

## **Ochratoxin A: A Persistent Menace in Nigerian Stored Cocoa Beans**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author OOK conceived and designed the experiment. Authors OOK and ARS performed the experiment. Authors OOK, AFO and SOA analyzed the data and wrote the paper. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aim:** To assess the levels of OTA in stored cocoa beans in southwest Nigeria.

**Study Design:** The cocoa beans samples for this study were collected randomly from seemingly healthy cocoa beans in six cocoa producing states.

**Methodology:** A solid phase direct enzyme immunoassay in Helica Biosystems kit was used to quantify OTA in 95 cocoa beans samples.

**Results:** The result showed that 82.1% of the samples were contaminated with OTA with concentration ranging from 1.08 µg/kg - 15.24 µg/kg. Out of 95 samples analyzed, 51.6% exceeded the proposed European Commission 2 µg/kg maximum limit of OTA in cocoa powder. There was significant difference in OTA levels observed in cocoa beans collected in the states surveyed ( $p = 0.05$ ).

**Conclusion:** The knowledge of OTA incidences in stored cocoa beans is imperative and insightful

to reducing OTA contaminations in cocoa beans through intervention schemes that take into cognizance periodic creation of awareness and training of farmers and other stakeholders on best pre and post-harvest practices that ensure the production of quality cocoa beans.

*Keywords: Cocoa beans; post-harvest; ochratoxin-A; mycotoxin; Nigeria.*

## 1. INTRODUCTION

The widespread occurrence of mycotoxins in foods and feeds is of public health importance and has become a huge threat to global trade. Mycotoxins are a group of low weight secondary metabolites produced by filamentous fungi [1]. These filamentous fungi are mainly members of the fungal genera *Aspergillus*, *Fusarium*, and *Penicillium* [2]. More than 300 mycotoxins have been reported, but only a few causes contamination frequently in food chains. Ochratoxins (OT) are among the important mycotoxins that cause contamination in wide variety of agricultural commodities, including cereals, cereal products, fruits, dairy products, coffee, cocoa and wine [3,4,5]. Ochratoxins are of different types; Ochratoxin A (OTA), Ochratoxin-B (dechlorinated OTA), and Ochratoxin-C (ethylated OTA), and are often produced jointly [6]. Nonetheless, among the ochratoxins, OTA is the most prevalent and known as the most toxic [2]. Ochratoxin A is produced by species of *Aspergillus* and *Penicillium* [7,8]. Ochratoxin A is nephrotoxic, hepatotoxic, immunotoxic, genotoxic, neurotoxic, teratogenic, embryotoxic and can inhibit both RNA and DNA synthesis [9,10,11,12], thus it has been classified as possible human carcinogen by International Agency for Research on Cancer (IARC).

Ochratoxin A is an important mycotoxin occurring in cocoa beans. It has been reported severally and widely in cocoa beans and cocoa beans products in many cocoa producing countries [13,14,15,16,17,18,19,20,21]. In recent years, concern about exposure and potential damages of OTA contaminated cocoa beans on human and animal health has been on the increase. This is a huge threat to cocoa beans exports. This is rather unfortunate as cocoa is very important to the livelihoods of about 50 million people worldwide, including over 5 million smallholder cocoa farmers [22]. Cocoa production contributes significantly to the economies of cocoa producing countries in West Africa region, as well as many other countries in Central America and South East Asia [23]. In Africa, cocoa beans are a major export for producing countries. Examples

include Ghana, where cocoa sector has played an important role in the nation's economic growth [24,25] as well as poverty reduction among cocoa-producing households [26]. Cocoa sector also contributes to the Nigerian GDP - Gross Domestic Product [27] and likewise in other West African countries, such Côte d'Ivoire, Cameroun and Togo.

In Nigeria, OTA has been encountered in stored cocoa beans in different quantities beyond acceptable limits [18,20,28]. A study was conducted by [29] on the effect of fermentation and storage on mycotoxigenic fungi, OTA and aflatoxin B1. The study revealed significant contamination by OTA in cocoa beans from south western Nigeria. The unsystematic act of cocoa processing among some farmers brought the quality of the cocoa beans produced under scrutiny with major concern on levels of OTA. This spurred intervention programmes from the Federal Government via Cocoa Research Institute of Nigeria (CRIN) in collaboration with other agencies to monitor and train farmers and other stakeholders on ways to prevent the incidences of OTA in cocoa beans. Occurrence of OTA in stored cocoa beans could be as a result of many factors at every stage of the cocoa beans processing, the high tropical temperature and relative humidity are contributing factors for the prevalence and high incidence of OTA in cocoa beans during fermentation and drying [29]. However, it can also be due to inadequate drying process as a result of prevailing climatic conditions during the drying periods. Nonetheless, there are other predisposing factors embedded in the pre- and post-harvest practices along cocoa beans production chain. These include damaged cocoa pods [21], irregular turning of cocoa bean mass during fermentation [30], storage at high humidity, prolonged drying due to adverse weather [30] and excessively prolonged storage [31]. Notwithstanding, a substantial amount of OTA, is produced during drying and storage [8]. Therefore special emphasis is laid on these two phases of processing. Considering the different training programmes undergone by cocoa farmers and other stakeholders over time, this study was intended to check the degree of OTA

in stored cocoa beans across the south-western region of Nigeria to ascertain if there are improvements in quality of cocoa beans produced in relation to the extent of OTA incidences.

## 2. MATERIALS AND METHODS

### 2.1 Cocoa Beans Sampling

The cocoa beans samples for this study were collected during the main cocoa harvest/crop season in 2018. Ninety five (95) samples of cocoa beans of about 200g each were collected randomly from different traders' stores and farmers in southwest Nigeria. The samples were collected from Lagos, Oyo, Ogun, Ondo, Osun and Ekiti State (Table 1). The cocoa beans samples collected from all the states were seemingly healthy and dry.

### 2.2 Sample Preparation and Quantification of OTA

Ten grams of milled cocoa beans was weighed into 250 ml conical flasks and 50 ml of 70% methanol was added. The mixture was placed on a shaker for 5 minutes to homogenize and then centrifuged for 5 min at 14,000 rpm until it became clear. The clear extract was diluted at ratio 10:1 with 70% methanol in water. The quantification of OTA was done using the Helica Biosystems kit. The quantification was carried out according to manufacturer's instructions. The data obtained were subjected to ANOVA.

## 3. RESULTS AND DISCUSSION

The degrees of OTA contamination in stored cocoa beans were examined in samples obtained across cocoa producing states in southwest Nigeria (Table 2). A high percentage of cocoa beans sampled were positive for OTA

contamination. Out of the 95 samples analyzed, 82.1% (78 samples) was found positive for OTA. The results suggest widespread contamination of stored cocoa beans by OTA in southwest Nigeria. Fig. 1 compares the incidences of OTA in the cocoa beans across the states. Cocoa beans samples from Oyo show a wide range of OTA level. The range of OTA level in cocoa beans samples from Ondo State also shows a similar trend. On the other hand, cocoa beans samples from Lagos, Osun, Ekiti and Ogun State have a very close range of OTA level. Nonetheless, more than 50% of the cocoa beans samples contained relatively low levels of OTA (Table 2) compared to earlier reported studies [18,20,28,29].

Previously, wide range of OTA in cocoa beans samples obtained from southwest Nigeria, with varying levels of OTA contaminations have been reported in different studies [18,20,28,29], notwithstanding, this new study present new data, which give new insights into the safety and quality of cocoa beans produced in relation to the OTA contaminations. The data show different levels of OTA contaminations, with OTA concentration ranging from 1.08  $\mu\text{g}/\text{kg}$  - 15.24  $\mu\text{g}/\text{kg}$ .

Ochratoxin A concentrations in cocoa beans differ with respect to origin and handling. Different levels of OTA have been reported in cocoa beans from the same regions in Nigeria and other West African countries [18,20,28,32]. Likewise in this study, the levels of OTA observed varied with different samples and locations. The disparity could be due to methods used for fermentation, primary storage and drying of cocoa beans in some locations. Perhaps these factors have contributed significantly to the different levels of OTA contamination at different locations recorded in this study.

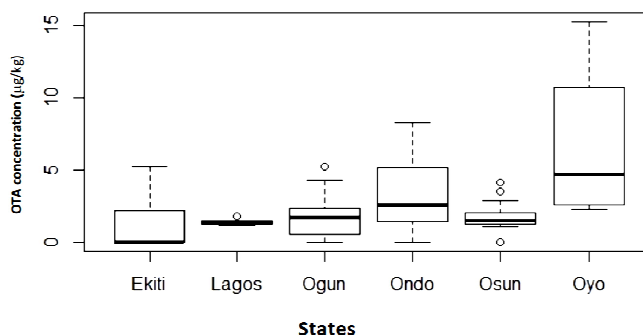


Fig. 1. Comparison of the distribution of OTA in Southwest Nigeria by State

**Table 1. Cocoa beans samples collected across southwest cocoa producing states in Nigeria**

S/N	State	Local Government Area	No of Sample	Remarks
1	Lagos		5	All cocoa beans samples were obtained from traders' stores. They were stored in jute bags at room temperature and ready for export.
2	Ondo	Akure South	4	All cocoa beans samples were obtained from farmers, stored in jute bags, stacked on raised platforms at room temperature
		Irele	5	
		Idanre	5	
		Ondo West	5	
		Odigbo	5	
3	Osun	Boluwaduro	5	Most cocoa beans samples were obtained from farmers, stored in jute bags at room temperature. A few samples were obtained from farmers who are still drying, spread on raised platform in the sun.
		Ife North	5	
		Aiyedade	5	
		Obokun	3	
		Atakumasa West	5	
4	Ekiti	Gbonyin	5	All cocoa beans samples were obtained from farmers, stored in jute bags, stacked on raised platforms at room temperature
		Irepodun/Ifelodun	5	
		Emure	5	
5	Ogun	Obafemi Owode	4	Most cocoa beans samples were obtained from farmers, stored in jute bags at room temperature. A few samples were obtained from farmers who are still drying, spread on raised platform in the sun. All samples were dry.
		Egbado	3	
		Ijebu North	4	
6	Oyo	Oluyole	4	All cocoa beans samples were obtained from farmers, stored in jute bags, stacked on raised platforms at room temperature
		Afijio	5	
		Akinyele	3	

**Table 2. Occurrence of Ochratoxin A in stored cocoa beans samples from Southwest Nigeria**

States	No of samples	No of samples positive for OTA	No of samples with OTA above 2 µg/kg	Mean concentration (µg/kg)	Concentration range (µg/kg)
Lagos	5	5 (100.0%)	0 (0.0%)	1.38	1.45 - 1.78
Ondo	29	27 (93.1%)	17 (58.6%)	3.69	1.08 - 8.26
Osun	23	20 (87.0%)	6 (26.1%)	1.88	1.16 - 4.12
Ekiti	15	6 (40.0%)	4 (26.7%)	3.41	1.36 - 5.20
Ogun	11	8 (72.7%)	5 (45.5%)	2.58	1.11 - 5.25
Oyo	12	12 (100.0%)	12 (100.0%)	6.50	2.28 - 15.24
<b>Total</b>	<b>95</b>	<b>78 (82.1%)</b>	<b>44 (46.3%)</b>		

The preponderance of OTA contaminated cocoa beans has been reported severally and widely, even so, less than 20% of the reports had OTA concentration above 2 µg/kg limit proposed by European Commission [8,32,33,34]. In this study, the data show that 56.4% of the 82.1% OTA positive samples contained OTA higher than 2 µg/kg proposed standard set by the European Commission. Taking into consideration the

toxicity of OTA and its negative health impacts, the quality of cocoa beans produced must be improved upon to safe guard the health of millions of cocoa and cocoa product consumers all over the world.

Considering the fact that drying and storage are major critical control points in production of quality cocoa beans, if OTA contamination is to

be eradicated or reduced to acceptable minimal level, huge attention must be given to these steps to ensure adequate care is taken. We observed that the cocoa beans were properly bagged and stored in appropriate bags during our sampling, so we envisage that the OTA incidences may have occurred largely during drying periods. Previously, the use of elevated plastic roof solar dryers has been recommended, seeing that this technology proffers solution to the stress and cumbersomeness encountered while drying cocoa beans during unfavourable weather condition. The plastic roof solar dryer also ensures increased efficiency of the solar radiation as well as shield the cocoa beans from rainfall during the drying period. However, farmers do not readily take up this new technology, this could be a reason why there is still a wide spread of OTA contamination in the cocoa beans. Personal communication with farmers on sites during sample collection revealed that farmers find the recommended new technology expensive and burdensome, thus unable to support the cost involved.

#### 4. CONCLUSION

In order to curtail OTA menace, we must encourage and ensure that farmers readily take up new technologies which seek to redress inadequacies that contribute to OTA incidences during cocoa beans production. Access to soft loans to alleviate the burden of cost of purchasing improved plastic roof solar dryer might be a proactive step towards assisting cocoa farmers fight OTA incidences in cocoa beans. Although concerted efforts have been made in the past to educate cocoa farmers and other stakeholders on the consequences of OTA contaminations in cocoa beans, notwithstanding creation of awareness and further training are still very much needed, as regards good practices that ensures production of quality cocoa beans including pre and post-harvest practices.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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