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Occurrence of Free Fatty Acids (FFA) in Cocoa Beans with Various Main Geographical Origin of Côte d'Ivoire in the Climate Change Context

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Authors' contributions

This work was carried out in collaboration among all authors. Author KRH has made substantial contributions to conception and design, or acquisition of data or analysis and interpretation of data. Author JST has contributed to conception and design, or acquisition of data or analysis and interpretation of data. Author KMY has been involved in drafting the manuscript or revising it critically for important intellectual content. Author KBDK has contributed to the isolation and the identification of fungal moulds strains. Author PM has contributed to the correction and improvement of this manuscript. She has been involved in drafting the manuscript or revising it critically for important intellectual content. Author DM has contributed to the correction of this manuscript as the co-supervisor of the author KRH. Author TSG as the corresponding author of this article project, has given final approval of the version to be published. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Free fatty acids (FFA) result from hydrolysis of cocoa butter triacylglycerides due to various abiotic and biotic factors. The resurgence of high FFA contents above 1.75% in cocoa beans depended on Ivorian geographical origin. High FFA contents have become a serious quality problem because they induced serious consequences for some beneficial properties of cocoa butter and chocolate. This study investigated the improvement of quality of Ivorian cocoa beans by reducing their FFA contents.
Methodology: Samples: 240 cocoa beans batches were sampled from cocoa farmers of some big cocoa producing regions for their FFA contents analysis. Also, influence of primary post-harvest processing such as pod's sanitary status, pod opening delay, fermentation duration and dry cocoa beans storage under various relative humidity (RH) ranged 80-90% on FFA's formation of Ivorian cocoa beans was investigated. Data interpretations were performed according to the Fisher's test.
Results: Main results showed that dry cocoa beans recorded FFA content varying from 0.99 to 14% in function of the geographical origin. Beside, 53% of tested cocoa bean samples exhibited FFA content up to 1.75%. Poor sanitary status of pods and 7-days fermented cocoa beans recorded FFA content above 1.75%. Dry cocoa beans stored at RH above 85% promoted more the FFA's formation (14%) than those stored under lower RH values. 53% of tested cocoa beans samples presented FFA contents up to 1.75%. Controlled primary post-harvest processing highlighted that poor sanitary status of pods, 7-days fermentation and storage of dry cocoa beans at RH above 85% lead to FFA content above 1.75%.
Conclusion: So, in order to produce fermented and dried cocoa beans containing a suitable FFA content in the context of climate change, mitigation strategies need to integrate good crop conditions regarding postharvest processing and to improve beneficial ones.

Keywords: Côte d'Ivoire; cocoa beans; postharvest processing; free fatty acids contents; relative humidity.

1. INTRODUCTION

Cocoa beans are reported to be the main raw material for cocoa butter and chocolate manufacture. Cocoa butter (CB) is one of the main industrial by-product with cocoa liquor and powder [1]. These fatty components are constituted of 97% of glycerolipids as tri-, di- and mono-acylglycerols, phospholipids, glycolipids and in the low proportion, unsaponifiable matter [2]. CB is one of the most important and expensive ingredients for chocolate formulation in the economic view [3]. Chocolate is a complex suspension of cocoa powder in the continuous lipid phase constituted currently by CB [4]. During the formulation of chocolate, the properties of crystallization of CB play an important role in the global quality of the end products. The desired luster and the consumers' appreciation of the "crack" of chocolate require perfect crystallization and high-quality CB [5]. This crystallization of CB could be affected by the monoglycerides (MG), diglycerides (DG), phospholipids, hydrocarbons, sterols and sterol esters, tocopherols and particularly FFA content [6]. FFA are naturally present and remain in low concentration in cocoa butter. However, FFA could occurred abundantly in high concentration in fat matrices due to the enzymatic lipolysis produced by the growth of molds [7]. High FFA content could be due to various factors including high values of RH, level of ripeness of cocoa pods, post-harvest processing on-farm level and microbial lipolytic activity [8]. The resurgence of high FFA content of cocoa beans sourced from Côte d'Ivoire in the last decade could be ascribed to both the crop practices and climate changes. Regarding the climate, Delgado-Ospina et al. [9] though that weather (RH, température) in cocoa producing countries could support fungal growth and, consequently, product quality deterioration. The temperature of cocoa producing countries varies from 15 to 34 °C, usually at an altitude ranged from 300 to 1100 m.a.s.l. For proper growth, the cocoa tree requires rain throughout the year, ranging between 1500 and 3000 mm. Since a long time, European Union Directive 2000/36/EC [10] set International legal maximum limit of FFA content of cocoa butter at 1.75 % because FFA content above1.75% could provoke rancidity taste of cocoa butter, serious technological quality and the decrease of cocoa beans commercial value [1]. According to Barel [11], cocoa beans has an intrinsic potential of quality depending on the plant variety or genotype, the terroir, the cropping conditions know-how and primary post-harvest processing. However, Guehi et al. [7] have reported that the high concentrations of polyphenol components inside cocoa beans constituted greater inhibitors against the endogenous lipolysis. So, the occurrence of high FFA concentration in dry cocoa beans could not due to the typical endogenous enzymatic lipase before and during postharvest processing of cocoa bean [12]. Cocoa primary postharvest processing are mediated by a dynamic of biochemical reactions catalyzed by a microbial succession such as yeasts, lactic, acetic bacteria and *Bacilli* contaminated spontaneously cocoa bean, and produce various organic metabolites [13]. Many researches highlighted the abundant growth of specific filamentous fungi particularly during the fermentation process, drying and storage steps [14,15]. Yet, Côte d'Ivoire is the world's largest cocoa producer [9], primary postharvest processing of cocoa beans vary considerably towards the main cocoa producing regions [16]. Consequently, cocoa beans sourced from Côte d'Ivoire are currently subjected to various defective quality problems including high FFA content for several years [7], contamination by ochratoxin A [17]. In order to allow Ivorian cocoa farmer to adapt and to be resilient to the climate and ecophysiological conditions of cocoa production chain changes, cocoa farmers may change their crop practices. Up today, few complete and comprehensive study really dealt with the influence of primary postharvest processing on the occurrence of high FFA concentrations in Ivorian cocoa beans in the climate change context. Therefore, this study aimed to identify the abiotic factors that promote the FFA formation through successive steps processing of cocoa beans from harvest pods to storage of fermented and dried cocoa beans.

2. MATERIALS AND METHODS

2.1 Materials

Peasant dry cocoa beans and controlled cocoa beans issued from specific postharvest processing were sampled. Fresh cocoa beans from healthy pods or poor health status pods were submitted to controlled post-harvest processing. Cocoa pods were opened using clubs for 0, 7 and 10 days storage. Extracted fresh cocoa beans from pods, and then fermented in heaps in banana leaves for 4 and 7 days. Fermented cocoa seeds were sun-dried for 6 days until moisture about 7-8 % [18]. Otherwise, 240 peasant cocoa beans samples were collected from cocoa farmers during the cocoa season 2019-2020 in Akoupé, Aniassué and Yakassé-Attobrou, 3 Ivorian cocoa producing locations. All final dry cocoa beans samples (1 kg) were stored in the jute bags at 15°C for further analysis. Table 1 summarizes the experimental design and the quality of samples.

2.2 Methods

2.2.1 Effect of relative humidity on FFA formation during cocoa beans storage

Saturated salts solutions were prepared according to the method previously described by Ahmat et al. [19] to set the RH at constant value inside a desiccator. The changes in FFA contents of dry cocoa beans from traditional farmers (KKO 1) and dry cocoa beans from controlled post-harvest processing (KKO 2) stored under 3 RH values including 80, 85 and 90% were measured. Eighteen samples of each dry cocoa bean samples (50 g) placed in aluminum cups for storage (Fig. 1). Stability of RH value was followed using a thermo-hygrometer. A sample of each type of dry cocoa beans was collected each week for measurement of the FFA content.

2.2.2 Determination of water content and water activity of cocoa beans

Moisture content of dry cocoa beans was determined following ISO 1980-12-01 in triplicate using cocoa powder dried at 103°C for 16 h [2]. Their water activity were measured using an electronic hygrometer AquaLab Series 4TE by introducing 3 g of cocoa powder into a suitable container for digital reading [20].

Table 1. Experimental design summarizing number of cocoa beans samples and their quality

Cocoa pods storage times (day)	Pods opening tools	Cocoa fermentation duration (day)						Number of cocoa beans samples
		Healthy pods			Damaged pods			
		0	4	7	0	4	7	
0	Clubs	01	01	01	00	00	00	03
	Cutlass	01	01	01	00	00	00	03
7	Clubs	01	01	01	00	00	00	03
	Cutlass	01	01	01	01	01	01	06
10	Clubs	01	01	01	00	00	00	03
	Cutlass	01	01	01	01	01	01	06
Number of samples		**06**	**06**	**06**	**02**	**02**	**02**	**24**

Fig. 1. Experimental cocoa beans storage system in climate chamber

2.2.3 Quantification of FFA concentration of dry cocoa beans

FFA concentrations of cocoa beans were assayed on 5 g of cocoa butter. Dry cocoa beans were freezed in liquid nitrogen and then ground using a Warring blender (SEB, Stockholm, Suède). Cocoa butter was extracted from 30g of cocoa powder using speedy solvent method by an appropriate equipement ASE Dionex modèle 350 (Sunnyvale, CA, USA). For this, 5g of cocoa butter were homogenised in 50 mL of 95 % Ethanol/Petroleum ether (1/1) solution containing phenolphthalein as indicator using the ISO 660-2020 method. The final mixture was titrated with KOH 0.1 N and FFA content was calculated as following:

$$\text{FFA (\%)} = \frac{\text{Volume of KOH used} \times \text{Normality of KOH (0.1N)} \times \text{Equivalent factor (28.2)}}{\text{Weight of cocoa beans sample}}$$

2.3 Statistical Analysis

The correlation coefficient (r) was calculated to determine the incidence of the cocoa post-harvest treatments on the FFA formation. Statistical analysis of the correlation were carried out by using the software XLSTAT® 2022.1.1.1265, analysis of variance (ANOVA). Interpretations of values were performed according to the test of Fisher least significant difference (LSD) with 95% confidence interval (tolerance 0.0001).

3. RESULTS

3.1 Effect of Post-harvest Treatments on FFA Formation in Cocoa Beans

Fig. 2 indicates the effect of each post-harvest treatment on the FFA formation. Variable FFA contents were found in all tested cocoa beans sample. Fig. 2a presents the influence of sanitary status of cocoa pods on the FFA formation. FFA concentration reached 1.81% for cocoa beans originated from damaged pods, while those of cocoa beans sourced from healthy pods reached 0.90 %. Statistical analysis indicates that poor sanitary status of cocoa pods promote significantly FFA formation in seeds (*p-value* =0.05). Fig. 2b indicates that FFA content of cocoa beans sourced from healthy pods remained constant and low (0.27-0.29%) whatever the opening delay (*p-value* =0.05). Fig. 2c indicates that the FFA content of cocoa beans was about 0.85-0.87% whatever using clubs or cutlass (*p-value* =0.05). Fig. 2d reveals that FFA content changed from 0.30 to 0.99% in cocoa beans from the start to the end of the fermentation process So, the results of this study indicated that the greater is the fermentation duration, the greater is the increase of FFA concentration (*p-value* =0.0001). However, the FFA concentration of cocoa beans did not exceed the international standards (1.75%).

3.2 Effect of Interaction of Post-harvest Treatments on FFA Content of Cocoa Beans

The results about the interactions between sanitary status of cocoa pods and the opening delay showed that cocoa beans from 7 days opening-delay damaged pods recorded higher FFA content (1.35%) than those from healthy pods (0.63%). Otherwise, cocoa beans extracted from 10 days opening-delay poor sanitary status and healthy pods exhibited lowest FFA content of 1.03 and 0.52% respectively (Fig. 3a). Interacting sanitary status of pods with fermentation duration showed that cocoa beans from poor sanitary status pods recorded FFA concentration above 1.75%. However, the changes in FFA content according to the fermentation duration of cocoa beans from healthy pods varied from 0.29 to 0.81 % (Fig. 3b). Interacting pods storage with fermentation duration indicated that cocoa beans from 7 days opening-delay pods recorded higher FFA content (1.41%) than those measured for cocoa beans from immediately opened pods (0.57%). Changes in FFA content dropped for cocoa beans from 10-days opening-delay pods regardless the fermentation duration (Fig. 3c). ANOVA test (*p-value* =0.05) revealed influence of sanitary status of pods, pods opening delay and fermentation duration on the FFA formation without exceeding 1.75%.

3.3 Changes in FFA Content of Cocoa Beans According to their Production's Regions

Table 2 indicates that average FFA content of cocoa beans was between 0.72 and 8.05% with the median FFA content about 2.33±0.63%. Cocoa beans from Yakassé-Attobrou and Akoupé exhibited highest FFA contents 11.02%. Peasant cocoa beans from Aniassué recorded FFA contents below the tolerable limit with an average content about 1.62±1.05 %. Analysis of variance according Tukey's test showed that FFA contents of cocoa bean samples from Akoupé and Yakassé-Attobrou were not significantly different (*p =0.05*). However, these values were significantly different from those recorded in cocoa beans collected Aniassué (*p =0.05*). 53 % of cocoa beans samples collected exhibited FFA content above international specification (1.75 %). Among them 31.65 and 61% of cocoa beans samples collected from Akoupé and Yakassé-Attobrou recorded FFA content above tolerable limit of 1.75%.

Table 2. Distribution of cocoa beans sampled from Southeast cocoa producing locations of Côte d'Ivoire according to the cardinal values of their FFA contents

Cocoa producing locations	Number of cocoa beans samples	Cardinale values of FFA contents (%)		
		Minimum	Mean	Maximum
Aniassué	77	0.38	1.62 ± 1.05^a	4.76
Akoupé	80	0.79	2.79 ± 1.73^b	8.36
Yakassé -Attobrou	79	0.99	2.59 ± 1.62^b	11.02
Total	**236**	**0.72**	**2.33 ± 0.63**	**8.05**

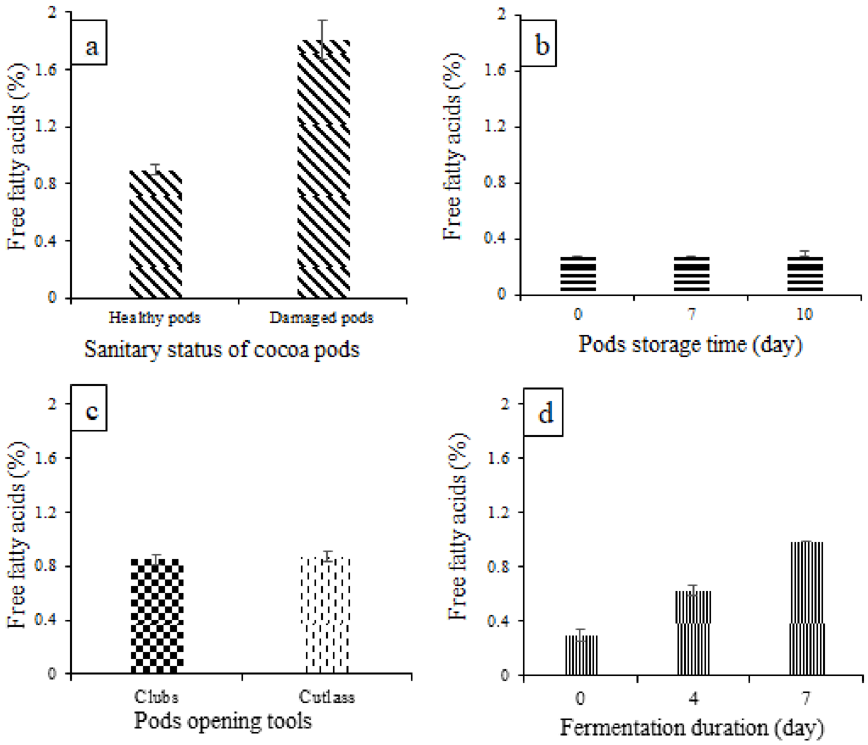

Fig. 2. Influence of sanitary status of pods a), pods storage times b), pods opening tools c) and fermentation duration d) on FFA formation in farmers post-harvest processing conditions

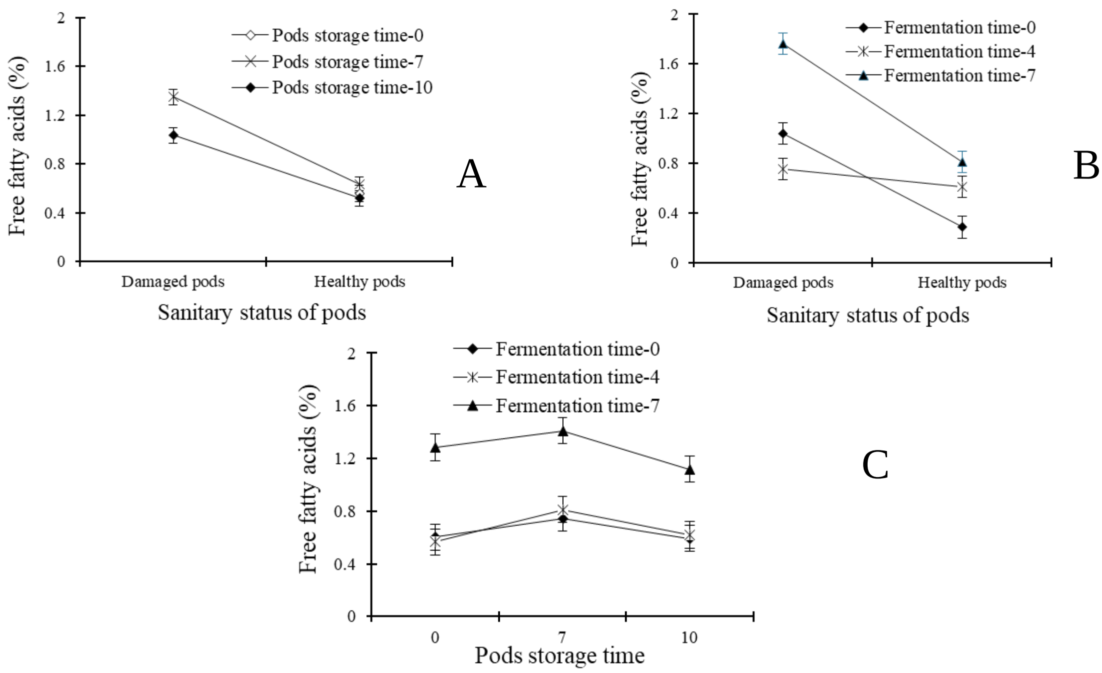

Fig. 3. Changes in FFA content of fermented and dried cocoa beans according to the post-harvest processing. A) Effect of interaction between pods sanitary status and storage time of pods, B) Effect of interaction between pods sanitary status and fermentation time, C) Effect of interaction between pods storage and fermentation times

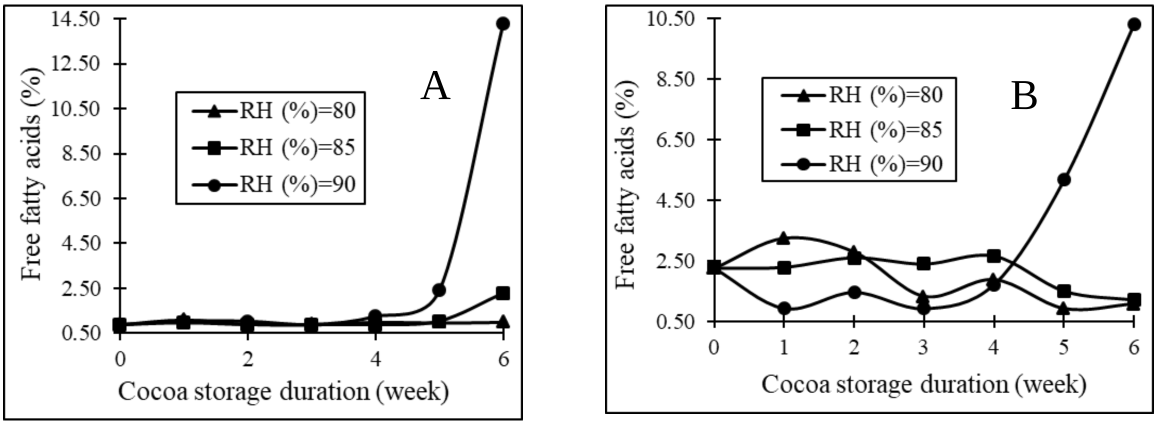

Fig. 4. Changes in FFA contents in stored fermented and dried cocoa beans according to the relative humidity (RH) value : A) cocoa beans sourced from controlled post-harvest processing (KKO1) ; B) cocoa beans sourced from paysant post-harvest processing (KKO2)

In a column, values of FFA content assigned with the same letter are not significantly different with a confidence interval of 95% according to Tukey's test.

3.4 Changes in FFA Content of Stored Cocoa Beans in Function of Relative Humidity (RH)

Results related to the changes in FFA content of stored cocoa beans according to the RH value are presented in Fig. 4A-B. FFA content of cocoa beans (KKO1) was remained around 1% during 6 weeks at 80 % RH whereas those of cocoa beans stored at 85 % RH increase from 0.89±0.00 to 2.30 ± 0.02 %. FFA content of cocoa beans stored at RH 90% (Fig. 4A). Cocoa beans sample recorded FFA content decreasing from 2.24 ± 0.03 to 1.2 ± 0.03 % except for cocoa beans samples stored at 90% RH which reached 10.3 ± 0.21% FFA content (Fig. 4B). According to ANOVA test (*p-value* =0.0001), RH above 85 % favored FFA formation in stored cocoa beans.

4. DISCUSSION

4.1 Effect of Post-harvest Treatments on FFA

The study of the influence of pod's sanitary status on the formation of FFA revealed that lower average FFA content was measured in cocoa beans from healthy pods than those of cocoa beans from damaged pods. According to Delgado-Ospina et al. [9] healthy cocoa pods provide safe beans and pulp. The FFA contents of these cocoa beans are below 1.75%. However high FFA content measured in cocoa beans from damaged pods could be due to the fungal contamination [17] inside pods before [21] or during the post-harvest processing [11]. Consequently, fungal enzymatic lipolysis lead to the formation of FFA in poor quality cocoa beans [7]. Among, the microbial communities, it has been reported that high fungal contamination level may cause an increase FFA content above 1.75%, compromising the quality of cocoa butter [9]. Constant and low FFA content of cocoa beans indicated that long pods opening delay did not affect the FFA formation as previously concluded Guehi et al. [7] when the seeds were from healthy cocoa pods. However, the FFA contents of cocoa beans extracted from 7-day pods opening delay were optimum because of the mycelial growth spoiled the product and the activities of fungal extracellular lipase produced by lipolytic mold strains such as *Rhizopus oryzae* Went & Prins.

Geerl. (Mucoraceae) and *Absidia corymbifera* (Cohn) Sacc. & Trotter (Mucoraceae) [8]. Otherwise, the increase of FFA content of cocoa beans from excessive ripened pods could be ascribed to the significant reduction of sugar content of mucilaginous pulp during pods opening delay [22]. The decrease of FFA contents of cocoa beans from above 10-days cocoa pod opening delay or poor quality cocoa pods could be due to its consumption as organic substrate by various microorganisms such yeast, filamentous fungi and both lactic and acetic bacteria for their growth [23]. So, reducing concentration of fermentable sugars, great substrates for the yeast [13] due to the storage of pods before opening limit the production of lactic and acetic acids which inhibited the growth of mold during cocoa fermentation. Otherwise, several researches have concluded storage of cocoa pods promoted the generation of cocoa flavor precursor compounds [24]. The using of clubs or cutlass did not influenced the formation of FFA in cocoa beans. This observation could be due to the absence of injury of cocoa beans [7]. However, use a cutlass for pod opening could damage cocoa beans [15] and consequently promote the significant increase of FFA content of cocoa beans later [25] favored the growth of ochratoxin A producing molds strains [26].

4.2 Effect of interaction of post-harvest treatments on FFA content

The interaction between these post-harvest processing conditions showed that damaged pods, long pod opening delay and long fermentation duration promote significantly the incidence of FFA. Poor quality of cocoa pods was the most parameter promoting the generation of FFA [15] because damaged pods exposed the cocoa beans to the fungal contamination early before extraction [21]. As shown by Seddek et al. [27] the growth of lipolytic molds could cause lipolysis that provoke deterioration of both nutritional and commercial values of cocoa beans. Otherwise, according to Kedjebo et al. [17], the interaction between the sanitary status of pods, long pod-opening delays and fermentation methods contributed significantly to the OTA's occurrence. However, we have to observe a drop of FFA content regardless of fermentation duration due to the using of FFA as a carbon source by fermentative microorganisms. Indeed, Papanikolaou and Aggelis [28] reported that numerous microorganisms are able to grow by consuming and conversion FFA in bio ernegy, regardless of their lipolytic activities.

4.3 Changes in FFA Content of Cocoa Beans with the Terroir

Cocoa samples from Yakassé-Attobrou and Akoupé location recorded the highest FFA contents (>1.75%). These results could be due to the differences in crop post-harvest practices. Indeed, according to Dano et al. [16] cocoa primary post-harvest processing vary considerably from a cocoa producing region to another and between cocoa producers in the same region in Côte d'Ivoire. Moreover, survey of cocoa farmers in Côte d'Ivoire (result not published) showed that a majority of cocoa farmers did not discard the poor quality cocoa beans before the fermentation process. So high FFA content could be due to the poor post-harvest [19] particularly poor storage conditions which could lead to rapid and excessive fungal invasion of stored cocoa beans [29]. According to Delgado-Ospina et al. [9] fermented and dried cocoa beans could harbor the fungi for a long time and then be able to activate their lipolysis under favorable moisture content above 8% due to the inadequate RH conditions [30].

4.4 Effect of Relative Humidity of the FFA Content of Stored Cocoa Beans

Moreover, absorption of water at 90% RH promoted the germination of fungal conidia, which stayed latent until storage (results not shown). Whatever the factors promoting the FFA generation, this chemical parameter affects and alter seriously the fundamentally technological and rheological quality such as solidification kinetic and soften of cocoa butter fraction and chocolate [1] FFA contents of cocoa beans increase exponentially from the fourth week of storage under RH 90% to reach values above 10% regardless post-harvest processing applied. These alarming changes in FFA contents could be due to the microbial lipolysis. Indeed, fermented and dried cocoa beans stored under high relative humidity may absorb water due to their strong hygroscopic properties [20] like table salt [31]. Cocoa beans that reached the hygroscopic balance represent an ideal environment for the growth of molds that cause lipolysis [9]. According to Mabbetti [31] mold contamination is triggered by high moisture which accelerated lipolytic activities production. Since a longtime, Barel [11] has shown that the moisture content of fermented and dried cocoa beans set at 8% (tolerable limit) guaranteed good conservation by ensuring a good balance with a relative humidity of 70% at 30°C. However, in cocoa production areas, this RH is very often above 70% increasing in moisture and poor preservation of cocoa beans [31]. After fermentation of the cocoa beans, moisture content (m.c.) is usually high that poses a risk for the rise of FFA in the beans. So it was recommanded relative humidity of 69% was recommended fro storage of dry cococa beans in order to avoid the great increse of FFA contents [32]. Fast and effective invasions of stored cocoa beans by filamentous fungi are triggered by poor primary post-harvest processing in particular poor storage conditions [29] who showed that

Eurotium species are cocoa spoiling fungi growing at water activities below 0.75 at 25°C. In general, filamentous fungi are invariably present during primary post-haverst processing especially pods opening delay, cocoa fermentation, solar drying and storage of cocoa beans but require the interactive physical factors before growing into economically-damaging problems [31].

5. CONCLUSION

This study has proved that primary post-harvest processing such as poor status of cocoa pods, long pod opening delays above 10 days, and fermentation duration of cocoa beans sourced from damage cocoa pods above 4 days could affect significantly the formation of FFA in raw cocoa beans. No significant relationship was found between pods opening tools and FFA formation. Peasant cocoa beans from Akoupé and Yakassé-Attobrou regions recorded highest FFA content above 10%. Storage of raw cocoa beans at RH above 85% promotes FFA formation. Storing cocoa beans under relative humidity of 85% inhibits of mould development and production of extracellular lipase ; as a consequence the FFA deriving from microflora development was inhibited. Results of this study indicate that cocoa farmers may adapt their crop practises to the climate change by use only cocoa beans from health status pods, limit fermentation duration about 5 days, solar drying fermented cocoa beans until 7-8% and avoid storing raw cocoa beans at RH below 85% in order to produce cocoa with low and consequently acceptable FFA content. So, the results of this study indicated that in the context of climate change, mitigation strategies need to integrate good crop practices during the postharvest processing and improve beneficial ones in order to improvement the quality of fermented and dried cocoa beans with low FFA contents below international standards of 1.75%.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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