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FUNGAL AND AFLATOXIN CONTAMINATION OF MAIZE STORED IN SILOS AND WAREHOUSES IN GHANA

A PROJECT REPORT

BY

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ABSTRACT

Samples of maize stored in silos and warehouses by the Ghana Food Distribution Co-operation were examined for fungal flora and aflatoxin content. Among six main types of fungi found in the samples were *Aspergillus flavus* Link, *Aspergillus ochraceus* and *Penicillium expansum* which are known to produce aflatoxins, ochratoxin A and patulin respectively. All the samples analysed showed aflatoxin contamination, with 80% containing aflatoxin concentrations above the FAO/WHO recommended maximum permissible limit of 30ug/kg. All the maize samples however had moisture contents below 13.5% suggesting that fungal growth and aflatoxin contamination occurred before storage.

INTRODUCTION

The prevailing humidity and temperature in tropical countries often provide ideal conditions for rapid fungal growth. Several thousand species of fungi exist in nature and are responsible for the spoilage of a wide range of both raw and manufactured materials. Growth may occur on staple agricultural products both in the field before harvest and during storage when basic principles of sound storage are neglected (Coker et al, 1984). Two main types of fungi are associated with cereal grains. These are field fungi which invade the commodity before harvest, and storage fungi which attack after harvest, during drying and in storage. These include mainly species of Aspergillus, Penicillium, Fusarium, Rhizopus and Mucor. The invasion of foods by these fungi results in the loss of viability, deterioration in quality and the production of mycotoxins.

Several species of Aspergillus, Penicillium and Fusarium are known to produce mycotoxins such as aflatoxins, ochratoxin A, citrinin, zearalenone, and the trichothecenes. The ingestion of these mycotoxins in particular aflatoxins has been linked to some diseases in animals and Man and evidence exists which implies that mycotoxins may be responsible for a wide range of diseases including primary liver cancer in man (WHO, 1979).

Aflatoxin contamination of foods and feeds has been detected in all parts of the world and aflatoxins have been found in oilseeds, crude vegetable oils, treenuts, fruits and cereal grains such as maize, sorghum, rice, wheat, barley, millet and oats (FAO 1979b).

Maize is a dietary staple for over 40% of the Ghanaian population and provides between 90 and 95% of the total calories in the diet of people in the coastal areas of Ghana (National Food and Nutrition Board, 1962). However, as high as 30% of the total annual crop harvest of maize and other grains are lost after harvest (Adams, 1977). The main responsible factor being poor storage facilities resulting in infestation by insects, moulds and rodents. The Government of

Ghana has therefore initiated a programme to invest in longterm infrastructure to hold large stocks of grain to reduce post-harvest losses as well as for price stabilization and food security purposes. This programme involves the building of silos and warehouses for storing food grains such as maize, rice, sorghum and millet. Consequently, aluminium metal silos of 250 ton capacity have been erected at various locations in the country.

Several problems are known to be encountered with such aluminium metal silos particularly if they are not well insulated. Apart from the fact that these structures must be constructed to prevent leaking or diffusion of moisture through the roofs, walls or floor, convection currents can produce pockets or layers of high moisture grain even in properly dried grain put into storage. In a tropical country such as Ghana, moisture migration may be a serious problem. Spoilage of grain can also occur from the creation of temperature gradients caused by metabolic activities of insects within the bulk of the grain which may give rise to condensation of moisture on the surface of the coller grains (Joffe, 1958). Localized increases of moisture content may therefore occur resulting in conditions favourable for fungal growth and ultimate production of mycotoxins.

Considering the facts that apart from groundnuts, maize constitute the highest potential aflatoxin hazard, and also the positive association of aflatoxin ingestion and liver cancer in Man in studies done in regions of sub-saharan Africa, (Peers and Linsell, 1973; Peersetal; 1976), there is the need to establish that stored maize which will eventually be utilized as food by the Ghanaian populace is not contaminated by aflatoxins.

The purpose of this study therefore was to isolate and identify the fungal flora and determine the extent of aflatoxin contamination of maize stored in silos and warehouses in Ghana.

MATERIALS AND METHODS

Maize Sample Collection

Samples were taken from all storage sites which had maize in store. These were silos at Techiman, Nkoranza, Mampong (Ashanti) and Abofour and warehouses at Wenchi, Balduzzi and Abuakwa. Several samples were drawn from each section of a silo such as the peripheral middle and central cores. These were pooled together such that for each silo there were three samples.

At the warehouses, samples were taken from the sides and tops of stacks using sampling spears. Several samples were taken and pooled to obtain a minimum of 500 gram sample for each warehouse.

Extraction and Estimation Of Aflatoxins

The method of extraction was based on that of Romer (1975). Ground samples were extracted with 250ml acetone: water (85:15v/v). The extract was filtered through Whatman No. 1 filter paper. Clean-up of filtrate was carried out using cupric carbonate and ferric gel (170mL sodium hydroxide + 30 mL ferric chloride). After a second filtration, the first 250mL of filtrate were collected and aflatoxins extracted into chloroform (2 x 10mL). The chloroform layer was run off into 100mL potassium hydroxide wash solution in a separating funnel which was gently swirled for 15 seconds and the layers allowed to separate. The chloroform layer was run through a bed of anhydrous sodium sulphate then evaporated to dryness under a stream of nitrogen in a sample concentrator (Model SC - 3 Philip Harris Scientific Co. Ltd., UK). The residue was picked up in 200uL chloroform.

Thin-Layer chromatography was carried out on silica gel 60 aluminium-backed TLC plates (Merck No. 5553 BDH Ltd, Dorset, UK). Bi-directional development first in diethyl ether to remove interferences followed by chloroform:acetone (9:1v/v) was carried out.

Quantification was by visual comparison of the intensity of the fluorescence under ultraviolet light using a chromato-vue ultra violet light cabinet fitted with a UVL 56 Blakray lamp (Ultra Violet Products Ltd., Cambridge U.K.) of sample aliquots and aflatoxin standards (Sigma Chemical Co. Ltd., U.S.A.). Confirmation of identity of aflatoxin was by spraying with aqueous sulphuric acid (50:50 v/v). All chemicals and reagents used were of the AnalaR grade (BDH Chemicals Ltd., Poole, U.K.).

Microbiological Analysis

Five hundred grams of grain of each sample were taken for determination of grain microflora, using the solid medium method. Sub-samples were taken from each sample of grain by the coning and quartering method. Out of this sub-sample, five hundred grains were taken and plated on solid agar plates. Ten surface sterilized (Sodium hypochlorite solution). The grains were placed on Malt agar containing 20% sodium chloride in 9.0cm. Petri dishes without any further treatment. There were 50 replicates for each treatment and plates were incubated at 26 + 3°C (ambient temperature) until fungi grew. Sodium hypochlorite treatment was used for surface sterilization with the aim of removing completely external sagrophytes which compete with pathogens.

Moisture determinations

Moisture content of all samples was determined by the oven-drying method (A.A.C.C. Method 44 - 15A).

RESULTS

Aflatoxin Contamination

All the maize samples collected from the silos and warehouses were positive for aflatoxins with a range of 19.7ug/kg to 355.3ug/kg. They all contained aflatoxins B_1 and B_2 with the exception of those from the middle core of Techiman silo and the periphery of

the Nkroranza silo which contained 18.5 and 50.2ug/kg of aflatoxin G_1 respectively in addition to aflatoxin B_1.

The mean aflatoxin content of samples of maize collected from the three sections i.e periphery, middle core and central core of each silo varied from 117.1 ug/kg in Nkoranza to 286.0 ug/kg in Abofour and for samples from warehouses, from 19.7 ug/kg in Abuakwa Kifoom farm to 355.3 ug/kg in Abuakwa mixed farms (Tables 1 and 2). The mean aflatoxin content for samples from warehouses was 171.58± 147.88 ug/kg. All the maize samples collected from silos had high levels of aflatoxins (Table 1). There was however a marked variation in aflatoxin content for samples obtained from warehouses (Table 2)

Fungal Contamination

Microbiological analysis of samples from silos gave six main types of fungi on the grains (Table 3). These were Aspergillus flavus Link, Aspergillus ochraceus, Aspergillus sp. 1, Aspergillus sp 2, Penicillium expansum and Fusarium spp. Generally the percentages of surface disinfested grains yielding fungi were low for all the samples. Penicillium expansum and Aspergillus sp. 1 were the only fungi isolated from a maximum of 18 to 20 percent of the grains.

Samples from warehouses showed growth of five species of Aspergillus, Penicillium and Fusarium. These were Aspergillus flavus Link, Asperillus ochraceus, Aspergillus sp. 1 Aspergillus sp. 2 Aspergillus niger, Penicillium expansum and Fusarium spp. Two main fungi namely Penicillium expansum and Aspergillus sp. 1 were isolated from 10 to 20 percent of the grains and therefore found to be more dominant.

Moisture Determinations

The moisture contents of samples taken from various sections of the silos i.e periphery, middle core and central core, showed no significant differences (Table 1). The means of moisture contents from the three sections of each silo ranged from 11.3 percent in Techiman to 13.0 percent in Abofour.

For samples from the warehouses, the moisture content ranged from 12.4 percent in Wenchi Warehouse 1 to 13.5 percent in Balduzzi warehouse. The mean moisture content for warehouse samples was 12.75 $\pm$ 0.39.

TABLE 1

AFLATOXIN AND MOISTURE CONTENTS OF MAIZE SAMPLES COLLECTED FROM GHANA FOOD DISTRIBUTION SILOS

LOCATION	SILO SECTION	INDIVIDUAL AFLATOXIN CONTENTS (ug/kg)				TOTAL AFLATOXIN CONTENT (ug/kg)	MOISTURE CONTENT (%)
		B_1	B_2	G_1	G_2		
Techiman	Periphery	300.6	98.4	ND	ND	399.0	10.8
	Middle Core	61.4	ND	18.5	ND	79.9	11.0
	Central Core	55.7	14.8	ND	ND	70.5	11.6
	Mean	-	-	-	-	183.1	11.3
	Standard deviation	-	-	-	-	187.0	0.4
Nkoranza	Periphery	167.0	ND	50.2	ND	217.2	12.5
	Middle Core	71.6	ND	ND	ND	71.6	12.6
	Central Core	54.4	8.0	ND	ND	62.4	12.4
	Mean	-	-	-	-	117.1	12.5
	Standard deviation	-	-	-	-	86.7	0.1
Mampong	Periphery	150.0	12.4	ND	ND	162.4	12.2
	Middle Core	30.5	6.0	ND	ND	36.5	12.4
	Central Core	290.7	28.8	ND	ND	319.5	12.3
	Mean	-	-	-	-	172.8	12.3
	Standard deviation	-	-	-	-	141.8	0.1
Abofour	Periphery	140.2	46.1	ND	ND	186.3	12.9
	Middle Core	214.0	58.8	ND	ND	272.8	13.0
	Central Core	300.6	98.4	ND	ND	399.0	13.0
	Mean	-	-	-	-	286.0	13.0
	Standard deviation	-	-	-	-	107.0	0.1

ND = None detected.

TABLE 2

AFLATOXIN AND MOISTURE CONTENTS OF MAIZE SAMPLES COLLECTED FROM GHANA FOOD DISTRIBUTION WAREHOUSES

WAREHOUSE	INDIVIDUAL AFLATOXIN CONTENTS (ug/kg)				TOTAL AFLATOXIN CONTENT (ug/kg)	MOISTURE CONTENT (%)
	B_1	B_2	G_1	G_2		
Wenchi (1)	277.8	13.7	ND	ND	291.5	12.4
Wenchi (2)	75.0	7.4	ND	ND	82.4	12.6
Balduzzi	222.7	36.4	ND	ND	259.1	13.5
Abuakwa (Ejura farms)	21.5	ND	ND	ND	21.5	12.8
Abuakwa (Kifoom farm)	13.2	6.5	ND	ND	19.7	12.5
Abuakwa (Mixed farms)	333.3	22.0	ND	ND	355.3	12.7

ND = None detected.

TABLE III: PERCENTAGE FREQUENCY OF FUNGI ISOLATED FROM MAIZE SAMPLES COLLECTED FROM SILOS AND WARE HOUSES IN GHANA

LOCATION	FUNGI						
	ASPERGILLUS FLAVUS LINK	ASPERGILLUS SP. 1	ASPERGILLUS SP. 2	ASPERGILLUS OCHRACEUS	ASPERGILLUS NIGER	PENICILLUIM EXPANSUM	FUSARUIM SPP.
+Nkorenza	5.3	8.5	1.3	5.5	0.0	7.3	1.0
+Mampong	3.7	11.0	2.3	2.0	0.0	8.5	3.0
+Abofour	8.0	12.0	2.0	7.0	0.0	9.0	2.0
+Techiman	4.0	9.0	2.0	2.0	0.0	11.0	0.0
-Wenchi (1)	3.0	12.0	2.0	4.0	0.0	8.0	2.0
-Wenchi (2)	7.0	17.0	2.0	7.0	0.0	10.0	2.0
-Balduzzi	4.0	20.0	1.0	0.0	0.0	11.0	3.0
-Abuakwa (Ejura farms)	5.0	16.0	2.0	0.0	0.0	14.0	2.0
-Abuakwa (Effoom farms)	4.0	6.0	1.0	5.0	2.0	14.0	0.0
-Abuakwa (Mixed farms)	4.0	11.0	4.0	0.0	0.0	18.0	6.0

+ Silo Sites

- Ware houses

DISCUSSION

In this study, microbiological examination of maize samples revealed the presence of various storage fungi namely *Aspergillus flavus* Link, *Aspergillus ochraceus*, *Penicillium expansum* as well as a field fungi (*Fusarium* spp.). It is known that prevention of damage by fungi is an important element in the safe storage of any commodity. Damage by fungi may render food inedible and hence cause a total loss, or down-grading of produce quality with a consequent loss in value and may give rise to toxic substances (mycotoxins) which can affect both humans and domestic animals.

Aspergillus flavus is known to produce aflatoxins, *Aspergillus ochraceus* produces ochratoxin A. *Penicillium expansum* produces patulin whilst some Fusarium species such as *F. graminearum* and *F. roseum* are responsible for the production of another mycotoxin, zearalenone. In addition, various other species of Aspergillus are also known to produce other mycotoxins such as sterigmatocystin, maltoryzine and cytochalasin E (FAO, 1969b; Coker et al. 1984). In this study however, samples were analysed for only aflatoxins and the results showed that all the samples were contaminated with aflatoxins. Only two samples namely those from Abuakwa Ejura farms and Abuakwa Kifoom farms had levels below the FAO/WHO recommended maximum permissible level of 30ug/kg (FAO, 1979a). Aflatoxins are known to cause several toxic effects such as liver and kidney carcinoma, bileduct proliferation and fatty infiltration of liver of animals. In humans, epidemiological data available does suggest a significant association between aflatoxin intake and liver cancer incidence in Man (WHO, 1979). Aflatoxin contamination of maize stored in silos and warehouses in the country should therefore be a matter for concern in view of the fact that maize is a dietary staple for over 40% of the Ghanaian population and provides between 90 and 95% of the total calories in the diet of people in the coastal areas of Ghana (National Food and Nutrition Board, 1962). It is of utmost importance therefore that measures are taken to prevent contamination of maize by aflatoxins.

Drying is an essential step in the preservation of grains against fungal attack. At harvest, cereal grains often contain too much moisture for safe storage it is known that in places where harvesting is done in very humid weather such as pertains in Ghana, the problem of mycotoxin contamination may reach alarming proportions. Maize stored by the Ghana Food Distribution Co-operation in silos and warehouses is purchased from farmers at various purchasing points. The moisture content of maize normally bought ranges from 13% to 24%. Soon after harvest, the moisture content of maize purchased is between 18 and 24% but getting to the end of the purchasing season such as in February, maize of about 13% moisture content can be obtained from the farmers implying that they have used their own methods to dry the maize down to this moisture level. It may be noted that harvesting of maize in the country coincides with the rainy season. The temperature and relative humidity during this period make it difficult for rapid sun-drying of maize to "safe" moisture levels. Mould growth and mycotoxin contamination can occur even before maize is purchased by the co-operation. Once purchased by the co-operation, maize is cleaned and dried down to a moisture content of 12% in Kongskilde batch dryers (Kongskilde Drying Units) or in Alvan Blanche continous flow dryers after which grains are sprayed to prevent infestation by insects, then stored in storage bins. During storage, there is regular monitoring of moisture content, temperatures and relative humidities both inside and outside the bins. In addition, measures are taken to maintain the moisture content and temperatures of stored maize at required levels to avoid mould growth. These measures include aeration and turning over from one bin to the other. Maize is later bagged and the bags stitched and transported to warehouses for storage and subsequently to Sales Depots.

To ensure safe storage, the maximum moisture content (wet basis) for maize is 13.5% (FAO 1979a). An abnormal moisture content may occur if the grain has been excessively dried or if it has been exposed to rain and condensation. A high moisture content is known to encourage

infestation by micro-organisms especially if grain is kept under poorly ventilated conditions. In this work, all the samples analysed had moisture contents within the limits required for safe storage of maize. This implies that mould growth and aflatoxin production probably occured even before maize was put into storage by the co-operation.

From the foregoing, it can be said that the Ghana Food Distribution Co-operation has the facilities to ensure the proper storage of maize to prevent fungal growth and subsequent aflatoxin/mycotoxin production after the commodity has been purchased from the farmer. What remains to be done however, is to assess the initial quality of grain bought from the farmers. This is a very important factor since the tendency for grain to deteriorate in storage is affected by the soundness of the grain initially put in store. Currently, the co-operation has little control over the quality of maize bought from the farmers. Except for obviously damaged grains which are sometimes rejected the bulk of the maize bought is of varied quality with varying degrees of moisture levels depending on the time after harvest. There is therefore the need for an official legislation and grain specifications for maize and other grains in the country.

CONCLUSION

Fungal and aflatoxin contamination of maize can occur before harvest, at harvest, during drying, storage or even during the transportation periods. This work has revealed the fact that maize in storage at the silos and warehouses of the Ghana Food Distribution Co-operation during the period of this work was contaminated with aflatoxins in addition to fungi known to be responsible for the production of aflatoxins and other mycotoxins. This work does not however give any conclusive indication regarding the point/s of contamination.

RECOMMENDATIONS

1. The presence of other fungi such as Aspergillus ochraceus and Penicillium expansum suggests that other mycotoxins such as ochratoxin A and patulin may have been present in the maize samples. Further work is therefore required to determine their presence.

2. Simple qualitative or semi-quantitative screening tests for aflatoxins should be used to ensure that maize is not contaminated before it is purchased from the farmers. To this effect, tests such as the "BGYF" (Blue-green-yellow flourescence) presumptive method or or the Holaday-Velasco semi-quantitative mini-column method may be used.

3. Regular checks for fungal and aflatoxin contamination should be carried out especially at the filling, emptying, turning over and bagging stages. Bagged maize stored in the warehouses should also be subjected to similar checks.

4. Further research work needs to be carried out to identify the contamination points for fungal growth and aflatoxin production in the distribution chain of maize in the country.

REFERENCES

1. Adams, J.M. 1977. A review of the literature concerning losses in stored cereals and pulses published since 1964. Trop. Sci. (19) 1:1 - 28.

2. American Association of Cereal Chemists, 1955. St. Paul, Minnesota, U.S.A.

3. Coker, R.D., Jones, B.D., Nagler, M.J., Gilman, G.A., Wallbridge, A.J. and Panigrahi, S. 1984. Mycotoxin Training Manual, Tropical Development and Research Institute, London.

4. FAO, 1979a. Prevention of Mycotoxins. FAO Food and Nutrition Paper No. 10, Rome.

5. FAO, 1979b. Perspective on Mycotoxins. FAO Food and Nutrition Paper NO. 13, Rome.

6. Joffe, A. 1958. Moisture migration in horizontally stored bulk maize: the influence of grain infesting insects under South African conditions. S. Afr. J. Agric Sci., 1:175 - 193.

7. National Food and Nutrition Board, 1962. Report of the National Nutrition Survey, Accra, Ghana.

8. Peers, F.G. and Linsell, C.A. 1973. Dietary Aflatoxins and liver cancer: A population-based study in Kenya. Brit. J. Cancer, 27:473 - 484.

9. Peers, F.G., Gilman, G.A. and Linsell, C.A. 1976. Dietary Aflatoxins and human liver cancer. A study in Swaziland. Int. J. Cancer, 17, 167 - 176.

10. Romer, T. 1975. Qualitative/Quantitative analysis for detection and estimation of aflatoxin. J. Ass. Off. Anal. Chem., 58: 500 - 506.

11. WHO, 1979. Environmental Health Criteria II, Mycotoxins. Geneva.