Original Research Article

Title : Occurrence of Free FattyAcids (FFA) in cocoabeanswithvariousmain geographicaloriginof Côte d'Ivoire in the climate changecontext**.**

Running title: Formation of Free Fatty Acids in cocoa beans

Abstract

Aims:FFA resultfromhydrolysis of triacylglycerides constituted cocoa butter due to various abiotic and biotic factors. The resurgence of high free fatty acids (FFA) content above 1.75% in cocoa has become a seriousqualityproblem in Côte d'Ivoirebecauseitinducedseriousconsequences for some beneficial properties of cocoa butter and chocolate.This study investigated the improvement of quality of fermented and driedcocoabeanssourcedfrom Côte d'Ivoire by reducing the FFA occurrencce.

Material and methods: 240 fermented and dried cocoa beans were sampled at the on-farm level from three big cocoa producing regions. They were analyzed for the FFA content. Influence of primary post-harvest processing such as interacting sanitary status, pod opening delay, fermentation duration and storage of raw cocoa beans samples under relative humidity (RH) ranged 80-90% on the formation of FFA inside cocoa beans from Côte d'Ivoire was investigated.

Results: Main results showed that fermented and dried cocoa beans recorded FFA content varying from 0.99 to 14% in function of the geographical origin. Beside, 53% of tested cocoa beans samples exhibited FFA content up to 1.75%. Poor sanitary status of cocoa pods and 7-days fermented cocoa beans showed FFA content above 1.75%. Fermented and dried cocoa beans stored at relative humidity (RH) above 85% promoted more the FFA's formation (14%) than those stored under low RH value. 53% of tested cocoa beans samples presented FFA contents up to 1.75%. Controlled primary post-harvest processing highlighted that poor sanitary status,7-days fermentation and storage of cocoa beans atRH above 85% lead to FFA content above the standards.

Conclusion: So, in order to producefermented and driedcocoabeanscontaining a suitable FFA content in the context of climate change, mitigation strategiesneed to integrate good crop practices during the post harvestprocessing and improvebeneficialones.

Keywords : Cocoa,post-harvest processing, free fatty acids,climate changes.

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-productwithcocoa liquor and powder[1]. These fatty components areconstituted 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 crystallization properties of CB play an important role in the global quality of the end-product. The desired luster and the consumers' appreciation of the "crack" of chocolate requires perfect crystallization and high-qualityCB[5].This crystallization 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 in low contents in cocoa butter. However, FFA occurred in high concentration in lipidsdue to the enzymatic lipolysis from molds [7].High FFA content could be due tovarious factors including relative humidity, level of ripeness of cocoa pods, post-harvest processing on-farm level and microbial lipolytic activity[8].The temperature of cocoaproducing countries ranges from 15 to 32 °C, usually at an altitude rangedfrom 300 to 1100 m.a.s.l. For propergrowth, the cocoatreerequiresrainthroughout the year, rangingbetween1500 and 3000 mm. The resurgence of high FFA content ofcocoabeansfrom Côte d'Ivoire couldbeascribed to boththe crop practices and climate changes. Delgado-Ospina *et al*. [9] though thatweather (RH, température) in cocoaproducing countries could support fungalgrowth and, consequently, productqualitydeterioration. 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 tasteof cocoa butter, serious technologicalquality and the decrease ofcocoa beans commercial value [1].According to Barel[11] cocoa bean has an intrinsic potential of quality depending on the variety of plant, the terroir, the agronomic condition know-how and primary post-harvest processing. However, Guehi*et al.* [7] have reported that the high contentof polyphenol components in cocoa beans inhibits strongly the endogenous lipolysis. So, the occurrence of high FFA contents could not due to the typical endogenouslipolysisstepsafterharvest in the inner of the cocoabean or before primary post-harvest [12].Cocoa primarypost-harvest 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 research activities highlighted the considerable growth of specific filamentous fungi particularly during fermentation,drying and storage steps [14; 15]. Yet Côte d'Ivoire is the world'slargestcocoa producer [9], primary post-harvest treatments of cocoa beans vary considerably towards the world and particularly in Côte d'Ivoire [16]. Consequently, cocoa beans sourced from Côte d'Ivoire are currently subjected to various defective quality 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 andecophysiological conditions of cocoa production chain changes, the cocoafarmersmay change theircrop practices. Up today, fewcomplete and comprehensive studyreallydealtwith the influence ofprimarypost harvestprocessingonthe occurrence of FFA inIvoriancocoabeansin the climate changecontext. Therefore, thisstudyaims to identify theabioticfactorsthatpromote FFA formationincocoabeansfromharvestpods to fermented and driedbeans.

2. Materials and Methods

2.1. Material

Peasant cocoa and weresampled and controlled cocoa beansissuedfrom specific post-harvest processing. Cocoa beans samples from healthy podsand frompoor status pods were obtained by controlled post-harvest processing. Cocoapodswereopened for 0, 7 and 10 daysstorageusing clubs.Cocoa beans were fermented in heaps in banana leaves for 4 and 7 days. The fermented seeds were sun-dried for 6 days until moisture about 7%[18].Otherwise, 240 peasant cocoa beans produced by farmers were sampled during the cocoa season 2019-2020 in Akoupé, Aniassué and Yakassé-Attobrou locations. All 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.

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

Cocoa pods storage times (day)	**Podsopeningtools**	**Cocoa fermentation duration (day)**						**Number of cocoabeanssamples**
		Healthypods			**Damagedpods**			
		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**

2.2. Methods

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

Saturated salts solutions were prepared according to method described by Ahmat*et al.* [19] to set the RH values inside 3 desiccators. The changes in FFA contents of cocoa beans from traditional farmers (KKO 1) and from controlled post-harvest treatments (KKO 2) stored under 3RHvalues including 80, 85 and 90% were measured. Eighteensamplesof each cocoa bean (50 g) placed in aluminum cupsfor storage (Figure 1).Stability of RHvalue was followed using a thermo-hygrometer. A sample of each type of cocoa beans was collected each weekfor measurement ofthe FFA content.

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

2.2.2. Determination of water content and water activity of cocoa beans

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

2.2.3. Free fatty acids quantification of cocoa beans

FFA contentwas assayed on 5 g of cocoa butter homogenized in 50 mL of 95 % Ethanol/Petroleum ether (1/1) solution containing phenolphthalein as indicatorusing the ISO660-2020 method.The final mixture was titrated with KOH 0.1 N and FFA contentwas calculated as following:

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

3. Statisticalanalysis

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 werecarried 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).

4. Results

4.1. Effect of post-harvest treatments on FFA formation in cocoa beans.

Figure 2 indicates the effect of each post-harvest treatment on the FFA formation. Variable FFA contents were foundin all tested cocoa beans sample. Fig. 2a presents the influence of sanitary status of cocoa pods on the FFA formation. FFA content reached 1.81% for cocoa beans from damagedpods, while those of cocoa beans from healthy pods reached 0.90 %. Statistical analysis indicate that poor status of cocoa pods promote significantly (*p-value* =0.05)FFA formation in seeds. Fig. 2b indicates that FFA content of cocoa beans 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 ranged in 0.85-0.87% whatever using clubs or cutlass(*p-value* =0.05).Fig. 2d revealstheglobal increase in FFAcontent with increase of fermentation duration. FFA contentchanged from 0.30 to 0.99% in cocoa beans from the start to the end of the fermentation process. The results of our study indicated that FFA contents increased significantly according to the fermentation durations (*p-value* =0.0001).However, this FFA content did not exceed the international standards (1.75%).

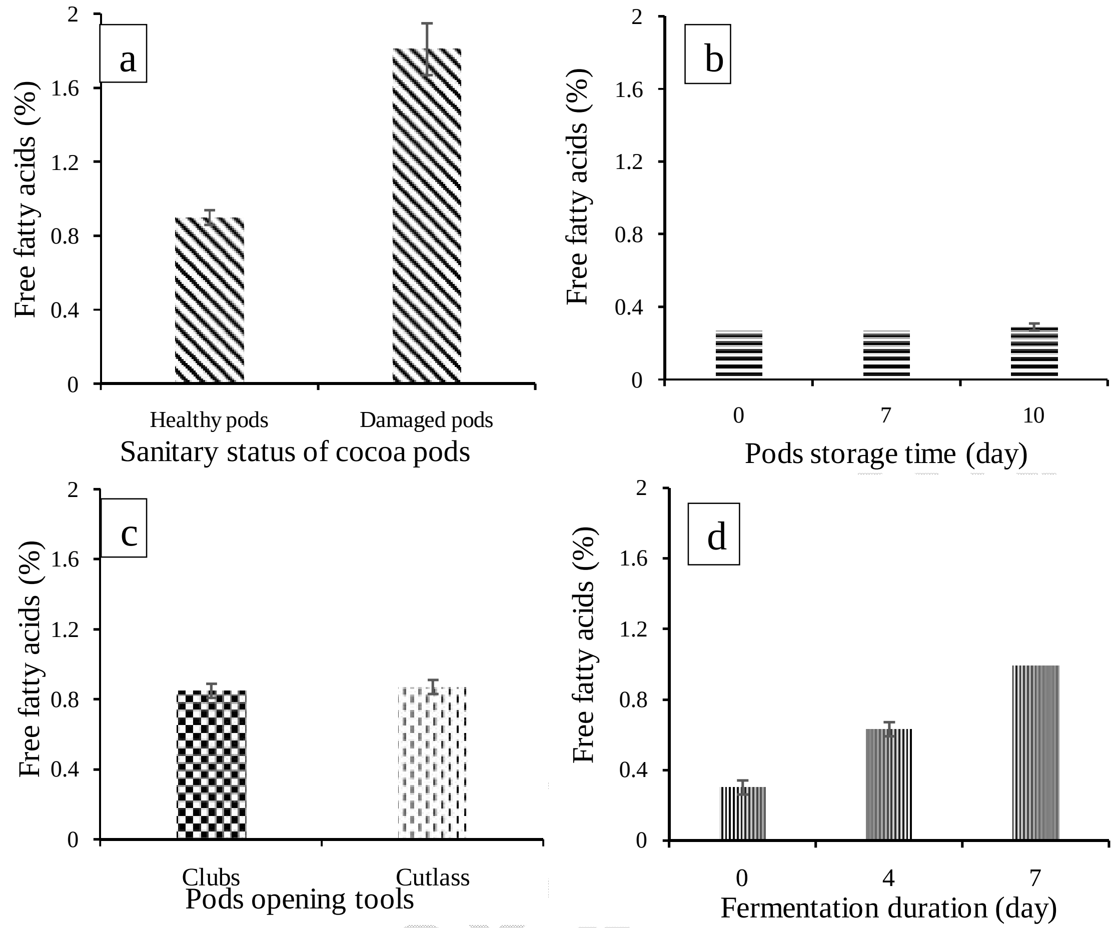

Fig. 2. Influence of sanitary status of pods a), pods storage times b), pods opening tools c) and fermentation duration d) on FFA formation in farmers post-harvest processing conditions

4.2. 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 delayshowed that cocoa beans from 7 days opening-delay damaged pods recorded higher FFA content (1.35%) than those from healthy pods,which was characterized by only 0.63%. Otherwise, cocoa beans from 10 days opening-delay damaged and healthy pods exhibited lowest FFA content of 1.03and 0.52%respectively(Figure3a). Interacting sanitary status of pods with fermentation duration showed that cocoa beans from damaged pods recorded FFA levels above 1.75%. However, the changes in FFA content according to the fermentation duration ofcocoa beans from healthy pods variedfrom 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 thosemeasuredforcocoa beans from immediately opened pods (0.57%).Changes in FFA content dropped for cocoa beans from 10-days opening-delay pods regardless thefermentation duration(Fig 3c). ANOVA test (*p-value*=0.05)revealedinfluence of sanitary status of pods, pods opening delay and fermentation duration on the FFA formationwithout exceeding 1.75%.

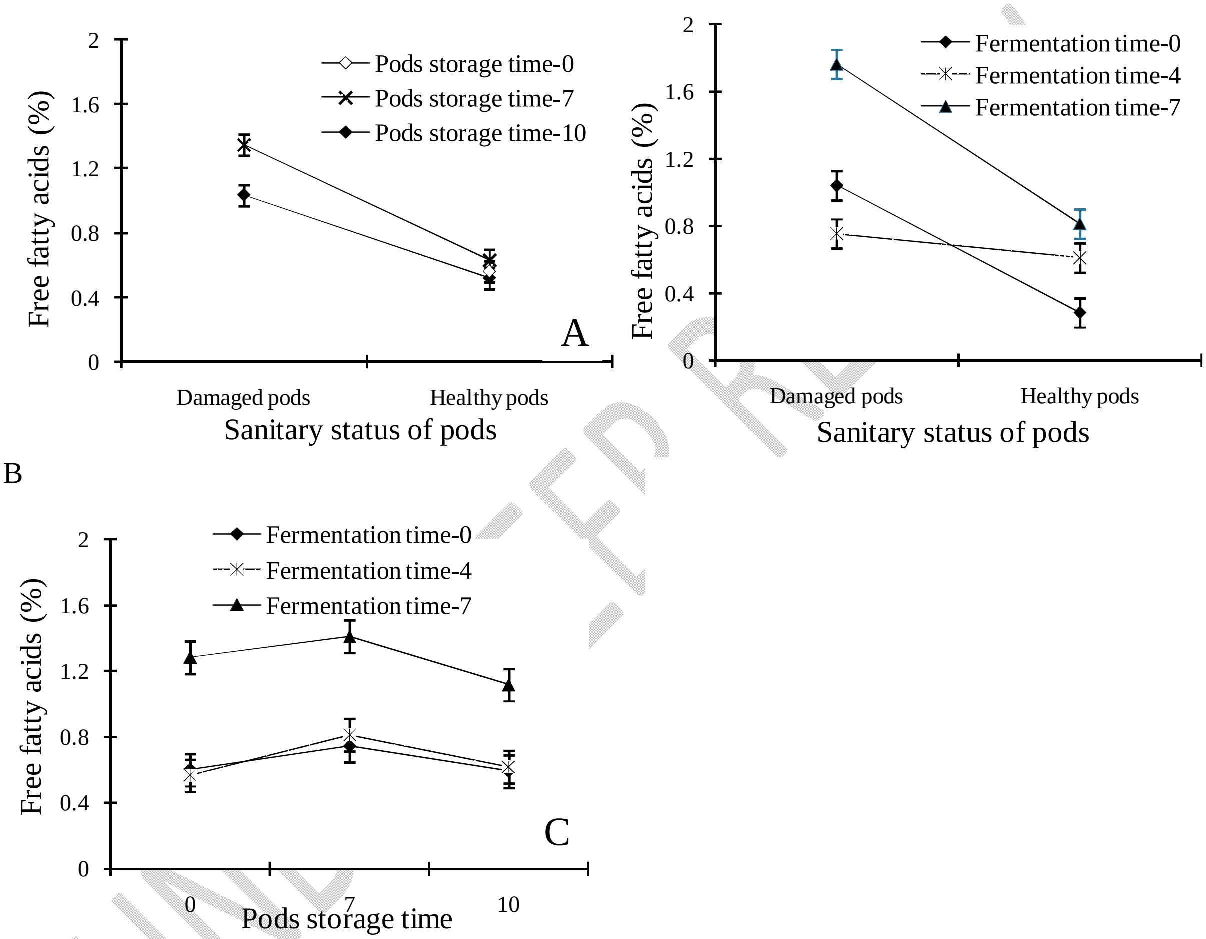

Fig. 3. Changes in FFA content of fermented and dried cocoa beans according to the post-harvest processing. A) Effect of interaction between pods sanitary status and storage time of pods, B) Effect of interaction between pods sanitary status and fermentation time, C) Effect of interaction between pods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

4.3. Changes in FFA content of beans sampled according to the cocoa producing regions

Table 2 indicates that averageFFA content of cocoa beans was between0.72 and 8.05% with the median FFA content about 2.33±0.63%. Cocoa beans fromYakassé-Attobrou and Akoupéexhibited highest FFA contents 11.02%. Peasant cocoa beansfrom Aniassuérecorded 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 differentfromthose recorded in cocoa beans collected Aniassué (*p=0.05*).A total of 53% of cocoa beans samples collectedexhibited FFA contentaboveinternational specification (1.75%). Among them31.65 and 61% of cocoa beans samples collected fromAkoupé and Yakassé-AttobrourecordedFFA 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 cocoabeanssamples**	**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***

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.

4.4. Influence of relative humidity (RH) value onFFA content of cocoa beans.

Results related to the changes in FFA content of stored cocoa beans according to the RH value are presented in Fig. 4A-B.FFA contentof 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 samplerecorded 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), RHabove 85% favored FFA formation in stored cocoa beans.

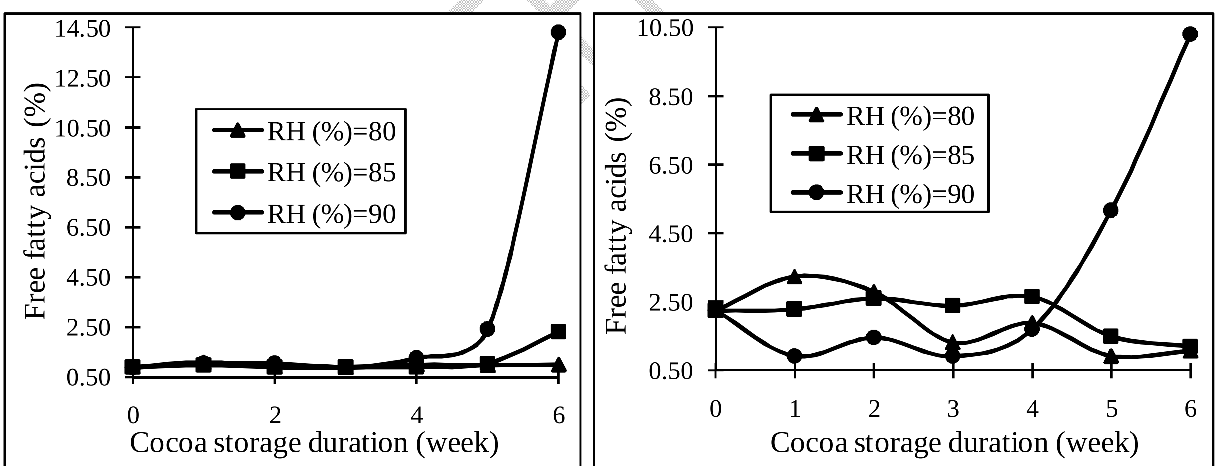

Fig. 4. Changes inFFA contents in storedfermented and driedcocoabeansaccording tothe relative humidity (RH) value : **A**) cocoabeanssourcedfromcontrolled post-harvestprocessing (**KKO1**) ; **B**)cocoabeanssourcedfrompaysantpost-harvestprocessing(**KKO2**).

5.Discussion

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 thoseof 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 FFAcontent measured in cocoa beans from damaged pods could be due tothe 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 fungalcontamination level may cause an increase FFA contentabove 1.75%, compromising the quality of cocoa butter [9].Constant and low FFA content of cocoa beansindicated that long pods opening delay did not affect the FFA formation as previously concludedGuehi*et al*. [7] when the seeds were from healthy cocoa pods.However, if they was from above 10-days cocoa pod opening delay orpoor quality cocoa pods they could record excessive formation of FFA. 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]. So, reducing concentration of fermentable sugars, great substrates for the yeast [13] due the storage of pods before opening limit the production of lactic and acetic acidswhich inhibited the growth of mold during cocoa fermentation. Otherwise, several researches have concludedstorage of cocoa pods promoted the generation of cocoa flavor precursor compounds [23].The usingof clubs or cutlass did not influencedthe 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 [24] favored the growth ofochratoxin A producingmoldsstrains [25].The interaction between some main post-harvest technological treatments 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 promotingthe generation of FFA[15] becausedamaged pods exposed the cocoa beans to thefungal contaminationearly before extraction [21].As shown by Seddek*et al*. [26] the growth oflipolytic molds could causelipolysisthat 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'soccurrence. However, we have to observe a drop of FFA contentregardless of fermentation duration due to the using of FFA as a carbon source by fermentative microorganisms.Indeed, Papanikolaou and Aggelis[27] reported that numerous microorganisms are able to grow by consuming and conversion FFA in bioernegy, regardless of their lipolytic activities.Cocoa samples fromYakassé-Attobrou and Akoupé locationrecorded the highest FFA contents (>1.75%). These resultscould 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 acocoa 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 contentcould be due to the poor post-harvest [19] particularly poor storage conditionswhich could lead to rapid and excessive fungal invasion of stored cocoa beans [28].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 favorablemoisture contentabove 8% due to the inadequate RH conditions. 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, thischemicalparameter 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 tothe microbial lipolysis. Indeed, fermented and dried cocoa beans stored under high relative humidity may absorb water due to their strong hygroscopic properties[29] like table salt[30].Cocoa beans that reached the hygroscopic balance represent an ideal environment for the growth of molds that cause lipolysis[9].According to Mabbetti[30] moldcontamination istriggered by high moisturewhichacceleratedlipolyticactivities 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 [30].Fast and effective invasions of stored cocoa beans by filamentous fungiare triggered by poor primary post-harvest processing in particular poor storage conditions [28] who showed that *Eurotium* species are cocoa spoiling fungi growing at water activities below 0.75 at 25°C. In general, filamentous fungi are invariablypresentduringprimary post-haverstprocessingespecially pods opening delay, cocoa fermentation, solar drying and storage of cocoa beans but require the interactive physicalfactorsbeforegrowingintoeconomically-damagingproblems[30].

Conclusion

This study has proved that primary post-harvest processingsuch 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 significantlythe formation of FFA in raw cocoa beans. No significant relationship was foundbetween pods opening tools and FFA formation. Peasant cocoa beans fromAkoupé and Yakassé-Attobrouregions recorded highest FFA content above 10%. Storage of raw cocoa beans at RH above 85% promotes FFA formation.Results of thisstudyindicatethatcocoafarmersmayadapttheircroppractisesto 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 storingraw cocoa beans at RH below 85% in order to produce cocoa with acceptable FFA content.So, the resultsobtainedfromthisstudyindicatedthatin the context of climate change, mitigation strategiesneed to integrate good crop practices during the post harvestprocessing and improvebeneficialonesin order ton improvement the mechantablequality offermented and driedcocoabeansby reducing of FFA occurrence.

References

[1] Afoakwa EO, Kongor JE, Takrama JF, Budu AS. Changes in nib acidification and biochemical composition during fermentation of pulp preconditioned cocoa (Theobroma cacao) beans. International Food Research Journal, 2013; 20(4):843-1853.

[2] Servent A, Boulanger R, Davrieux F, Pinot M, Tardand E, Forestier-ChironaN, Hue C. (2018). Assessment of cocoa (Theobroma cacao L.) butter content and composition throughout fermentations. Food Research International, 2018;107:675-682.

[3] Lannes SCS, Medeiros ML, Gioielli LA. Physical interactions between cupuassu and cocoa fats. Grasas y Aceites, 2013;54 (3): 253-258.

[4] Liendo R, Padilla FC, Quintana A. Characterization of cocoa butter extracted from Criollo cultivars of Theobroma cacao L. Food Research International, 1997;30(9): 727-731.

[5] Müller M, Careglio E. Influence of Free Fatty Acids as Additives on the Crystallization Kinetics of Cocoa Butter. Journal of Food Research, 2018;7(5):85-97.

[6] Lipp EM, Anklam, E. (1998). Review of cocoa butter and alternative fats for use in chocolate part A. Compositional data. Food Chemistry, 1998;62(1):73-97.

[7] Guehi ST, Dingkuhn M, Cros E, Fourny G, Ratomahenina R, Moulin G, Clement-Vidal A. Impact of cocoa processing technologies in free fatty acids formation in stored raw cocoa beans. African Journal of Agricultural Research, 2008;3(3):174-179.

[8] Guehi TS, Dingkuh, M, Cros E, Fourny G, Ratomahenina R, Moulin G,Clement-Vidal A. Identification and lipase-producing abilities of molds isolated from Ivorian raw cocoa beans. Research Journal of Agriculture and Biological Sciences, 2007;3(6): 838-843.

[9] Delgado-Ospina J, Molina-Hernández JB, Chaves-López C, Romanazzi G, Paparella A. The role of fungi in the cocoa production chain and the challenge of climate change. Journal of Fungi, 2021;7(3):202.

[10] Directive 2000/36/EC of the EuropeanParliament and of the Council of 23 June 2000 relating to cocoa and chocolateproductsintended for humanconsumption. Official Journal L197, 03/08/2000: 19–25.

[11] Barel M Qualité du cacao. L'impact des traitements post-récolte. Edition Quae. Versailles, France. 2013.

[12] Kedjebo KBD, Aka KEA, Boli, ZA, Sika AE, Guehi TS, (2022). Effect of germination, mucilaginouspulp and mold contamination of cocoabeans on the formation of free fattyacids. 1er CIBIOSDA: 1er Colloque International des Biosciences et Développement en Afrique Université Félix-Houphouët-Boigny Abidjan, 2022; Cote d'Ivoire, 02-04 Novembre 2022.

[13] Muñoz SM, Cortina RJ, Vaillant FE, Parra ES. An overview of the physical and biochemical transformation of cocoa seeds to beans and to chocolate: Flavor formation. Critical Reviews in Food Science and Nutrition, 2020;60(10):1593-1613.

[14] Sánchez-Hervás M, Gil JV, Bisbal F, Ramón D, Martínez-Culebras PV. Mycobiota and mycotoxin producing fungi from cocoa beans. International Journal of Food Microbiology, 2008;125(3):336-340.

[15] Mounjouenpou, P., Gueule, D., Maboune, T. S. A., Guyot, B., Fontana-Tachon, A. & Guiraud, J. P. (2012). Incidence of pod integrity on the fungal microflora and Ochratoxin-A production in cocoa. Journal of Biology and Life Science, 2012;3(1):254-265.

[16] DanoSD, Manda P, Dembélé A, Abla AMJK, Bibaud JH, Gouet JZ, Sika CBZM. Influence of fermentation and drying materials on the contamination of cocoa beans by ochratoxin A. Toxins. 2013;5(12):2310-2323.

[17] Kedjebo KBD, Guehi TS, Kouakou B, Durand N, Aguilar P, Fontana A, Montet D. Effect of post-harvesttreatments on the occurrence of ochratoxin A in rawcocoabeans. Food Additives & Contaminants : Part A, 2016 ;33(1):157-166.

[18] Guehi TS, Dadie AT, Koffi KPB, Dabonne S, Ban-Koffi L, Kedjebo KD, Nemlin GJ. Performance of different fermentation methods and the effect of their duration on the quality of raw cocoa beans. International Journal of Food Science and Technology, 2010;45(12):2508-2514.

[19] Ahmat T, Bruneau D, Kuitche A, Aregba AW. Desorption isotherms for fresh beef: An experimental and modeling approach. Meat science, 2014;96(4):1417-1424.

[20] Nganou DN, Sokamte TA, Tchinda SE, Ntsamo BTM,Nodem SFS, Douanla NNF, Noumo NT, Tatsadjieu NL. Fungal diversity and occurrence of Aflatoxin B1, Citrinine, and Ochratoxin A in rice of Cameroon. Journal of Food Processing and Preservation, 2022;46(4):e16429.

[21] Copetti MV, Iamanaka BT, Mororó RC, Pereira JL,Frisvad JC, Taniwaki MH. The effect of cocoa fermentation and weak organic acids on growth and ochratoxin A production by Aspergillus species. International Journal of Food Microbiology, 2012;155(3):158-164.

[22] Hinneh M, Semanhyia E, Van de Walle D, De Winne A, Tzompa-Sosa DA, ScaloneGL.L, De Meulenaer B, Messens K, Durme JV, Afoakwa EO, De Cooman L, Dewettinck K. Assessing the influence of pod storage on sugar and free amino acid profiles and the implications on some Maillard reaction related flavor volatiles in Forastero cocoa beans. Food Research International, 2019;111:607-620.

[23] KonéKM, Assi-Clair BJ, Kouassi ADD, Yao AK, Ban-Koffi L, Durand N, Lebrun M, Maraval I, Bonlanger R, Guehi TS. Pod storage time and spontaneous fermentation treatments and their impact on the generation of cocoa flavour precursor compounds. International Journal of Food Science & Technology, 2021;56(5):2516-2529.

[24] Tee YK, Bariah K, HisyamZainudin B, Samuel Yap KC, Ong NG. Impacts of cocoa pod maturity at harvest and bean fermentation period on the production of chocolate with potential health benefits. Journal of the Science of Food and Agriculture, 2022;102(4):1576-1585.

[25] Pires PN, Vargas EA, Gomes MB, Vieira CBM, Santos EAD, Bicalho AAC, Silva SDC, Rezende RP, De Oliviera IS, Luz Newman EDMN, TrovattiUetanabaro AP. Aflatoxins and ochratoxinA : occurrence and contamination levels in cocoabeansfrom Brazil. Food Additives & Contaminants : Part A, 2019;36(5):815-824.

[26] Seddek NH, Gomah NH, Osman DM. Fungal flora contaminating Egyptian Ras cheese with reference to their toxins and enzymes. Food Science and Technology, 2016;4(4):64-68.

[27] Papanikolaou S, Aggelis G. Yarrowialipolytica: A model microorganism used for the production of tailor-made lipids. European Journal of Lipid Science and Technology, 2010;112(6), 639-654.

[28] Akinfala TO, HoubrakenJ, Sulyok M, Adedeji AR, Odebode AC, Krska R, Ezekiel CN. Molds and their secondary metabolites associated with the fermentation and storage of two cocoa bean hybrids in Nigeria. International Journal of Food Microbiology, 2013;316:108490.

[29] Medeiros ML, Ayrosa AMIB, de MoraesPitombo RN, da Silva Lannes SC. Sorption isotherms of cocoa and cupuassuproducts. Journal of Food Engineering, 2006;73(4), 402-406.

[30] Mabbetti T. Quality control for stored coffee and cocoa. International Pest Control Magasine, 2, 2013;55(2).