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Surveillance of ochratoxin A in cocoa beans from cocoa-growing regions of Ghana

Joel Cox Menka Banahene [a,b,*], Isaac Williams Ofosu [a], Bernard Tawiah Odai [c]

[a] Department of Food Science and Technology, Kwame Nkrumah University of Science and Technology, Kumasi, Ashanti, Ghana
[b] Research Department, Quality Control Company Limited – Ghana Cocoa Board, Tema, Greater Accra, Ghana
[c] Radiation Technology Centre - BNARI, Ghana Atomic Energy Commission, Kwabenya, Accra, Ghana

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ABSTRACT

Cocoa is one of the agricultural commodities which is highly susceptible to mycotoxin contamination. During two crop/harvest seasons, the occurrence and distribution of ochratoxin A (OTA) in viable commercial cocoa beans were investigated. The cocoa bean samples were collected randomly from farmers across cocoa-growing regions of Ghana. OTA concentrations in the samples were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods following purification on immunoaffinity solid phase column. The result showed that 21.7% of all samples analyzed were contaminated with OTA at concentrations ranging from 0.01 μg/kg to 12.36 μg/kg. The Western South region had the highest occurrence of OTA-positive samples at 32.5%, followed by the Western North region at 28.75%, the Eastern and Volta regions at 25% each, Brong Ahafo (16.25%), Central (15%) and the Ashanti region at 11.25%. However, 0.9% and 3.5% of the total OTA-positive samples exceeded the OTA maximum limits of 10 μg/kg for cocoa beans and 3 μg/kg for cocoa powder, set by the Brazilian National Health Surveillance Agency and the European Commission, respectively. During the Main and Light crop seasons, the highest concentrations of OTA were detected in the Western North region, reaching up to 12.36 μg/kg and 3.45 μg/kg, respectively. OTA concentrations between the cocoa-growing regions in the Main crop season were not significantly different ($p > 0.05$), however, the Light crop season indicated a significant difference ($p < 0.05$). There was a significant difference ($p < 0.05$) between the two crop seasons. The need for regular monitoring and careful adherence to agronomic strategies such as good agricultural practices (GAPs), recommended code of practices (COPs) and good manufacturing practices (GMPs) for the prevention and reduction of OTA throughout the cocoa value chain cannot be overemphasized.

1. Introduction

In Ghana, cocoa (*Theobroma cacao* L.) is a widely cultivated crop, and as the nation's primary export commodity, it has a significant economic impact on the nation and an even greater socioeconomic impact on the cocoa-growing farmers and villages [1–4]. Ghana is the second-largest cocoa-producing country in the world, and merged with Côte d'Ivoire, produces about 70% of the world's cocoa [5,6]. Cocoa beans is the main ingredient for the production of chocolate and other confectionaries, a delightful treat enjoyed by the

* Corresponding author. Department of Food Science and Technology, Kwame Nkrumah University of Science and Technology, Kumasi, Ashanti, Ghana.
E-mail address: coxjmb@yahoo.com (J.C.M. Banahene).

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majority of people and sold worldwide. It is well known that cocoa beans contain various bioactive compounds, such as polyphenols (especially flavonoid – catechins and epicatechins), methylxanthines (theobromine and caffeine), and other beneficial components which are associated with a variety of potential health benefits [7–9]. However, it can be contaminated by mycotoxigenic fungi, affecting the quality of the product and posing a significant risk to human and animal health due to mycotoxin production [10]. The contamination of cocoa beans by filamentous fungi can occur at various stages of the production chain, including harvesting, transportation, fermentation, drying and storage [11].

Mycotoxins of great concern in food safety are those produced by various filamentous fungi of the genera *Aspergillus*, *Penicillium*, and *Fusarium*. Ochratoxin A (OTA) is one of the most significant and major naturally occurring foodborne mycotoxin produced primarily by the genera *Aspergillus* and *Penicillium* [12–14]. These frequently contaminate a wide variety of agricultural commodities such as grains, seeds and beans, dried fruits, spices, and roots [15–17]. Due to its stability at moderate heating, OTA can persist throughout the food production chain after its production during sun drying and storage of crops [18–20]. However, up to 90% of OTA reduction has been observed at temperatures above 180 °C [21].

Several toxic effects associated with OTA have been reported over the years. The International Agency for Research on Cancer (IARC) has included OTA in its Group 2B classification (possible human carcinogen), due to its kidney carcinogenicity [22]. Similarly, the National Toxicology Program (NTP) reported that OTA is the most potent kidney carcinogen ever studied in animal species [23]. In addition, the Committee on Toxicity of Chemicals in Food, Consumer Products, and the Environment (COT) and the European Food Safety Authority (EFSA) have reported that OTA is genotoxic, although the mechanisms of genotoxicity are unclear, and have recommended that OTA concentration in foods be reduced to the lowest level scientifically achievable [21,24].

Nations increasingly recognise that tackling mycotoxin contamination in agricultural commodities and food products would not only protect consumer health but also boost trade and access to high-value markets. As a result, legislation and regulations are continually evolving. The EU expert panel suggested a maximum limit of 1 μg/kg for OTA in chocolate, and chocolate beverages, whereas 2 μg/kg is established for cocoa beans, cocoa mass, and cocoa powder [25,26]. However, in May 2020, EFSA produced a revised OTA risk assessment that incorporated new scientific findings. Based on this new risk assessment, the EU Commission adopted Regulation (EU) 2022/1370, which revised the maximum limits for OTA in Regulation (EC) 1881/2006 and a limit of 3 μg/kg for cocoa powder [27] was set. The new regulation took full effect on January 1, 2023, after entering into force in August 2022. In Brazil, the National Health Surveillance Agency (ANVISA) set OTA limits of 10 μg/kg for cocoa beans and 5 μg/kg for cocoa products, and chocolate sold commercially [20,28].

Food contaminated with OTA is a major risk to consumer health [29], and removing non-compliant food commodities and food

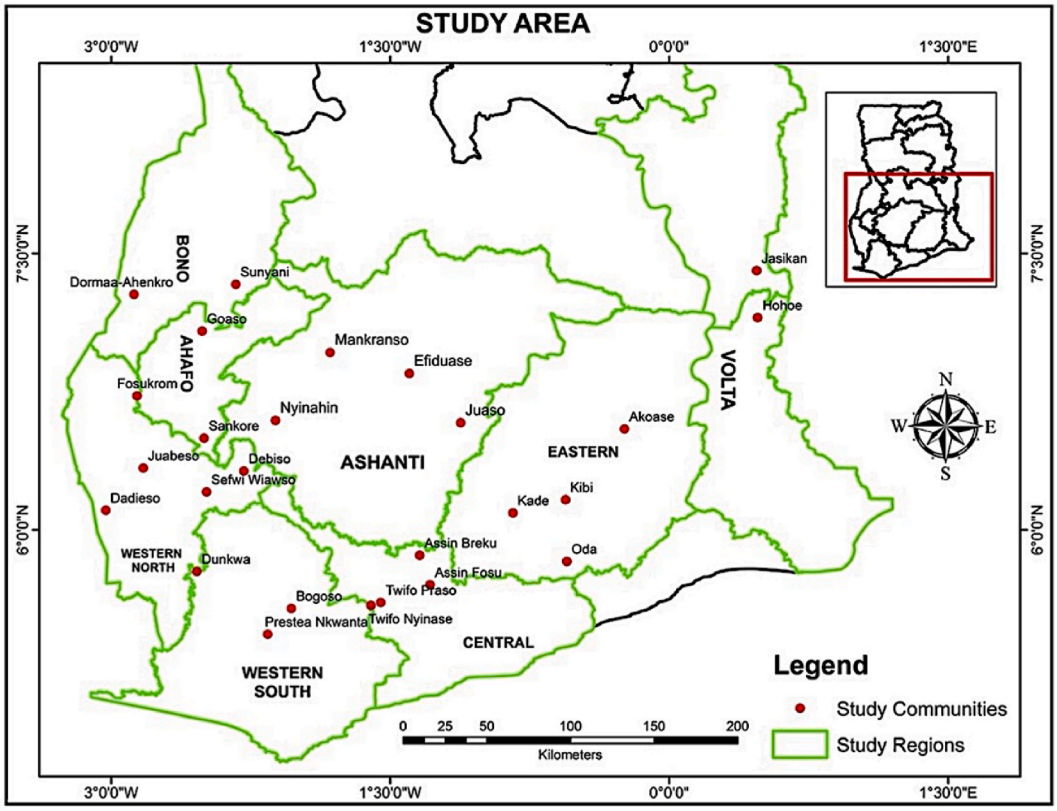

Fig. 1. Map of Ghana showing the cocoa-growing regions where the cocoa beans were sampled from.

products from the market imposes a substantial cost on exporting countries and food businesses alike. Consequently, minimizing OTA contamination in agricultural commodities and food products has the potential to promote both public health and economic growth. To manage OTA contamination and minimize human exposure, however, it is necessary to develop control and preventive measures [30]. Pre-harvest methods, such as farm management, the use of chemical and biological agents, harvesting with the appropriate tools and under the right conditions, and post-harvest practices (such as optimizing drying and storage conditions), are essential for preventing the contamination of OTA. In addition, the Codex Alimentarius Commission (CAC) has established a code of practice for the prevention and reduction of OTA in various agricultural commodities and products based on good agricultural practices (GAPs) and good manufacturing practices (GMPs) [31]. Specifically, by combining various control measures, reliable procedures can be established that can be used to reduce the occurrence and frequency of OTA-contaminated agricultural commodities and food products worldwide [32].

Cocoa beans and products derived from cocoa have previously been reported as agricultural commodities and foods that may contain OTA [20,33–38]. Bonhevi, in 2004, detected OTA in cocoa beans, cocoa butter, cocoa nibs, and cocoa mass from Côte d'Ivoire, Ghana, and Nigeria [39]. In 2011, a study by de Magalhães et al. [26] found that approximately 92.5% of cocoa beans evaluated in Brazil had OTA, however, the concentrations detected were below the European Union's limit (2 μg/kg). Fairly recently, Manda et al. [40] reported OTA contamination in cocoa, based on pod quality, ranging from 0.16 to 1.56 μg/kg. Currently, reports on OTA occurrence and concentration in cocoa beans from Ghana remain insufficient [41]. In the present study, OTA concentration in cocoa beans over two seasons in a crop year from all seven cocoa-growing regions of Ghana was investigated using a validated liquid chromatography-tandem mass spectrometry analytical method (LC-MS/MS). This study enables the investigation of the occurrence and distribution of OTA in cocoa beans from Ghana. In addition, this provides information for the assessment of mycotoxin risk to minimize economic losses, promote trade, and reduce and prevent the hazard to human and animal health.

2. Materials and methods

2.1. Materials

2.1.1. Sample collection and treatment

From October 2020 to September 2021, during the Main (October–June) and Light (July–September) crop seasons, samples of fermented and dried cocoa beans were collected from farm gates of farmers across the cocoa-growing regions of Ghana (Ashanti, Brong Ahafo, Central, Eastern, Western North, Western South, and Volta) (Fig. 1), thus the sampled regions account for 100% of cocoa beans production in Ghana. Apart from the Volta region, which has only 2 cocoa-growing districts, 4 districts each were selected from the regions, representing the main cocoa-growing districts. 10 samples collected randomly from different farmers were taken from each of the 26 district locations, thus representing 260 samples per crop season, and a total of 520 samples of about 1 kg each in the two crop seasons. The dried beans samples were transported to the laboratory using sterile zip-lock polyethylene bags. The sampled cocoa beans were ground in a laboratory blender (MX1500XTSSEE, Waring Commercial, Pennsylvania, USA) and stored at −20 °C until analysis. The concentrations of OTA in these samples were then determined using liquid chromatography-tandem mass spectrometry. After OTA quantification, results < LOD were considered as 50% LOD in calculating mean values.

2.1.2. Reagents

Solvents and chemicals used for the extraction and clean-up solutions were ACS grade or equivalent (Carlo Erba, Val-de-Reuil, France). For UHPLC-MS/MS analysis, methanol, acetonitrile, formic acid, and 2-propanol were LC/MS grade (Chem-Lab Analytical,

Table 1
Acquisition parameters for the analysis of OTA in cocoa beans.

UHPLC			Nexera 40 LC system					
Column temperature			40 °C					
Flow rate			0.4 mL/min					
Injection volume			2 μL					
Solvent A			0.15 mM NH_4F aqueous solution					
Solvent B			0.15 mM NH_4F methanol solution					
Binary gradient			Solvent B conc. 15% (0–1 min) – 100% (6–8 min) – 15% (8.01 min) – Stop (12 min)					
Needle wash			500 μL methanol/acetonitrile/2-propanol/water (1:1:1:1) 1% formic acid					
Mass spectrometer			LCMS-8060NX					
Ionization mode			Heated electrospray					
Source temperatures (interface; heat block; DL)			400 °C; 400 °C; 250 °C					
Gas flows (nebulizing; heating; drying)			2 L/min; 15 L/min; 3 L/min					
Detector voltage			4.0 kV					
MRM-parameters								
Compound	Retention time	Polarity	MRM transitions	Q1 (V)	CE (V)	Q3 (V)	MS1 Res.	MS2 Res.
OTA		Positive	404.30 > 239.00	−12.0	−25.0	−28.0	Unit	Unit
	4.85	Positive	404.30 > 358.15	−20.0	−15.0	−27.0	Unit	Unit
		Positive	404.30 > 221.10	−26.0	−34.0	−24.0	Unit	Unit

Zedelgem, Belgium), and water (Ultra Clear 18.2 MΩ/cm resistivity; Evoqua Water Technologies, Barsbüttel, Germany). Sodium bicarbonate (Sigma-Aldrich, Steinheim, Germany) was used for sample extraction and phosphate-buffered saline (PBS) was prepared as follows NaCl 8 g/L, KCl 0.2 g/L, Na_2HPO_4 1.15 g/L, KH_2PO_4 0.2 g/L, pH 7.4. NH_4F (Park Scientific Limited, Northampton, UK) was used as a mobile phase additive. OTA standard solutions used for spiking and calibration were prepared by diluting 10 μg/mL stock solution (Biopure, Tulin, Austria).

2.2. Analytical methods

2.2.1. LC-MS/MS conditions

The LC-MS/MS system was a Shimadzu Scientific, equipped with a binary pump (LC-40D X3), degasser (DGU-403), autosampler (SIL-40C X3), column oven (CTO-40C) and a triple quadrupole mass spectrometer (LCMS-8060NX) with electrospray ionization (Kyoto, Japan). Chromatographic conditions included the use of a stationary phase support material Mastro2 PFP 3 μm particle size, 150 × 2.0 mm i.d., p/n 370-0105-84 (Shimadzu, Kyoto, Japan), a mobile phase composition of 0.15 mM NH_4F aqueous solution (Solvent A; approx. pH 6) and 0.15 mM NH_4F methanol solution (Solvent B). OTA was eluted in gradient mode at a flow rate of 0.4 mL/min (Table 1). The mass spectrometric analysis was performed in multiple reaction monitoring (MRM) mode. For fragmentation of the $[M+H]^+$ ion (*m/z* 404.30), argon was used as a collision gas at the pressure of 270 kPa, and three transitions were measured, *m/z* 239.00 (quantifier), 358.15 and 221.10 (qualifiers). The LC-MS/MS data from the OTA analysis was acquired and processed using LabSolutions (version 5.99) and LabSolutions Insight (version 3.7) software (Shimadzu, Kyoto, Japan).

2.2.2. Ochratoxin A determination

OTA was extracted from 10 g of sample homogenized (Polytron PT 3100 D, Kinematica AG, Malters, Switzerland) with 100 mL of 3% sodium bicarbonate-methanol (50 + 50 v/v) at 17000 rpm for 180 s and centrifuged (K-3, Centurion Scientific Limited, West Sussex, UK) at 3000×*g* for 5 min according to the procedure of [42] with slight modifications. Following filtration with folded filter paper (Whatman, GE Healthcare, Buckinghamshire, UK), an aliquot of the filtrate (5 mL) was diluted with PBS (20 mL) and purified through an immunoaffinity column (Ochratest, WB, Vicam, Watertown, MA, USA). The column was washed with PBS (5 mL), allowed to air-dry using two 10 mL syringe air volumes, and OTA was slowly eluted (0.5 mL/min) using methanol acidified with 2% acetic acid (2 mL) followed by water (2 mL) into a graduated amber vial. Three 10 mL syringe air volumes were passed through the column after collecting the eluate. The eluate was brought to 5 mL using methanol with 2% acetic acid: water (1:1 v/v) and vortex-mixed for a few seconds. The extract was filtered (Uniflo 0.45 μm, Whatman) before LC-MS/MS analysis. Fig. 2 provides a summary of the sample preparation steps followed to analyse the cocoa bean samples. It is worth emphasizing that blank cocoa bean samples were analyzed to guarantee they were free of OTA.

2.2.3. Performance evaluation of the analytical methods

To evaluate the performance of the analytical method to comply with the performance criteria required by Regulation EC no. 401/

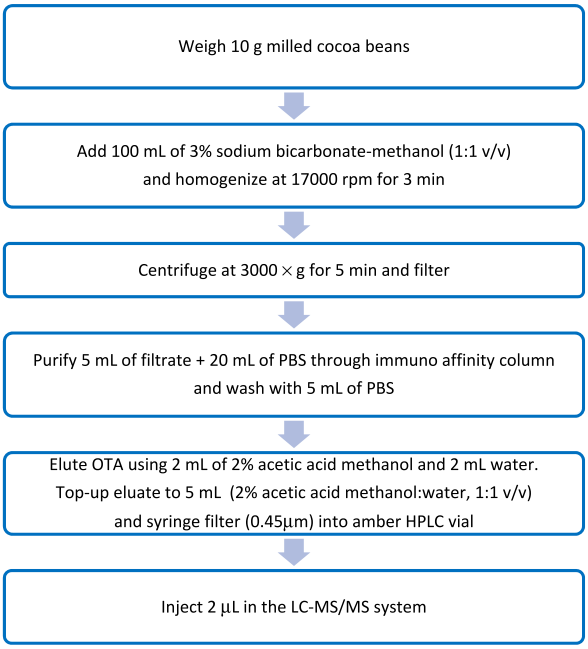

Fig. 2. Sample preparation workflow for the analysis of OTA in cocoa beans.

2006 [43] and Regulation EC no. 882/2004 [44], OTA standard was spiked into a blank sample at concentration levels of 1 μg/kg (low), 20 μg/kg (medium), and 50 μg/kg (high). Two blank samples and two spiked samples of each concentration were analyzed in parallel per day, and this procedure was repeated for 5 days. All blank and spiked samples were prepared and analyzed using the same procedure. The method's trueness, repeatability, and intralaboratory reproducibility were estimated based on the analytical data. The limit of detection (LOD) and the limit of quantification (LOQ) were determined by the signal-to-noise approach, which was defined as those concentrations resulting in signal-to-noise ratios of 3 and 10, respectively. The linearity of the instrumental analyses was established through eight calibration standards, between 0.05 and 40 μg/L. The calibration curve model was determined by Equation (1):

$$y = ax + b \tag{1}$$

least squares weighted (1/x) linear regression, where y - peak area and x – analyte concentration. The respective calibration curve was generated, and the coefficients of determination were calculated. If R^2 was >0.99, the linearity of the calibration curves was considered satisfactory. In addition, the analytical method was evaluated to establish that OTA could be identified and quantified in the presence of the sample matrix without significant interferences from the matrix. The blank sample was injected first, followed by 0.05 μg/L of OTA in sample extract (blank sample spiked with standard solution).

2.2.3.1. Assessment of matrix effect. Matrix effect was investigated for the LC-MS/MS analysis of OTA following the procedure of [45]. Briefly, blank cocoa bean extracts spiked at two concentration levels of 0.05 and 40 μg/kg were compared to neat solvent spiked at the same concentration level (n = 3). Matrix effect was estimated by Equation (2):

$$ME = (A_{matrix} / A_{standard}) \times 100 \tag{2}$$

where A_{matrix} is the peak response area of the matrix standard solution and $A_{standard}$ is the peak response area of the neat solvent standard solution [46]. Usually, ME is acceptable if the ME value is between 85 and 115%, while it was regarded to be a matrix suppression or enhancement effect when the value is less than 85% or greater than 115%, respectively [45,47].

2.2.3.2. Assessment of extract stability. The stability of extracts used for OTA analysis was investigated by comparing the peak response area of freshly prepared extracts obtained from the cocoa bean samples spiked at 1 μg/kg versus the same samples after 24, 48, and 72 h in the autosampler trays. Extracts were kept at 10 °C throughout the times under investigation.

2.2.3.3. Assessment of carryover. The highest calibration standard level (40 μg/L) was injected 5 times in a row followed by the analysis of post-spiked cocoa bean extract containing OTA at the LOQ according to the procedure of [48]. This procedure was repeated 3 times. There would be no carryover if the coefficient of variation (CV) of the low-level sample extract at the LOQ differed by <20% [48].

2.3. Statistical analysis

Data are expressed as mean ± standard error. Statistical analyses were performed using SPSS v.23 (SPSS Inc., Armonk, NY, USA, 2012). To highlight the significant variations between means, the OTA data for the cocoa-growing regions were statistically compared by one-way ANOVA and *t*-test. Significant differences were compared at a level of $p < 0.05$.

3. Results and discussion

3.1. Validation of the analytical methods

Table 2 shows the estimated trueness, repeatability, and intralaboratory reproducibility based on the analytical results for the spiked samples at three distinct concentrations. The estimated trueness ranged between 80 and 104% across the three concentrations.

Table 2
Accuracy estimated by analysis of spiked cocoa bean samples.

Spiked concentration (μg/kg)	Replicate	Concentration per day (μg/kg)					Average (μg/kg)	Accuracy		
								Trueness (%)	Precision	
		Day 1	Day 2	Day 3	Day 4	Day 5			Repeatability (RSD_r%)	Intralaboratory reproducibility (RSD_R%)
1.00	1	1.04	0.91	1.10	0.95	0.98	1.04	104.00	8.40	7.30
	2	1.12	1.08	1.09	1.15	1.03				
20.00	1	13.95	14.88	14.69	17.77	17.29	15.91	80.00	9.60	8.70
	2	16.20	17.13	15.77	14.27	17.17				
50.00	1	43.54	41.18	43.29	42.78	48.91	43.81	88.00	6.00	7.70
	2	41.01	48.32	40.86	40.54	47.69				

The repeatability (RSD_r) and intralaboratory reproducibility (RSD_R) expressed as RSD% for the three concentrations were estimated to vary between 6.0 and 9.6% and 7.3 and 8.7%, respectively.

The LOD and LOQ were 0.01 and 0.05 μg/kg, respectively. The linearity of calibration curves was satisfactory, with the coefficient of determination (R^2) values consistently exceeding 0.999. Finally, no interfering peaks were observed in the spiked samples and no significant peaks were found at the specified retention time in the blank samples, indicating a good degree of specificity (Fig. 3A–C). Based on these results, we considered that this method can be used to analyse OTA in cocoa beans.

3.1.1. Matrix effect

It is generally known that the electrospray ionization technique can exhibit ionization effects when analyzing complex matrices. Depending on the nature and concentration of the interference and target analyte, the co-elution of matrix interferences with the target analyte could result in response suppression or enhancement. Absolute matrix effect estimation should ideally be 100%, however, 85% to 115% is an acceptable range. As demonstrated, OTA did not exhibit significant suppression or enhancement, with a mean matrix effect of 106.2 ± 6.15%. This could be due to the tenfold (10x) dilution of the sample extract used in the sample preparation procedure, resulting in matrix interference at a very low concentration with minimum or acceptable ionization effect. For the two different concentration levels of 0.05 μg/kg and 40 μg/kg, the matrix effect was 112.3 ± 0.09% and 100 ± 0.01%, respectively. This was consistent across replicates within each series of studies.

3.1.2. Extract stability

Results corresponding to the assessment of extract stability after 24, 48 and 72 h of storage in the LC instrument autosampler are depicted in Fig. 4. OTA exhibited a decrease in response within the range of 1.5–5% after 72 h of storage. Although OTA is stable at the proposed storage conditions and the final extract composition chosen for this method, these findings indicate that storage time is a parameter that could significantly affect the accuracy and precision of the analytical method. Setting a lower temperature in the autosampler may help to preserve the integrity of OTA, however, these parameters should be further evaluated taking into account different matrices and final extract compositions.

3.1.3. Carryover

Quantitative analysis of test samples by LC-MS/MS is an established technique used throughout analytical laboratories for the determination of analyte concentration [49,50]. The accuracy of sample measurement can be affected by carryover, which is the contamination of a sample by the analyte of interest coming from a previous sample injection [51]. No significant carryover was observed from high OTA concentration standard into low-level sample extract spiked at the LOQ (CV < 5%). While carryover was insignificant at the conditions employed for this method, the result shows that this parameter could impact the accuracy and precision of the analytical method. Evaluating carryover is important when validating analytical methods since it is considered a ubiquitous problem [52,53].

3.2. Occurrence of ochratoxin A in the cocoa bean samples

The high-efficiency liquid chromatography-tandem mass spectrometry (LC-MS/MS) with previous immunoaffinity column clean-up method was used to evaluate the occurrence and concentration of OTA in 520 samples of cocoa beans collected from farmers in the seven cocoa-growing regions of Ghana. In the present study, the sample was considered positive when OTA concentration was ≥ LOD. The descriptive statistics (occurrence, 95th percentile and range of contamination) of OTA in all the samples analyzed and comparison to the European Commission and ANVISA maximum limits are reported in Table 3.

A relatively low percentage of cocoa beans sampled were positive for OTA contamination. Out of the 520 samples analyzed, 21.7%

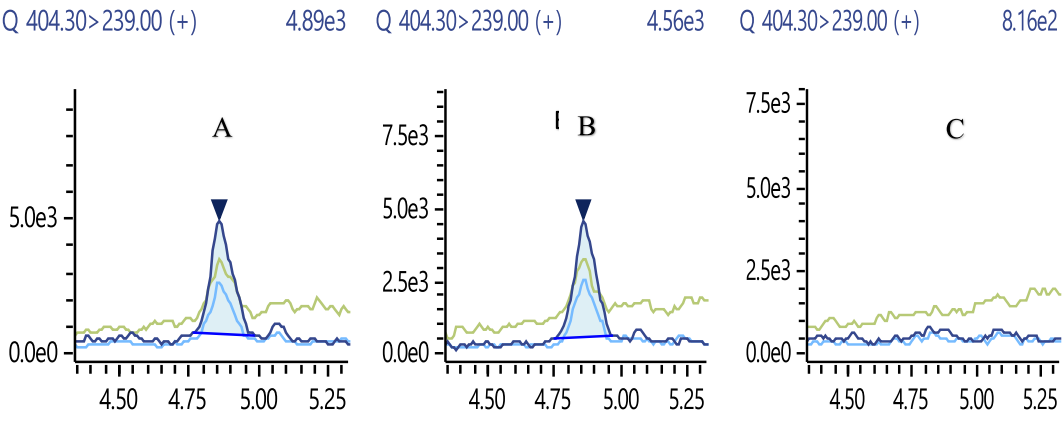

Fig. 3. Representative chromatogram corresponding to LC-MS/MS analysis of OTA; (A) in standard solution at LOQ, (B) in sample spiked at 1 μg/kg, (C) in the blank sample.

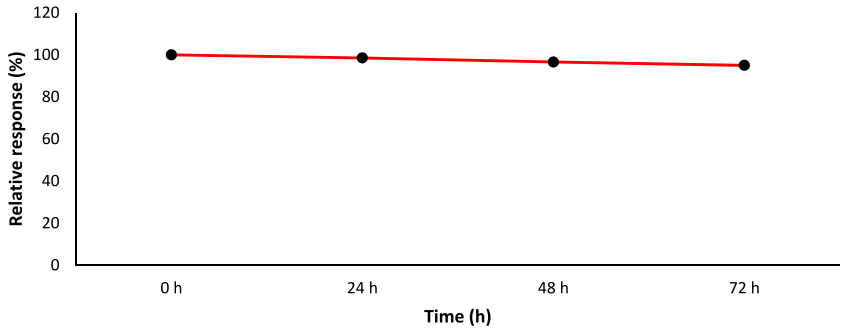

Fig. 4. Assessment of stability of OTA samples after 24, 48, and 72 h of storage in the autosampler at 10 °C.

(113 samples) were found positive for OTA in the range of <0.01–12.36 μg/kg within the cocoa-growing regions (Table 3). Fig. 5 shows the mean distribution of the occurrence of OTA in the cocoa bean samples throughout the cocoa-growing regions in the Main and Light crop seasons. The results suggest narrow contamination of cocoa beans by OTA across the cocoa-growing regions of Ghana. In the Main crop season, the data showed moderate widespread contamination of OTA in cocoa beans across the cocoa-growing regions except for the Volta region. The occurrence of OTA in the Western North region was 35% and the contamination level was high, which ranged from <0.01 to 12.36 μg/kg. Similarly, Brong Ahafo, Western South, Eastern, and Ashanti regions recorded varied contamination levels ranging from <0.01 to 3.30 μg/kg, <0.01–1.86 μg/kg, <0.01–2.71 μg/kg, and <0.01–7.66 μg/kg, respectively. Regarding the Volta region, OTA was detected in 9 out of 20 samples (occurrence 45%), however, the contamination level was very low and more uniform in the range of <0.01–0.47 μg/kg. On the other hand, OTA contamination of cocoa beans in the Light crop season was fairly limited and in close range across the cocoa-growing regions.

To investigate the effect of geographical location (sample region) on concentrations of OTA in cocoa beans, the mean levels of OTA in the individual regions were subjected to a one-way ANOVA and *t*-test comparison between regions in the Main and Light crop seasons. There was no statistically significant difference p-($0.82 > 0.05$) in the mean OTA concentration between locations during the Main crop season. Nevertheless, a significant difference p-($0.004 < 0.05$) was identified in the mean OTA levels between locations throughout the Light crop season. Consequently, cocoa beans would contain different levels of OTA during the Light crop season, depending on the region from where the samples were collected. Moreover, a *t*-test comparison of the OTA concentrations between the two crop seasons revealed a statistically significant difference p-($0.01 < 0.05$).

Various studies have previously reported varied ranges and contamination levels of OTA in cocoa beans. In 2020 [55], investigated 95 cocoa bean samples in Southwest Nigeria and found that 82.1% of the total samples analyzed were contaminated with OTA with concentrations ranging from 1.08 μg/kg to 15.24 μg/kg [36]. reported considerably varied ranges and levels of contamination of OTA in cocoa. These authors analyzed 130 cocoa clones developed in Brazil and observed that 18% of the samples had OTA between < LOD and 274.9 μg/kg. Similarly [34], found OTA in 54 of 59 ready-to-sell cocoa bean samples from Nigeria, with concentrations ranging

Table 3
Frequency of occurrence (positives) and range of concentration of OTA in cocoa bean samples collected from the cocoa-growing regions of Ghana.

Crop Season	Region	Positive	95th Percentile (μg/kg)	Range (μg/kg)	No. of positive with OTA above 3 μg/kg[a]	No. of positive with OTA above 10 μg/kg[b]
Main	Ashanti	7 (17.5%)	1.28	<0.01–7.66	1 (14.3%)	0 (0.0%)
	Brong Ahafo	10 (25.0%)	0.84	<0.01–3.30	1 (10.0%)	0 (0.0%)
	Central	9 (22.0%)	1.83	<0.01–2.46	0 (0.0%)	0 (0.0%)
	Easter	15 (37.5%)	1.96	<0.01–2.71	0 (0.0%)	0 (0.0%)
	Western North	14 (35.0%)	1.84	<0.01–12.36	1 (7.1%)	1 (7.1%)
	Western South	18 (42.5%)	1.83	<0.01–1.86	0 (0.0%)	0 (0.0%)
	Volta	9 (45.0%)	0.47	<0.01–0.47	0 (0.0%)	0 (0.0%)
Light	Ashanti	2 (5.0%)	0.01	<0.01–0.42	0 (0.0%)	0 (0.0%)
	Brong Ahafo	3 (7.5%)	0.22	<0.01–0.66	0 (0.0%)	0 (0.0%)
	Central	3 (7.5%)	0.9	<0.01–1.32	0 (0.0%)	0 (0.0%)
	Eastern	5 (12.5%)	0.89	<0.01–2.02	0 (0.0%)	0 (0.0%)
	Western North	9 (22.5%)	1.61	<0.01–3.45	1 (11.1%)	0 (0.0%)
	Western South	8 (20.0%)	0.94	<0.01–1.64	0 (0.0%)	0 (0.0%)
	Volta	1 (5.0%)	0.03	<0.01–0.57	0 (0.0%)	0 (0.0%)
	Total	**113 (21.7%)**	-	**<0.01–12.36**	**4 (3.5%)**	**1 (0.9%)**

[a] European Commission maximum limit of OTA in cocoa powder [54].
[b] ANVISA maximum limit of OTA in cocoa beans [20,28].

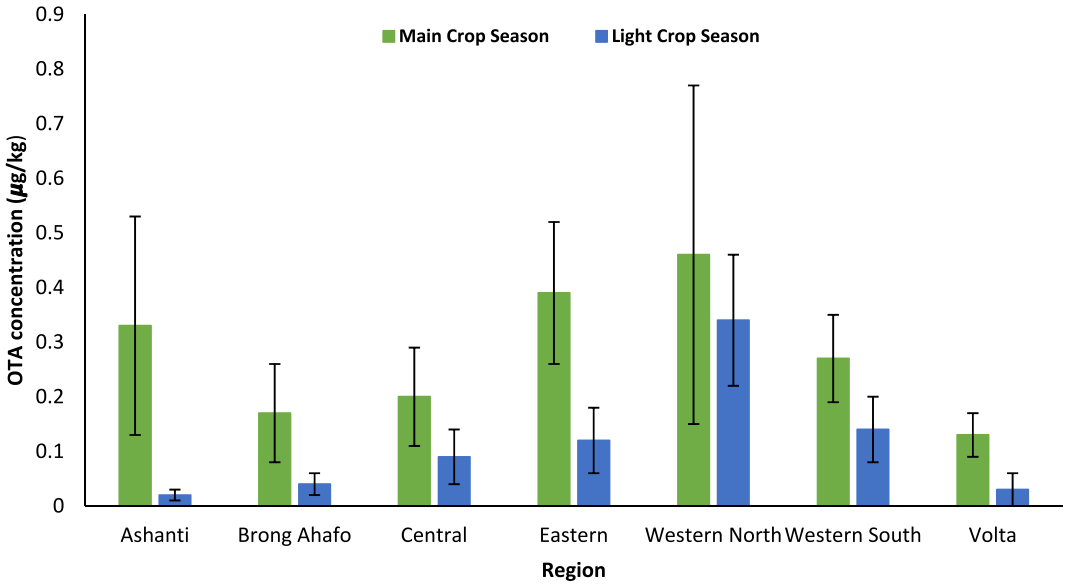

Fig. 5. Distribution of OTA in cocoa beans from the regions during Main and Light crop seasons.

from 1.0 µg/kg to 277.5 µg/kg. Additionally [56], examined cocoa bean samples from Trinidad and Tobago between 2007 and 2010 and found OTA contamination ranging from 2.67 ± 0.18 µg/kg to 14.61 ± 0.39 µg/kg. Conversely [41], indicated that the range and levels of OTA contamination in cocoa beans varied relatively narrowly. These authors analyzed 32 cocoa bean samples from the Western North and Western South regions of Ghana and found OTA contamination ranging from 0.186 µg/kg to 4.650 µg/kg. In agreement with previously reported studies, the present study demonstrated comparable variability in the range and levels of OTA contamination across the cocoa-growing regions and crop seasons [34,36,41,55,56].

Ochratoxin A levels in cocoa beans vary based on a variety of factors, including but not limited to origin and handling. Several studies have found variable concentrations of OTA in cocoa beans from the same areas in Ghana, West Africa, and elsewhere [34,36,41,55–57]. Similarly, the observed concentrations of OTA differed between samples, regions, and seasons in this study. The difference may be attributable to the pre-harvest, post-harvest practices and cocoa bean storage practiced in some regions. Moreover, levels of cocoa contamination by OTA are linked to seasonal changes, agricultural practices, and phytosanitary conditions, such as cocoa pods that are damaged by pests, physically injured, rotting, or mummified [57]. Possibly, these factors have contributed to the observed variability in OTA contamination levels across the regions and seasons.

The occurrence of OTA contamination in cocoa beans has been widely reported, with OTA concentrations regularly exceeding European Commission and ANVISA regulatory limits [26,39,41,55]. In this study, our results indicate that 3.5% and 0.9% of the 21.7% OTA-positive samples exceeded the 3 µg/kg and 10 µg/kg limits established by the European Commission and ANVISA, respectively. In addition, the 95th percentile of the OTA-positive samples ranging from 0.01 µg/kg to 1.96 µg/kg were below the European Commission and ANVISA regulatory limits during the two crop seasons. However, a larger sample size compared to the present study's total of 40 per region could result in more reliable and accurate 95th percentile results in the two seasons. Considering the possible carcinogenic properties of OTA and the negative effects it has on human health, the quality and safety of cocoa beans produced must be monitored to protect the health of people who consume cocoa and cocoa-derived products in different parts of the world.

Considering that certain pre-harvest, post-harvest, and storage practices are critical control points in the production of high-quality cocoa beans, considerable attention must be paid to these factors to prevent and reduce OTA contamination. Particularly, strategies for preventing and reducing OTA contamination, including good agricultural practices (GAPs), good manufacturing practices (GMPs), appropriate environmental factors, favourable storage practices, and the Codex recommended code of practices, must be implemented throughout the cocoa value chain [58,59]. GAPs include the use of licensed fungicides, herbicides, and insecticides for fungal infection control, weed eradication, and insect damage control. In addition, storage conditions play a significant role in the management of OTA, as they influence the total proliferation of ochratoxigenic fungi. OTA contamination is reduced through proper storage conditions, including temperature regulation, ventilation, and adequate humidity [59].

4. Conclusions

Employing a validated LC-MS/MS with an immunoaffinity clean-up method, the OTA levels in Ghanaian cocoa beans were determined. The method was sensitive, selective, accurate, and produced outstanding OTA recoveries. The present study provides information on the occurrence and distribution of OTA in cocoa beans from cocoa-growing regions of Ghana during the Main and Light crop seasons. OTA contamination was found in 21.7% of the 520 samples examined. In addition, all cocoa-growing regions had varying concentrations of OTA contamination in the range of 0.01 µg/kg to 12.36 µg/kg in the studied samples. However, 0.9% and 3.5% of the

OTA-contaminated samples exceeded the 10 μg/kg and 3 μg/kg corresponding MLs of ANVISA and the European Commission. Farmers' and other stakeholders' knowledge of the consequences of OTA contamination of cocoa beans should be strengthened through increased awareness and education. This could potentially aid in preventing and reducing OTA contamination in cocoa beans production, protecting human and animal health, boosting economic value, and promoting trade.

Author contribution statement

Joel Cox Menka Banahene; Isaac Williams Ofosu; Bernard Tawiah Odai: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data will be made available on request.

Additional information

No additional information is available for this paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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