



2016 HAWAII UNIVERSITY INTERNATIONAL CONFERENCES
SCIENCE, TECHNOLOGY, ENGINEERING, ART, MATH & EDUCATION JUNE 10 - 12, 2016
HAWAII PRINCE HOTEL WAIKIKI, HONOLULU

ANALYSIS OF AFLATOXIN IN PEANUTS

STUKES, JAMES & ET AL
SOUTH CAROLINA STATE UNIVERSITY
1890 RESEARCH
DEPARTMENT OF BIOLOGICAL & PHYSICAL SCIENCES

Dr. James Stukes
Ms. Ebony Dyson
Mr. David Karemara
Mr. Nazimuddin Mohammed
Mr. Isa Musa

1890 Research
Department of Biological and Physical Sciences
South Carolina State University.

The Analysis of Aflatoxin Levels Found in Peanuts

Synopsis:

The objective of this study is to test the aflatoxin levels of processed peanuts compared to those obtained directly from small farms in S.C. Furthermore, the farmers of South Carolina will be informed and educated on the seriousness of aflatoxin in their crops.

ANALYSIS OF AFLATOXINS IN PEANUTS

Stukes, James, Ebony Dyson, Nazimuddin Mohammed, Isa Musa, and David Karemera
1890 Research
Department of Biological and Physical Sciences
South Carolina State University
Orangeburg, SC 29117

ABSTRACT

Aflatoxins are highly toxic fungal compounds produced by the mold *Aspergillus* which grows on a number of raw food commodities. Farmers in the U.S. and throughout the world face the impact of this toxin on crops such as peanuts and corn. Animals and humans may be affected by the consumption of these contaminated crops. In 2004, Kenya suffered from 317 people getting ill and 125 casualties due to consuming Aflatoxin B1 in corn. Aflatoxins have been found to damage and affect the lungs, kidneys, brain and heart. In this study, the level of aflatoxins found in peanuts was investigated. To determine the presence of aflatoxin levels, the Vicam Afla-V test reader was used. This device accurately detects the presence of aflatoxins B1, B2, G1 and G2 levels ranging from 2 ppb to 100 ppb. To analyze the samples, peanuts were blended finely and weighed to 5 g. The ground samples were then inserted into an extraction tube containing 25 ml of 70% MeOH and vortexed for 2 minutes. Samples were filtered and 100 μ l were placed on the Afla-V test strips which were placed into the reader; aflatoxin levels were obtained within 5 minutes. Results indicated that although the ppm levels of aflatoxin varied in the samples tested, there were some samples within the tolerant level of less than 25 ppb, while others exceeded the level established by U.S. Department of Agriculture (USDA). Future experiments will test for the presence of aflatoxin in various crops located within 4 regions of South Carolina.

INTRODUCTION

South Carolina grows and exports products such as peanuts, pecans, corn, and wheat. However, these products may contain the mold *Aspergillus flavus* or *Aspergillus parasiticus*, species of fungi that produce aflatoxin. *Aspergillus spp.* can grow on raw food commodities including nuts, cereals, and grains (Pattern, R.C.1981) and produces aflatoxin. Aflatoxin is toxic and among the most carcinogenic substances known (Hudler, 1998). Once these toxins enter the body, they can cause damage to the liver, such as cirrhosis or carcinoma of the liver. Among naturally occurring aflatoxins, aflatoxin B1 is the most toxic (Culler and Newberne 1994). Aflatoxins are naturally fluorescent producing a bright blue or blue green color due to their oxygenated pentacyclic

structure (Fente et al., 2001). Because it affects a number of farm products, aflatoxin is regarded as one of the most important food safety problems in the world, and is regulated by over 100 countries (Reuben, 2008). The emergence of aflatoxin contamination in foods, feed and agricultural commodities has made aflatoxin research a rapidly developing area (Rassaghi-Abyaneh et al., 2014). The objective of this study is to test the aflatoxin levels of processed peanuts compared to those obtained directly from small farms in S.C.

MATERIALS AND METHODS

Sample extraction

Five grams of ground sample were 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 2 minutes at maximum speed. Lastly, the sample was filtered and put into a clean extraction tube.

Analysis of Aflatoxin levels using the Vicam Vertu Reader

One hundred microliters of Afla-V diluent were transferred to the strip test vial as well as 100 μ L of the sample extract. The mixture was mixed well by vortexing. Then, 100 μ L of the sample was 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", the sample was diluted to extract 1 to 6 with 70% MeOH (100 μ L extract +500 μ L 70% MeOH). Then previous steps were repeated and results were multiplied by 6 to obtain the true level of contamination.

RESULTS

The samples described as processed peanuts were obtained from various grocery stores in Orangeburg, SC. The level of aflatoxins were 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 all six samples (A-F) tested for the processed peanuts have aflatoxin readings below 25 ppb, thereby placing them in an acceptable range for consumption. Moreover, there was little variation in their ppb levels of aflatoxin.

Table 1. Aflatoxin levels of processed peanuts

Samples	PPB (parts per billion)
A	11.57 ppb
B	10.24 ppb
C	9.61 ppb
D	11.35 ppb
E	12.66 ppb
F	11.30 ppb

Figure 1. below, depicts the test strips used for each sample. Ten drops of the samples were placed in the opening shown on the left of the strip at one drop per second. The strips were allowed to react for 5 min. prior to placing them in the Vicam reader. The red line on the strip shows the reaction of the sample. Similar strips were used to detect aflatoxin readings in peanuts obtained from farms.

Figure 1. Test strips used for processed peanuts

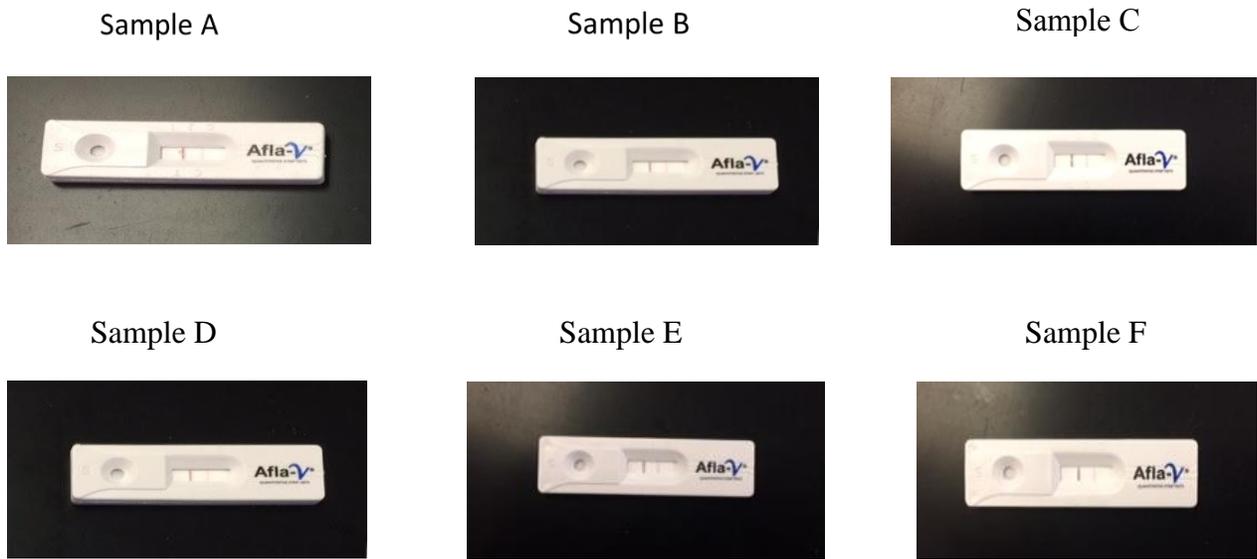


Table 2. Aflatoxin levels of farm peanuts

Peanut Samples From Lot # 521720264X South Carolina	
Sample #	Vicam Reading (PPB) Parts Per Billion
3p,c	9.25 ppb
1p	10.50 ppb
4p	51.29 ppb*
6p	96.40 ppb*
5p	22.40 ppb
2c	0.00 ppb

The results of Table 2 indicated that 4 of the samples obtained from farms were below the acceptable range of 25 ppb and did not pose a threat to human consumption. In contrast, two of the samples obtained from farms were over the acceptable range, 51.29 ppb and 96.40 ppb respectively. Furthermore, there was a high degree of variation in the aflatoxin levels 0 ppb in sample 2c to 96.4 ppb in sample 6p.

DISCUSSION

This study shows that the Vicam Vertu Afla-V test reader is a fast and effective device for determining aflatoxin levels in peanuts. Five grams is the minimum amount that is needed to conduct the experiment. When grinding samples less than 15 grams, a smaller size blender will need to be utilized in order for the peanuts to be thoroughly blended. All of the processed peanuts tested demonstrated levels within the safe consumption range. In contrast, two of the six samples obtained from storage bins from the farms showed levels 2.1 and 3.9 times higher than 25 ppb recommended by the USDA. Furthermore, these samples were above readable range and had to be diluted. These peanut samples were obtained from farmers during their off growing seasons. Therefore, they were stored in silos. These results indicate that the storage conditions need to be modified to decrease the likelihood of aflatoxin contamination. It has been suggested that the decontamination of aflatoxin consists of physical removal, treatment with heat, chemical or radiation treatment (Beaver, 1991). Although all of 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. Future studies will involve testing aflatoxin levels of corn from various farms in the state of South Carolina.

REFERENCES

- Beaver, R.W. (1991). Decontamination of mycotoxin-containing foods, and feedstuffs. *Trends in Food Science and Technology*, 2, 170-173.
- Fente, CA , Jaimex, J., Vazques, B.I., Franco, C.M., & Cepeda, A. (2001). New additive for culture media for rapid identification of aflatoxin producing aspergillus. *Applied and Environmental Microbiology*, 67(10), 4858-4862.
- Hudler, G. W. (1998). *Magical mushrooms, mischievous molds: The remarkable story of the fungus kingdom and its impact on human affairs*. Princeton University Press. ISBN 978-0-691-07016-2.
- Razzaghi-Abyaneh, M., Perng-Kuang C., Masoomeh S. & Mahendra. R. (2014). Global health issues of aflatoxins in food and agriculture: Challenges and opportunities. *Frontiers in Microbiology*, 5(420), 1-3.
- Pattern, R.C. (1981). Aflatoxin and Disease. *American Journal of Tropical Medicine and Hygiene*. 30(2), 522-425.
- Reuben, J. (2008). Aflatoxin Recognition, Understanding and Control with Particular Emphasis on the Role of the Agricultural Research Service. *Toxin Rev.*, 27, 143-169.