Detoxification of Aflatoxin in Artificially Contaminated Maize Crop by Ammoniation Procedures

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Abstract

Aflatoxin contamination is a common occurrence in corn-based agricultural commodities such as grains and oilseed crops that are normally stored in warm moist places. Since aflatoxins are highly toxic and carcinogenic, this study was undertaken to determine the use of ammoniation procedures to destroy aflatoxin in contaminated maize samples. Strains of *Aspergillus parasiticus* were used to contaminate maize samples collected from market centers in Eldoret Municipality. They were then decontaminated by ammoniation procedure under normal temperature and pressure. High performance liquid chromatography was used for analysis of aflatoxin content in maize crop samples. The results revealed that ammoniation procedure can destroy over 90% of the aflatoxin in maize and may be considered economically viable especially where alternative foodstuffs are not available or affordable.

Key words: Aflatoxin destruction, contaminated maize samples, ammoniation procedures.

Résumé

La contamination par l'aflatoxine est un phénomène fréquent dans des produits agricoles à base de maïs à base, des céréales par exemple et les oléagineux, normalement stockés dans des endroits chauds et humides. Étant donné que les aflatoxines sont fortement toxiques et cancérigènes, cette étude a été entreprise pour déterminer l'utilisation de procédures d'ammonisation destinés à détruire l'aflatoxine dans les échantillons de maïs contaminés. Les souches d'Aspergillus parasiticus ont été utilisées pour contaminer les échantillons de maïs prélevés prélevés des marchés de la municipalité d'Eldoret. Ces échantillons ont alors été décontaminés par la procédure ammoniation dans des conditions de température et de pression normales. La chromatographie liquide à haute performance a été utilisée pour analyser le contenu d'aflatoxine dans les échantillons de maïs. Les résultats ont révélé que la procédure ammoniation peut détruire plus de 90% de l'aflatoxine contenue dans le maïs et pourrait donc être considérée une activité économiquement rentable en particulier lorsque d'autres denrées alimentaires ne sont pas disponibles ou accessibles

Mots clés: Aflatoxine destruction, contamination des échantillons de maïs, procédures d'ammoniation.

Introduction

Every year a significant percentage of the farmers' maize crop stored in warm moist places is often contaminated with aflatoxin produced as secondary metabolites by *Aspergillus parasiticus* (Alpert *et al*., 1971). These toxins are known to be hepatotoxic and hepatocarcinogenic in man and animals (Newberne and Butler, 1969; Ngindu *et al*., 1982; Nyandieka *et al*., 1990). A positive correlation between aflatoxin levels in the food eaten and the frequency of human liver cancer in East Africa has been established (Nyandieka and Wakhisi, 1996; Nyandieka, 1998; Nyandieka and Nyamwange, 2000).

Consumption of aflatoxin-contaminated maize crop is, therefore, a public health hazard in countries where maize production and storage conditions are conducive to mold spoilage and consequent mycotoxin production. Discontinuation of consumption of maize meal contaminated with aflatoxin is also not always practicable especially when alternative food commodities are neither available nor affordable. This study was, therefore, carried out to determine the potential use of ammoniation technique to detoxify aflatoxin in maize crop sold for consumption within Eldoret Municipality in Kenya. Ammonia reagent was chosen for this study because the toxicity from the products of its interactions with aflatoxin is negligible (Park, 1993).

Materials and Methods

Culture of Fungal Parasites

Strains of *Aspergillus parasiticus* were cultured in potato dextrose agar media at 25°C for 7 days. The

media consisted of 100g of pealed potatoes, 10g of dextrose and 10g of agar in 500ml of water. The mixture was cooled to 4°C before it was utilized.

Contamination Procedures

White dry maize samples (50kg) were purchased from market centers in Eldoret Municipality. Then they were artificially infected with *Aspergillus parasiticus* strain as described by Codner *et al*. (1963) and Gomaa *et al*. (1997). A final concentration of aflatoxin contaminated maize sample was prepared by mixing the contaminated sample with aflatoxin-free sample and milling the final mixture to produce 1000μg/kg contamination level.

Detoxification Procedure

Detoxification of aflatoxin-contaminated maize samples was carried out by ammoniation procedure described by Gomaa *et al*. (1997). The moisture content of 50kg contaminated maize was adjusted to 18% wet matter. They were then divided into 5 batches of 10kg each and sprayed with 0.25%, 0.5%, 1.0%, 1.5% and 2.0% concentrations of ammonia, respectively. Each sample was packed in polyethylene bags and stored at room temperature for 24 hrs. Aflatoxin residues were extracted and determined by the use of High Pressure Liquid Chromatography (HPLC).

Extraction and Determination of Aflatoxins

The extraction and purification of aflatoxin residues were performed according to the method of Park *et al*. (1990). Quantitative estimation of aflatoxin B_1, B_2, G_1 and G_2 was performed as described by Gomaa *et al*. (1997). The same procedure was applied on aflatoxin standards. Aflatoxin concentration in μg/kg (ppb) of maize sample were calculated using standard curves for each toxin (Table 1).

Table 1. Aflatoxin levels in artificially contaminated maize samples

Toxins	Weight (kg)	Concentration (μg/Kg) Mean ± SE
AFB_1	10	960 ± 1.8
AFB_2	10	920 ± 1.6
AFG_1	10	876 ± 1.4
AFG_2	10	864 ± 1.2
Total	10	905 ± 1.5

The amount of AFB_1, AFB_2, AFG_1 and AFG_2 were statistically analyzed by Excel Software using aflatoxin standards. Each standard contained 10μl/ml.
Values are means ± SE of the mean of 10kg samples per toxin.

HPLC Analysis

HPLC analysis was carried out with Waters Liquid Chromatography. It was equipped with solvent delivery systems, system controller, data module, injector and fluorescence detector with excitation and emission wavelengths of 338 nm and 455 nm.

The effects of different concentrations of ammonia on the destruction of aflatoxin were statistically analyzed using Excel software. Statistical differences were assessed using students t-test and values for $P < 0.05$ were considered significant. The mean values were used to calculate the percent decontamination of different ammonia concentrations.

Results

The results of the effects of ammonia concentration on aflatoxin destruction in contaminated maize, regardless of ammoniation pressure, are presented in Table 2 and illustrated by Figure 1. A proportional increase in destruction of AFB_1, AFB_2, AFG_1 and AFG_2

Table 2. Effect of ammonia concentration on aflatoxin detoxification regardless of ammoniation pressure

NH_4 Conc %	Weight (Kg)	All Toxins	AFB_1	AFB_2	AFG_1	AFG_2
0.25	10	50 ± 0.5*	40 ± 0.4*	30 ± 0.3*	70 ± 0.7*	60 ± 0.6*
0.50	10	77 ± 0.9*	70 ± 0.7*	65 ± 0.6*	88 ± 1.9*	86 ± 1.7*
1.00	10	87 ± 1.8*	83 ± 1.4*	80 ± 1.2*	93 ± 2.5	91 ± 2.2
1.50	10	88 ± 1.9	84 ± 1.5	82 ± 1.3	94 ± 2.6	92 ± 2.4
2.00	10	89 ± 2.0	85 ± 1.5	83 ± 1.4	95 ± 2.7	93 ± 2.5

* 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 10μg/ml. The values are expressed as percent mean ± SE of the mean from 10kg sample per toxin.

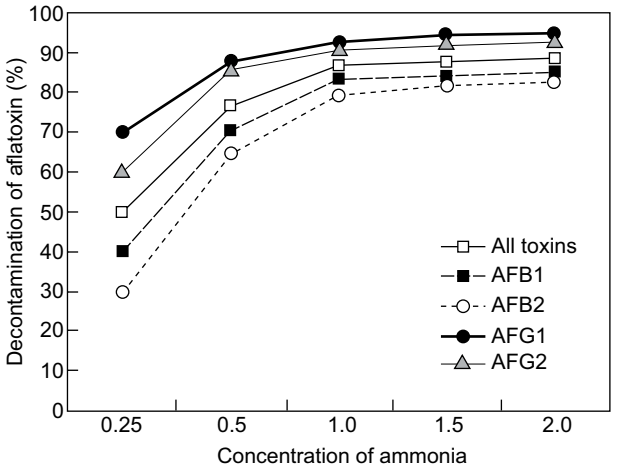

Figure 1. Effects of different concentrations of Ammonia on aflatoxin percent decontamination

was noted with the increase in the concentration of ammonia (0.25, 0.5, 1.5 and 2.0%). However, this relationship came to a plateau between 1.0% and 2.0% of ammonia concentrations (Figure 1).

Statistical analysis revealed the existence of significant differences among the effects of 0.25%, 0.5% and 1.0% ammonia concentration on aflatoxin destruction. On the other hand, no significant differences were observed between the effects of 1.5 and 2.0% ammonia concentration for all aflatoxins. A similar observation was also noticed with the use of 1.0% ammonia concentration in respect of AFG_1 and AFG_2.

Discussion

The above results concerning the detoxification of aflatoxins by different concentrations of ammonia were in agreement with those reported by Kolton *et al*. (1979). Current results have clearly shown that detoxification of aflatoxin in maize samples increases progressively with a corresponding increase in the concentration of ammonia until a plateau is reached. These observations have confirmed the findings of Bagley (1979) at least in respect with aflatoxin B_1. Similar results were reported by Jorgensen and Ralph (1981), Norred (1982), and Mahalingam *et al*. (1990) who found that ammoniation treatment under high pressure could reduce the content of aflatoxin in corn to undetectable levels.

This study has further demonstrated that the effect of ammonia is more destructive to aflatoxins G_1 and G_2 compared with aflatoxin B_1 and B_2. The highest detoxification rates were 95% for aflatoxin G_1 and 93% for aflatoxin G_2. This effect was slightly lower for aflatoxin B_1 (85%) and aflatoxin B_2 (83%). Similar findings have been reported by Gomaa et al (1997) who found that the effect of different ammonia concentrations was slightly destructive to aflatoxins B_1 and B_2 compared with aflatoxins G_1 and G_2 in yellow corn.

Therefore, these observations may support the view that the use of ammoniation procedure to detoxify aflatoxin in food products may be a viable option especially when alternative foodstuffs are not readily available or affordable. These findings should stimulate more active research into similar methods to preserve food commodities from hazardous aflatoxins.

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