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Destruction of Aflatoxins in Contaminated Maize Samples using Ammoniation Procedures

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Because of widespread occurrence of aflatoxins and the potential hazards associated with consumption of aflatoxin-contaminated foodstuffs, a study was undertaken to determine how best to destroy aflatoxin in contaminated maize samples. Strains of *Aspergillus parasiticus* were used to contaminate maize to produce 1000 µg/kg of contaminated test sample. Ammoniation procedure in different concentrations of ammonia was adopted for aflatoxin destruction. Aflatoxin concentrations were determined by HPLC using fluorescence detection. The results obtained showed a proportional increase in aflatoxin destruction with the increase of ammonia concentrations. This study therefore established that ammoniation treatment under high pressure and at high ammonia concentration is more destructive to aflatoxins than treatment under atmospheric or low pressure. Ammoniation procedures may be considered suitable for large scale destruction of aflatoxins that contaminate foodstuffs stored in warm moist places.

Key words: Aflatoxins, contaminated foodstuffs, ammoniation procedures.

INTRODUCTION

Aflatoxins are mold metabolites of *Aspergillus flavus* and *Aspergillus parasiticus* that contaminate foodstuffs stored in warm moist places [1]. These toxins are not only hepatotoxic but are also hepatocarcinogenic in a wide variety of animals and humans, causing acute hepatitis, cirrhosis and hepatoma [2-3]. A positive correlation between aflatoxin levels in the food eaten and the pattern of liver cancer incidence in humans has been demonstrated [3-5]. Exposure to these toxins is therefore a public health hazard in areas where food production and storage conditions are conducive to mold spoilage and consequent aflatoxin production [1].

Aflatoxin contamination is not only a potential source of health hazards but is also involved in the spoilage of agricultural commodities such as nuts and cereal grains that are usually preserved by reduction of moisture content drying processes [6]. Contamination of these commodities often occurs in areas where the prevailing temperatures are high and favored by warmth and high humidity [6]. According to estimates of Food and Agriculture Organization (FAO) about 25% of the world's food crops are affected by aflatoxin contamination every year [7].

Although aflatoxins are frequent contaminants of a wide variety of cereal grains and groundnuts, contaminated maize staples are the main source of dietary aflatoxins consumed daily, especially in developing countries [3-9]. For example significant aflatoxin contamination levels were detected in several corn-based commodities such as corn on cob, corn drink, corn flour and corn flakes which were ready for consumption [8]. Since discontinuing the feeding of aflatoxin-contaminated maize staples is not always practicable, especially when alternative foodstuffs are not readily available or affordable, this study was undertaken to assess the efficiency of ammoniation procedure in the destruction of aflatoxins in maize samples. Ammonia reagent was chosen for this study because the products formed by ammoniation reaction do not revert back to aflatoxins and their toxicity is negligible [10].

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MATERIALS AND METHODS

Culture of Fungal Parasites

Aspergillus parasiticus strain was subcultured on potato dextrose agar for 7 days at 25°C. The fungal strain was then activated on potato dextrose agar media which consisted of 100 g pealed potatoes, 10 g dextrose and 10 g agar in 500 ml distilled water.

Contamination of maize samples

White dry maize sample (50 kg) was artificially infected with *Aspergillus parasiticus* strain as previously described [11-12]. A final concentration of contaminated maize sample was prepared by adding aflatoxin-free maize and blended the final mixture to produce 1000 μg/kg contamination level.

The destruction procedures

Destruction of aflatoxin in contaminated maize was carried out by ammoniation procedure as previously described [12]. The moisture content of 50 kg contaminated maize was adjusted to 18% wet matter. They were divided into five batches of 10 kg each. Then ammonia was sprayed to provide a level of 0.25%, 0.5%, 1.0%, 1.5% and 2.0% ammonia on dry matter. Each ammonia concentration was used to spray 10 kg contaminated maize under low pressure (5 kg) and high pressure (5 kg) ammonium treatment.

Extraction and determination of aflatoxins

Extraction and clean up of aflatoxins in all samples were performed using well established procedures [13]. The final residues of aflatoxins AFB_1, AFB_2, AFG_1 and AFG_2 were determined by a slightly modified method of High Pressure Liquid Chromatography (HPLC) described previously [14]. All solvents used were of the HPLC grade.

The procedure can be summarized as follows: to a final aflatoxin residue, 200 μl hexane was added followed by 50 μl trifluoroacetic acid (TFA) and mixed by a vortex shaker and then left to stand for 5 min. A mixture of 1.0 ml distilled water and 1.0 ml acetonitrile was added and then left to stand for 10 min. The hexane layer was discarded. A 20 μl of TFA-treated sample was used for determination of aflatoxins by HPLC technique.

For these analytical procedures aflatoxin standards AFB_1, AFB_2, AFG_1 and AFG_2 at concentrations of AFB_1 (0.76 μM), AFB_2 (0.48 μM), AFG_1 (0.55 μM), and AFG_2 (0.75 μM) were dissolved in methanol. The methanol was evaporated before the HPLC analysis of the samples.

The aflatoxin concentration in μg/kg (ppb) of maize samples were calculated using standard curves for each of the aflatoxin standard used.
The amount of aflatoxins: AFB_1, AFB_2, AFG_1 and AFG_2 were statistically analyzed by Microsoft Excel software 2003) using aflatoxin standards for each toxin as shown on table 1. Each standard contained 10 μl/ml.

Table 1: Aflatoxin levels in artificially contaminated maize by *Aspergillus parasiticus.*

Toxins	Sample	Conc.μg/kg
AFB_1	10 kg	96.0 ± 1.2
AFB_2	10 kg	92.0 ± 1.4
AFG_1	10 kg	87.6 ± 1.6
AFG_2	10 kg	86.4 ± 1.8
Mean	10 kg	90.5 ± 1.5

Values are means ± SE of the mean of 10 kg maize sample.

The effect of ammoniation treatment under high pressure on aflatoxin destruction were statistically analyzed by Excel software using aflatoxin standards containing 10μg/ml. Values are expressed as percent means ± SE of the mean for 10kg of maize sample (Table 2)

Table 2: Effect of ammoniation treatment under atmospheric or low pressure on destruction of aflatoxins. (% Mean ± S.E)

Ammonia Conc.	0.25%	0.5%	1.0%	1.5%	2.0%
Sample	**10 kg**	**10 kg**	**10 kg**	**10 kg**	**10 kg**
Total toxin	41±3.6*	71±2.9*	81±1.8*	86±1.4	90±0.9
AFB_1	40±3.7*	65±3.2*	77±2.1*	80±1.9	88±1.0
AFB_2	35±3.8*	65±3.2*	75±2.4*	78±2.0	85±1.3
AFG_1	58±3.4*	75±2.4*	85±1.5*	95±0.7*	96±0.6
AFG_2	30±3.9*	74±2.5*	84±1.6*	90±0.9	93±0.8

* Values statistically significant where $P<0.05$

The effect of different concentrations of ammonia on aflatoxin destruction were statistically analyzed by Excel software using aflatoxin standards containing 10 μg/ml.

Values are expressed as percent mean ± SE of the mean from 10 kg of maize sample (Table 3).

Table 3: Effect of ammoniation treatment under high pressure on aflatoxin destruction (% Mean ±S.E)

Ammonia Conc.	0.25%	0.5%	1.0%	1.5%	2.0%
Sample	10 kg	10 kg	10 kg	10 kg	10 kg
Total toxin	71±2.9*	90±0.9*	95±0.7	97±0.5	97±0.5
AFB_1	53±3.5*	75±2.4*	98±0.3*	98±0.3	98±0.3
AFB_2	81±1.8*	90±0.9*	98±0.3	98±0.3	98±0.3
AFG_1	85±1.5*	95±0.7*	98±0.3	99±0.2	99±0.2
AFG_2	81±1.8*	94±0.8*	98±0.3	99±0.2	99±0.2

* Values statistically significant where $P<0.05$

RESULTS AND DISCUSSION

The efficiency of ammoniation procedures for quantitative determination and destruction of aflatoxins in contaminated maize samples were assessed. The results of quantitative analysis of aflatoxins AFB_1, AFB_2, AFG_1 and AFG_2 are presented in Table 1. All four aflatoxins were recorded with the highest set of values being AFB_1 (96%) and AFB_2 (92%) compared with AFG_1 and AFG_2. This observation is in agreement with the previous results indicating detection of higher values of group B aflatoxins in a wide variety of foodstuffs compared with group G aflatoxins [3]. Although it was not possible to establish the origin of high levels of aflatoxins, appropriate storage conditions may have caused rapid mold growth and consequent aflatoxin production.

The effect of ammoniation treatment low atmospheric pressure on aflatoxin destruction is shown in table 2. A proportional increase in the destruction of all four types of aflatoxins was observed with the increase of ammonia concentration Statistical analysis revealed significant differences among the effects

of 0.25%, 0.5% and 1.0% ammonia concentration for aflatoxin destruction. No significant differences were noted between 1.5% and 2.0% ammonia concentration. Comparable results were reported by other investigators who found that ammoniation treatment under low pressure reduced aflatoxin content in corn to undetectable levels [12, 15].

Data presented in table 3 contains the results of the effect of ammoniation treatment under high pressure on aflatoxin destruction. The results revealed that ammoniation treatment under high pressure was more destructive to aflatoxins than treatment under low pressure. AFB_1 recorded the lowest rate of destruction at 0.25% and 0.5% ammonia concentrations. The results also indicated that 1.0% ammoniation treatment resulted in destruction of 98% of all four types of aflatoxins and remained unchanged for group B aflatoxins regardless of high ammonia concentration. In general, group G aflatoxins recorded the maximum destruction percent rate both at low and high treatment pressure compared with group B aflatoxins. These effects were enhanced with an increase in the concentration of ammonia.

CONCLUSION

In the present study the efficiency of ammoniation technique to decontaminate aflatoxin-contaminated maize samples was assessed in terms of percentage destruction of aflatoxins. A proportional increase in aflatoxin destruction with the increase in ammonia concentrations was observed. Ammoniation treatment under high pressure and at high ammonia concentration offered the maximum percent aflatoxin destruction than treatment under low atmospheric pressure. Therefore the use of ammoniation technique to destroy aflatoxins that contaminate a variety of foodstuffs may be economically viable for commercial application.

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