A Study to Evaluate Aflatoxin Contamination in Food from Gauteng Province

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Abstract

Aflatoxins (AFs) and their producers have been found to be problematic and is still an issue. This is due to their role in influencing food quality, health and economy loss. In maintaining good food quality, minimize AFs occurrence and its deleterious effects, it is essential that issues relating to mycotoxin production and contamination are monitored, and addressed on a regular basis. This will proffer suggestions for immediate actions to be taken, in order to achieve and maintain regulatory limit before situations are out of control. Hence the case study to evaluate the concentration of AFs in food, by way of thin layer chromatography and high performance liquid chromatography was attempted. This is to monitor concentrations of the emerging mycotoxin in relation to international regulatory limits. Concentrations levels ranging 0.06 - 77.97 ppb was observed, (mean: 16.8 ppb) and measured out in the food samples of study, exceeding the ISO limits (0.05 ppb). This calls for proper monitoring of pre and postproduction stages in food, by employing easy to use chromatographic techniques as a routine process to screen and ascertain concentrations do not exceed regulatory limits.

Introduction

Foods and feeds generally have the potential of being invaded by fungi, particularly *Aspergillus flavus* and *Aspergillus parasiticus* during pre-production or post- production, resulting to contamination due to proliferation. In the process, metabolic activities take place within the fungi, in and on the food produce or product. The biochemical actions results to production of secondary metabolites in the food produce/product, which can be toxic, particularly in the case of aflatoxins. Evidences that foods are frequently subjected to *Aspergillus* fungi spoilage and subsequently aflatoxins AF(s) contaminations cannot be over emphasised. Reports of [13,12,3] indicated the metabolic activities of *Aspergillus* fungi to yield AFs, which have been found in various agricultural commodities. This contamination results in poor quality food produce/products. Consumption of these, results in reduced health and economic losses. Presence of AFs in under developed and developing countries is increasingly recognised, owing to their high concentrations in food produce and products [7]. Data on prevalence of AFs that causes aflatoxicosis; a disease caused by ingestion of food contaminated with aflatoxins in Africa is limited. There have been reports of AF contamination and the disease outbreak in human, from stapled agricultural products in Kenya [16] and in dogs in South Africa [15] due to AFs exposures. This incident did arise quality check in relation to AF contaminations.

Study Method

Materials and Reagents

Aflatoxins levels in food samples from Gauteng province were estimated by High Performance Liquid Chromatography (HPLC) and thin layer chromatography following an immune-affinity clean up. Aflatoxins extraction, detection and quantification were reached using the following materials and reagents that were of MERCK specifications except otherwise. They include: Separating funnels fitted with stoppers, large test tubes (15x25cm)/boiling tubes, wash bottles, elastic bands, methanol (HPLC grade), sodium chloride, nitric acid, potassium bromide, anhydrous sodium bicarbonate solution (saturated), acetonitrile, toluene, formic acid, Propan-2-ol, Ethyl acetate, dichloromethane, variable hot air drier (FENICI, 41512), TLC tank (Camag Ltd), Amber vials, HPLC vials, UV box, rotary blade blender (Torrington, CT. USA), phosphate buffered saline tablets (Prod codes: RP202), microfiber filter paper (Whatmann N° 113 (Prod codes: P66 and P67), aflaprep immunoaffinity columns (Prod code: AP01; R-Biopharm AG; Darmstadt, Germany), mobile phases, standards of aflatoxins (AFs) i.e. aflatoxin G₁ (AFG₁), aflatoxin G₂ (AFG₂), aflatoxin B₁ (AFB₁) and aflatoxin B₂ (AFB₂) (ARC, South Africa), 20 x 20 cm pre-coated aluminium backed silica gel G TLC plates Merck Art 5553, Aldrich), HPLC Spectra Physics SCM400 SYSTEM (Shimadzu Corporation, Kyoto,

Japan) equipped with a LiChrospher 100 RP-18 column (250 mm x 4 mm i.d and 5 μ m particle size) (Merck, Darmstadt, Germany), Waters Sentry™ guard column and a fluorescent detector (Shimadzu Corporation, Kyoto, Japan), , thermostatically controlled hot plate (University of Natal 198364), visking dialysis tubing (8/32) (Sigma), water pump with trap to supply vacuum, Virtis homogeniser (Sigma).

Aflatoxin Analysis

Aflatoxin extraction and clean-up

12.5g of milled sample and 1 gram of NaCl into a solvent resistant blender jar. Aflatoxins extraction from samples using an immuno-affinity column was by an extraction and clean-up protocol, using the version PO7/V15/26.01.05 aflaprep kit. AFs extracts were dried using N₂ gas and stored at 0°C until used analysed.

Thin Layer Chromatography

Thin layer chromatography technique employed at room temperature using modified method of [6] two solvent mixtures; dichloromethane/ethyl acetate/2-propanol and Toluene/ethyl acetate/formic acid. A 10x10 aluminium backed silica gel TLC plate (Merck) was used for TLC runs.

Validation of the TLC results

Validation of the TLC results and the determination of the concentrations of AFs in food were achieved by HPLC analysis from immuno- affinity column extracts. This was based on the peak area of chromatograms of AFs standards (Figure 1) in comparison to those of extracts (Figure 1.1) AFs.

Figure 1.0: HPLC Calibration curves of aflatoxins standards.

High Performance Liquid Chromatography

The HPLC system used for this assay was a Shimadzu Corporation (Kyoto, Japan) LC-20AB liquid chromatograph equipped with CBM-20A communication bus module, LC-20AB degasser, CTO-20A column oven, SIL-20A auto sampler, RF-10A_{XL} fluorescence detector, Kobra cell RID-10A refractive index detector and SPD-M20A photodiode array detector linked to an LC solutions version 1.22 Software Release. The experimental calibration curves (Figure 1.0) were obtained with known concentrations of standards and the equations describing these calibrated curves; $Y = 2312588x$, $8938199x$,

$120465x$ and $1466470x$ for standards of AFB₁, AFB₂, AFG₁ and AFG₂, respectively, (where x is the peak area of chromatogram and Y is the AF concentration) with correlation coefficient values of (R^2) of ≥ 0.9993 obtained and all at the y-intercept of zero showing a linearity of the method used.

Figure 1.1: HPLC Chromatograms of aflatoxins standards.

Figure 1.2: HPLC chromatograms of aflatoxins in food extracts showing presence of aflatoxins ([1]:AFB₁, [2]:AFB₂, [3]:AFG₁ & [4]:AFG₂).

Validation of Reaction System

Validation of the analysis and reaction system was determined by recoveries of AFs spiking on 12.5 g clean feed sample using aflaprep immuno-affinity extraction and clean-up with known concentrations of AFB₁, AFB₂, AFG₁ and AFG₂ standards. The spiked samples was thoroughly mixed and incubated at room temperature in a fume cupboard for at least an hour in triplicates and AFs extracted.

Discussion/Conclusion

Thin layer chromatography data in the study showed the presence of AFs, although the limits of detection (LOD) were higher for HPLC (83%) compared to TLC (27%). This could be attributed to the fact that the LOD of AFs by HPLC methods is much sensitive compared to TLC. The levels of AFs in the food based on the HPLC

results obtained in the present study varied from 0.06 to 77.97 ppb. These results were in line with those of [5,2,12,17] in agricultural products, with AFB₁ being the most abundant of the AFs recovered and detected. Aflatoxins, principally produced by *Aspergillus flavus* and *A. parasiticus* represent a group of potent mycotoxins (especially AFB₁) that contaminate food and feed commodities worldwide [9]. Exposure to significant amounts of these toxins, seriously affect health [10], but also economic losses [8,4] due to weight loss, poor immune function [18], decreased reproduction and even death in severe circumstances. Aflatoxicosis, in animal and man due to exposure to AFs is often reported in literature. Several reasons can be advanced for this but more importantly, is the fact that there is lack of proper management strategies and process routine checks being put in place to limit AFs contaminations. Limiting these contaminations can be achieved if there is continuous routine monitoring of feeds for AFs and the fungi responsible in producing them. In evaluating AF contamination in feeds, it is may be imperative to identify fungal species responsible for producing them. In which case, the producing fungi can be identified and quantified morphologically. However, this can be time consuming and requires expatriates in taxonomic skills [11]. Approach of [1], also presented a model system that could easily be adapted for aflatoxin detection in a variety of food and feed samples. It is in this light that the study related to toxigenic fungi attendant mycotoxins in feed is reported.

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