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Research Article

Determination of Aflatoxins in Groundnut and Sesame Oils from three States of Sudan and Experiments for Decontamination of Aflatoxin B1

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Abstract

In this work 36 samples of groundnut oil and 18 samples of sesame oil from Khartoum, Kordofan and Gadarif states in Sudan were investigated for contamination by different aflatoxins including AFB1, AFB2, AFG1 and AFG2 using liquid chromatography with detector of fluorescence. According to the method limit of detections (LODs) were found to be in range between 0.01 - 0.017 µg kg^{-1} but limit of quantifications (LOQs) were found to be in range between 0.03 - 0.05 µg kg^{-1}. The percentages of contaminated groundnut oil samples with AFB1 were 58.3%, 41.6%, and 50.0% from Khartoum, Gadarif and Kordofan state, respectively. Only one samples of sesame oil from Khartoum state was found to be contaminated with AFB1 (16.6%). The efficiency of decontamination of aflatoxin B1 (AFB1) residues in groundnut oil using different detoxification methods namely zeolite, activated charcoal, lumen juice and UV radiation were investigated. Lumen juice and UV radiation have been found to cause a significant decrease of AFB1. AFB1 was decreased by 37.9% and 55.1% when the groundnut oil was treated with lumen juice and UV radiation, respectively.

Keywords: *Mycotoxins; Aflatoxins; Groundnut Oil; Sesame Oil; Decontamination*

Introduction

Different aflatoxins (AF) can be produced by *Aspergillus flavus* and A. *parasiticus*, and they are a group of naturally fungal metabolites which occurred in food and feed samples, aflatoxins have long been recognized as significant food and feed contaminants. Aflatoxin B1 (AFB1) is the most frequent metabolite present in contaminated food and feed samples, Aflatoxin B1 (AFB1) was recognizing as a group I carcinogen by the International Agency for Research on Cancer as it is the cause of human primary hepatocellular carcinoma [1]. In general, aflatoxins are carcinogenic, mutagenic, teratogenic, and genotoxic posing a significant threat to human and animal health through ingestion [2].

Aflatoxins occur in many countries, especially in tropical and subtropical regions, and many agricultural commodities and important crops are susceptible to such contamination, especially peanuts and peanut-based foods [3]. Therefore, removal or inactivation in food and feed stuff already contaminated with toxic fungal metabolites is a major concern [4-6].

For reducing aflatoxins contamination of feeds and foods, some different strategies have been used and can classified into chemical decontamination, physical sorting and biological inhabiting methods [7]. Ammoniation is the method that has received the most attention

for detoxification of aflatoxin contamination in animal's feeds and has been adopted and successful strategies in many countries. Other chemical treatments with hydrochloric acid, hydrogen peroxide, sodium hypochlorite, ascorbic acid and ammonium carbonate [8].

In previous work [9], the presence of aflatoxins in groundnut and groundnut product from three states from Sudan were investigated. Some of the samples were found to contain AFB1 concentrations above the EU regulatory limits. Therefore the aims of this study were to determination of different aflatoxins in groundnut and sesame oils and to investigate the detoxification of AFB1 from groundnut oils using, zeolite, activated charcoal, UV irradiation and lemon juice (citric acid).

Material and Methods

Samples

Groundnut and sesame oils samples collected from markets in Khartoum, Kordofan and Gadarif, each oil sample contain about one litre. The sample was mixed well to before analysis [5,8].

All method steps for determined different aflatoxins were done according to official method 990.33 in AOAC with some modifications done in some steps [10].

Chemicals

Different chemical contain Methanol HPLC grade, Acetonitrile HPLC grade, n-Hexane, Hydrochloric acid, Dichloromethane were obtained from Scharlau, (Barcelona, Spain). Sodium chloride, Acetone, Benzene, Trifluroacetic acid and chloroform were purchased from (ROMIL, UK). Phosphoric acid and Acetic acid were obtained from (Riedel-de Haën, Hannover, Germany). Sodium sulphate anhydrous, Citric acid and diethyl ether were all profiled by El Walidien Trade and Investment Co. (Khartoum, Sudan). Standard of different aflatoxins were purchased from Immunolab GmbH (Kassel, Germany).

Apparatus

Shimadzu HPLC system with Fluorescence detector used to determination of different aflatoxins.

Analysis of aflatoxins (AFB1; AFB2, AFG1, and AFG2)

In the extraction procedure 50 gram of oil sample was transferred to 500 ml separation funnel, containing 200 ml methanol and 50 ml 0.1N hydrochloric acid and shacked for 30 min at high speed in mechanical shaker. The aqueous solution was filtered through 24 cm Whatman No 1. 50 ml of the filtrate was transferred into 250 ml separation funnel; 50 ml of 10% sodium chloride solution was added and the solution was swirled. 50 ml hexane was added and the solution was shaken gently for 30s. The two phases were separated and lower layer was drained into 250 ml separation funnel and was extracted three times with 25 ml dichloromethane. In clean up the dichloromethane extracts was then carefully transferred into silica gel chromatography column and 30 ml ether: hexane (3: 1) v (volume)/v (volume) used to wash the column. For elution aflatoxins 100 ml of dichloromethane: acetone (90: 10) v/v were added to the column then dried. To derivatize the aflatoxins trifluroacetic acid (TFA) were added and1.95 ml acetonitrile: water 1: 9 (v/v) was added and left for 10 minutes to separate. The aqueous layer used for HPLC analysis.

To detect aflatoxins HPLC operating conditions contain Supelcosil LC18; 150 x 4.6mm (id) using Mobile phase contain water: acetonitrile: methanol (700:170:170) with flow rate 1.0 ml min^{-1} and Oven temperature 40°C. and excitation 360 nm and emission 440 nm for the detector, the Injection volume was 20 µL.

Decontamination procedure for AFB1

Zeolite

0, 5, 10, 15, 20 and 25 g of zeolite were added to each 50g of groundnut oil samples and each was mixed well and was left to stand for 24 hour then the samples were analyzed for AFB1.

Activated charcoal

0, 5,10,15,20 and 25 g of Activated charcoal were added to each 50g of groundnut oil samples. The samples were mixed well and were left for 24 hour; then the samples were analyzed for AFB1.

UV

Each 50g of groundnut oil samples were exposured to UV light for different time 0, 0.5, 1, 2, 3, and 4 hours. The samples were mixed well and analyzed for AFB1.

Citric Acid (lemon juice)

0, 2.5, 5 and 10g of lemon juice were added to each 50g of groundnut oil then mixed well and let them for hour. The samples were analyzed for AFB1.

Result

Method performance

The calibration curves were generated using aflatoxins peak areas against the aflatoxins concentrations. The coefficient of correlation (R) from 0.991 to 0.996 for different Aflatoxins. The LOD was 0.017 ng/g for AFB1 and AFG1 and 0.01 ng/g for AFB2 and AFG2; respectively. The LOQ was 0.05 ng/g for AFB1 and AFG1 and 0.03ng/g for AFB2 and AFG2; respectively (Table 1).

Analyte	Linear Range (Ng/Ml)	Slope	Intercept	R^2	Lod (Ng/G)	Loq (Ng/G)
AFB_1	2-18	3434627.02	+52628.24	0.991	0.017	0.05
AFB_2	0.4- 3.6	5911589.33	+12887.47	0.995	0.01	0.03
AFG_1	2-18	2053862.12	+12733.01	0.995	0.017	0.05
AFG_2	0.4- 3.6	2695521.41	+2974.84	0.996	0.01	0.03

Table1*: Linearity range, limit of detection and limit of quantification for different aflatoxins.*

LOD: Limit of Detection, LOQ: Limit of Quantification, R^2: Correlation coefficients.

In analysis of groundnut oil samples, AFB_1 was detected in 7 (58.3%), 5 (41.7%) and 6 (50%) out of each 12 samples analyzed from Khartoum, Gadarif and Kordofan states, respectively; AFB_2 was detected in 6 (50%) from Khartoum and Kordofan, and 5 (41.7%) in Gadarif; AFG_1 was detected in 6 (50%), 3 (25%) and 4 (33.3%) in groundnut oil samples from Khartoum, Gadarif and Kordofan states, respectively. The mean values of AFB_1, AFB_2 and AFG_1 in Khartoum state were 25.56 µg kg^{-1}, 3.75 µg $kg^{-1}$and 3.59 µg kg^{-1}; respectively. They

are higher compared to the other states, no groundnut oil samples have been found contaminated with AFG_2 (Table 2 and Figure 1). From all sesame oil samples analyzes from the three states only one sample has been found contaminated with aflatoxin B1 from Khartoum state.

State		AFB_1	AFB_2	AFG_1
Khartoum	N	7	6	6
	Mean	25.56	3.75	3.59
	Std. D.	18.85	2.09	2.06
Gadarif	N	5	5	3
	Mean	11.57	1.25	1.35
	Std. D.	4.32	0.35	0.26
Kordofan	N	6	6	4
	Mean	14.87	2.48	1.34
	Std. D.	10.20	1.62	0.31

Table 2: *The mean and standard deviation for groundnut oil among the three studied states.*

N: Number of positive samples, Std. D: standard deviation.

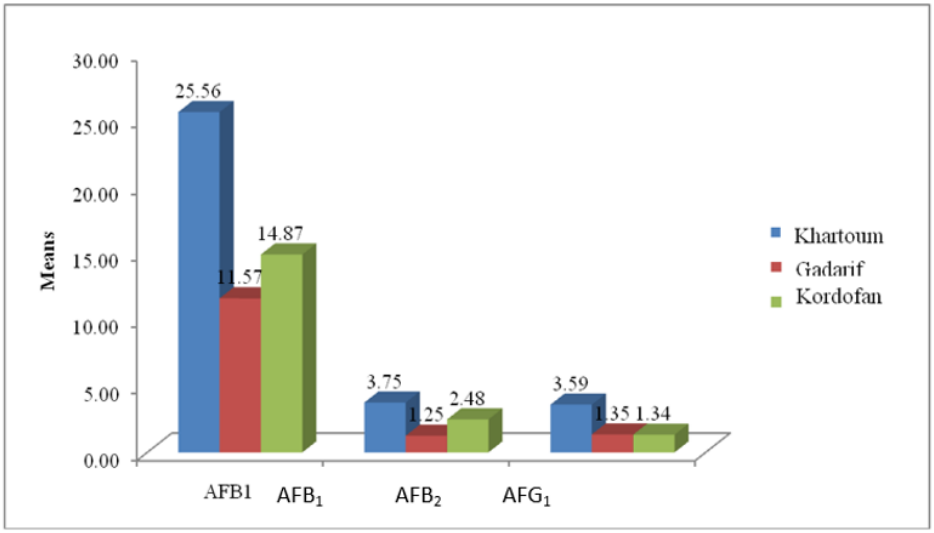

Figure 1: *The means of AFB1, AFB2 and AFG1 in groundnut oil from the three studied states.*

The detoxification of AFB_1 was carried out to contaminated samples of groundnut oil product by addition different amount of zeolite, activated charcoal and citric acid, or exposure to UV light for different times. The high percent of AFB1 decreasing by zeolite, activated charcoal, limon juice and UV light were 15.8%, 21.3%, 37.9.4% and 55.1%; respectively (Table 3 and Figure 2).

Materials for decontamination		Decreasing percent of AFB_1
Type	**Amount Added (%)**	
Zeolite	2	6.3
	5	9.7
	10	11.5
	20	13.7
	25	15.8
Activated charcoal	2	7.5
	5	10.4
	10	14.8
	20	18.2
	25	21.3
Lemon Juice (Citric acid)	2	17.1
	5	19.4
	10	25.5
	20	32.4
	25	37.9

***Table 3**: Decreasing percent of AFB_1 in groundnut oil using different materials for decontamination.*

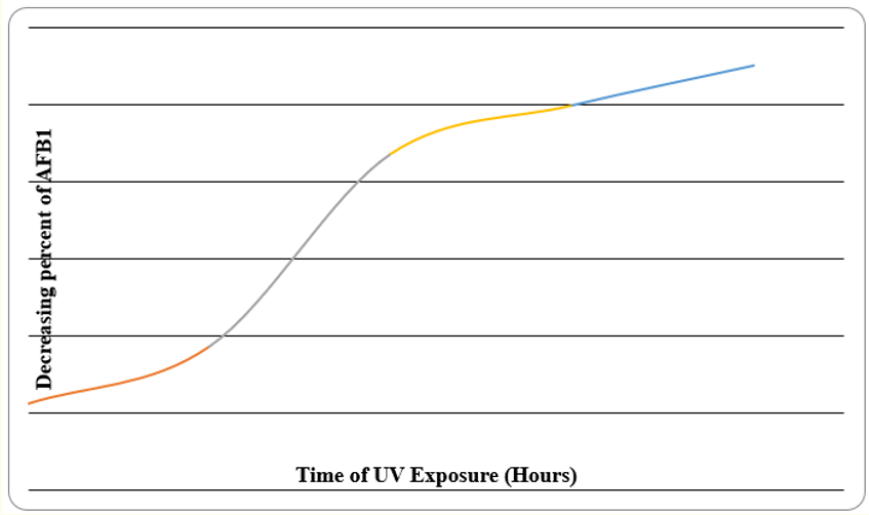

***Figure 2:** Decrease of AFB1 in groundnut oil using UV light.*

Discussion

The most frequently found aflatoxins in groundnut oil were AFB_1 followed by AFB_2 and AFG_1. This order of the AFB_1 and AFB_2 is consistent with the results found in peanut and peanut products in Sudan [9-12]. In a study done by Yousif., *et al*. [13], AFB_1 was detected in eight

samples representing 14.3%, and the highest incidence of aflatoxin contamination occurred in sesame oil in the range between 0.2-0.8 µg kg^{-1}, followed by groundnut while no aflatoxin contamination was detected in cotton seeds oil in his study.

Elzupir and others [14] has studied the contamination of groundnut cake and sesame cake using in animal feeding in Khartoum state by aflatoxins and ochratoxin A and he found that 66.67% of the samples analyzed were contaminated by aflatoxins at concentration ranged between 2.79 to 147.13 µg kg^{-1}. In another study by Salah and others [15] found that 60% of the samples analyzed from groundnut cake were contaminated with AFB_1 and AFB_2 with a mean values of 16.75 and 3.43 µg kg-1; respectively.

The obtained variation in the current study as compared to the one performed in Khartoum state by Elzupir and others [14] and that by Elamin and others [16] might be due to the difference in the test materials used and/or the applied techniques. Elamin and others [17] performed analysis of groundnut cake for aflatoxins using HPLC with UV Detector.

A photo degradation study done to AFB_1 in peanut oil by Ruijie and others [18], was performed under UV irradiation at different AFB_1 initial concentrations and UV irradiation intensities they found that the UV intensity and the time of exposure are more effective on AFB_1 photo degradation ratio, and AFB_1 with initial concentration of 2 mg/kg can be degraded thoroughly within 30 min under the intensity of 800 $\mu w/cm^2$. In other study by Qian and others [19] they decontaminated AFB_1 in peanuts with different solutions of sodium hypochlorite and found that the decontaminated rate of AFB_1 are 60%, 70% and more than 80% in alkaline, neutral and acid solution of sodium hypochlorite; respectively.

Ammonization, mainly used to decrease the level of aflatoxins in feed, is an efficient method for detoxifying feed for several years. Park and others [20] found that ammonization is effective against AFB_1 when done at high temperature and pressure. AFD_1 id the product when using ammonia in detoxification and this aflatoxin is less toxic than AFB_1.

Conclusion

The percent of aflatoxins in groundnut and sesame oils from Gadarif, Kordofan, and Khartoum were investigated. The higher incidence of AFB1 contamination of groundnut oil warrants further investigation as this oil is widely consumed in Sudan. A wide investigation of aflatoxin levels in edible oils consumed in Sudan is necessary. The occurrence of mycotoxins in human foods and animal feeds is a large-scale problem for the agricultural industry. The issue of mycotoxins in foods and animal feeds is a major challenge for the scientific community.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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