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Analysis of Aflatoxins in South Carolina Farm's Corn, Peanut, Wheat, Soybean, and Cottonseed

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ABSTRACT

The mold Aspergillus grows on several raw food commodities and produces highly toxic compounds known as aflatoxins. These compounds can cause developmental and immune system suppression, cancer, and death if ingested. The aim of this study was to determine the aflatoxin levels in various crops obtained from farms in South Carolina, USA. Aflatoxin levels were measured using the Vicam Virtu Reader and High-Performance Liquid Chromatography (HPLC). The Vicam Virtu Reader utilized five grams of corn and peanuts blended and placed into an extraction tube containing 25 ml of 70% methanol. The sample mixture was placed on the AlfaV test strip for readings. For use of the HPLC, the samples were analyzed by isocratic using 60:20:20 water/methanol/acetonitrile mixture as the mobile phase. Results from the Vicam Virtu Reader indicated corn samples and peanut samples had aflatoxin levels below 25 ppb established by the USDA. When the HPLC analysis was done on soybean, wheat, and cottonseed, all results were below 25 ppb as well. A food safety survey was administered to 190 farmers to ascertain their familiarity with aflatoxins. Sixteen percent (16%) reported they heard about it. In conclusion, storage conditions of the crops can affect the level of aflatoxins. The Vicam Virtu Reader is a fast method to identify aflatoxin levels in crops. The HPLC has the advantage of separating aflatoxins into subgroups even at low levels. The aflatoxin levels were low and safe for export and consumption.

Keywords

Aflatoxin, *Aspergillus*, Contamination, Crops.

Introduction

Mycotoxins are secondary metabolites produced by many filamentous fungi and its contamination of food and feed is an ongoing global problem. Although good agricultural, storage, and processing practices are implemented, mycotoxin contamination is considered an unavoidable and unpredictable problem, and poses a difficult challenge to food safety. Furthermore, many mycotoxins are not easily eliminated during food processing because of their stability against heat, physical, and chemical treatments [1]. Mycotoxin contamination of grain is a complex and frustrating situation affecting producers, grain elevators, food and feed processors, and consumers. Although over 300 mycotoxins have been identified and reported; however, aflatoxins (AF), ochratoxins, fumonisins, patulin, zearalenone, and trichothecenes including deoxynivalenol and T-2 toxin contaminate food and animal feedstuffs, and these mycotoxins are of greatest importance from food safety and regulatory viewpoints [2,3].

Among the mycotoxins, AFs are considered the most toxic, with a significant economic burden to agriculture [4,5]. Favourable conditions for growth of AFs include high moisture content and high temperature. AFs can contaminate agricultural commodities including corn, wheat, rice, peanut, and many other crops [6,7]. AFs are primarily an economic concern in the United States and European Union countries, whereas in the developing countries

of Asia and Africa, AFs contribute to hundreds of hepatocellular carcinoma cases each year [4,8,9]. The total estimated annual losses to the US corn industry is from US $52.1 million to US $1.68 billion due to aflatoxin contamination [4].

AFs are a group of structurally related, toxic, secondary metabolites produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus* that are present normally in soil and various organic materials [8,10,11]. While *A. flavus* strains produce only AFB1 and AFB2, *A. parasiticus* strains can produce AFB1, AFB2, AFG1, and AFG2 [12]. AFB1, B2, G1, G2 and M1 can be regarded as the most important mycotoxins due to their genotoxic carcinogenic properties and potent mutagenic and carcinogenic substances; AFB1 is the most potent followed by AFG1 and AFM1 [11]. AFB1 have been found in most staple foods, e.g., cereal grains such as maize, wheat, oats, rice, etc., ground nuts, peanut butter, beans, Brazilian nuts, almonds, cottonseed, cayenne pepper, Indian chili powder, bread, eggs and meat [13,14]. The chronic AF-exposure induces liver cancer, infections, and growth impairment in humans, while high exposures cause acute symptoms, and even death [15,16]. AFB1 is one of the most potent hepatocarcinogens, teratogen and mutagen to humans and animals and has been listed as a group I human carcinogen by the International Agency for Research on Cancer (16-18) which causing damage such as toxic hepatitis, hemorrhage, edema, immunosuppression, and hepatic carcinoma [19-21]. Since AFs affect several farm products, they are regarded as one the most important food safety problems in the world and are regulated by over 100 countries [22].

Economically important crops such as maize, rice, cottonseed, peanuts, and spices are all susceptible to contamination of aflatoxin resulting in a major global challenge to manage aflatoxin contamination in crops and other food products [23]. There are reports of creating a large economical loss of aflatoxins in the developed and developing countries [24-26]. Unfortunately, about 25% of the world's harvested crops are contaminated by mycotoxins each year, leading to huge agricultural and industrial losses in the billions of dollars [1]. Significant economic losses are associated with the impact of mycotoxins on human health, animal welfare and productivity, and both domestic and international trade [4,8,27]. The Food and Agricultural Organization of the United Nations (FAO) estimated that at least 25% of the world's cereal grains are contaminated by mycotoxins, including aflatoxins [28]. In the US, it was reported that income losses due to AFs contamination cost an average of more than $100 million per year to US producers [29].

The aim of this study was to analyze the level of aflatoxins in South Carolina farm's corn, peanuts, wheat, soybean, and cottonseed. This study will determine the prevalence of AFs in South Carolina farm's crop as well as provide useful information to the farmers, producers, and consumers.

Materials and Methods

Samples

Samples of corn, peanut, wheat, cottonseed, and soybeans were collected from farmers in Hampton County, Orangeburg County, Williamsburg County, and Charleston County during the Spring and Fall of each year. The extension agent coordinated with farmers to obtain the sample crops. The samples were labeled, packaged in sterile polyethylene bags, transferred to the laboratory, and kept in a cool place (3-5°C) until aflatoxins analysis and ozone studies were performed.

Chemicals and reagents

Seventy percent (70%) methanol and Afla-V strips were purchased from Aqua Solutions, Inc. (Deer Park, Texas), and Vicam, a Waters Corporation (Nixa, Missouri, USA), respectively.

Apparatus

The equipment used in this study were the Vicam Vertu™ reader (Nixa, Missouri, USA) and HighPerformance Liquid Chromatography (HPLC) (Shimadzu Inc, Osaka, Japan).

Sample preparation

All samples were ground by using an Osterizer blender (Sunbeam Products Inc., Boca Raton, FL, USA). For Vicam Vertu Reader, twenty-five grams of corn and peanut samples were placed in the jar and blended at Grate mode for 2 minutes. Five grams of ground sample was weighed and placed in an extraction tube. Twenty-five milliliters of 70% MeOH were measured with a graduated cylinder and poured into the extraction tube. Next, the extraction tube was covered and vortexed for the next 2 minutes at maximum speed. Lastly, the sample was filtered through Whatman number one filter paper (Whatman International Ltd., Maidstone, Kent, UK) and placed into a clean extraction tube.

For HPLC analysis, twenty-five grams of each homogenized and pulverized samples were mixed with 125 mL MeOH: H_2O (70:30 v/v). The sample suspensions were blended, and the extracts were filtered through Whatman Number 1 filter paper and the clear supernatants were collected in separate airtight amber vials. Sample purification was carried out using immunoaffinity column. Briefly, ten milliliters of the filtrate were diluted with 30 ml of deionized water and filtered through glass fiber filter. Ten milliliters of deionized water were passed through the aflatest immunoaffinity column for 1 drop per second, followed by 20 ml of diluted filtrate. Then ten milliliters of deionized water were passed through the column again. Aflatoxins were eluted with 1 ml of HPLC grade methanol and 1 ml of deionized water in a test tube. The sample was mixed and filtered by 0.45 µm syringe filter and 20 µl was injected into HPLC for analysis.

HPLC and Vicam Vertu Reader Analyses

For Vicam Vertu reader, one hundred microliters of Afla-V diluent were transferred to the strip test vial as well as 100 µL of the sample extract. The mixture was mixed well by vortexing. Then, 100 µL of the sample were transferred to the Afla-V strip test by dropping (1 drop per second) vertically into the circular opening. The strip test was allowed to develop for 5 minutes on a flat surface (such as a countertop). Lastly, the Afla-V strip test was inserted into the Vertu reader (circular opening side in first) and results were retrieved. However, If the reader displayed"> Range", sample was

diluted to extract 1 to 6 with 70% MeOH (100 µL extract +500 µL 70% MeOH). Then previous steps were repeated, and results were then multiplied by 6 to obtain the true level of contamination.

For HPLC analysis, samples were analyzed for aflatoxins using the HPLC system consisting of a degasser, auto sampler, and quaternary pump, and fluorescence detector. The chromatographic separation was performed with a reverse-phase column (Extend-C18, Zorbax column, 4.6 mm i.d., 250 mm, 5 µm, Agilent Co.). The samples were analyzed by isocratic using 60:20:20 water/methanol/acetonitrile mixture as the mobile phase. The column temperature was adjusted at 40°C at a flow rate of 1.0 mL/min to achieve the optimum resolution of the aflatoxins. The injection volume was maintained at 20 µL for both the sample and standard solutions.

All experiments were carried out at least in triplicate. The results were expressed as mean ± standard deviation for each sample.

Results

Analysis of farm peanuts and corn by the Vicam Virtue Reader

Samples designated as farm corn and peanut were obtained from Orangeburg, Williamsburg, Dorchester, and Calhoun counties in South Carolina. The level of aflatoxin was tested using the Vicam Vertu Reader, an instrument capable of giving results in 5 min. The aflatoxin levels for these samples are depicted in Table 1. The results indicate that the corn samples had readings in line with the USDA recommended reading of 25 ppb making them acceptable for export and consumption. The data indicates there was little variation in their ppb levels of aflatoxin. Furthermore, the Vicam Vertu Afla-V test reader is a fast and effective device that determines aflatoxin levels in corn. Table 2 reveals that corn obtained from local county farms SC #S1, SC #S2, SC #S1A and SC #S1B had acceptable ranges.

Table 1: Corn samples from local County farms in South Carolina.

Samples	Aflatoxin, ppb (mean ± SD)
12c	5.46 ± 0.45
9c	2.42 ± 0.22
15c	5.28 ± 0.27
13c	14.45 ± 1.54
8c	23.61 ± 3.27
10c	4.11 ± 0.32
7c	5.54 ± 0.55
3c	6.72 ± 0.52

Table 2: Determination of Aflatoxin from corn in local County farms of South Carolina.

Location	Aflatoxin, ppb (mean ± SD)
SC, #S1	14.08 ± 2.340
SC, #S2	2.04 ± 0.136
SC, #S1A	10.985 ± 1.81
SC, #S1B	12.13 ± 2.30

Table 3 is an illustration of the peanut samples obtained from farms in South Carolina. The results indicate an acceptable range. Results showed that the highest amount of aflatoxin found in sample 5p (22.40 ppb) which is also below the recommended value 25 ppb. Figure 1 represents the results of 190 farmers' knowledge of aflatoxins. Results indicate that 16% definitely knew what they were.

Table 3: Aflatoxin Levels of Farm Peanut.

Samples	Aflatoxin, ppb (mean ± SD)
3p,c	9.25 ± 1.23
1p	10.50 ± 1.11
5p	22.40 ± 2.45
2c	0.00 ± 0.00

Table 4: Amount of aflatoxin in farm 5824 Wheat.

Aflatoxin types	Aflatoxin, ppb (mean ± SD)
G2	0.00 ± 0.00
G1	0.00 ± 0.00
B2	0.06 ± 0.02
B1	0.40 ± 0.08

Analysis of farm soybean, cottonseed and wheat by HPLC

Samples designated as farm wheat were obtained from farm 5824. Table 4 shows the amount of aflatoxin in farm 5824. Farm wheat has only aflatoxin B2 (0.06 ppb) and B1 (0.40 ppb). Samples designated as farm cottonseed were obtained from cotton gin, mixed from mainly Williamsburg County, and some from Berkeley and Clarendon County). Table 5 shows the amount of aflatoxin in cottonseed obtained from the cotton gin. Cottonseed has aflatoxin G2 (0.01 ppb), B2 (0.01 ppb) and B1 (0.15 ppb).

Table 5: Amount of aflatoxin in cottonseed (cotton gin, mixed from mainly Williamsburg County, and some from Berkeley and Clarendon County).

Aflatoxin types	Aflatoxin, ppb (mean ± SD)
G2	0.01 ± 0.002
G1	0.00 ± 0.00
B2	0.01 ± 0.004
B1	0.15 ± 0.010

Table 6: Amount of aflatoxin in farm 5824 Corn.

Aflatoxin types	Aflatoxin, ppb (mean ± SD)
G2	0.000 ± 0.000
G1	0.000 ± 0.000
B2	0.372 ± 0.057
B1	5.575 ± 0.415

Table 7: Amount of aflatoxin in farm 5824 Soybean.

Aflatoxin types	Aflatoxin, ppb (mean ± SD)
G2	0.01 ± 0.02
G1	0.00 ± 0.00
B2	0.12 ± 0.01
B1	1.29 ± 0.19

Samples designated as farm corn were obtained from farm 5824. Table 6 shows the amount of aflatoxin in corn. Corn has aflatoxin

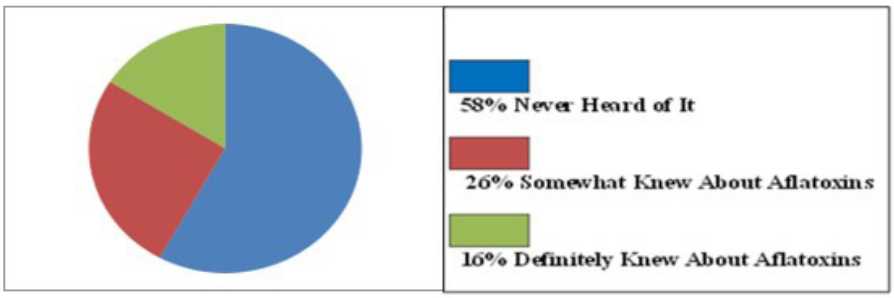

Fig. 1. Survey of Farmer's Knowledge of Aflatoxins

G1 (0.009 ppb), B2 (0.372 ppb) and B1 (5.575 ppb). Samples labeled as farm soybean were obtained from farm 5824. Table 7 shows the amount of aflatoxin in corn. Soybean has aflatoxin G2 (0.01 ppb), B2 (0.12 ppb) and B1 (1.29 ppb).

Discussion

South Carolina continues to play a significant role in the production of crops that are needed in the U.S. and the world. It is reassuring, that the results presented in this study, indicate that the low levels of aflatoxin make the products safe for export and consumption. They have set a high bar to produce crops that are of excellent quality. This study also shows that the Vicam Vertu Afla-V test reader is a fast and effective device for determining aflatoxin levels in corn and peanuts. Five grams was the minimum amount that was needed to conduct the experiment. When grinding samples less than 15 grams, a smaller size blender was used for the peanuts to be thoroughly blended. The readings represent levels that are in conjunction with the recommended USDA concentration of 25 ppb. The famers did an excellent job making sure their levels were low. However, there are several factors that could come into play to contribute to high levels aflatoxins in crops. These factors are the irrigation procedure, storage conditions or the moisture content at harvest and storage. Therefore, these conditions should be closely monitored to reduce the risk of aflatoxin contamination.

The HPLC data found in Figure 6 demonstrated that the B1 levels of cottonseed were the lowest (0.15 ppb), when compared to that of wheat, corn, and soybean found in tables 4,6, and 7, respectively. Furthermore, G1 levels were 0.00 ppb for all the crops tested. This indicates there is little concern for G1 contamination in the crops tested. Although this instrument is more costly and requires more time to obtain the results, it does offer the advantage of detecting the various subgroups of G2, G1, B2, and B1 aflatoxins present. The data indicates these crops had no issues with aflatoxin contamination. Since the levels of aflatoxins detected in the crops were so low, this indicates that the harvesting and storage conditions were more than sufficient to decrease the probability of aflatoxin contamination. If aflatoxin levels had been high, it has been suggested that the decontamination of aflatoxin should consist of physical removal, treatment with heat, chemical or radiation treatment. These methods, however, may cause a significant modification to the taste and structure of the crops harvested. The results from the farmers' survey were surprising. These findings indicate that there is room for more educational training.

Conclusion

Overall, the information obtained in performing the studies proved to be quite informative. We now have a baseline level of the aflatoxins found on farms growing corn, peanuts, wheat, cottonseed, and soybean in South Carolia, USA. Although all the samples tested were performed in the laboratory, the Vicam test reader does have the capability of being used for on-site testing of aflatoxins in peanuts and corn. Because aflatoxins pose such a health concern for the farming industry, the necessary steps must remain in place to maintain the quality of its products.

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