# Study on the origin of lipolytic activity involved in free fatty acid formation in cocoa beans

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***For several years, certain batches of cocoa from Côte d'Ivoire have been considered to have a free fatty acid (FFA) content well over the official standard (1.75% oleic acid equivalent). This leads to a decline in the quality and commercial value of Ivorian cocoa. The origin and optimum conditions of the lipase activity involved in this phenomenon were studied.***

## Material and methods

Lipase activity in cocoa was studied on acetone powder of shelled cocoa (source: exporters). The FFA formed were determined by the rhodamine 6G method of Chakrabarty *et al.* modified by Van Autryve *et al.*
The effect of the cocoa genotype, post-harvest processing and storage time on lipase activity and FFA content was studied on healthy cocoa harvested at the beginning and end of the 2000-2001 season in a private plantation at Soubré (Côte d'Ivoire). The microfermentation technique in 65 x 50 x 50 cm boxes (turning after 48 and 96h) was used, followed by sun drying.

- Assay 1. Genotype: Amelonado, 1st generation hybrids, open-pollinated populations.
- Assay 2. Ripeness: Cocoa from underripe, ripe and overripe pods of 1st generation hybrids.
- Assay 3. Pod opening delay: Cocoa from ripe pods of 1st generation hybrids harvested and opened the day of harvesting, 5 days after harvesting and 9 days after harvesting.
- Assay 4. Fermentation time: Cocoa from an open-pollinated progeny fermented for between 0 and 6 days.
- Assay 5. Storage time (climatic cabinet, 27°C, RH 75%): Monthly samples of 30 to 40 g.

The effect of bean quality and physical integrity on FFA formation was studied on samples (300 g) of healthy, clustered, black and naturally broken beans (source: exporters). The effect of cocoa decontamination was studied on broken black beans. FFA contents were determined every two weeks using the official method ICCO 42-1993.

## Results and discussion

Low lipase activity was found. Of the 5 substrates studied, it displayed greater affinity for cocoa butter and had two optimum pH values (Table 1). Irrespective of the harvesting period, there was no variation, be it for the genotype, post-harvest processing of storage time. The FFA content of healthy cocoa was very low and not affected by any of these factors; only fermentation revealed fluctuations in FFA contents (Cros, 2001).

| Characteristics | |
|---|---|
| Optimum number of successive cocoa powder defatting operations | 3 |
| Optimum defatted cocoa powder concentration (mg.ml$^{-1}$) | 20 |
| Optimum substrate concentration (g.ml$^{-1}$) | 0.25 |
| Optimum pH (2 optimums were observed) | 5,2 and 7,4 |
| Reaction mixture incubation time (min) | 10 |
| Reaction mixture incubation temperature (°C) | 40 |
| Preferential substrate out of 5 (olive oil, soybean oil, cocoa butter, triolein, tricetin) | Cocoa butter |

The initially low FFA content of healthy beans remained unchanged during storage. Only the initially high FFA content of defective beans (clustered and black) and of naturally broken beans, increased substantially (Fig. 1).

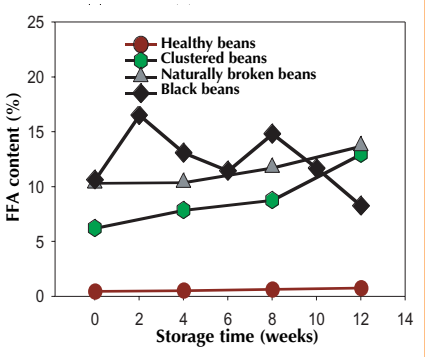
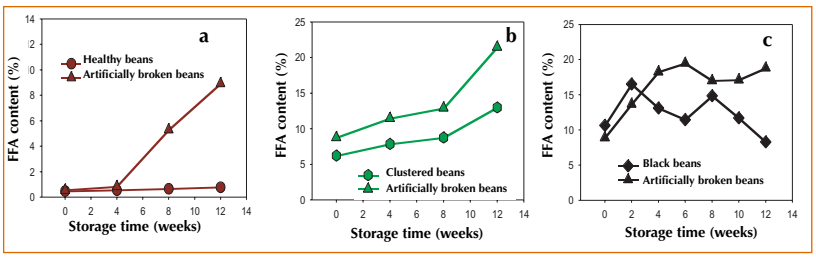



**Figure 1. Variation in FFA content during storage depending on bean quality.**

Irrespective of bean quality, an increase in FFA content was seen during storage for artificially broken beans (Fig. 2a, b, et c). The physical integrity of beans (existence of shells) therefore acts as a barrier to lipase activity involved in FFA formation in cocoa.

The FFA content of decontaminated broken black beans remained stable (from 8.17 to 8.66%), whereas that of the control broken beans increased significantly ($P < 0.05$-confidence interval determined with SigmaPlot V8.0) from 8.17 to 18.8% over 12 weeks' storage (Fig. 3).

**Figure 2. Effect of artificially broken beans on the variation in FFA content during bean storage: healthy (a), clustered (b), black (c).**



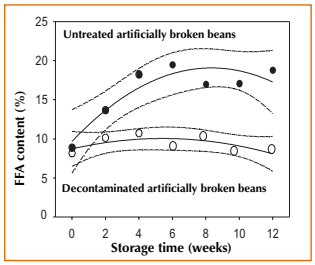




**Figure 3. Quadratic regression (solid lines) of the variation in FFA formation during storage in decontaminated broken black beans, and untreated beans. The dotted lines represent the confidence intervals for each function at $P < 0.05$.**

## Conclusion

FFA formation in cocoa does not arise from enzyme autolysis, but involves lipolytic activity of microbial origin combined with other factors such as bean quality, physical integrity and storage conditions.

**Reference**
Cros E., 2001. FIRC project: Search for factors responsible for high free fatty acid contents in Ivorian cocoa. Annual report, year 2, 42 p.

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