



Alice Y, Jonfia-Essien WA, Voegborlo BR (2016) Levels of Ochratoxin A in cocoa beans (*Theobroma cocoa*) from Western regions of Ghana. Pp. 88–91. In: Navarro S, Jayas DS, Alagusundaram K, (Eds.) Proceedings of the 10th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2016), CAF Permanent Committee Secretariat, Winnipeg, Canada.



Levels of Ochratoxin A in cocoa beans (*Theobroma cocoa*) from Western regions of Ghana

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ABSTRACT

Ochratoxin A (OTA), a potent toxin, is a secondary metabolite produced by filamentous fungi, *Aspergillus* and *Penicillium* present in a wide variety of foodstuffs. Ochratoxin A can contaminate a wide variety of foods as a result of fungal infection in crops, in the field during growth, at harvest, in storage and in shipment, depending on environmental conditions especially when they are not properly dried, causing health concerns. Thirty-two samples of cocoa beans (*Theobroma cocoa* L.) obtained from Western North (WN) and Western South (WS) regions of Ghana, were analysed using HPLC with a fluorescence detector. The range of concentrations obtained were 0.186 to 1.557 µg/kg (mean 0.928) for WS and 0.393 to 4.650 µg/kg (mean 1.802) for WN region. From the results of the study, 80% of the samples had OTA concentrations below the draft standard of 2 µg/kg proposed by the European Union (EU) for cocoa beans and 20% had concentrations above the draft standard proposed by the EU. The WN region recorded the highest OTA level of 4.650 µg/kg for samples from Sefwi Kaase from WN region and the lowest OTA concentration of 0.186 µg/kg was recorded for samples from Manso Amenfi from the WS region. The low levels of OTA detected in this study indicate that exposure of OTA to humans through consumption of cocoa beans from the areas under study is unlikely to be of health concern.

Key words: Cocoa beans, European Union proposed standard, Fluorescence detector, HPLC, Ochratoxin A

Ochratoxin A (OTA) can contaminate a wide variety of foods due to fungal infection in crops, in the field during growth, at harvest, in storage and in shipment under favourable environmental conditions especially when they are not properly dried. Cocoa beans (*Theobroma cocoa* L.) are normally placed in heaps and fermented prior to drying and then transported for processing. Thus during the fermentation phase the cocoa beans can be colonized by *Aspergillus ochraceus* Wilhelm resulting in OTA contamination. The OTA may be present in a foodstuff even when the visible mould is not seen. It is a potent toxin affecting mainly the kidney (Van der Merwe et al., 1965; Li et al., 1997). These fungi are ubiquitous and can occur in

tropical and temperate climates. Besides the presence of nutrients, the most important factors for growth and mycotoxin production are temperature, water activity (a_w) and oxygen.

The presence of OTA in food is of great concern due to its chronic effects at low levels of exposure, in humans; severe dietary exposure to Ochratoxin A has been associated with chronic, progressive, Balkan endemic nephropathy which is a kidney disease (Chukwuka, 1997; Badru, 2005; Ogunledun, 2007).

Consumer exposure to OTA is reported to be increasing gradually and in order to protect consumers the European Union has drawn up a standard to define tolerable contamination limits (European Commission, 1995). As these toxicants can never be completely removed from the food supply, many countries have defined levels in food (tolerances, guideline levels, maximum residue levels) that are unlikely to be of health concern (Stoloff et al., 1991).

The lack of data pertaining to OTA intakes from

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cocoa products makes it difficult to assess the health risks to consumers, thereby crippling governmental and international agencies regulatory safety measures. Since Ghana is one of the leading exporters of cocoa worldwide, it is therefore necessary to monitor the levels of OTA in cocoa beans to determine whether the cocoa beans produced in Ghana conforms to international standards.

MATERIALS AND METHODS

Acetonitrile and methanol were of HPLC grade (Park Scientific Limited, UK). Acetic acid, sodium bicarbonate and phosphate buffered saline were all analytical grade. Immunoaffinity columns for OTA (AFLAOCHRA PREP) and OTA standard were from R-Biopharm Ltd, Scotland and filter paper from Whatman Int. Ltd.

Analysis of Ochratoxin A by HPLC

The analysis was carried out by a reversed-phase HPLC system using Mediterranean SEA18 5 μm (25 cm \times 0.46 mm). The separation was performed using isocratic mode at excitation and emission wavelengths of 333 nm and 460 nm respectively. The mobile phase was a mixture of acetonitrile/water/glacial acetic acid (55:43:2, v/v) with a flow rate of 1 ml/min and was injected at 100 μl maintaining a column temperature at 40°C. For creating calibration curve, five calibration points were obtained from 2 ng/ml, 4 ng/ml, 6 ng/ml, 8 ng/ml and 10 ng/ml concentrations. Standard curve was plotted from the peak areas against concentrations.

Extraction of Ochratoxin A

Extraction of OTA was performed in alkaline conditions as per the method described by Tafuri et al., (2004) and Amézqueta et al. (2005). Approximately 200 g cocoa beans were weighed and deshelled. The hammer mill was used in milling the cocoa beans sampled for Ochratoxin A analysis. Exactly 15 g of each sample was weighed into a 250 ml beaker. Then 150 ml aqueous solution of a mixture (50 : 50 v/v) of methanol/ sodium bicarbonate 3% (m/v) was added and the mixture homogenized using the ultraturax for 2 minutes. The mixture was decanted and filtered using the Whatman filter paper no. 4 into a 250 ml conical flask. Then 11 ml aliquot of the filtrate was pipetted into a 100 ml beaker and an equivalent volume of Phosphate Buffered Saline (PBS) was added, ready for clean up using immunoaffinity column. The immunoaffinity column which was specific for OTA containing antibodies was placed on an SPE manifold (Supelco) and conditioned with 5 ml of

PBS at approximately one to two drops per second, maintaining approximately 1 cm of solvent above the IAC antibodies at all times. A receiving flask was placed under the column to collect the eluate. Then 20 ml of the sample mixture was taken and loaded onto an empty glass column connected to the immunoaffinity column and released onto the column at a flow rate of 1–2 ml/min. The immunoaffinity column was washed with 10 ml distilled water and then with 20 ml PBS to remove non-specific components. The OTA was slowly eluted using 1.5 ml mixture of acetic acid and methanol (2:98, v/v) at a rate of 1–2 drops/s. Then the column was washed with 1.5 ml distilled water and added to the mixture to obtain a final volume of 3.0 ml. This was then analysed using HPLC with fluorescence detector.

Recovery of Ochratoxin A

Recovery test was performed by spiking cocoa samples with 0.5 ng/ml, 0.2 ng/ml and 0.1 ng/ml OTA standards. Exactly 15 g milled cocoa samples were spiked with 1 ml OTA standards. The samples were extracted after standing for 15 min. The spiked samples and blank samples without standard OTA were then extracted and analyzed by HPLC.

RESULTS AND DISCUSSION

The HPLC chromatogram showing the retention time of the standard OTA and the calibration curve of the standard OTA are presented in Fig. 1a and Fig. 1b respectively. Retention time for OTA was 7.17 min, the limit of detection (LOD) was 0.1 $\mu\text{g}/\text{kg}$ and the limit for quantification (LOQ) was 0.4 $\mu\text{g}/\text{kg}$. Percentage recoveries of the various analytes ranged from 84 to 95%.

The OTA was detected in 10 out of the 18 samples analysed from the Western North Region. The highest concentration of OTA (4.650 $\mu\text{g}/\text{kg}$) was found in cocoa beans sampled from Sefwi Kaase and the lowest (0.393 $\mu\text{g}/\text{kg}$) in cocoa beans sampled from Debiso 'A' (Fig. 2). The levels ranged from ND to 4.650 $\mu\text{g}/\text{kg}$ and the mean concentration was 1.802 $\mu\text{g}/\text{kg}$. All samples analysed were below EU permissible limit of 2 $\mu\text{g}/\text{kg}$ except for samples from Bonso Nkwanta 'A', Sefwi Kaase, Sefwi Asawinso which were 4.221, 4.650 and 2.595 $\mu\text{g}/\text{kg}$ respectively.

The OTA was detected in five (5) out of the fourteen (14) samples analyzed from the Western South region. The highest concentration of OTA (1.557 $\mu\text{g}/\text{kg}$) was found in cocoa beans sampled from Tarkwa and the lowest (0.186 $\mu\text{g}/\text{kg}$) in cocoa beans sampled from Manso Amenfi. The levels ranged from ND to

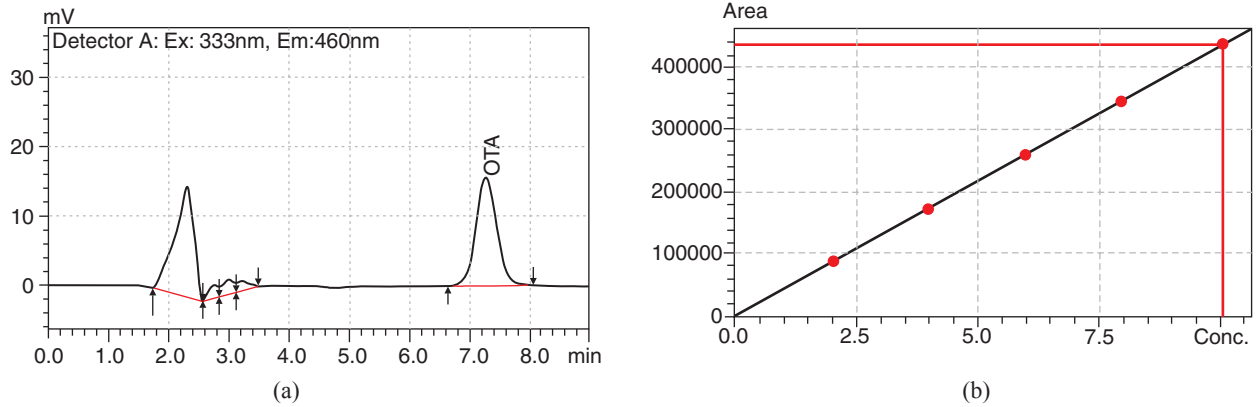


Fig. 1. (a) HPLC chromatogram showing the retention time of the standard Ochratoxin A ($\mu\text{g}/\text{kg}$), (b) Calibration curve of Ochratoxin A ($\mu\text{g}/\text{kg}$)

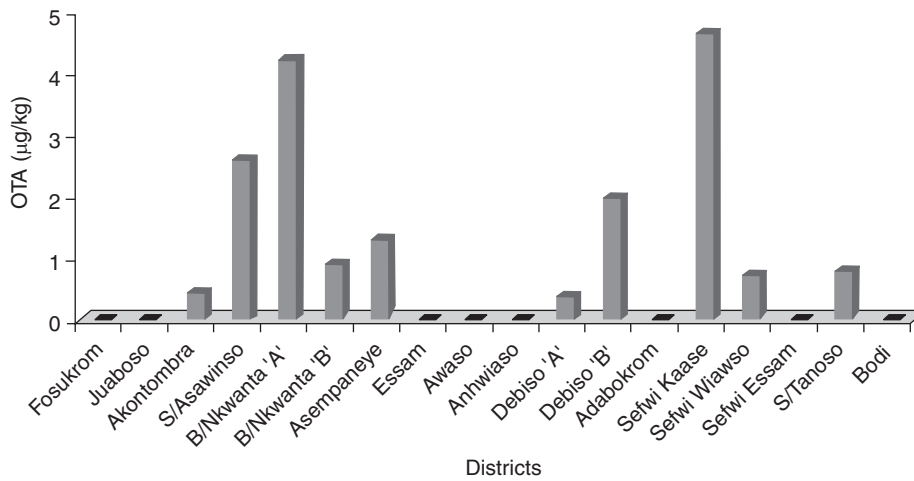


Fig. 2. Concentration of Ochratoxin A ($\mu\text{g}/\text{kg}$) in samples of cocoa beans from the Western North region

1.557 $\mu\text{g}/\text{kg}$ and the mean concentration was 0.928 $\mu\text{g}/\text{kg}$ (Fig. 3). All samples analysed were below proposed EU level of 2 $\mu\text{g}/\text{kg}$ for cocoa beans.

Ochratoxin A contamination appears to be associated with the integrity (intactness) of the pods from which they were obtained, duration and conditions

of drying, farm to farm practices and season of primary processing. Ghana Cocoa Board recommendation to farmers is that damaged and diseased pods should not be fermented with wholesome pods, and that beans should be dried to completion in maximum of seven days. The study shows that if these conditions are

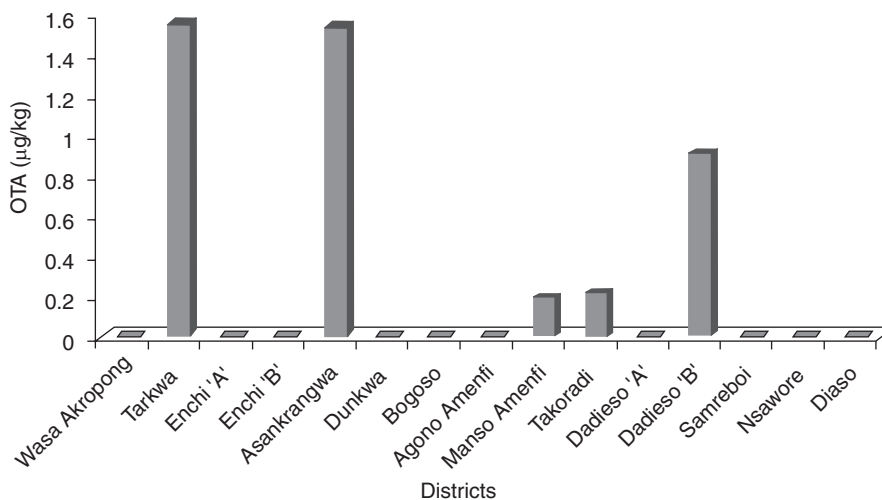


Fig. 3. Concentration of Ochratoxin A ($\mu\text{g}/\text{kg}$) in samples of cocoa beans from the Western South region

adhered to, contamination of Ghana's Cocoa beans by Ochratoxin A can be entirely prevented (Abrokwah et al., 2013).

The low levels of Ochratoxin A reported in this study indicated that the quality of cocoa in most of the districts are good. The low incidence in Ochratoxin A contamination indicated a low rate of infection of the Ochratoxin A producing fungi which may be due to good storage and weather conditions in the food supply. Since Ochratoxin A is stable and generally resistant to heat and processing, control of Ochratoxin A contamination lies in the control of the growth of the toxin-producing fungi. Effective prevention of Ochratoxin A contamination therefore depends on good farming and agricultural practices. Good agricultural practices (GAP) including methods to reduce fungal infection and growth during harvesting, storage, transport and processing provide the primary line of defense against contamination of crops with Ochratoxin A.

The average concentrations of OTA found in cocoa beans (exportable, non-exportable and total) are below the maximum residue of OTA (2 µg/kg), proposed by the regulation of the European Communities (Codex Alimentarius Commission, 2008). This low rate of OTA indicates the application of good production practices and marketing of cocoa beans.

CONCLUSION

The results from the study indicated that, out of the 32 samples analysed, OTA was detected in 15 samples, and 3 samples had levels above EU proposed limit of 2 µg/kg. From the results of the study, 80% of the samples had OTA concentrations below the draft standard of 2 µg/kg proposed by EU for cocoa beans and 20% had concentrations above the draft standard of 2 µg/kg proposed by EU.

The Western North region recorded the highest OTA level of 4.650 µg/kg for samples from Sefwi Kaase and the lowest OTA concentration of 0.186 µg/kg was recorded for samples from Manso Amenfi from the Western South region. The low levels of OTA detected in this study indicated that exposure of OTA to humans through consumption of cocoa

beans from the areas under study is unlikely to be of health concern.

REFERENCES

- Abrokwah FK, Takramah J, Ocloo A, Sackey ST (2013) Studies on factors which predispose fermented cocoa (*Theobroma cacao*) beans to mycotoxin contamination. *Journal of Experimental Biology and Agricultural Sciences* **1**: 174–180.
- Amezqueta S, Gonzalez-Penas E, Murillo M, Lopez de Cerfin A (2005) Occurrence of Ochratoxin A in cocoa beans: effect of shelling. *Food Additives and Contaminants* **22**: 590–595.
- Badru MA (2005) Mycoflora and Ochratoxins in local cocoa based beverages in Lagos, Nigeria. M.Sc. Thesis, OOU, Nigeria.
- Chukwuka B (1997) Bacteriology of cocoa powder drinks. B.Sc. dissertation, University of Lagos, Lagos Nigeria.
- Codex Alimentarius Commission (2008) Discussion paper on Ochratoxin A in cocoa. Joint FAO/WHO Food Standards Programme. Codex Committee on Contaminants in Foods. Second Session, The Hague, the Netherlands. CX/CF 08/2/15ftp://ftp.fao.org/codex/cccf2/cf02_15e.pdf
- European Commission (1995) SCOOP task 3.2.2 Assessment of dietary intake of Ochratoxin A by the population in EU member states. Task coordinator: Denmark. EUR Report 17523 EN.
- Li S, Marquardt RR, Frohlich AA, Vitti TG, Crow G (1997) Pharmacokinetics of Ochratoxin A and its metabolites in rats. *Toxicology and Applied Pharmacology* **145**: 82.
- Ogunledun DS (2007) Incidence of microbial contaminant and nutrient composition of selected cocoa based beverages in Ibadan, Nigeria. Ph D Thesis, University of Ibadan, Nigeria.
- Stoloff L, Van Egmond HP, Park DL (1991) Rationales for establishment of limits and regulations for mycotoxins. *Food Additives and Contaminant* **8**: 213–221.
- Tafuri A, Ferracane R, Ritieni A (2004) Ochratoxin A in Italian marketed cocoa products. *Food Chemistry* **88**: 487–494.
- Van der Merwe KJ, Steyn PS, Fourie L (1965) Mycotoxins. Part II. The constitution of Ochratoxin A, B and C, metabolites of *Aspergillus ochraceus* Wilhelm. *Journal of the Chemical Society* **5**: 7083–7088.