

# **Mycotoxins in Foodstuffs: Case of Aflatoxin B1 and Ochratoxin A in Dry Okra and Cassava Chips Marketed in Daloa (Côte d'Ivoire)**

**Ehouman Ano Guy Serge<sup>1\*</sup>, Kouassi Kouassi Clément<sup>1</sup>,  
Kouassi Kra Athanase<sup>1</sup>, Ehui Edi Jean Fréjus<sup>1</sup>, Beugre Grah Avit Maxwell<sup>1</sup>  
and Traore Karim Sory<sup>2</sup>**

<sup>1</sup>Agroforestry Training and Research Unit, Biochemistry-microbiology Department, Agrovalorisation laboratory, University Jean Lorougnon GUEDE, BP 150 Daloa, Côte d'Ivoire.

<sup>2</sup>Laboratory of Environmental Sciences, University Nangui Abrogoua, 02 BP 801 Abidjan 02, Côte d'Ivoire.

## **Authors' contributions:**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## **ABSTRACT**

The mycotoxins are toxic composites frequently found in foodstuffs causing some damages to consumers. This current study has been realised in order to estimate the content of Aflatoxin B1 and Ochratoxin A in dry okra and cassava chips frequently consumed by the population of Daloa. The analysis by chromatography in the liquid phase at high performance has shown that these foodstuffs contain Aflatoxin B1 and Ochratoxin A. These results reveal an average content of :  $2.58 \pm 3.65$   $\mu\text{g/Kg}$  and  $3.08 \pm 5.04$   $\mu\text{g/Kg}$  for the Aflatoxin B1 ;  $1.06 \pm 0.86$   $\mu\text{g/Kg}$  and  $0.61 \pm 0.24$   $\mu\text{g/Kg}$  for the Ochratoxin A respectively in the flour of cassava chips and the powder of dry okra. These concentrations are somewhat to higher than those allowed by the norm of Codex Alimentarius. Under this form, the consumption of these foodstuffs by the population expose them at risk of diseases.

\*Corresponding author: E-mail: [ehoumangserge@gmail.com](mailto:ehoumangserge@gmail.com);

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## 1. INTRODUCTION

The mycotoxins are natural toxins produced by some molds. They are part of natural contaminants of human and animal feeding [1, 2].

These toxins are found on numerous vegetal foodstuffs, notably cereals, but also the fruits and composite or manufactured from these products and intend to feeding [3]. They provoke many diseases in human and animals [4, 5]. In fact, these toxins can have carcinogen, mutagen, teratogen effects and immunosuppressors to the consumer. The mycotoxins are a real plague for hmanity [6]. They draw attention all over the world because of the great economic losses linked to their effects on the human health, the animal productivity and the international commerce.

In the developing countries, where foods are capable of being contaminated, the losses are more important in human population. This is why the present study is planned to estimate the content in Aflatoxin B1 and Ochratoxin A,

(cancerogen and nephrotoxin mycotoxins) in dry okra and cassava chips, foodstuffs too much consumed in Côte d'Ivoire in general and particularly in the department of Daloa.

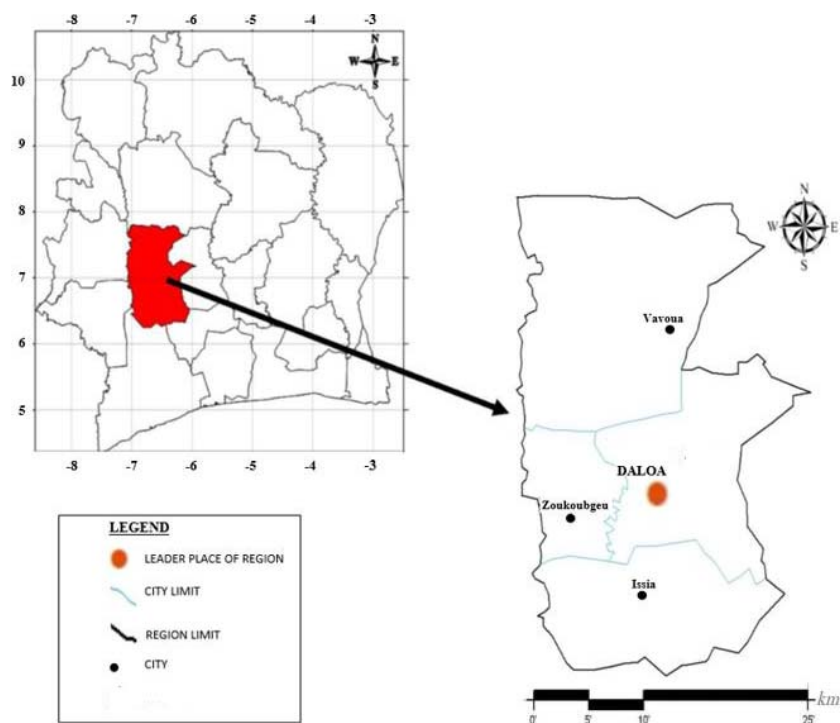
## 2. MATERIALS AND METHODS

### 2.1 Presentation of the Study Zone

This present study has been realised in Daloa, city located in center West of Côte d'Ivoire, in West Africa (Fig. 1). Main city of this department and of the administrative region of Haut-Sassandra. Daloa is an agricultural zone with a population of 591 633 inhabitants [7].

### 2.2 Choice of Foodstuffs

The mycotoxins (Aflatoxin B1 and Ochratoxin A) have been checked out in samples of dry okra and cassava chips (Fig. 2). These two foodstuffs are too much consumed by local population. With the treatments after harvest (drying and conservation techniques), these foods would represent a good indicator of contamination by the mycotoxins.



**Fig. 1. Geographical situation of the zone of study [8]**



**Fig. 2. Biological materials of study**

### **2.3 Determining the Content of Aflatoxins and Ochratoxin A in the Matrixes**

#### **2.3.1 Sampling**

Thirty (30) samples (15 samples per foodstuffs) have been taken on Daloa market with women sellers. This sampling has been done according to the mycotoxins analysis in accordance with the rule CE N° 401/2006 of February 23 2006 [9] and the technical accreditation guide LAB GTA 21 revised of the French committee accreditation (COFRAC) [10].

#### **2.3.2 Protocol of mycotoxins dose**

The mycotoxins have been analysed according to ISO 16050/2003 (F) and AFNOR NF norms [11, 12].

Twenty (20) g of grinded samples have been taken and put in a ball of 200 mL. Then a mixture of methanol/ distilled water (80/20; v/v) were added. The mixture has been made homogenous with a ultra-turax (OMNI MIXER) for four (4) minutes and then centrifuged to 5000 Tr/min for 5 min to 4 °C by a centrifuge (ZENTRIFUGEN 320R Helich). The lingering on liquid has been taken and filtered on a wattman paper N°4 in some erlenmeyers. Ten (10) mL of the filtrate has been taken and diluted in a 40 mL pad solution PBS (Salty solution of padded phosphate, 10 drops / liter water pH 7, 3). The immunoaffinity columns AFLAPREP R-Biopharm specific to aflatoxins and ochratoxins have been set out of the fridge and kept for 30 minutes at the normal temperature before use. These columns then emptied from their conservation liquid and conditioned with a 10 mL PBS Pad with a vacuum pump with a capacity of 1mL/min

to prevent the columns from drying. Then after 10 mL of the filtrate have been taken and purified on the immunoaffinity column with a flow rate of 1mL/min. The columns were washed with 10 mL of the same Pad. The mycotoxins washed out with 2 mL of methanol, and then 2 mL of distilled water added to the taken quantity. A 3 mL quantity put in a recuperation tub of 4 mL. A volume (20µL) of purified extract has been taken into a vial and injected in the chromatography in liquid phase at a high performance (SHIMADZU JAPON) with a fluorescence detector (RF-10AXL). The contents have been obtained with a computer provided with an integrator (software LC solution)

#### **2.3.3 Statistic analysis of data**

For the exploitation of data, statistic analysis have been done with the STATISTICA software 7.1. The descriptive statistic (elementary statistic) permitted to determine the maximum and minimum concentrations, the average concentrations and the standard deviation of mycotoxins concentration detected into the samples analysed. The similarity of the concentrations has been possible owing to a comparison from an average to the standard.

## **3. RESULTS**

### **3.1 Contents in Aflatoxin B1 in the Matrixes Studied**

The chromatographic analysis realised for the check of Aflatoxin B1 (AFB1) in the powder of dry okra and the flour of cassava chips has revealed that these two foodstuffs contain this toxin. The results of this analysis are reported in the following table (Table 1).

The Aflatoxin B1 has been detected in all the thirty (30) samples analysed (the powder of dry okra and the flour of cassava chips). The average contents of this toxin vary from a foodstuff to another. Concerning the powder of dry okra, the average concentration is 3.08 µg /Kg, with a maximum of 18.65 µg /Kg and a minimum of 0.02 µg /Kg. Almost half of the samples measured presented a content in Aflatoxin B1 higher than the normal.

Concerning the flour of cassava chips, the average content in Aflatoxin B1 is 2.58 µg /Kg, with a maximum of 12.43 µg /Kg and a minimum of 0.04 µg /Kg.

### 3.2 Contents in Ochratoxin A in the Matrixes Studied

Like the Aflatoxin B1, the Ochratoxin A (OTA) has been detected in the foodstuffs analysed. The average concentrations range from 0.61 µg/Kg (powder of dry okra) to 1.06 µg/Kg (flour of cassava chips). Once again, all the samples

analysed contain Ochratoxin A (100%). Nevertheless, the contents quantified in the foodstuffs (power of dry okra and flour of cassava chips) are under the norm agreed on by European Commission [13]. In no sample taken, the content has been higher to the prescribed norm (Table 2).

### 3.3 Comparison of Aflatoxin B1 and Ochratoxin A Levels in the Matrixes Studied against the Norms

The results of analyses on the dry okra and the cassava chips revealed that these foodstuffs contain some mycotoxins (AFB1 and OTA). In fact, for the fifteen (15) samples analysed for each matrix, some contamination rates up to 100% are observed. These results reveal an average content of : 2.58 ± 3.65 µg/Kg and 3.08 ± 5.04 µg/Kg for the Aflatoxin B1 ; 1.06 ± 0.86 µg/Kg and 0.61 ± 0.24 µg/Kg for the Ochratoxin A respectively in the flour of cassava chips and the powder of dry okra.

**Table 1. Contents of Aflatoxin B1 in the foodstuffs studied**

N= 15	Contents of AFB1 in the studied foodstuffs	
	Powder of dry okra	Flour of cassava chips
Average	3.08 ± 5.04	2.58 ± 3.65
Maximum	18.65	12.43
Minimum	0.02	0.04
Rate of contaminated samples	100	100
Number of contaminated samples > the norm	7	4

*N= Number of samples.*

**Table 2. Contents of Ochratoxin A in the foodstuffs analysed**

N= 15	Contents of AFB1 in the studied foodstuffs	
	Powder of dry okra	Flour of cassava chips
Average	0.61 ± 0.24	1.06 ± 0.86
Maximum	1.07	3.05
Minimum	0.22	0.29
Rate of contaminated samples	100	100
Number of contaminated samples > the norm	0	0

**Table 3. Comparison of average concentrations in AFB1 and OTA in the powder of dry okra and the flour of cassava chips with the norms (LMR).**

Mycotoxins detected	Samples	Average contents (µg /kg)	Norm (µg/kg)	Amount of samples	p
Aflatoxin B1	PDO	3,084±5,041	2,000	15	0,418
	FCC	2,581±3,649	2,000	15	0,547
Ochratoxin A	PDO	0,608±0,241	5,000	15	0,000
	FCC	1,062±0,861	5,000	15	0,000

*PDO: power of dry okra; FCC: flour of cassava chips; P: probability (Tukey's test,  $p \leq 0.05$ ,  $N= 15$ ). ( $P \leq 0.05$ ): difference between the average concentrations and the norms*

#### 4. DISCUSSION

The objective of this study was to evaluate the levels of aflatoxin B1 (AFB1) and Ochratoxin A (OTA) in dry okra and cassava pods, widely consumed foodstuffs in the department of Daloa (Côte d'Ivoire). This study is in the context of those [14].

For the latter, 25% of foodstuffs produced worldwide are contaminated by toxins.

The results of the analyses on dry okra and cassava chips revealed that these foodstuffs contain mycotoxins (AFB1 and OTA). Indeed, on fifteen (15) samples analyzed for each matrix, contamination rates of 100% were observed. These results reveal an average content of aflatoxin B1 and ochratoxin A of  $2.58 \pm 3.65 \mu\text{g}/\text{kg}$  and  $1.06 \pm 0.86 \mu\text{g}/\text{kg}$  respectively in cassava flour and  $3.08 \pm 5.04 \mu\text{g}/\text{kg}$  and  $0.61 \pm 0.24 \mu\text{g}/\text{kg}$  in dry okra powder.

Similar results were obtained by [15] with aflatoxin B1 contamination rates also of 100% in dry Okra powder, cassava flour, peanut paste and Corn flour. These high contamination rates would be linked to poor agricultural practices (field, storage, processing ...) [16, 17]. However, it should be noted that very few studies have been conducted on the same matrices. Nevertheless, matrices with similar compositions to cassava husks such as rice and other cereals have been the subject of several studies. In Vietnam, a study on rice gave contamination levels of 25% for AFB1, OTA as well as CIT [18]. In 2007, studies by [19]. on barley, corn and wheat gave AFB1 positivity rates of 55%, 40% and 40% respectively. In Tunisia, studies by [20] on sorghum gave contamination rates of 59.37% AFB1, 37.5% OTA and 32.81% ZEN. These contaminations would probably be related to insufficient heat treatment (drying) thus high moisture content [21, 22]. According to [14] this contamination occurs during harvest. For these authors, the harvesting of roots and tubers such as cassava is done during the rainy season. Water would facilitate the harvest but would favor the proliferation of these mycotoxins.

Like cassava flour, contamination of dry okra flour is also the result of inappropriate drying [18, 22] Added to this is the cutting of okra. A study conducted by Ouoba et al. [23] showed that women with different sizes and shapes of cuts generally practice traditional drying in Africa. Cutting the okra lengthwise by dividing it into four

pieces dries better than cutting it into slices thicker than 10 mm. All cut samples dry better than whole okra. Also, storage of foods with moisture content >10% for prolonged periods and in inadequate facilities leads to mold growth [24] Knowledge of the carcinogenicity of aflatoxins and ochratoxins prompts comparisons between these levels and those recommended by the standard. These standards are  $2 \mu\text{g}/\text{kg}$  for aflatoxin and  $5 \mu\text{g}/\text{kg}$  for ochratoxin (EC regulation N° 1881/2006). The statistical analysis carried out showed that only the cassava chips flour contained Aflatoxin B1 in quantity higher than the standard. This concentration is  $2.581 \pm 3.649 \mu\text{g}/\text{kg}$ . These results are in agreement with those of [15]. Indeed, in their study, only dry okra flour has a concentration ( $2.16 \mu\text{g}/\text{kg}$ ) of AFB1 above the standard. Those of [19] fall within the same framework. Slightly more worrying results are observed [25] in peanut and pistachio pastes with average concentrations of  $18.4 \mu\text{g}/\text{kg}$  and  $30.6 \mu\text{g}/\text{kg}$  respectively. In Egypt, a higher concentration of about  $1076.5 \mu\text{g}/\text{kg}$  is observed in cocoa paste [26]. The low concentration of mycotoxins in dry okra and cassava chips would be related to the fact that these commodities are dehydrated and the climate of the study area [27]. These results are far from those of [28] for whom the temperature that the study area offers is conducive to the development of AFB1 and OTA. According to Dieme et al. [29], it is in African, South Asian, and South American countries, where the climate is hot and humid, that mycotoxin contamination is more common.

#### 5. CONCLUSION

The chromatographic analysis done on the dry okra and the cassava chips consumed in the department of Daloa has shown that these foodstuffs are contaminated with the mycotoxins (Aflatoxin B& and Ochratoxin A). These different mycotoxins are present with  $2.58 \pm 3.65 \mu\text{g}/\text{Kg}$  and  $3.08 \pm 5.04 \mu\text{g}/\text{Kg}$  for the Aflatoxin B1 ;  $1.06 \pm 0.86 \mu\text{g}/\text{Kg}$  and  $0.61 \pm 0.24 \mu\text{g}/\text{Kg}$  for the Ochratoxin A respectively in the flour of cassava chips and the powder of dry okra. These concentrations are sometimes superior to those ordered by the norm. These toxins provoke too many diseases in humans and animals. They create important economic lost linked to their effects on the human health, the animal productivity and the international commerce. We should particularly pay attention to this plaque in order to reduce the risks and preserve the consumers' health.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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