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Ochratoxinogenic fungi And Ochratoxin A contamination Of Cocoa Beans

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Abstract

Introduction*:Ochratoxin A is a mycotoxin produced by Aspergillus and Penicilliumfungi. It is a food contaminant found in cereals, coffee, dried fruits, beer, wine, and cocoa. The aim of this study was to identify potentially ochratoxinogenic fungi and OTA contamination depending on pod quality and post-harvest operations of cocoa beans.*

Methodology*: Collected and sorted cocoa pods were divided into four categories depending on their quality (intact, pricked, rotten, and injured). Cocoa bean samples were taken at different steps of production: pod-opening, fermentation, and drying. The molds were identified after growing them on Malt Extract Agar medium. The ochratoxin A was extracted from samples using a mixture of methanol/3% sodium hydrogen carbonate in water (50/50), followed by purification using an immunoaffinity column and quantified by high performance liquid chromatography and fluorescence detection.*

Results*: The average level of ochratoxin A contamination varied depending on the quality of the pods: 0.16 µg/kg (intact pod); 1.10 µg/kg (rotted pod); 1.51 µg/kg (pricked pod), and 1.56 µg/kg (injured pod). We identified four genera offungi:Aspergillus(51.3%), Penicillium(13.01%), Alternaria(13.01%), and Fusarium(9.23%). There was a significant association between damaged cocoa pods and the presence of ochratoxinogenic fungi and the level of ochratoxinA contamination in cocoa beans.*

Conclusion: *Properagricultural practices could reduce ochratoxinAcontaminationin cocoa.*

Key Words*:cocoa, ochratoxinogenic fungi, Ochratoxin A, post-harvest treatment*

I. INTRODUCTION

OchratoxinA (OTA) is a mycotoxin produced mainly byfungi species of the genera *Aspergillus* and *Penicillium* **[1].** OTA contaminates a myriad of agricultural products, such as cereals, dried fruits, coffee, and cocoa, as well as manufactured agricultural products, including chocolate, wine, beer, andbread [2-6]. The consumption of OTA-contaminated foods may lead to human and animal diseases. Indeed, OTA was involved in endemic nephropathy in the Balkan countries [7]. It is genotoxic, immunotoxic, and carcinogenic [8-10]. The health risk from consuming OTA-contaminated foods has led to worldwide preventive measures to protect consumer health. For example, foodstuffs in the European market are subject to directives and regulations. EU regulation 1881/2006**[11]** sets maximum levels of OTA in foodstuffs: 5 µg/kg in cereals, 3 µg/kg in processed cereal-based products, 5 µg/kg in raisins, 5 µg/kg in roasted and ground coffee, 10 µg/kg in instant coffee, and 2 µg/kg in wine and spices. The European Food Safety Authority (EFSA) established an acceptable weekly intake of OTA of 120 ng/kg body weight in 2006[12]. The European Commission has not set a maximum acceptable level for OTA in cocoa and cocoa-derived products because they do not significantly contribute to OTA exposure and high levels of OTA are rarely detected in these products [13].

Nonetheless, cocoa-producing countries should develop post-harvest treatment guidelines to maximally reduce OTA contamination of cocoa. Indeed, the analysis of cocoa bean samples originating from various countries has revealed the presence of variable levels of OTA for samples collected from different regions and even within the same region **[6,14-19]**. The contamination of cocoa beans with OTA occurs mostly during post-harvest treatment: harvesting, pod-opening, fermentation, drying, storage, and transportation.Our study aimed to establish the relationship between the integrity of cocoa pods and OTA contamination of dry cocoa beans, according to the stage of post-harvest treatment, to identify potentially ochratoxinogenic fungi responsible for this contamination.

II. MATERIAL AND METHODS

2.1. Collection of samples

Cocoa is grown in Côte d'Ivoire in forested zones (13 zones). For this study, we collected samples from three different zones: east: Abengourou (YakasséFeyassé); central: Gagnoa (Tehiri); and south – west: San Pedro (Gabiadji). For each test, ripe cocoa pods were harvested, sorted, and classified depending on their quality: We defined four quality levels: (1) intact pods: ripe pods (yellow or orange in color) with no defects (lesions, insect attacks) on the outer cortex which should have no colored spots orfungion the beans when they are opened; (2) pricked pods: ripe pods with lesions caused by insects, birds, or other pests; (3) rotten pods: adult pods with brown traces of rot, and (4) injured pods: adult pods with lesions due to machetes or sickles as well as lesions of the cortex due the falling of pods from the cocoa trees during harvesting. Various mixtures of different pod types were prepared to obtain five lots of 500 pods each (figure 1):

lot 1: 500 intact pods
lot 2: 250 intact pods + 250 pricked pods
lot 3: 250 intact pods + 250 rotted pods
lot 4: 250 intact pods + 250 injured pods
lot 5: 500 unsorted pods (similar to those of the farmers).

2.2. Pod-opening

The cocoa pods were assembled for five days. Each pod was opened using a club and the beans manually removed. A 4-kg sample of fresh beans from each lot was collected and stored at −22°C at the site.

2.3. Fermentation

The beans from each lot were fermented for five days under banana leaves (*Musa paradisiaca*L). This step aids removal of the beans from the mucilaginous pulp which surrounds them and prevents their germination. A 4-kg sample of fermented beans from each lot was collected and stored at −22°C.

2.4. Drying

The beans were then dried in the sun. The fermented beans were spread on a tarpaulin and stirred from time to time. Drying lasted from 5 to 10 days, depending on the weather conditions. The aim of this stageis to reduce moisture or water content to less than or equalto8%. At the end of drying, a 4-kg sample of dried beans from each lot was collected and stored at −22°C. Eleven test batches, including the steps of cocoa harvesting, pod-opening, fermentation, and drying were prepared for each quality level (Abengourou: n=3, Gagnoa: n=4 San Pedro: n=4). In addition, 165 samples of cocoa beans were collected from the three sites.

2.5 Treatment of samples in the laboratory

Fresh samples (fresh and fermented beans) taken from the three sites were dried at 30°C to below 8% moisture content. All dried bean samples were crushed and homogenized. The final samples (200 g) were labeled and stored at -22°C until analysis.

2.6. Quantification of OTA

The method to quantify OTA has been previously described in Manda et *al.*[20]. Approximately 200 g of beans and nibs were thinly ground. Fifteen grams of each sample were transferred to a Waring blender and 150 ml aqueous solution (50:50, v/v) of methanol/sodium hydrogen carbonate 3% (m/v) added and the mixture blended for 2 min. After decanting and filtering through Whatman paper no. 4, 11 ml filtrate were added to an equivalent volume of PBS buffer. An immunoaffinity column was pre-conditioned with 10 ml PBS buffer at a flow-rate of 3 ml/min. Twenty milliliters of extract were loaded onto the immunoaffinity column at a flow-rate of 1–2 ml/min and the OTA present in the samples captured by antibodies contained in the agar suspension. The immunoaffinity column was washed with 20 ml PBS buffer to remove non-specific components. OTA was slowly eluted with 1.5 ml of a mixture of acetic acid/methanol (2:98, v/v) at a rate of 1–2 drops/s. The column was then washed with 1.5 ml distilled water to obtain a final volume of 2.8 ml. After stirring, the analysis was performed by HPLC.

HPLC analysis was carried out in an isocratic mode using fluorimetric detection at excitation and emission wavelengths of 333 and 460 nm, respectively. The mobile phase consisted of a mixture of acetonitrile/water/glacial acetic acid (55:43:2, v/v) and the stationary phase a 2.5 mm (25 cm_4.6mm) C18 S5 ODS column equipped with a pre-column. The injection volume was 100 µl. The retention time was approximately 8 to 9 min. The OTA peak in the samples was identified by comparison with standards. OTA was quantified by measuring the peak area, taking into account the dilution performed during OTA extraction and purification.

2.7. Fungiidentification

The cocoa beans were sterilely soaked in a diluent (peptonal water 0.1%) at a ratio of 1:10 between sample and diluent for 10 min and successive 10-fold dilutions performed. The resulting solutions were cultured on PDA or DRBC medium (enumeration medium) at 25°C for five to seven days. Detected molds were then cultured on Czapek or MEA (identification media) at 25°C for five to seven days [12]. One or more filaments were captured with a dissectingneedlefor glabrous cultures or with scotch tape for filamentous cultures and then placed in a drop of lactic blue between a slide and coverslip for microscopic examination.

2.8. Statistical Analysis

Statistical analysis of the OTA data was performed using STATA 11a software. A difference was considered to be significant if $P < 0.05$.Logistic regression was carried out using Eviews 7.1 software to evaluate the influence of the pod quality levels and post-harvest operations on the presence of the ochratoxinogenic fungi. Coefficients associated with an independent variable are significant at a threshold of 5% when the z-stat is greater than 1.95.

III. RESULTS AND DISCUSSION

3.1. OTA concentrations depending on quality levels of the pods and post-harvest operations

OTA concentrations were determined at all stages of post-harvest transformation (pod-opening, fermentation, and drying) for all pod quality levels (intact, pricked, rotten, or injured). The average OTA concentration depending on the quality level of the pods are reported in table 1 and ranged from 0.13 ± 0.1 µg/kg (intact pod, fermentation) to 2.68 ±2 µg/kg (rotten pod, fermentation).The lowest OTA concentrations were observed in intact podsirrespective of the post-harvest transformation stage: pod-opening (0.16± 0.1 µg/kg), fermentation (0.13 ± 0.1µg/kg), and drying (0.18 ±0.1 µg/kg). The average OTA concentrations for the pricked pods were from 1.34 ± 2 µg/kg at the pod-opening stage to 2.49 ± 2 µg/kg at the drying stage. The OTA concentrations in the other pod types varied. At the end of the drying process, there was a significant difference between the average OTA concentration in the intact pods (0.18 ± 0.2 µg/kg) and those of the cocoa podsof the other quality levels (rotten, pricked, injured, unsorted) where the average OTA concentrations were: 1.87 ± 2 µg/kg for the rotten pods, 2.49 ± 2 µg/kg for the pricked pods, 2.32 ± 2 µg/kg for theinjured pods, and 1.16 ± 1 µg/kg for the unsorted pods.

Our results show that OTA is primarily found in damaged pods: rotten, injured, or pricked. Our results corroborate those of Bastide et *al*.[22], Dembélé et *al*[16]and Mounjouenpou et *al*.[23]. These authors showed that OTA concentrations depended on the type of damage suffered by the pod. According to Bastideet *al*.[22]varying OTA concentrations also depended on the cocoa season and the type of damage suffered by the pods: healthy (2.3µg/kg), damaged by insects (4.2µg/kg), mutilated (19.8µg/kg), rotten (7.2 µg/kg), or mummified (3.4 µg/kg). The OTA concentration at the end of drying is the most important because cocoa beans at this stage are destined for commercialization. At the end of drying, the OTA concentrations varied from 0.18 ± 0.2 µg/kg (intact pods) to 2.49 ± 0,2 µg/kg (injured pods). Our results are in agreement with those of Adama et al.[24]. These authors analyzed 1895 cocoa bean samples collected in the ports and cocoa-producing zones of Côte d'Ivoire and reported that OTA contamination ranged from 0.19 ± 0.16 to 1.90 ± 1.87 µg/kg for an average of 1.40 ± 1.32 µg/kg. These results show that OTA contamination of cocoa beans is mainly due to the condition of the pods. Cacao farmers should apply good agricultural practices to produce cocoa with little OTA content.

3.2. Fungiidentification

We identified ten genera and 16 fungi species:

- Six species of *Aspergillus*:*Aspergillusniger*, *Aspergillusflavus*, *Aspergillusfumigatus*, *Aspergillusterreus*, *Aspergilluscandidus*, and *Aspergillusglaucus*;
- Two species of *Fusarium*:*Fusariumsolani*and *Fusariumoxysporum*;
- *Penicillium, Alternaria, Acremonium, Cladosporium, Exophiala, Bipolaris*, *Scytalidium,*and*Aureobasidium genera.*

The frequency of the fungiisolated from the three study sites was almost equal: 35.2% in Gagnoa, 33.9% in Abengourou, and 30.8 % in San Pedro. The most frequently isolated generafrom the three sites were:*Aspergillus*(51.3%), *Penicillium*(13.0%), *Alternaria*(13.0%), and *Fusarium* (9.2%).These results partly corroborate those of Mounjouenpou et al.[24]and Appiah et al.[25]. These authors isolated three of the four principal fungi that we isolated in our study from cocoa beans: *Aspergillus*, *Penicillium*, and *Fusarium.Aspergillus* (51.3%) was the most frequently isolated genus. We identified several fungi species from this genus: *Aspergillusniger*, *Aspergillusflavus*, *Aspergillusfumigatus*, *Aspergillusglaucus*, *Aspergillusterreus*, and *Aspergilluscandidus*. Among them, *Aspergillusniger*was the most frequently isolated at 22.9 %. Two of the identified fungi (*Aspergillusniger*and *Penicilliumsp*)have been reported to be potentially ochratoxinogen[26]. We found these species in almost all the contaminated samples of our study with a frequency of 22.9%

for*Aspergillusniger*and 13.0%for*Penicillium sp*. (Table 2). Copetti et *al.*[19] identified two species in cocoa beans from Brazil as ochratoxinogenic fungi:*Aspergilluscarbonarius*and *Aspergillusniger*.

3.3. Influence of cocoa pod quality and post-harvest handling on the presence of ochratoxinogenic fungi

Ochratoxinogenic fungi were identified irrespective of cocoa pod quality (table 2).However, they were more abundant in rotten pods and in those of the unsorted lot than in intact pods (table 3). These results are in accordance with those of Mounjouenpou et *al*.[24] who showed that the fungal microflora was qualitatively identical, irrespective of the quality of the treated pod and the type of post-harvest treatment. However, cocoa from damaged pods was more highly contaminated by toxic fungi. According to Bastide et *al*.[22], rotten pods present the greatest risk of contamination by ochratoxinogenicfungi. In addition to the qualityof the pods, we also studied the dependence of the presence of fungi on the post-harvest operations of pod opening, fermentation, and drying. We found ochratoxinogenicfungi to be present, irrespective of the type of post-harvest treatment. Logistic regression showed that drying did not further promote the proliferation of ochratoxinogenic fungi (z-Stat = -2.35; table 3). Indeed, most fungi prefer a water activity (w_a) between 0.85 and 0.99 for their proliferation. The reduction in water activity of the fermented beans brought about by drying prevented proliferation of the fungi.Beuchat[26] previously showed that there is a progressive reduction of water content to a water activity below 0.88 during the drying process that inhibited the growth of both bacteria and yeast.Kouadioet *al*.[27]showed that a temperature higher than 42°C and a water activity of 0.75 inhibited the proliferation of the ochratoxinogenic fungi, notably *Aspergillusniger*, *Aspergillusochraceus*, and *Aspergilluscarbonarius.* In contrast, Copettiet *al*.[19]showed the proliferation of *Aspergillusniger andAspergillusflavus*, fungithat produce ochratoxin and aflatoxin, respectively, during drying.

IV. CONCLUSION

The presence of these fungi in cocoa beans is due to the poor quality of the cocoa pods. All damaged pods (rotten, pricked, and injured) are contaminated by OTA. The application of good agricultural practices by the producer is a suitable measure to reduce OTA contamination of cocoa.

REFERENCES

[1] M. Suarez-Quiroz, O. Gonzalez-Rios, M. Barel, B. Guyot, S. Schorr-Galindo, J. Guiraud,Effect of the post-harvest processing procedure on OTA occurrence in artificially contaminated coffee. *International Journal of Food Microbiology*103, 2005,339– 345.

[2] K. Jorgensen, Survey of pork, poultry, coffee, beer and pulses for Ochratoxin A. *Food Additives and Contaminants, 15*, 1998, 550–554.

[3] B. Sangare-Tigori, S. Moukha, JH. Kouadio, DS. Dano, AM. Betbeder, A. Achour, EE. Creppy,Ochratoxin A in human blood in Abidjan, Côted'Ivoire. *Toxicon, 47*,2006,894 – 900

[4] E. Chiavaro, A. Lepiani, F. Colla, P. Bettoni, E. Pari, E. Spotti,Ochratoxin A determination in ham by immunoafiinitty clean up and quick fluorimetric method. *Food Additives and Contammants, 19*, 2002, 575-581

[5] M. Miraglia, C. Brera, Assessment of dietary intake of ochratoxin A by the population of EU member states. *Rapport Task force 3.2.7*. 2002

[6] PA. Burdaspal, TM. Legarda, Ochratoxin A in samples of different types of chocolate and cacao powder, marketed in Spain and fifteen foreign countries. *Alimentaria, 347*, 2003,143-153

[7] P. Krogh, NH. Axelsen, F. Elling, N. Gyrd-Hansen, B. Hald, J. Hyldegaard-Hensen, AE. Larsen, A. Madsen, HP. Mortensen, T. Möller, OK. Petersen, U. Ravnskov, M. Rostgaard, O. Alund, Experimental porcine nephropathy: Changes of renal function and structure per orally induced by christallineOchratoxin A, *ActaPathologicaMicrobiologicascandinavica Section A suppl, 246*, 1974, 1-21.

[8] International Agency for Research on Cancer (IARC), IARC monographs on the evaluation of carcinogenicrisks to humans; *IARC Working Group, WHO: Lyon, France, vol. 56*, 1993

[9] JECFA, Evaluation of certain food additives and contaminants. Forty-fourth Report of the Joint FAO/WHO Expert Committee on Food Additives. *WHO Technical Report Series No. 859*, 1995

[10] M. O'Brien, KF. Nielsen, P. O'Kiely, PD. Forristal, HT. Fuller, JC. Frisvad, Mycotoxins and other secondary metabolites produced in vitro by *Penicilliumpaneum*Frisvad and *Penicilliumroqueforti*Thomisolatedfrombaledgrasssilage in Ireland. *Journal of Agricultural and Food Chemistry. 54*, 2006,9268-9276.

[11] European Communities Commission (ECC.) Regulation (EC) No 1881/2006 of the commission of bearing 19 December 2006 fixing of maximum contents for certain contaminants in the foodstuffs.*Official Journal of the European Union, L 364/4 - 364/24*,2006.

[12] EFSA, Opinion of the scientific panel on contaminants in foodchain on a requestfrom the commission related to ochratoxinA in food. *The EFSA Journal, 365,* 2006,1-56

[13] European Communities Commission (ECC.) Regulation (UE) No 105/2010 of the Commission of 5 February 2010 modifying regulation (EC) N 1881/2006 bearing fixing of maximum contents for certain contaminants in the foodstuffs with regard to ochratoxine A.*Official Journal Of the European Union L 035,*2010, pp. 0007 – 0008

[14] SJ. Bonvehi, Occurrence of ochratoxin A in cocoa products and chocolate. *J. Agric. Food Chem.52,* 2004,6347–6352

[15] S. Amézqueta, E. Gonzalez-Penas, M. Murillo, A. Lopez de Cerain, Occurrence of ochratoxin A in cocoa beans: effect of shelling. *Food Additives and Contaminants,22,* 2005,590-595

[16] A. Dembele, F. Gerard, P. Manda, JG. Nemlin, Research studies results of the estimate programs OTA No DP/IVC/2005/16:Contamination of the coffee and the cocoa by ochratoxine A (OTA) inCote d'Ivoire Dimension. Report/ratio of the Ministry of Agriculture, General Direction of the Agricultural Productions and Diversification , Republic of Côte d'Ivoire, 2007.

[17] L. Dongo, R.Bandyopadhyay, M. Kumar, PS. Ojiambo, Occurrence of ochratoxinA in Nigerian ready for sale cocoa beans. *Agricultural J. 3,*2008, 4 – 9.

[18] M. Gilmour, M. Lindblom, Management of ochratoxin A in the cocoasupplychain: A summary of work by the CAOBISCO/ECA/FCC working group on ochratoxine A. In: Leslie, R., Bandyopadhyay, R., Visconti, A. (Eds.), Mycotoxins: DetectionMethods, Management, Public Health and Agricultural Trade. CABI, Wallinford,2008, pp. 231–243

[19] MV. Copetti, JL. Pereira, BT. Lamanaka, JI. Pitt, MH. Taniwaki,Ochratoxigenic fungi and ochratoxin A in cocoa during farm processing. *International Journal of Food Microbiology,* 143, 2010,67-70.

[20] P. Manda, DS. Dano, JH. Kouadio, A. Diakité, B. Sangaré-Tigori, MJM. Ezoulin, A. Soumahoro, A. Dembele, G. Fourny,Impact of industrial treatments on ochratoxin A content in artificially contaminated cocoa beans. *Food Additives & Contaminants: Part A.* 26, 7,2009,1081 — 1088

[21] RA. Samson, Current taxonomic schemes of the genus *Aspergillus* and its teleomorphs. In: Bennett JW, Klich MA (eds). Aspergillus Biology and Industrial Applications. Boston MA: Butterworth-Heinemann, 1992,329-353

[22] P. Bastide, G. Fourny, N. Durand, P. Petithuguenin, B. Guyot, M. Gilmour, M. Lindblom, Identification of Ochratoxin A sources during cocoa post-harvest processing: influence of harvest quality and climatic factors. *15th Intl Cocoa Res. Conf*., San Jose, Costa Rica. 9-17 October 2006.

[23] P. Mounjouenpou, D. Gueule, M. Ntoupka, N. Durand, A. Fontana-Tachon, B. Guyot, FP. Guiraud,lnfluence of post-harvestprocessing on ochratoxin A content in cocoa and on consumer exposure in Cameroon. *World Mycotoxin Journal. 4 (2),* 2011,141-146

[24] C. Adama, HMB. Godi, D. Ardjouma, MB. Kouassi, T. Abba, Cocoa beans and cocoa derivatives from Côte- d'Ivoire: investigating ochratoxinA level and assessing dietary intake adults. *Sustainable Agriculture Research 2(1),*2013: 173-180

[25] P. Mounjouenpou, D. Gueule, A. Fontana-Tachon, B. Guyot, PR. Tondje, JP. Guiraud, Filamentous fungi producing ochratoxin A during cocoa processing in Cameroon. *International Journal of Food Microbiology, 128,* 2008,234–24.

[26] MR. Appiah, K. Ofori-frimpong, AA. Afrifa, Cocoa variety X fertilizer trial ($K6\text{-}O_2$). Annual Report of the Cacao Research Institute of Ghana 1998/99.Tafo,Ghana: CRIG, 2001,46-49.

[27] A. Pittet, D. Royer, Layer thin fast method, to weak chromatographic tracking cost for the detection of the ochratoxine A in the raw coffee on a level of 10 µg/kg of order *J. Agric. Food. Chem., 50,*2002, 243 - 247.

[28] LR. Beuchat, Influence of water activity on sporulation, germination, out growth, and toxin production. In: Rockland, L.D., Beuchat, L.R. (Eds.), *Water activity: theory and applications to food.* Marcel Dekker, New York, 1987pp: 153-172.

[29] J. Kouadio, Ochratoxin A in Côte d'Ivoire:ochratoxinogenmoulds, human exposure and food detoxification.*Rev. Ivoir. Sci. Technol., 20,*2012, 87 – 103

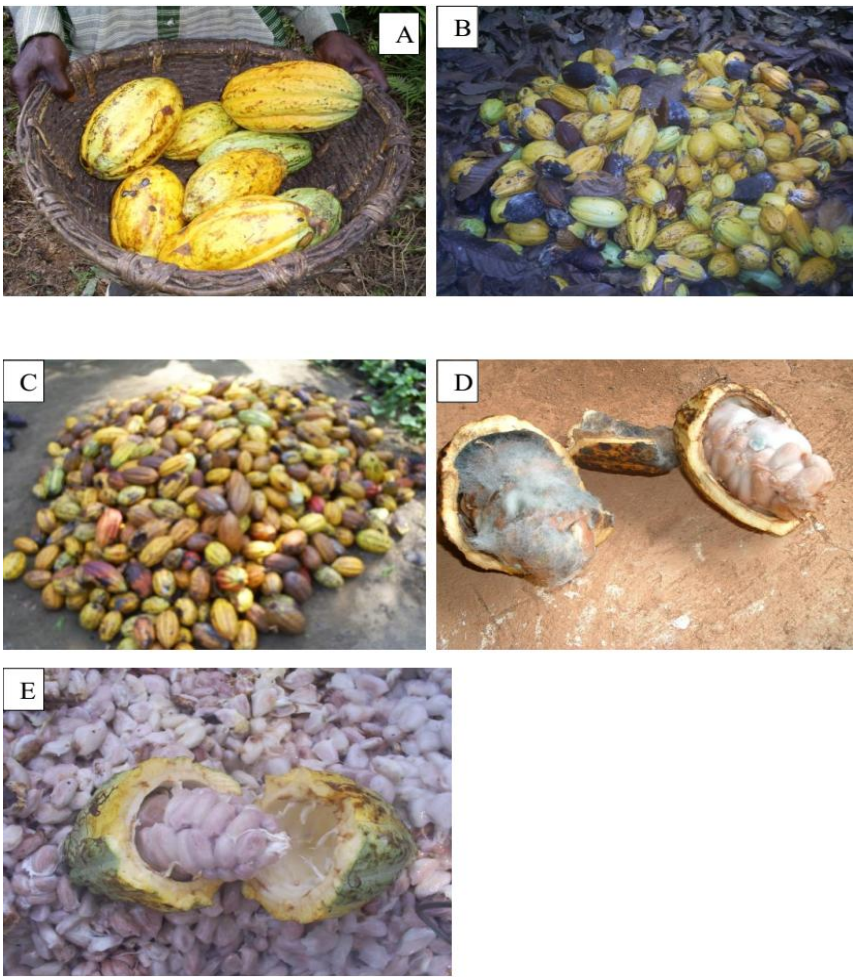

Figure 1: An intact pods, B: unsorted pods, C: unsorted pods, D: opened rotten pods, E: opened intact pods.

Table 1: AverageOTA concentrations in µg/kg depending on cocoa pod quality and post-harvest treatment

	Cocoa pod quality levels				
	Intact	Pricked	Rotten	Injured	Unsorted
Podopening	0.16±0.16[a]	1.34±2.77[a, b]	1.15±1.87[a, b]	1.72±2.64[a, b]	1.34±1.34[b]
Fermentation	0.13±0.10[c]	0.96±1.06[d]	2.68±2.85[d]	0.90±0.76[c, d]	1.37±0.88[d]
Drying	0.18±0.21[e]	2.49±2.94[f]	1.77±2.41[f]	2.32±2.94[f]	1.16±1.25[f]
Averageconcentration	0.15±0.16	1.6±2.43	1.87±2.42	1.65±2.32	1.29±1.14
Median	0.09	0.69	0.83	1.20	1.06
Extended	LD-0.63	LD-9.54	LD-9.69	LD-9.09	LD-4.2

Averages with the same letter in the same line are not significantly different at 5% risk. Cocoa beans from rotten and wounded pods were the most highly contaminated by OTA.

Table 2: Molds according to post-harvest treatment

Post-harvest treatment	Isolated molds	Total	Frequency (%)
Pod-opening	***Aspergillus niger***	**22**	**7.5**
	Aspergillus flavus	10	3.4
	Aspergillus fumigatus	4	1.3
	Aspergillus terreus	4	1.3
	Aspergillus glaucus	3	1.0
	Aspergillus candidus	1	0.34
	Bipolarissp	4	1.3
	Penicillium sp	**12**	**4.1**
	Alternariasp	16	5.4
	Fusariumsolani	4	1.3
	Fusariumoxysporum	3	1.0
	Acremoniumsp	2	0.68
	Scytalidiumsp	1	0.34
	Cladosporiumsp	2	0.68

Fermentation	***Aspergillus niger***	**24**	**8.2**
	Aspergillus flavus	13	4.4
	Aspergillus fumigatus	14	4.7
	Aspergillus glaucus	1	0.34
	Aspergillus candidus	1	0.34
	Aspergillus terreus	2	0.68
	Bipolarissp	4	1.3
	Penicillium sp	**16**	**5.4**
	Alternariasp	10	3.4
	Fusariumsolani	4	1.3
	Fusariumoxysporum	6	2.0
	Acremoniumsp	3	1.0
	Scytalidiumsp	1	0.34
	Cladosporiumsp	3	1.0
	Exophialasp	1	0.34
	Aureobasidiumpullulans	1	0.34
Drying	***Aspergillus niger***	**15**	**5.1**
	Aspergillus flavus	15	5.1
	Aspergillus fumigatus	11	3.7
	Aspergillus terreus	1	0.34
	Bipolarissp	9	3.0
	Penicillium sp	**11**	**3.7**
	Alternariasp	9	3.0
	Fusariumsolani	4	1.3
	Fusariumoxysporum	6	2.0
	Acremoniumsp	4	1.3
	Scytalidiumsp	1	0.34
	Cladosporiumsp	3	1.0

Aspergillusniger and *Penicillium sp*. are two potentially ochratoxinogenic moldspresent in cocoa beans, irrespective of pod quality

Table 3: Influenceof pod quality on the presence of ochratoxinogenicmolds

Independent variables	**Coefficient**	**z-Stat**
C*	-0.26	-0.53
Injuredpods	1.14	1.95
Prickedpods	0.67	1.14
Rottenpods	1.30	2.20
Unsortedpods	1.30	2.20
Fermentedbeans	0.86	1.94
Dry beans	-1.06	-2.35

* Comparisonswere made with beans from intact pods.There was a significant association between rotten or unsorted pods and the presence of ochratoxinogenic molds.