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Full Length Research Paper

Impact of cocoa processing technologies in free fatty acids formation in stored raw cocoa beans

Simplice Tagro Guehi^{1*}, Michael Dingkuhn², Emile Cros³, Gérard Fourny³, Robert Ratomahenina⁴, Guy Moulin⁴ and Anne Clement Vidal²

¹Laboratoire de Biochimie et de Technologie Alimentaire. UFR Sciences et Technologies des Aliments. Université d'Abobo-Adjame. 02 Bp 801 Abidjan 02. Côte d'Ivoire.

²CIRAD-AMIS, Equipe Ecotrop, TA 40/01, 34398 Montpellier Cedex 5, France.

³CIRAD-CP/Programme Cacao, Laboratoire de Chimie-Technologie du cacao, TA 80/16, 34398 Montpellier Cedex 5, France.

⁴INRA-Montpellier, Laboratoire de microbiologie industrielle et de génétique des microorganismes, 2 Place Viala, 34000 Montpellier, France.

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The quality of raw cocoa beans depends widely on their free fatty acids (FFA) content. High FFA content is a serious quality defect and reduces the technical and economic value of the cocoa beans. The work investigates the influence of cocoa processing technologies on FFA formation during storage of raw cocoa beans. Different samples of ferment dried cocoa beans purchased from Côte d'Ivoire were stored and analysed for FFA content. Very low FFA contents were found in whole healthy cocoa beans generally complied with UE standards (1.75% oleic acid equivalent) throughout storage while high FFA content was found in poor quality and broken healthy beans. The formation of FFA did not depend on the genotype or on cocoa post-harvest processing technologies. However, high and increasing FFA contents were observed in defective cocoa beans and could be attributed probably to the activity of microflora which in turn were associated with initial quality and loss of physical integrity of the cocoa beans.

Key words: Raw cocoa beans, processing technologies, free fatty acids content.

INTRODUCTION

Cocoa beans are actually seed from the fruit of *Theobroma cacao* tree. Cocoa butter, the naturally fat, contained in cocoa beans is the only continuous phase of chocolate. It is responsible for dispersion of the other constituents of chocolate properties during storage, handling and tasting (Nickless, 1996). Cocoa butter was currently reported to be the main vegetable fat used in the chocolate manufactures because of its rheological, textural and chemical characteristics such as triglycerides fatty acids composition, (Awua, 2002; Lipp and Anklam, 1998; Whitfield, 2005). Its hardness depends on the saturated and unsaturated fatty acid contents bound in triglycerides, and on free fatty acids (FFA) content. The

general opinion is that higher FFA content leads to a decrease in hardness of cocoa butter (Pontillon, 1998) and must be considered as a raw cocoa commercial value reducing factor both for producers and chocolate manufacturers. For reasons of quality therefore, the directive 73/241/EEC (EEC, 1973) limits the maximum FFA content to 1.75% oleic acid equivalent in cocoa butter. Yet, for several years, from 15 to 20% of annual Ivorian cocoa production is considered to have excessive FFA contents (exporters' statistics) recurrently and seasonally, notably at the end of the main season.

FFA are carboxylic acids released from triglycerides (Selamatet al., 1996) through the effect of a lipase (E.C. 3.1.1.3) or an oxidation. The risks of oxidation are negligible in cocoa butter due to its low unsaturated fatty acid content (Whitfield, 2005) and high content of polyphenols, natural antioxidants, in cocoa beans (Nickless, 1996). To date, although no lipase activity has

*Corresponding author. E-mail: g_tagro@hotmail.com. Tel: +225 66 11 23 44. Fax : + 225 20 37 74 48.

yet been detected in fermented and dried cocoa beans to our knowledge, the role of a lipase is strongly suspected. Numerous studies have shown plant lipase activity is mostly detectable during seed germination (Wanasundara et al., 2001), except in some oil crops such as the castor-oil plant (Ory et al., 1962), nigella kernel (Mert et al., 1995) and rice (Raghavendra and Prakash, 2002). For Fowler (1999), high FFA contents in cocoa beans might result from black beans originating from rotten pods or germinated beans. Likewise, microflora, particularly moulds, can cause similar problems during storage (Hiol, 1999). Indeed, Wood and Lass (1985) and Pontillon (1998) suggested that FFA occurrence in stored cocoa beans is linked to the action of microbial lipases. Therefore, this study aims to determine the impact of cocoa processing technologies on FFA formation in raw cocoa beans and if microflora of the cocoa beans are implied in FFA formation.

MATERIALS AND PROCESSING

Cocoa beans used in this study were originated healthy from region of Soubré in Côte d'Ivoire. A total of 16 samples (25 pods each) were opened and beans of each protocol were fermented using the micro-fermentation technique in a wooden box measuring 65 x 50 x 50 cm (turned manually after 48 and 96 h). Cocoa beans were sun dried on plastic tarpaulins for one week, reducing bean moisture content from 60 to 8%. All samples were stored in a climatic cabinet (Firlabo, SB-BVEHF type). Temperature and relative humidity were fixed respectively at 27°C and 75% for 6 months. For storage, 50 g of cocoa beans were individually putted in bags to be removed at each sampling time, 0, 2, 3, 4 and 5 months later. FFA content was measured on carefully shelled, manually and finely ground beans (< 500 µm).

Effect of cocoa beans processing technologies on FFA's formation was studied. Genetic origin's effect was studied using beans from three cultivars ripe pods: Amelonado, Ivorian 1st generation of hybrids (Amelonado x West African Trinitario) and open pollinated progenies. Influence of pod degree ripeness was studied using beans from pre-ripe, ripe and over-ripe pods of Ivorian 1st generation of hybrids. Pods were opened 5 days after harvesting. Beans from 1st generation hybrids pods harvested the same day were used for the impact of the time lapse between pods harvesting and opening. Pods were divided into three parts and were opened respectively the day of harvesting, 5 and 9 days after harvesting. Ripe pods from open pollinated progenies were used for study of fermentation duration's effect. Beans samples were taken in the middle of the cocoa beans mass each day throughout fermentation period and then were sun dried.

Effect of raw cocoa beans quality on FFA formation was studied using 1200 g of uncontrolled cocoa samples of three different qualities: healthy beans which are brown and compact with no visible defects, clustered beans and black beans originated from Abidjan harbour. Clustered beans are the agglomerates of 2 to several beans. Each sample was divided into 4 fractions: beans of first 2 fractions were individually and roughly broken and those of second 2 fraction remained whole. And then both sub-samples were stored (27°C, RH 75%). FFA content was measured on 50 g sampled from each fraction every two weeks.

About 1800 g of black beans samples were used in order to determine influence of decontamination of the cocoa beans and beans physical integrity in the FFA's formation in raw cocoa beans. The samples were divided into 6 equal portions: 2 untreated cocoa

beans of first fraction were stored as previously described, the beans of the 2 others parts are individually and roughly broken (control) and cocoa beans of last 2 fractions were broken and then individually and carefully decontaminated by soaking in a 5% (v/w) hydrogen peroxide solution (Sigma) for 3 hours at 28°C. After steriley drying at 27°C during 3 days about 40 g of each decontaminated cocoa beans were individually sealed hermetically into sterile flasks prior to storage for 12 weeks as previously described. FFA content was measured every two weeks on samples from one flask per experimental protocol and from over corresponding sub-samples.

Physicochemical analyses

About 15 g of cocoa beans were carefully shelled manually. Cocoa nibs were frozen in liquid nitrogen before finely grinding in a kitchen- scale coffee grinding (Moulinex, France) to the smallest particle (size < 500 µm). Ten grams of cocoa powder were putted in Whatman cartridge and soaked in 350 ml of petroleum ether (Prolabo Normapur, type 40-60°C) for one night. Cocoa butter was extracted on a Soxhlet apparatus for 8 h. After eliminating of solvent in a rotary evaporator, FFA contents were quantified by the official method 42-1993 (IOCCC, 1996). About 5 g of extracted cocoa butter was weighed (W) and dissolved in 50 ml of a previously hot petroleum ether/absolute ethanol mixture (1:1, v/v) neutralized by adding phenolphthalein. Titration carried out with 0.1N alcoholic KOH solution used (V) was noted. FFA (% oleic acid) was calculated as follows:

$$FFA \left(\% \text{ oleic acid} \right) = \frac{(28.2 \times V \times N)}{W}$$

Statistical analysis

Data represent the means of triplicate determinations. Analysis of variance were conducted using JMP software Version 5 (SAS Institute, 2002) with mean separation performed by the LSD test (P<0.05). Quadratic regression function at 0.05 confidence intervals was conducted using SigmaPlot 8.0 software.

RESULTS AND DISCUSSION

Effect of cocoa processing technologies on the FFA content

FFA content in whole healthy beans was low and ranged from 0.2 to 0.8%, irrespective of the genotype (Figure 1). FFA contents are not significantly different whatever the genotype of cocoa. In the similar FFA content of pods ripeness degree (results not shown) or pods opening delay are around the same values (Figure 2).

Slighter increasing FFA depending on the fermentation duration indicates a difference (LSD test, P<0.05) within fermented cocoa beans. Initial and final FFA contents in cocoa beans fermented during over 3 days are higher than those in cocoa beans fermented below 3 days. Cocoa beans fermentation duration seemed to be critical to increase the chances for FFA formation. Furthermore after 5 months' storage FFA contents remained below

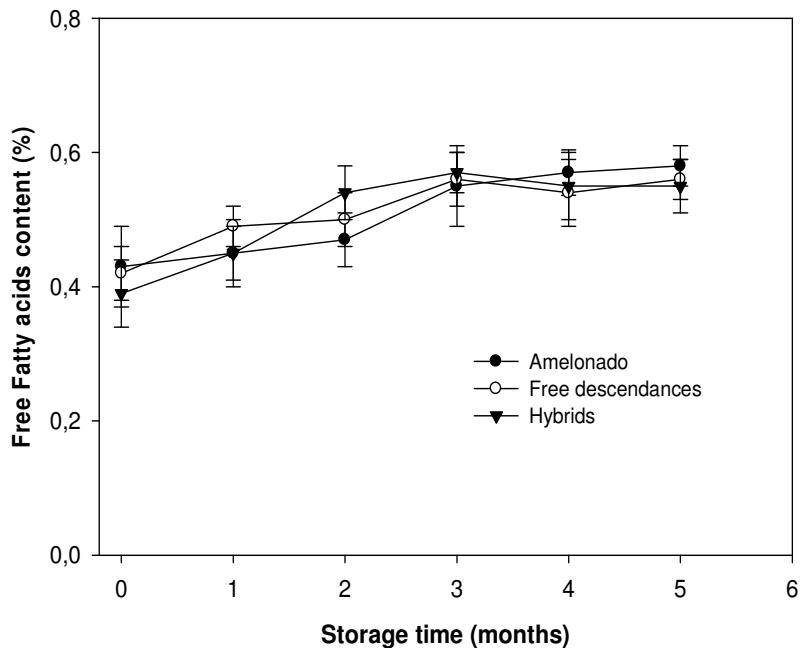


Figure 1. Effect of cocoa genotype on FFA content during storage at 27°C, 75% HR for up to 5 months. Bars represent the standard error of the mean (n=3).

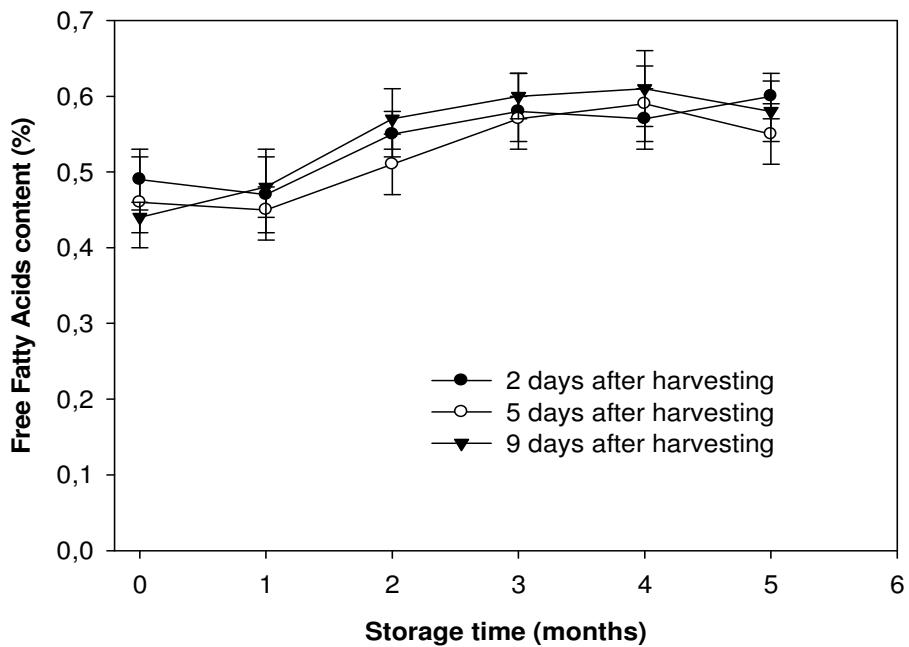


Figure 2. Effect of pod opening delay on FFA content in cocoa beans during storage at 27°C, 75% HR for up to 5 months. Bars represent the standard error of the mean (n=3).

1.75% (Figure 3). The small changes observed in FFA content in controlled cocoa samples indicate that any appropriate cocoa processing technology did not have an appreciable impact on FFA formation during correct storage.

Effect of cocoa beans quality, physical integrity and decontamination on FFA content

Initial FFA contents in uncontrolled cocoa beans sampled according their quality at Abidjan harbour were over 12-

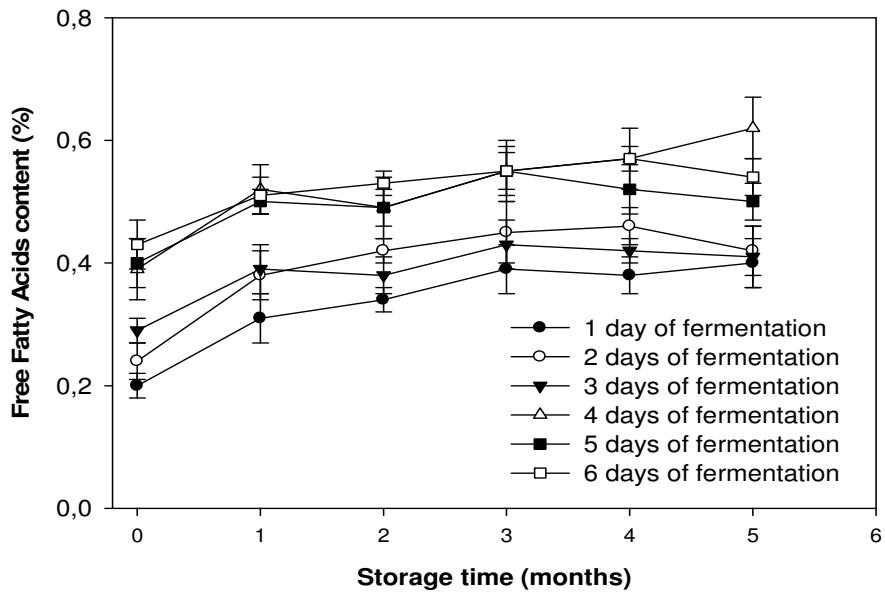


Figure 3. Effect of cocoa beans fermentation duration on FFA content during storage at 27°C, 75% HR for up to 5 months. Bars represent the standard error of the mean (n=3).

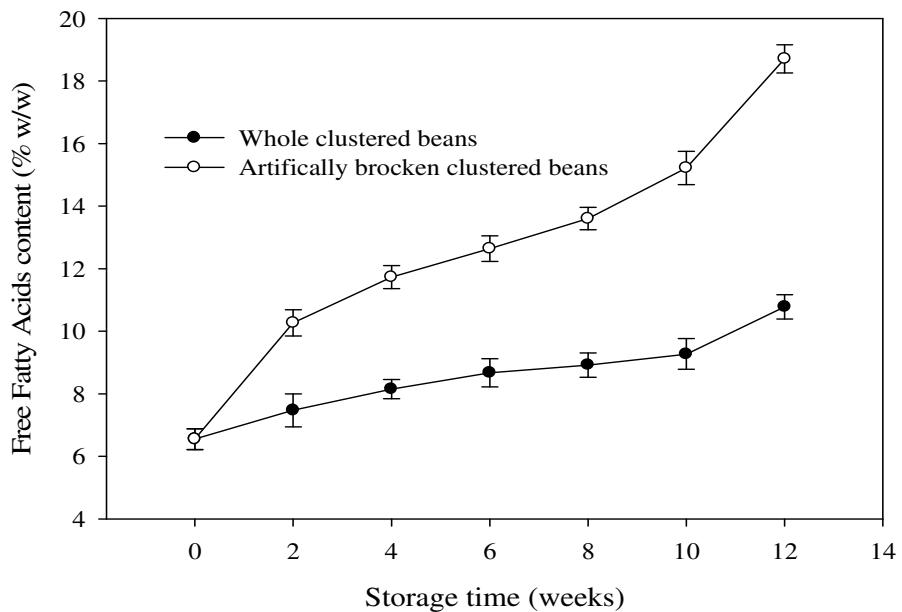


Figure 4. Influence of artificially broken clustered beans on FFA content during storage at 27°C, 75% HR for up to 2 months. Bars represent the standard error of the mean (n=3).

fold higher in both clustered beans (6.23%) and black beans (7.48%) than those in healthy and intact beans (0.48%). The low FFA content in whole healthy beans increased from 0.48 to 0.78% and did not exhibit an appreciable change during over 12 weeks' storage (results not shown). Considerable increasing FFA contents were observed in poor quality cocoa beans such as clustered beans where it increased from 6.23% to ca

11% (Figure 4). No variation of FFA content was noted in black beans where FFA content remained around 10% (results not shown).

Bean clustering could have been caused either by harvesting of unripe pods, or by poor separation of the beans and placentas during cocoa pods opening. So beans would be clustered in placentas during and after fermentation. Barel (1998) have mentioned that following

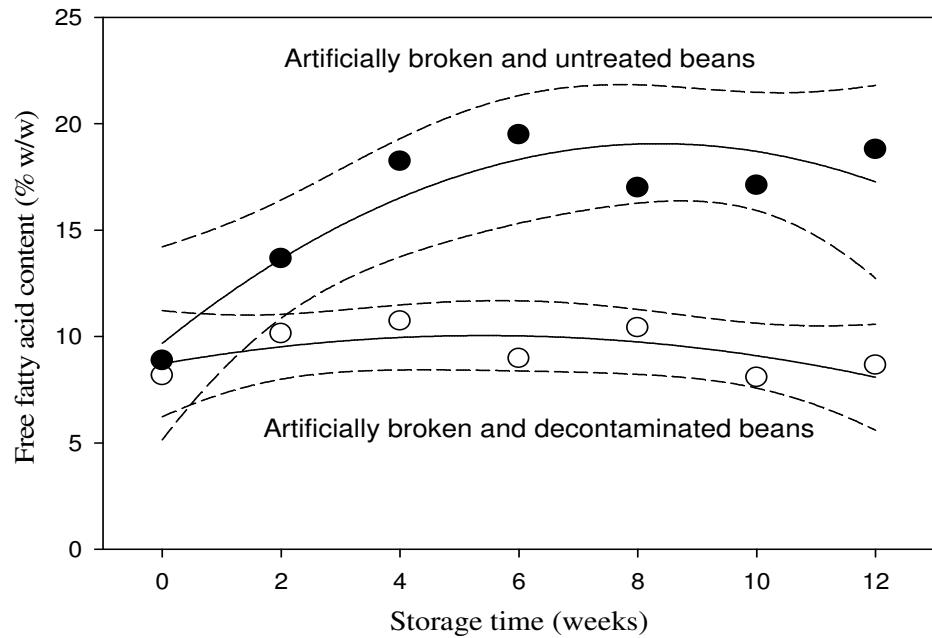


Figure 5. Variation of FFA content in decontaminated artificially broken black beans during storage. Solid lines indicate quadratic regression functions and broken lines indicate 0.05 confidence intervals for each function.

inadequate drying due to a surface crust clustered cocoa beans show always high moisture content which favoured moulds growth. According to Poisson and Cahagnier (1979) and Raghavendra and Prakash (2002) foodstuffs with high moisture content were easily attacked by moulds. These moulds could produce lipase (Wood and Lass, 1985) which in contact with cocoa butter of broken cocoa nibs released FFA from triglycerides. Black beans were probably originating from overripe pods infected by *Phytophthora* sp. Fungal diseases could create optimal conditions for the development of moulds (Renaud, 1954), which in turn would lead to FFA formation. However, variable FFA content of black bean sample was probably due high variation among individual beans and insufficient sample size. Given the variation of FFA content due probably to a highly heterogeneity of samples, it was not possible to characterize changes in FFA contents in storage conditions used. High FFA contents were due to poor quality of cocoa raw materials quality as previously demonstrated (Guénöt et al., 1976).

Slighter increasing of lower FFA contents in whole healthy beans than that in defective beans confirms that storage time did not affect FFA formation if processing technologies were previously appropriate. The greater the initial FFA content of the raw cocoa beans, or the lower the quality of the beans, the greater is this increase in FFA. And when cocoa beans were artificially broken, their FFA content increased strongly whatever cocoa beans initial quality. FFA content of decontaminated artificially broken beans remained unchanged and well below the considerably increasing FFA content in the

untreated cocoa beans. Using the quadratic regression functions at 0.05 confidence intervals indicates that the variations over storage time in FFA content of untreated cocoa beans sample and decontaminated sample are significantly different (Figure 5).

As decontamination prior to storage under sterile conditions stopped FFA formation in broken black beans only lipase activity of external origin can thus be considered. This conclusion was supported by many studies which demonstrated that plants in which lipases are detected during dormancy are rare. This is the case with the castor-oil plant (Ory et al., 1962) and nigella (Mert et al., 1995). Indeed, enzyme lipase is responsible for lipid breakdown in plants to produce the energy required for embryo growth (Imeson et al., 1993). Yet, during cocoa beans fermentation, the rise in temperature ($\geq 50^{\circ}\text{C}$) and diffusion of acid products through the cotyledons due to partial cell walls lyses, hence death of germ and prevent germination (Barel, 1998). In addition fermentation process induced contact between proteins and polyphenolic compounds hence inactivation of enzymatic proteins. Indeed many polyphenolic compounds in plants were already described as enzyme activities inhibition factor (Hagermann, 1992; Kallithraka et al., 2001; Misnawi et al., 2002). So cocoa beans endogenous enzymes could not be active in complete ferment dried cocoa beans (Yusep et al., 2002). Cocoa beans of poor quality deteriorate much faster than cocoa beans of high quality. Circumstantial evidence was presented that lipolytic activity leading to the FFA formation was due to microbes associated with other factors such as the quality, the phy-

sical condition, and the storage conditions of cocoa beans (Guénot et al., 1976, Oyeniran, 1979; 1980) and not to endogenous plant lipases by decontaminating part of the cocoa beans with hydrogen peroxide. Similar results were obtained by Hiol (1999) in previous study on formation of FFA in palm fruits caused by moulds. Thus cultivation and identification of microbes responsible of FFA formation in raw cocoa beans was not elucidated in this study, yet the relatively high FFA content exhibited by the poor quality beans and no change observed in FFA content of decontaminated beans were good evidence to support their potential lipase-producing abilities and their action on cocoa butter.

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