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Mycotoxins in Food: Evaluation of Aflatoxin B1 and Ochratoxin A in a Few Foodstuffs in Côte D'Ivoire

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Abstract

The objective of this work was to determine the occurrence of mycotoxins like aflatoxin B1 and ochratoxin A in a few foodstuffs consumed by the Ivorian population. The levels of AFB1 are higher than the contents of OTA. Dry gumbo is the most contaminated food in AFB1 with an average grade of 71.18 µg/kg. Adjo van has an average AFB1 concentration of 64.65 µg/kg. The levels of AFB1 of cassava are on average 4.17 µg/kg. Dried maize is the least contaminated commodity with an average concentration of 2.28 µg/kg. The mean levels of Aflatoxin B1 in the range of 4.17 to 71.18 µg⁻¹.kg⁻¹ exceed the maximum residue limit (MRL) of 2 µg/kg for cereals and vegetables and 5 µg/kg for spices. The most contaminated samples of ochratoxin A (OTA) samples were dry cassava and adjo van with average concentrations of 0.94 µg/kg and 0.92 µg/kg. Low levels are 0.60 µg/kg and 0.48 µg/kg respectively for dry gumbo and dry maize. These mean OTA values of the samples analyzed are well below the maximum residue limit (MRL) of 5 µg/kg for vegetables, cereals and spices. This study presents the results concerning the safety assessment of a few foodstuffs regarding mycotoxins in Côte d'Ivoire.

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Introduction

The word mycotoxin is derived from the Greek word 'mykes' meaning 'fungus' and the Latin word 'toxicum' meaning 'poison'. They are low molecular weight molecules produced as secondary metabolites by saprophytic fungi, especially *Aspergillus*, *Penicillium* and *Fusarium* (Aiko and Mehta, 2015). Mycotoxins are natural products, synthesized by fungi, capable of causing a toxic response when entering naturally (ingestion, inhalation or absorption through the skin) in

animals or the human body (Bennett, 1987). According to the World Health Organization, 25 % of the foodstuffs are contaminated with mycotoxins, which are responsible for significant economic losses (Mannon and Johnson, 1985). Good agricultural practices, plant disease management, and adequate storage conditions limit mycotoxin levels in the food chain yet do not eliminate mycotoxins completely. Aflatoxins, fumonisins, ochratoxin A, zearalenone and deoxynivalenol are mycotoxins that are detected in cereal crops (Ezekiel et al., 2014; Warth et al., 2012;

Juan et al., 2014; Zinedine and Idrissi, 2007) and in peanuts (Afolabi et al., 2015). Among dangerous mycotoxins, aflatoxins (AFs) and ochratoxin A (OTA) represent the greatest health risk in tropical Africa (Manjula et al., 2009), in Asia (Li et al., 2014) and the rest of the world (Alborch et al., 2012) due to their high toxicity. They are known to be carcinogenic, genotoxic, teratogenic, nephrotoxic, hepatotoxic and immunotoxic for humans (Creppy, 2002; Mahmoudi et al., 2012). Aflatoxins and ochratoxin A were classified in carcinogenic group 1 and 2B, respectively, by the International Agency for Research on Cancer in 1993 (IARC, 1993). Among aflatoxins, aflatoxin B1 (AFB1) is the most toxic form for mammals and causes damages such as toxic hepatitis, hemorrhage, edema, immunosuppression and hepatic carcinoma (Speijers and Speijers, 2004). In fact, various epidemiological studies have implicated the AFs and OTA in the increased incidence of gastrointestinal and liver cancer in Africa, Philippines and China (Zinedine and Idrissi, 2007). Recently, cases of acute poisoning affecting a large geographic area in Kenya causing many deaths were reported by Centers for Disease Control and Prevention

(CDC, 2004). In Côte d'Ivoire, drying of foodstuffs takes place on the ground, along roads, on mats, on plastic films, on flat rocks, on the roofs of dwellings. The purpose of drying is to greatly reduce the various reactions involved in the normal decomposition of the product. But the drying and fermentation of foodstuffs may be subject to the proliferation of molds producing mycotoxins (CTA, 2008). For this reason, this study aims to draw the attention of the ivoirien population to the existence of mycotoxins (ochratoxin A and aflatoxin B1) in dried or fermented foodstuffs consumed by populations. Specifically, it will be necessary to identify and quantify the mycotoxins likely to be present in these foodstuffs consumed by the ivoirien population.

Materials and methods

Biological materials

Mycotoxins (aflatoxin B1 and ochratoxin A) were searched in dry maize, dry Gumbo, dry cassava (chips) and fermented fish (adjowan) samples (Figs. 1a, 1b, 1c and 1d).



Fig. 1: Foodstuffs (a) Dry maize ; (b) Dry gumbo ; (c) Dry cassava ; (d) Fermented fish (Adjowan).

Methods

Sampling: Dried cassava, dried maize and dried Gumbo samples were collected from Abobo (Abidjan, Côte d'Ivoire) at the main market. Adjoan samples were taken from the commune of Port-Bouët (Abidjan, Côte d'Ivoire).

Methods of preparation and extraction of mycotoxins

General principle: Whatever the material used (dry maize, dry gumbo, dry cassava and adjoan), the principle of the technique is based on an acidified chloroform extraction in the presence of magnesium chloride. The mycotoxins are extracted under acidic conditions from the samples of the crushed matrices. The acidification and the ionic strength of the extraction solution make it possible to partly break the bonds of the toxins to the constituents of the matrix and thus promote their extraction. The purification of the extract is carried out by phase sharing. The mycotoxins are soluble, sometimes in the aqueous phase, sometimes in the organic phase depending on their ionization state.

Preparation of standard toxin solutions: The powdered mycotoxins (OTA, AF) are solubilized in ultra pure methanol. One (1) mg of toxin is weighed on the precision balance and dissolved in 1 mL of solvent. For OTA this stock solution is diluted one hundred times and the concentration of the diluted solution is determined precisely by assaying the absorbance to the spectrophotometer according to the following parameters:

- For OTA ($PM = 403.8 \text{ g}^{-1} \cdot \text{mole}^{-1}$): Absorbance measurement at 330 nm. At this wavelength, the molar absorption coefficient of Ochratoxin A in methanol is $5500 \text{ mol}^{-1} \text{ cm}^{-1}$.
- For AF: Measurement of the absorbance at 360 nm, the molar absorption coefficient in methanol ϵ is $21800 \text{ mol}^{-1} \text{ cm}^{-1}$.

Extraction of mycotoxins from different food matrices: The samples were analyzed by the method developed by Molinié et al. (2005). The extraction is carried out in an acid medium and the purification is carried out by liquid-liquid partition, in order to allow the simultaneous extraction of the OTA and AFB1, and to defect the mycotoxins from their binding with proteins. fifty (50) g of each matrix (dry maize, dry gumbo, dry cassava and adjoan) finely ground are

mixed with 400 mL of extraction solvent composed of acetonitrile/water (9:1). The water contains 4 % of potassium chloride and 0.8 mL of concentrated sulfuric acid. The mixture is placed in a 500 ml Erlenmeyer flask and placed on a rotary shaker for 20 minutes. The extract is filtered under vacuum on filter paper (Wattman No. 4). After filtration, an aliquot of 200 mL of this filtrate is defatted twice, by adding 100 mL of n-hexane. The defatted filtrate is placed in a separatory funnel for one minute. The lower phase is recovered in a 250 mL Erlenmeyer flask. The mycotoxins are extracted with 100 mL of chloroform from the lower acetonitrile/water phase to which 50 mL of ultra pure water have been added. The mixture of acetonitrile/water and chloroform is mechanically stirred. After a decantation of 10 minutes, the chloroform phase (lower) is recovered in an Erlenmeyer flask. The acetonitrile/water phase (upper phase) is added twice with 20 ml of chloroform. After stirring for 10 minutes and decanting also for 10 minutes, the chloroform phase capable of containing the mycotoxins is recovered. At the end of these three extractions, the mycotoxins dissolved in the chloroform fractions are extracted three times by the addition of 50 ml of a 5 % sodium bicarbonate solution. After stirring (10 minutes) and decantation (10 minutes), the upper bicarbonate phase containing the mycotoxins is recovered. At the end of the three extractions with bicarbonate, all the bicarbonate fractions are combined in the same Erlenmeyer flask.

The extract is acidified to pH 1.5 by the addition of concentrated hydrochloric acid. Finally mycotoxins are extracted from this mixture three times with chloroform (volumes of 100, 50 and 50 mL) after stirring (10; 5 and 5 minutes) and decantation (10 minutes at each extraction step). The chloroform extract (lower phase) containing the mycotoxins is recovered in an Erlenmeyer flask. The whole of the combined chloroform extracts are evaporated (under vacuum) with a rotavapor at 45 °C. The volume of chloroform is reduced to about 1 mL. The dry extract is taken up with 1 ml of methanol and placed in an ultrasonic bath for one minute. When the resuspension is complete, this mL of methanol is filtered over a 0.2 μm filter previously conditioned with methanol, that is to say that the filter is rinsed with 500 μL of methanol. The filtered methanol is placed in a hemolysis tube and the filtrate is dried in a water bath under a nitrogen stream to recover the mycotoxins. After drying, the extract resuspended in 500 μL of methanol is placed in a glass flask, stored at -20 °C before being analyzed by HPLC.

Chromatographic analysis (HPLC) of mycotoxins

High Performance Liquid Phase Chromatography (HPLC) allowed the separation of the various compounds contained in the mixture in order to characterize and quantify them. The detection and quantification of the OTA was carried out by fluorimetric detection. The reading was done by a fluorodensitometry reader. Thus, the fluorimetric detector had an excitation wavelength of 333 nm and an emission wavelength of 460 nm. Confirmation of the presence of aflatoxins in the sample by HPLC requires derivatization of aflatoxins B1 and G1. This is necessary in order to increase their natural fluorescence under UV light, and thus be able to detect them better. Aflatoxin B1 was detected at wavelengths of excitation at 362 nm and emission filters at 425 nm.

The mobile phase A was water-methanol (55:45; v/v), 119 mg of potassium bromide and 350 mL of nitric acid and the mobile phase B was water-methanol (20:80; v/v), 119 mg of potassium bromide and 350 mL of nitric acid. AFs and OTA standard solutions were used for the construction of a five-point calibration curve of peak areas versus concentration (ng/mL). The operating conditions were as follows: injection volume of 100 mL of sample and standard solutions; C18 reverse-phase HPLC column, Uptisphere type, ODS, 5 µm particle size, 5 ODB, 250 x 4.6 mm, with identical pre-column, thermo-

statically controlled at 40 °C; isocratic flow rate of 0.8 mL/min. Mobile phase gradient: mobile phase A: 0 % (0-26 min); 65 % (26-45 min); 0 % (45-50 min); 41 % (20-25). The detection and quantification limits on aflatoxins were 0.3 µg/kg and 1 µg/kg, respectively.

The detection and quantification limits on ochratoxin A were 0.05 µg/kg and 0.1 µg/kg, respectively. The contents were calculated from a calibration curve established with aflatoxins (TSL-108, Biopharm Rhône Ltd, Glasgow, UK) and ochratoxin standards (TSL-504, Biopharm Rhône Ltd, Glasgow, UK).

Statistical analysis

The statistical analysis was performed on the results using SPSS (version 10.0) software. The comparison of the variables measured during this study was done using the analysis of variance (ANOVA) and Duncan test. The differences were considered significant if $p \leq 0.05$. All the experiments were conducted in triplicate.

Results and discussion

The results showed that samples of dried cassava, dried maize, dried gumbo and adjoan harvested in the communes of Abobo and Port Bouet contained mycotoxins (Aflatoxin B1 and Ochratoxin A) at different concentrations. The levels of AFB1 are higher than the contents of OTA (Fig. 2).

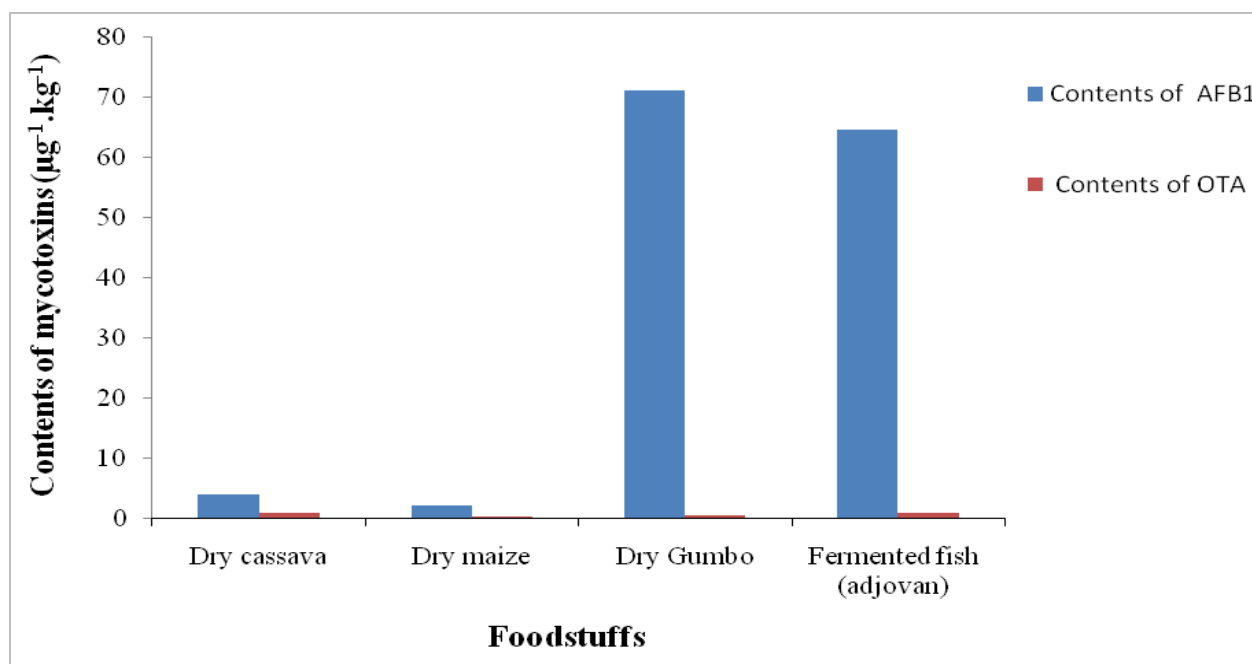


Fig. 2: Contents of AFB1 and OTA in foodstuffs.

Several authors have established that AFs have been detected in various commodities such as maize, wheat, barley, nuts, cocoa, dried fruits, wines and spices and other foodstuffs (Juan et al., 2014; Se and Nadir, 2003). OTA contaminates cereals such as barley, wheat, maize, oat, as well as green coffee, fruit juices (grape fruit), wines and spices (Juan et al., 2014; Zinedine and Idrissi, 2007). However, environmental factors which effect mold growth and mycotoxin production are temperature, pH, moisture content, oxygen levels, nutritional components, the mold strains and microbial competition (Jackson and Al-Taher, 2008). This explains the fact that the levels of AFB1 are higher than the contents of OTA. Dry gumbo is the most contaminated food in AFB1 with an average grade of 71.18 $\mu\text{g}/\text{kg}$ (Table 1).

Table 1. AFB1 and OTA content in dried gumbo.

Samples	AFB1 ($\mu\text{g}^{-1}.\text{kg}^{-1}$)	OTA ($\mu\text{g}^{-1}.\text{kg}^{-1}$)
1	160	0.59
2	49.6	0.57
3	156	0.68
4	70.6	0.71
5	48.7	0.59
6	89.4	0.64
7	48.4	nd
8	50.1	0.54
9	41.2	nd
10	37.8	0.51
11	31.2	nd
Total average concentration ($\mu\text{g}^{-1}.\text{kg}^{-1}$)	71.18	0.60
<i>nd: not detected.</i>		

Table 2. AFB1 and OTA content in Adjovan.

Samples	AFB1 ($\mu\text{g}^{-1}.\text{kg}^{-1}$)	OTA ($\mu\text{g}^{-1}.\text{kg}^{-1}$)
1	104	0.48
2	56.7	1.23
3	43.8	nd
4	53.7	0.31
5	89.5	1.32
6	42.7	nd
7	48.2	nd
8	87.4	1.48
9	71.2	0.72
10	49.3	nd
Total average concentration ($\mu\text{g}^{-1}.\text{kg}^{-1}$)	64.65	0.92
<i>nd: not detected.</i>		

Indeed, the high contamination of dry gumbo can be explained by the fact that gumbo is dried in its entirety

(high humidity). It dries hard because the sun's rays will take time to reach the interior and the food is prone to the proliferation of aflatoxin B1 producing molds. Sliced gumbo dries generally faster than whole food, but slices should be no more than 1 cm thick to dry completely and quickly to prevent mycotoxin proliferation (FAO, 2002). Adjovan has an average AFB1 concentration of 64.65 $\mu\text{g}/\text{kg}$ (Table 2). Yet before being dried the adjovan fish underwent a salting which prevents the microbial proliferation by decrease of the water of the product. It is thus protected from the ambient air by this envelope of salt. But the samples of adjovan could be contaminated with AFB1 when drying by the materials used, ambient air, rodents or even the geographical location (industrial zone). Indeed, the place of drying of the adjovan is near a garbage dump and stagnant water which are vectors of contamination of mycotoxins (Brochard and le Bâcle, 2009). The levels of AFB1 of cassava are on average 4.17 $\mu\text{g}/\text{kg}$ (Table 3).

Table 3. AFB1 and OTA content in dry casava.

Samples	AFB1 ($\mu\text{g}^{-1}.\text{kg}^{-1}$)	OTA ($\mu\text{g}^{-1}.\text{kg}^{-1}$)
1	29.82	2.36
2	4.44	0.35
3	12.43	0.87
4	0.65	0.29
5	0.75	nd
6	1.34	0.42
7	2.33	1.63
8	0.89	0.29
9	1.25	0.87
10	1.44	2.78
11	1.21	0.38
12	0.87	nd
13	0.58	nd
14	nd	0.12
15	0.02	nd
16	0.43	nd
Total average concentration ($\mu\text{g}^{-1}.\text{kg}^{-1}$)	4.17	0.94
<i>nd: not detected.</i>		

Dried maize is the least contaminated commodity with an average concentration of 2.28 $\mu\text{g}/\text{kg}$ (Table 4). Dried cassava (4.17 $\mu\text{g}/\text{kg}$) and dried maize (2.28 $\mu\text{g}/\text{kg}$) can be derived from the conditions under which they were harvested, dried and stored. According to Ilhame and Aziz (2006), mycotoxin contamination of food can occur during harvest and storage. Indeed, if the food is not well dried or rainfall occurs during drying, the high humidity may favor the proliferation of mycotoxins. Fluoxins are produced by the Aspergillii; Particularly

avored mushrooms in a hot and humid environment. Aflatoxin production by *Aspergillii* occurs at high humidity (above 0.7) and temperatures sometimes above 30°C (Ilhame and Aziz, 2006). These factors may also explain high levels of Aflatoxin B1 in the samples because Côte d'Ivoire is in a tropical humid climate; Where temperatures sometimes exceed 30°C and relative humidity is high (Rougerie, 1978).

Table 4. AFB1 and OTA content in dry maize.

Samples	AFB1 ($\mu\text{g}^{-1}.\text{kg}^{-1}$)	OTA ($\mu\text{g}^{-1}.\text{kg}^{-1}$)
1	3.31	1.02
2	0.75	0.12
3	10.08	nd
4	1.77	nd
5	4.5	nd
6	0.58	0.35
7	1.34	1.03
8	0.95	0.29
9	0.89	0.85
10	2.31	nd
11	0.96	0.49
12	nd	0.22
13	nd	0.31
Total average concentration ($\mu\text{g}^{-1}.\text{kg}^{-1}$)	2.28	0.48

nd: not detected.

Also, in Côte d'Ivoire drying is usually done in the open air (on the ground, in the yard, at the roadside, etc.) and therefore within the reach of dust or animal dirt. In addition, foods are naturally in contact with fungal spores before, during and after harvest, during transport and storage. Rodents, birds, insects and mites etc. Are involved in the contamination process by causing physical damage to plant tissues that promote spore

penetration and mycotoxin production (Le Bars and Le Bars, 1982).

The mean levels of Aflatoxin B1 in the range of 4.17 to 71.18 $\mu\text{g}^{-1}.\text{kg}^{-1}$ (Table 5) exceed the maximum residue limit (MRL) of 2 $\mu\text{g}/\text{kg}$ for cereals and vegetables and 5 $\mu\text{g}/\text{kg}$ for spices (EC, 2001; 2002). Their consumption therefore poses a risk to human and animal health because AFB1 is carcinogenic to our organs and especially the liver (Lovelace and Aalbersbekg, 1989). A relationship between the consumption of foods contaminated by these molds and the development of liver cancer has been established (Shank et al., 1972).

The most contaminated samples of ochratoxin A (OTA) samples were dry cassava and adjovan with average concentrations of 0.94 $\mu\text{g}/\text{kg}$ (Table 2) and 0.92 $\mu\text{g}/\text{kg}$ (Table 3). Low levels are 0.60 $\mu\text{g}/\text{kg}$ (Table 1) and 0.48 $\mu\text{g}/\text{kg}$ (Table 4) respectively for dry gumbo and dry maize. These mean OTA values of the samples analyzed are well below the maximum residue limit (MRL) of 5 $\mu\text{g}/\text{kg}$ for vegetables, cereals and spices (EC, 2001; 2002). The OTA samples can be explained by the fact that the average temperatures in Côte d'Ivoire revolve around 28°C (Rougerie, 1978). This promotes the proliferation of molds producing OTA. Cereal-based food products such as maize sometimes contain ochratoxin A, and the amounts found are generally low. Families of *Aspergillus* and *Penicillium* mostly proliferate on the surface of grains (Ngundi et al., 2006). Thus, the drying could greatly reduce the water content of the samples analyzed; this has slowed down the proliferation of molds producing OTA. Even if the levels of these OTA samples are below the maximum residual limits, low OTA accumulation in the body can be toxic in the long term. These contents can be reduced if the foodstuffs are dried and stored under hygienic conditions.

Table 5. Comparison of the levels of foodstuffs in AFB1 and OTA with standards (CE, 2001; 2002).

	Dry casava		Dry maize		Dry gumbo		Adjovan	
	AFB1	OTA	AFB1	OTA	AFB1	OTA	AFB1	OTA
Total average concentration ($\mu\text{g}^{-1}.\text{kg}^{-1}$)	4.17	0.94	2.28	0.48	71.18	0.60	64.65	0.92
Standard ($\mu\text{g}^{-1}.\text{kg}^{-1}$)	2	5	2	5	2	5	5	5

Conclusion

Analysis of our samples of dried gumbo, dried cassava, fermented fish (adjovan) and dry maize showed that AFB1 and OTA producing molds develop in our foods in Côte d'Ivoire. These molds proliferate in conditions of

humidity and temperature which are favorable to them and produce mycotoxins which have toxic and even carcinogenic effects on the health of humans and animals. Aflatoxin B1 is the most toxic of aflatoxins while ochratoxin A is the most toxic of ochratoxins. The average concentrations of our samples are very high in

AFB1 and largely exceed the MRLs. These samples contain levels no less negligible in OTA. Knowing that these mycotoxins are carcinogenic; it would be time to worry about the drying and storage conditions of these foodstuffs in order to reduce the proliferation of mycotoxigenic molds. Awareness of the population and especially the rural world must be made on the methods of harvesting, drying and preserving foodstuffs. This study aims to draw the attention of the ivoirien population to the existence of mycotoxins (ochratoxin A and aflatoxin B1) in dried or fermented foodstuffs consumed by populations.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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