysis: The MINITAB.18 software was used for variance analysis (DUNNETT) and Hierarchical Ascending Classification (HAC) to compare the data of fermented beans on different supports with those of the control support. Principal component analyses (PCA) were performed in addition to hierarchical cluster analysis (HCA) using the R software (version 4.0.2, R Development Core Team, Boston, USA).

RESULTS

Influence of different substrates on fermentation temperature and pH: The fermentation temperature and pH had the same profile across all fermentation substrates with a variation from 26 to 46 °C and from 2 to 6 during the six days of fermentation. However, before mixing, the temperature gradually increased and reached the highest values in palm leaves (38.60 ± 0.69 °C) and polypropylene sheets (37.40 ± 1.51 °C), while it was, lower in cocoa pods (31.83 ± 0.76 °C). The pH remained acidic

with values below 3 across all substrates. After mixing, a more pronounced increase in temperature and pH was also observed, reaching its maximum with fermenting beans on banana leaves (control) at 46.73 ± 0.25 °C and 6.38 ± 0.03, followed by those on palm leaves (46.67 ± 1.52 °C; 6.21 ± 0.01) and polypropylene sheets (46.50 ± 0.50 °C; 4.81 ± 0.15) on day 4 of fermentation. The temperature remained below 38°C with a pH between 3 and 4 during fermentation.

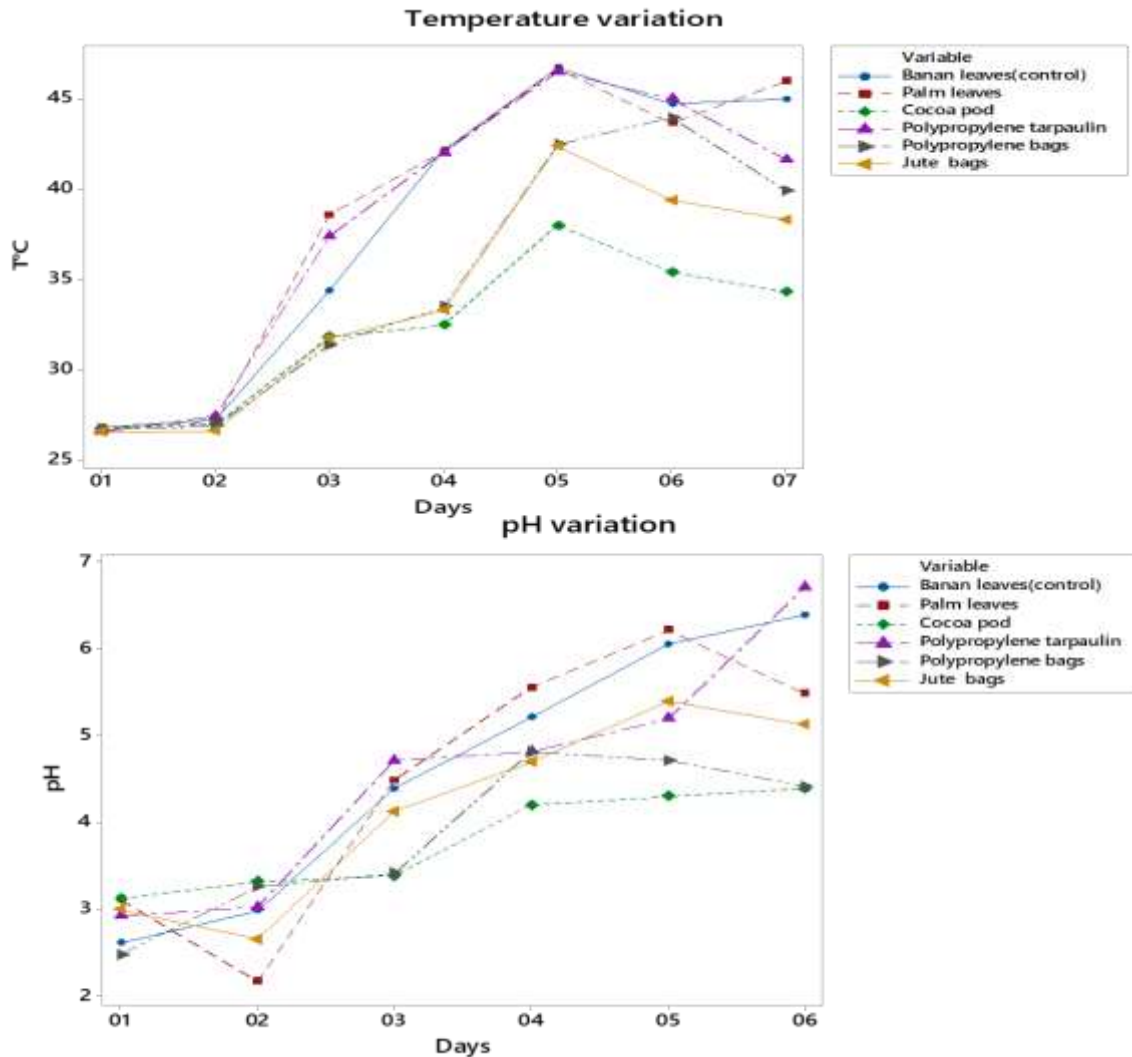


Figure 3: Variation of temperature and pH during fermentation

Physico-chemical composition of fermented and dried beans: The observed variations in temperature and pH parameters caused significant changes, both external and internal, in the final cocoa beans. Table 1 reveals that among all the fermentation substrates studied, the pH of the dried beans varied from 4 to 6, with significant differences ($P < 0.05$). Overall, all beans recorded a pH below the threshold value of 5.5, except for those fermented in cocoa pods (6.23 ± 0.04). However, variance analyses (Dunnett, $\alpha=0.05$) showed that the beans fermented on palm leaves (5.09 ± 0.01) had a pH closer to that of the control (5.08 ± 0.01),

with no significant difference ($P = 0.0001$). Regarding moisture content, it showed variable values from one substrate to another, ranging from 6.5 to 8.85% with significant differences ($P < 0.05$). The beans fermented on polypropylene bags had a higher moisture content in the final beans, 9.1 ± 0.26 , significantly higher than those on the control substrate (7.7 ± 0.36). Furthermore, the variation in fermentation substrates did not cause a significant difference in the final beans' water activity (0.43 ± 0.00 and 0.59 ± 0.13), ash content (2.46 to 2.84), or the bean count (75 to 80 beans per 100 grams) compared to the beans fermented on banana

leaves (control) according to Dunnett's test at $\alpha=0.05$. The shell percentage ranged from 9 to 12% with a significant difference (Dunnett's test; $\alpha = 0.05$). The beans fermented on polypropylene sheets recorded a higher shell percentage, $12.17 \pm 4.79\%$, compared to those in cocoa pods, which were

lower ($9.76 \pm 6.36\%$). However, the highest values were recorded with the beans fermented on polypropylene sheets ($12.17 \pm 4.79\%$). Finally, the yield of the final product ranged between 52% and 60%. The beans fermented on banana leaves showed the highest yield, at 60%.

Table 1: Physico-chemical composition of fermented and dried beans

Supports	pH	% water content	water activity	% Ash	seed	% hull	yield
Banana leaves (control)	5.08 ± 0.01 ^a	7.7 ± 0.36 ^a	0.52 ± 0.07 ^a	2.84 ± 0.03 ^a	75.33 ± 1.15 ^a	11.22 ± 5.08 ^a	60
palm leaves	5.09 ± 0.01 ^a	7.11 ± 0.81 ^a	0.56 ± 0.10 ^a	2.67 ± 0.2 ^a	76.00 ± 0.00 ^a	11.09 ± 5.09 ^a	52
cocoa pod	6.23 ± 0.04	6.46 ± 0.23	0.49 ± 0.09 ^a	2.56 ± 0.26 ^a	77.00 ± 2.64 ^a	9.76 ± 6.36	52
polyp tarpaulin	4.79 ± 0.06	7.83 ± 0.66 ^a	0.59 ± 0.13 ^a	2.76 ± 0.14 ^a	79.00 ± 20.00 ^a	12.17 ± 4.79 ^a	56
Polyp bags	5 ± 0.01	9.1 ± 0.26	0.49 ± 0.05 ^a	2.51 ± 0.09 ^a	80.00 ± 4.58 ^a	11.93 ± 5.0 ^a	56
Jute bags	5.48 ± 0.04	7.9 ± 0.4 ^a	0.49 ± 0.04 ^a	2.46 ± 0.58 ^a	76.66 ± 2.51 ^a	11.57 ± 5.48 ^a	56

For each component, the means ± standard deviations. Means not labelled with the letter "a" are significantly different from the mean of the control level at a threshold of $p \leq 0.05$ according to the DUNNETT test.

Degree of fermentation and level of bean degradation (Cut-test) as a function of fermentation substrate: The results of the parameters evaluated during the cutting test revealed significant differences ($P < 0.05$) (see Table 2). Overall, beans fermented inside cocoa pods showed the lowest proportion of fully brown beans, at $85.33 \pm 0.57\%$, compared to a proportion ranging between 93% and 98% for other substrates,

with significant differences compared to the control substrate (Dunnett test; $\alpha = 0.05$). Additionally, these beans recorded a higher percentage of violet beans, reaching $6.67 \pm 0.57\%$, with significant differences compared to beans showing signs of germination and mould exceeding 3%. The cutting test score was also lower for these beans, at 886.667 ± 7.6 , with a fermentation index below 1.

Table 2: Analysis of the cut-test and fermentation index

Supports	Moisture content	purple	Slate	sprouted	Sprouted	Mite	Mouldy	broad beans	IF	Score Cut test	Grade
Banana leaves (control)	93,33 ^a ±3,05	5,33 ±1,52 ^a	0,00 ±0,00 ^a	0,00 ±0,00 ^a	0,00 ±0,00 ^a	0,00 ±0,00 ^a	0,00 ±0,00	0,00 ±0,00 ^a	1,42 ±0,04 ^a	960,00 ± 22,91 ^a	I
palm leaves	95,00 ±1,00 ^a	4,66 ±0,57 ^a	0,00 ±0,00 ^a	0,00 ±0,00 ^a	0,00 ±0,00 ^a	0,00 ±0,00 ^a	0,33 ±0,57 ^a	0,00 ±0,00 ^a	1,60 ±0,14 ^a	973,33 ± 12,58 ^a	I
cocoa pod	85,33 ±0,57	6,67 ±0,57 ^a	0,00 ±0,00 ^a	0,33 ±0,57 ^a	3,00 ±1,00	1,00 ±1,00 ^a	3,67 ±0,57	0,00 ±0,00 ^a	0,93 ±0,06	886,667 ± 7,63	II
polyp tarpaulin	94,67 ±0,57 ^a	5,33 ±0,57 ^a	0,00 ±0,00 ^a	0,00 ±0,00 ^a	0,00 ±0,00 ^a	0,00 ±0,00 ^a	0,00 ±0,00 ^a	0,00 ±0,00 ^a	1,08 ±0,03	973,33 ± 2,88 ^a	I
Polyp bags	94,67 ±1,52	5,33 ±1,52 ^a	0,00 ±0,00 ^a	0,00 ±0,00 ^a	0,00 ±0,00 ^a	0,00 ±0,00 ^a	0,00 ±0,00 ^a	0,00 ±0,00 ^a	1,08 ±0,19	973,33 ± 7,63 ^a	I
Jute bags	96,00 ±2,64 ^a	4 ±2,64 ^a	0,00 ±0,00 ^a	0,00 ±0,00 ^a	0,00 ±0,00 ^a	0,00 ±0,00 ^a	0,00 ±0,00 ^a	0,00 ±0,00 ^a	1,11 ±0,24	980,00 ±13,23 ^a	I

For each component, the means ± standard deviations. Means not labelled with the letter "a" are significantly different from the mean of the control level at a threshold of $p \leq 0.05$ according to the DUNNETT test.

Multivariate Analysis: Observing the 'scree plot' graph reveals that three components can be extracted for principal component analysis. In fact, 90.12% of the information contained in the physico-chemical data is retained by the first three components (Figure 4a). Figure 4b presents the results of the principal component analysis (PCA) applied to the physico-chemical characteristics of the various samples. These results are represented based on the first two axes, PC1 and PC2, which together account for 79.7% of the total observed variability, thus providing a significant explanation of the PCA. Consequently, beans fermented in cocoa pods, jute bags, and polypropylene sheets are identified as being predominant in the formation of the first component, PC1 (62.9%). The second component, PC2 (16.8%), is mainly influenced by beans fermented on polypropylene sheets, palm leaves, and banana leaves (control). Four

groups were identified with the axes PC1 and PC2 in conjunction with the contribution of the variables listed in Table 1. The first group, exclusively consisting of beans fermented in cocoa pods, is negatively correlated with PC1 and is distinguished by higher values in pH, percentage of germinated, mouldy, slaty, mite-damaged, and purple beans. The second group includes beans fermented on palm leaves and control substrates, characterized by water activity, ash content, and the fermentation index. The third group is composed of beans fermented on polypropylene bags and jute bags, characterized by bean consistency (bean count), moisture content, and a higher percentage of brown beans. The last group consists of beans fermented on polypropylene sheets, which showed a higher proportion in yield, brown beans, cut test score, and ash content.

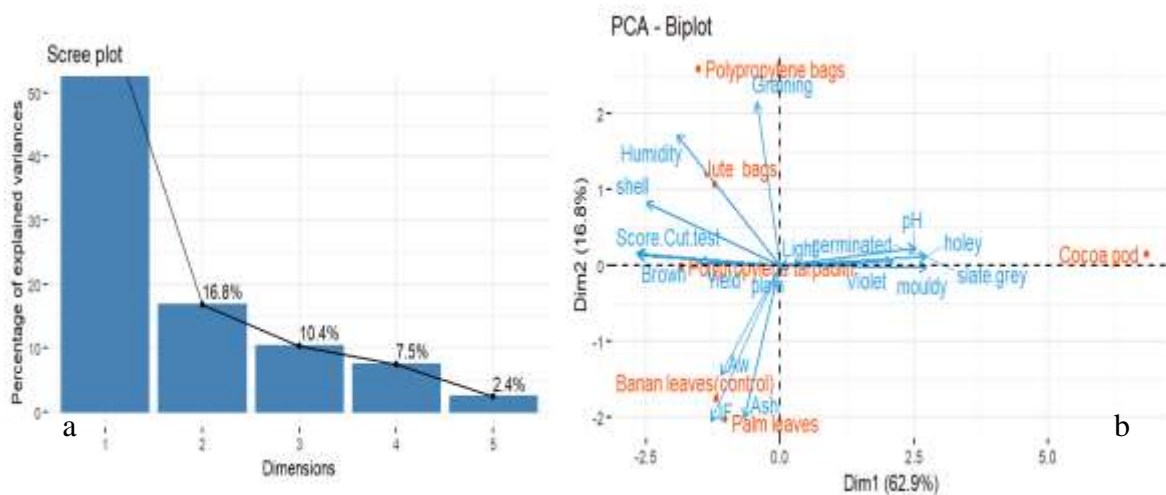


Figure 4: Applied Variance (a) and Contribution of Physicochemical Parameters (b) in the Representation of Dimensions

The dendrogram (CHA) encompassing all the studied parameters allowed for the differentiation of the various substrates and identified those closest to the control substrate (banana leaves). The results reveal that the beans fermented in cocoa pods exhibited a very different quality from those

of the control substrate. The beans fermented in the polypropylene sheet and polypropylene bags were more similar to each other. Finally, the beans fermented on jute bags and palm leaves showed qualities closer to those of the control substrate.

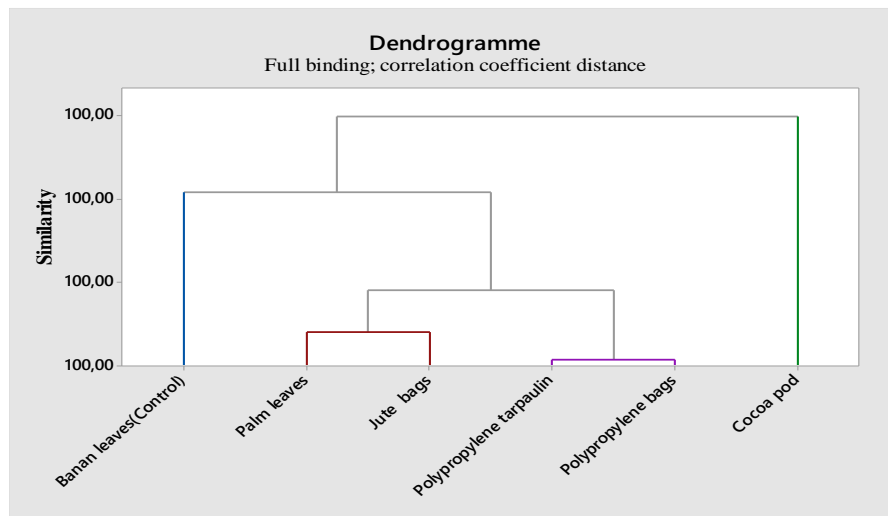


Figure 5: Dendrograms (HCA) Grouping All Studied Physicochemical Parameters

DISCUSSION

Measuring the temperature indicates the speed and degree of progress of the various chemical reactions necessary for fermentation. The results revealed a higher temperature, above 35°C, in palm leaves and polypropylene sheets before the first mixing, while the value recorded in the cocoa pod was below 32°C. The pH ranged between 2 and 4 with a dip observed in banana leaves (control) and palm leaves during the first two days of fermentation. This slight increase in temperature and pH before mixing observed in polypropylene sheets and palm leaves could be attributed to more favourable conditions for the alcoholic phase. According to Bankoff *et al.* (2013), this phase is accelerated by microbial activities followed by mucilage degradation, which acidifies the fermentation medium with a slight increase in temperature. However, the recorded pH variation is comparable to the values obtained by Sandra *et al.* (2007), who reported a pH profile between 3.2 and 4.75 in cocoa beans from various spontaneous fermentation substrates. After mixing, fermentation in palm leaves and polypropylene sheets maintained a temperature (23 to 48°C) and pH (2 to 6) profile close to that sought with banana leaves, consistent with previous

recommendations and comparable to the temperature values of 48°C observed by Aka *et al.* (2021). Indeed, according to studies conducted by De Vuyst and Weckx (2015), beans considered well-fermented are those whose maximum fermentation temperature is between 45 and 50°C. Additionally, Schwan & Wheals (2004) showed that a temperature above 45°C during fermentation is favourable for accelerating the death of the embryo and preventing bean germination. However, the lower temperature and pH levels observed in the cocoa pod could be due to the number of beans (40 beans) and the aerated space between the pods, which reduces microbial fermentation activity (Thompson *et al.*, 2001). The size of cocoa beans is a key factor in assessing their commercial quality. Quality standards require fewer than 100 beans per 100 grams, meaning each bean should weigh at least 1 gram (Guéhi *et al.*, 2007). Our results show that all analysed samples were uniform, with 75 to 80 beans per 100 grams, well within the standard of 100 beans and without significant differences (see Table 1). These results can be attributed to the Mercedes variety of cocoa, a hybrid, and the pre-sorting process of the beans. Our findings are consistent with those of Chen-Yen-Su

(2020), who also found 83 to 87 beans in his studies. The evaluated shell percentage ranged between 10 and 12%. The results for beans fermented in cocoa pods ($9.76 \pm 6.36\%$) were significantly lower ($P < 0.05$) compared to beans fermented on banana leaves ($11.22 \pm 5.08\%$), while those on polypropylene sheets ($12.17 \pm 4.79\%$) were higher and exceeded the standard (11%). This disparity could be attributed to the thick mucilaginous pulp adhering immediately to the testa before fermentation. According to the work of Afoakwa *et al.* (2008), it is recommended that the shell percentage of beans be as low as possible (10-12%), since it is removed during the processing stage. The beans fermented in polypropylene bags had a higher moisture content ($9.1 \pm 0.26\%$), significantly different from the beans fermented in banana leaves ($7.7 \pm 0.36\%$), and while the beans fermented in cocoa pods had a lower moisture content ($6.46 \pm 0.23\%$). This observation can be attributed to the structure of the polypropylene support, which potentially has a higher water retention capacity during the fermentation process. It is important to note that a moisture content of 8% constitutes a critical threshold beyond which fungal proliferation is favoured (Dharmaputra *et al.*, 1999). The pH of the beans after drying ranged between 4 and 6. Beans fermented inside cocoa pods had the highest pH, measuring 6.23 ± 0.04 , which significantly differs from that of beans fermented on banana leaves (5.08 ± 0.01) and other supports, indicating a lower level of fermentation. According to Beckett (2009), beans with a high pH (5.5 to 5.8) are generally considered unfermented or minimally fermented, while those with a lower pH (4.75 to 5.19) are classified as well-fermented. This difference could be attributed to temperature fluctuations during the fermentation process. As for the ash content, it ranged between 2.46 and 2.84% without showing a significant difference

compared to the beans fermented on the control support, which is comparable to the values obtained by Karim *et al.* (2020) for cocoa beans of the 'Mercedes' and 'Theobroma cacao' varieties from the Lôh-Djiboua (Divo) and Indénié-Djuablin (Abengourou) regions. According to these authors, the ash content of cocoa varies depending on the origin of the cocoa beans, genotype, and post-harvest processing methods. Nevertheless, these results demonstrate that these cocoa beans are excellent sources of minerals. The results of the visual evaluation showed that all samples met the defined quality criteria, with high percentages of brown beans indicating high-quality fermentation, ranging from 85% to 96%. According to the criteria established by Guehi *et al.* (2010), bean quality is assessed based on the proportion of fully superior brown beans, set at 60%. However, beans fermented inside cocoa pods exhibited higher rates of mouldy, germinated, and insect-damaged beans, exceeding 3%. This was associated with a lower fermentation index (835.83 ± 23.11), attributable to a higher proportion of germinated beans during fermentation. Additionally, these beans displayed a fermentation index below 1, with a score of 0.93 ± 0.06 , significantly different from that of the control substrate (1.42 ± 0.04). Consequently, these beans were classified as lower quality (grade II) according to international standards (ISO 2451) and ICCO (2008), while other substrates produced higher grade I beans. The results of Principal Component Analysis (PCA) coupled with Hierarchical Ascendant Clustering (HAC) corroborated the observations made on the different variables. Indeed, these findings revealed that beans fermented inside cocoa pods exhibited significantly different quality compared to the control substrate, potentially explained by their higher pH and a notable proportion of mouldy, germinated, and damaged beans.

Beans fermented on jute bags and palm leaves showed qualities relatively similar to

those of the control, possibly attributed to their closer cutting test profile.

CONCLUSION AND APPLICATION OF RESULTS

This study underscores the crucial importance of fermentation in the cocoa bean transformation process. The results reveal significant variations in temperature and pH conditions depending on the different substrates used during fermentation. At the end of the drying process, analyses showed modifications in the overall composition of the final beans. Beans fermented inside cocoa pods exhibited higher proportions of mouldy and germinated beans, pH levels exceeding standard norms, and a relatively low fermentation index, disqualifying them from meeting the criteria for high-quality final

beans. Statistical analyses based on Principal Component Analysis (PCA) and Hierarchical Ascendant Clustering (HAC) indicate that palm leaves and jute bags, in addition to their availability, exhibit qualities more closely resembling those of beans fermented on banana leaves (controls). Consequently, these substrates could serve as viable alternatives to banana leaves in ensuring optimal cocoa bean quality in Côte d'Ivoire. Furthermore, this study highlights the importance of further research to explore the relationship between different fermentation substrates and the aromatic profiles of cocoa beans.

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