3 of 8

PERSISTENCE OF AFLATOXINS DURING TRADITIONAL STEEPING AND FERMENTATION OF MAIZE IN GHANA

A PROJECT REPORT

BY

KAFUI A. KPODO

FOOD RESEARCH INSTITUTE
(C.S.I.R.)
P.O. BOX M.20
ACCRA, GHANA

MARCH, 1992

PERSISTENCE OF AFLATOXINS DURING TRADITIONAL STEEPING

AND

FERMENTATION OF MAIZE IN GHANA

KAFUI A. KPODO

ABSTRACT

The occurrence of aflatoxins in fermented maize dough and "Ga Kenkey" (an end product of maize dough fermentation) from some major processing sites and markets in Accra was investigated. Experiments were then conducted to study the fate of aflatoxins B_1 and B_2 during steeping and spontaneous fermentation under laboratory conditions in four lots of naturally contaminated maize. Total aflatoxin levels in fermented maize doughs and kenkey samples ranged from none detected to 312ug kg^{-1}. Of the thirty samples analysed, seventeen had total aflatoxin levels above 30ug kg^{-1}, the FAO/WHO maximum permissible level. High performance liquid chromatography (HPLC) analysis revealed that not only did aflatoxin persist throughout the steeping and fermentation procedures but there were significant increases in their levels during the first 48 hours of fermentation. The results suggest that the traditional steeping and spontaneous fermentation procedure for maize currently used in Ghana may not be effective in aflatoxins B_1 and B_2 destruction.

INTRODUCTION

Aflatoxins are a group of highly toxic metabolites produced by some strains of Aspergillus flavus and by most but not all strains of A. parasiticus (Diener and Davis, 1969). Studies on aflatoxins in mould contaminated foods and feeds began with the outbreak of turkey X disease in England (Asplin and Carnaghan, 1961; Allcroft and Carnaghan, 1962). Since that time, several studies have been carried out and established the frequent presence of aflatoxins in human foods. These studies have been reviewed (Bullerman, 1979; FAO, 1979) and several commodities found to be susceptible to aflatoxin contamination. These include maize, millet, groundnuts, wheat, barley rice, sorghum, vegetable oils and tree nuts among others.

The aflatoxins are known to be hepatotoxic, carcinogenic and teratogenic and a positive correlation has been established between the consumption of aflatoxin contaminated foods and the increased incidence of liver cancer in several Southeast Asian and African populations (Shank et al., 1972; Peers and Linsell, 1973; Shank, 1976; WHO, 1979; Groopman et al., 1988). In Ghana, no such studies have been carried out but it is known that primary liver cancer is one of the commonest cancers with a relative frequency of 14.5 percent and accounts for 20 percent of cancers in the male (Foli and Christian, 1976). The presence of aflatoxins in commodities for human and even animal consumption should therefore be prevented especially in areas where the commodities are staple foodstuffs for the populations.

Maize is a dietary staple in several West African countries (Odunfa, 1985). In Ghana, it contributes between 90% and 95% of the total calories in the diet of people in the coastal areas (National Food and Nutrition Board, 1962) and is used in various forms for staples, snacks and beverages (Dovlo, 1970). Studies carried out in the country have however showed that maize stored in some silos and warehouses are contaminated by aflatoxins (Kpodo and Halm, 1990) and could result in serious health problems for the consumer.

Spores of aflatoxin producing moulds are ubiquitous in nature and can infect growing maize as a result of drought stress or insect damage and produce the toxins prior to harvest, during harvesting, drying or storage. Proper agronomic and handling practices therefore play an important role in preventing contamination. Atmospheric conditions of

temperature and relative humidity in the country are favourable for the growth of these moulds on most agricultural produce. The problem is further compounded by the fact that harvesting of maize in the country coincides with the rainy season thus making drying to the safe moisture level of 13.5% within 72 hours impossible for most small-scale farmers who do not have access to mechanical dryers and need to rely on sun-drying.

On the basis of the foregoing and the fact that the small-scale farmer produces the bulk of the maize in the country, it has become necessary to ascertain the quality of maize products with respect to aflatoxins being consumed by the populace and also to establish ways of preventing or reducing aflatoxin levels in our foods.

In Ghana, a large proportion of the maize produced is consumed in the fermented form through traditional spontaneous and uncontrolled fermentations. Whilst it is known that studies have been carried out on the effects of several processing methods on aflatoxin levels in various commodities (Samarajeewa et al., 1990), no such work has been done in Ghana. Studies have also revealed that some substances such as p-aminobenzoic, benzoic, butyric, propionic, fluoroacetic acids and sucrose esters are able to prevent aflatoxin formation by inhibiting the growth or A.flavus and parasiticus (Zaika and Buchanan, 1987).

The objectives of this work therefore, are to ascertain the levels of aflatoxins in maize dough and Ga Kenkey (an end product of maize dough fermentation) on sale in some markets and processing sites in Accra and to investigate changes occurring in aflatoxin amounts during the steeping and fermentation procedure used in the production of fermented maize dough in the country.

MATERIALS AND METHODS

Samples

Fermented maize dough samples all at the advanced stage of fermentation were collected from three production sites in Accra at different times over a one and a half year period of time. Two of the sites have a capacity of several tons per week with the third site being a local market.

Ga kenkey samples were obtained from four production sites all in Accra over a four week period of time. All samples were promptly analysed for their moisture content (AOAC 1980) and aflatoxins B_1 B_2 G_1 and G_2 levels.

Steeping and Fermentation Procedure

A local variety of maize (Zea mays) was purchased from a market in Accra. The kernels were well mixed and a portion milled and analyzed for moisture and aflatoxin levels. Remaining grains were steeped as such (without prior sorting) at 28° C(ambient temperature) in water in the ratio 1:3 (w/v) for 48 hours. The steeped grains were then milled in a commercial disc attrition mill (No. 2A Premier). Maize dough of 50% moisture content was prepared by kneading the meal with the appropriate amount of water based on the initial meal moisture content. Duplicate samples were placed in containers and left to ferment spontaneously at 28° C for 3 days. Daily samples were taken for moisture, pH and aflatoxin analyses.

Chemicals, Reagents and Standards

Standard aflatoxin stock solutions were obtained from Sigma Chemical Co. Ltd., U.S.A. HPLC working standard aflatoxin solutions were prepared by appropriate dilution of the intermediate with acetonitrile and finally with HPLC mobile phase consisting of water, methanol, acetonitrile (6:1:3 v/v). All reagents for HPLC separations were HPLC grade (Merck Chemicals, D-6100 Darmstadt, Germany). All other chemicals and reagents used were of the analytical grade. Distilled and deionized water was used throughout and all mobile phase solutions were filtered through a 0.45um Millipore HV disc filter and degassed prior to use through a Millipore filtration unit (Millipore Co., USA).

Extraction of Aflatoxins

The extraction procedure was based on that of Pons (1979). Aflatoxins were extracted with methanol followed by precipitation of colour pigments using zinc acetate then extraction into dicholoromethane and further clean-up by column chromatography using cellulose and silica gel. Aflatoxins were eluted with dichloromethane: acetone (80:20 v/v) which was evaporated off and the residue quantitatively transferred into 10ml HPLC grade dichloromethane. 5ml was evaporated to dryness under a stream of nitrogen and the final residue dissolved in 0.1 to 1.0ml HPLC mobile phase and used for HPLC analysis.

High Performance Liquid Chromatography

The instrument system used was from Waters Associates (Milford, MA, USA) and included a Model 501 Solvent Delivery System fitted with a Rheodyne Model 7125 injector with a 20ul fixed volume loop, a Temperature Control System consisting of a temperature control module and two column heaters, a Model 470 Scanning fluorescence detector and a Model 746 Data Module.

Identification and quantification of aflatoxins was by reversed phase liquid chromatography with post column iodine derivatisation. Separation of aflatoxins was carried out on a Spherisorb S5 ODS-1 column of dimension 250mm x 4.6mm I.D packed with 5um particles (Phase Separations Inc., Norwalk, USA) maintained at 35°c. HPLC mobile phase flow rate was 1.2ml/min and postcolumn iodine derivatisation of aflatoxins B_1 and G_1 was achieved using saturated iodine solution (Shepherd and Gilbert, 1984) pumped at a flow rate of 0.4ml/min using an Eldex precision metering pump (Eldex Laboratories Inc., San Carlos, USA). Derivatisation tube consisted of stainless steel tubing (5m x 0.3mm) maintained at 75°c.

The excitation and emission wavelengths used were 360nm and 440nm respectively. The aflatoxins were identified by their retention times, and peak areas were used to determine their concentrations in the samples by reference to standard curves obtained by chromatographing pure aflatoxin standard solutions under identical conditions. To compensate for any day-to-day variation, aflatoxin standards were routinely run at the beginning, in-between and after samples have been run.

Statistical Analysis

Data obtained from steeping and fermentation studies were subjected to analysis of variance according to standard method of statistical analysis (Snedecor and Cochran, 1976). Differences in aflatoxin levels were analyzed by Duncan's (1955) multiple range test where F values were significant. Comparisons between the treatment levels were also made.

RESULTS AND DISCUSSION

TABLE I

AFLATOXIN LEVELS OF FERMENTED MAIZE DOUGH FROM PROCESSING SITES IN ACCRA

Collection period (Month/Year)	Processing site	Aflatoxin type and conc. (ppb)				Total Aflatoxin (ppb)
		B_1	B_2	G_1	G_2	
8/90	A	267.0	15.0	6.8	0.4	289.2
10/90	"	145.0	10.0	3.3	0.1	158.4
2/91	"	295.4	15.1	2.1	0.2	312.8
2/91	"	13.8	1.0	2.7	0.4	17.9
10/91	"	139.5	19.8	101.2	12.3	272.8
3/92	"	63.9	5.6	106.5	7.8	183.8
8/90	B	45.0	2.0	0.6	ND	47.6
10/90	"	88.0	7.8	5.4	0.4	101.6
2/91	"	3.8	0.3	0.8	0.2	5.1
2/91	"	0.6	0.1	ND	ND	0.7
10/91	"	211.8	7.2	12.1	2.2	233.3
3/92	"	0.5	0.1	0.4	ND	1.0
2/91	C	241.1	18.1	1.3	0.2	260.7
4/91	"	77.6	3.5	ND	ND	81.1

ND = None detected

TABLE II

AFLATOXIN LEVELS OF GA KENKEY FROM PROCESSING SITES IN ACCRA

Site	Week	Moisture	Aflatoxin type and conc.(ppb)				Total Aflatoxin (ppb)
		%	B_1	B_2	G_1	G_2	
A	1	70.1	152.6	43.5	ND	ND	196.1
"	2	67.7	57.1	22.6	23.1	5.7	108.5
"	3	64.4	39.3	16.9	6.3	1.6	64.1
"	4	66.7	49.8	8.8	10.4	ND	69.0
B	1	63.5	4.1	2.7	ND	ND	6.8
"	2	60.4	4.6	1.5	ND	ND	6.1
"	3	64.4	7.1	9.4	ND	3.5	20.1
"	4	61.2	11.1	0.9	1.0	ND	13.0
C	1	71.4	ND	ND	ND	ND	ND
"	2	68.6	32.6	7.2	4.0	2.8	46.6
"	3	67.5	6.1	1.0	9.2	ND	16.3
"	4	65.3	6.9	1.1	ND	ND	8.0
D	1	63.3	1.1	0.8	4.0	1.7	7.6
"	2	64.1	22.9	6.4	30.6	5.5	65.4

ND = None detected

TABLE III

EFFECT OF STEEPING AND FERMENTATION ON AFLATOXIN LEVELS IN NATURALLY CONTAMINATED MAIZE [a]

TREATMENT	AFLATOXIN TYPE AND LEVELS (UG/KG)[b]	
	B_1	B_2
Milled Maize Kernels	69.77a	4.47a
Kernels steeped 48 hrs. milled and doughed (0 hr. fermentation)	116.67a	11.47b
Maize dough fermented for 24 hr	206.32b	18.93c
Dough fermented for 48 hr.	270.37c	22.23d
Dough fermented for 72 hr.	290.63c	25.46e

[a]Calculations based on dry weight of maize or maize product.

[b]Means within column sharing the same letter are not significantly different ($P<0.05$).

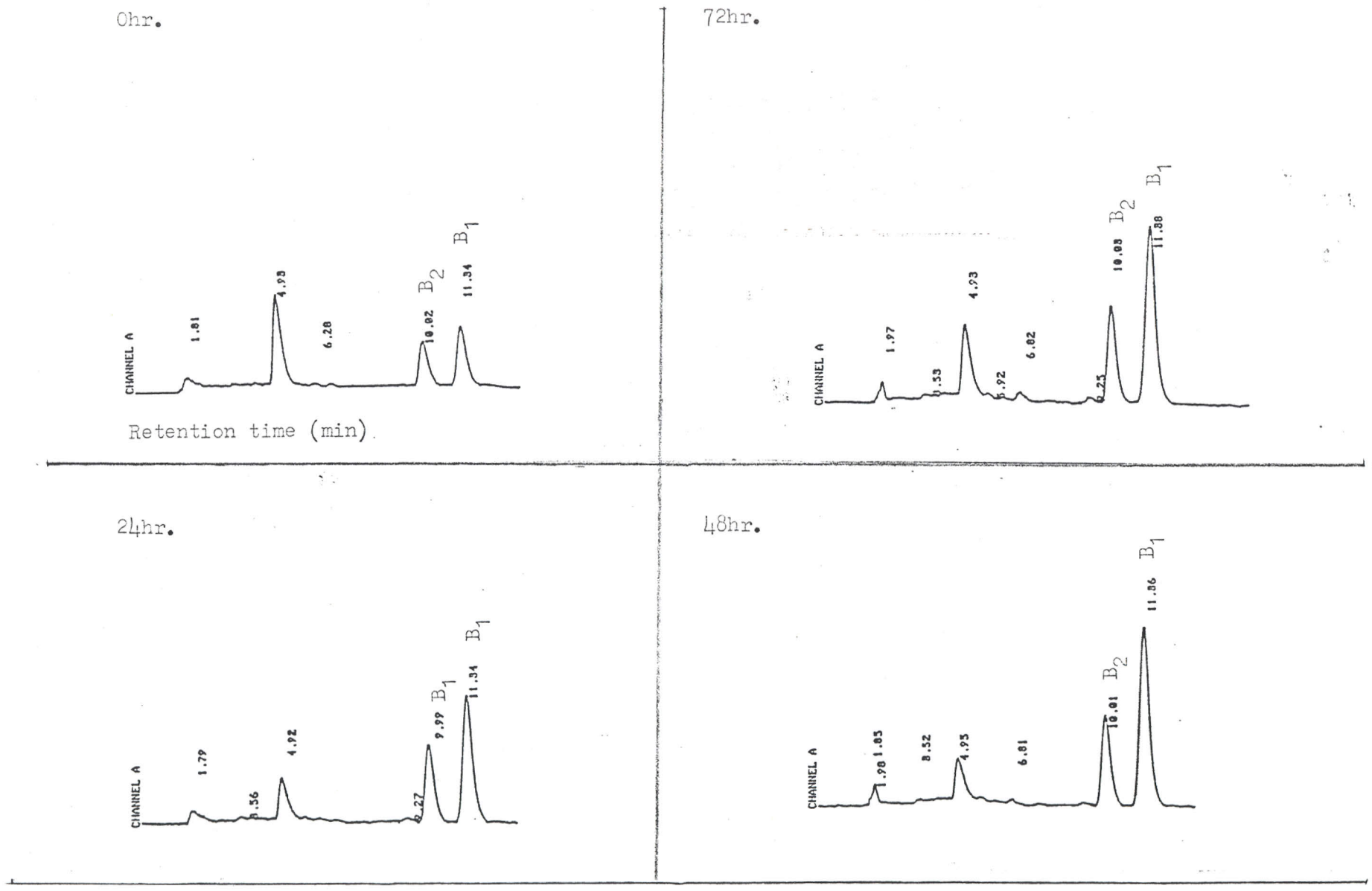

Fig. I: HPLC analysis of maize dough fermented for various times.

Table I shows aflatoxin levels of fermented maize dough samples from three processing sites in Accra over a one and a half year period of time. All the samples analysed contained aflatoxins at levels ranging from 0.7ug kg $^{-1}$ to 312.8ug kg $^{-1}$. Of the fourteen samples only four had total aflatoxin levels below 30ug kg $^{-1}$ which is the FAO/WHO maximum permissible total aflatoxin level.

Generally, all four types of aflatoxins (B_1 B_2 G_1 G_2) were present suggesting contamination resulting from invasion by both A.flavus and A. parasiticus. In most cases aflatoxin B_1 (the most toxic of all four aflatoxins) was present in the highest ratio.

The presence of aflatoxins in the fermented maize dough samples is a matter of concern especially when one considers the fact that processing sites A and B have capacities of the order of several tons per week and site C is one of the three largest markets in Accra.

Table II shows the aflatoxin levels of Ga Kenkey samples from four processing sites A,B,C and D over a four week period of time. Sixteen samples were analyzed and only one was "completely free" of aflatoxins. Detection levels for aflatoxins B_1 and B_2 was 0.04 ug kg-^{1} and for G_1 and G_2, 0.06 ug kg-^{1}. All the samples from site B however had total aflatoxin levels below the FAO/WHO maximum permissible level of 30ug kg-^{1}. On the contrary, all the site A samples had levels above the limit. Average total aflatoxin levels for sites A,B,C and D were 109.4, 11.5, 17.7 and 52.2 ug kg-^{1} respectively.

The fact that aflatoxins were still present in Ga Kenkey which is the end product of maize dough fermentation and is eaten in that form without further processing poses a health hazard to consumers. Preparation of Ga Kenkey involves steeping maize for between 24 and 48 hours, milling, doughing, and fermentation for 72 hours. A portion of the fermented dough is cooked and mixed with fresh fermented dough. The mixture is then moulded into balls, wrapped with maize husks and boiled for about three hours or till cooked.

It was hoped that the prolonged cooking time of Ga Kenkey would destroy any aflatoxins present in the light of work done by Newberne et al. (1966) which showed that the presence of moisture in foods may enhance

degradation by hydrolyzing the lactone ring of the aflatoxin molecule. Further studies also revealed that whilst temperatures higher than 100°C are required to attain at least partial detoxification, normal food processing and preparation conditions appear to cause an average 60% degradation under laboratory conditions. Aflatoxin B_1 in the solid form is known to be stable to dry heat up to its melting point of 260°C (Ciegler and Vesonder, 1983). In the presence of moist heat however it undergoes a hydrolytic opening of the lactone ring to form a terminal carboxylic acid which then undergoes "heat-driven" decarboxylation (Coomes et al., 1966). From Table II moisture content of Ga Kenkey ranged from 60.4% to 71.4%. It is also known that fermented maize doughs from Ghanaian markets have moisture contents between 46 and 64% (Plahar and Osei Yaw, 1978). One would therefore expect some destruction or degradation of aflatoxins during the cooking of Ga Kenkey. In this work however, aflatoxin contents of Ga Kenkey before boiling are unknown therefore no conclusions can be made regarding the fate of aflatoxins during the cooking process. Further studies need to be carried out. In the interim, the fact that aflatoxins are still present in the end product, Ga Kenkey should be of concern to all.

Table III summarizes results obtained for aflatoxin B_1 and B_2 analyses during the steeping and fermentation processes (Results are means of four experiments using the same batch of maize kernels). Figure 1 shows the HPLC chromatograms of maize dough samples after 0, 24, 48, and 72 hour fermentation. Under the HPLC conditions applied retention times were 10.0 and 11.3 min. for aflatoxins B_1 and B_2 respectively.

From Table III statistically significant ($P<0.05$) increases occurred during the first 48hr. of fermentation for aflatoxin B_1 whilst significant increases in aflatoxin B_2 levels were recorded throughout both the steeping and fermentation processes. Comparison of results for the different treatment levels during the experiment were made and when aflatoxin B_1 level in maize kernels was compared to levels obtained during steeping and fermentation, highly significant ($F=50.51$, $P<0.01$) differences were observed. Comparison of the steeping procedure with fermentation for aflatoxin B_1 was also highly significant ($F = 87.54$, $P<0.01$). For aflatoxin B_2, extremely significant differences ($F = 321.89$, $P<0.01$) were observed when aflatoxin levels in maize kernels were compared to levels obtained during steeping and fermentation.

The significant increases in aflatoxin levels during steeping and fermentation may be attributed to pH changes occurring during fermentation, presence of aflatoxin precursors and production of stimulatory substances for aflatoxin formation.

During spontaneous fermentation of maize dough, lactic acid bacteria (L. plantarum, L. cellobious, L. fermentum) have been found to be the dominating organisms resulting in the production of high levels of lactic acid (Akinrele, 1970; Fields et al., 1981). Apart from lactic acid, acetic, butyric and propionic acids are known to be produced and are the main aroma components in fermented maize doughs (Banigo and Muller, 1972; Plahar and Leung, 1982). During this work, there was reduction of pH from 5.5 in 0hr. dough through 4.0, 3.7, 3.7 after 24, 48, and 72 hrs. fermentation respectively (data not shown).

The effects of acids and alkali on aflatoxins have been discussed by Price and Jorgensen (1985). In the presence of alkali, reduction of aflatoxin occurs with the opening of the lactone ring. In the presence of acid however a reformation takes place with the closing of the ring. This suggests that a reduction of pH such as occurred during maize dough fermentation could result in reformation of any reduced molecules to give higher aflatoxin levels as observed during the first 48 hours of fermentation. No significant increases were recorded for aflatoxin B_1 after 48 hours and this is reflected in the constant pH recorded.

The presence of aflatoxin precursors in the maize kernels and fermenting doughs may also play a role in elevating aflatoxin levels. The biosynthetic pathway for aflatoxin B_1 has been studied by several researchers (Townsend et al., 1982; Bennett and Christensen, 1983; Townsend and Christensen, 1983) and six compounds have been recognized as being intermediate compounds in the biosynthesis of aflatoxin B_1. These are norsolorinic acid, averantin, averufin, versiconal hemiacetal acetate, versicolorin A and sterigmatocystin. One of these compounds sterigmatocystin itself a mycotoxin can be produced by some Aspergillus species (Schroeder and Kelton, 1975., Davis, 1981).

In this work, the amounts of the individual precursors in the biosynthetic pathway for aflatoxin B_1 were not determined. It is therefore not known if these precursors were originally present in the maize kernels, and the steeping and fermentation procedures only enhanced their conversion to the end product aflatoxin B_1 which was

measured or whether they were actually produced by the responsible fungi during steeping and fermentation. Further work needs to be carried out to ascertain the levels of these precursors at various stages of the steeping and fermentation procedures. Furthermore regarding sterigmatocystin, the levels present were not determined nor were the fungi responsible for its production namely A. versicolor and A.nidulans isolated. Earlier work done (Akinrele, 1970; Fields et al., 1981) showed that although high numbers of moulds of Penicillium, Aspergillus, Fusarium and Cephalosporium spp. were detected on maize kernels, a significant reduction in mould numbers was observed during steeping and the early stapes of fermentation suggesting that increases in aflatoxin levels could be attributed more to the presence of precursors rather than aflatoxin producing fungi during traditional steeping and fermentation of maize in Ghana.

It has been established that several substances are able to inhibit or stimulate aflatoxin production either through their action on growth of A.flavus or A.parasiticus or by direct action on aflatoxins themselves. These compounds have been reviewed by Zaika and Buchanan (1987). During fermentation, several substances are produced. These include aromatic compounds such as esters, alcohols, aldehydes, lactones and terpenes produced by yeasts (Janssens et al., 1992) and lactic, acetic, butyric and propionic acids (Banigo and Muller, 1972; Plahar and Leung, 1982). Substances which stimulate growth and/or aflatoxin formation by A. flavus and A.parasiticus under certain conditions include acetone, ethanol, DL-ethionine, isoprothiolane, nisin, phytate and sodium chloride (Zaika and Buchanan, 1987). Further work needs to be carried out to establish the production of any of these substances during steeping and fermentation of maize and their respective roles in relation to aflatoxins.

During the steeping and fermentation processes there is some breakdown of starch to glucose and studies by Abdollahi and Buchanan (1981) have indicated that either glucose or a product of its metabolism may act as an inducer of one or more of the enzymes needed for aflatoxin synthesis. There is therefore a possibility that glucose produced during the steeping and fermentation processes plays a role in increasing aflatoxin levels.

Aspergilli are known to catabolize glucose either by the Embden-Meyerhof or the Hexose Monophosphate pathways. Aerobic conditions favour the latter pathway whilst anaerobic conditions which can be said to prevail inside the fermenting dough favour the Embden-Meyerhof pathway. Shih and Marth (1974) from studies further concluded that in anaerobic (or less aerobic) environments, oxidation of acetate (a precursor of aflatoxin) via the citric acid cycle would be decreased and more acetate would be available for the synthesis of more aflatoxin. Studies have also revealed that as the environment becomes less aerobic, formation of NADPH via the Hexose Monophosphate pathway would be decreased resulting in a low NADPH/NADP ratio which favours aflatoxin synthesis (Niehaus and Dilts, 1984).

CONCLUSIONS

Results of this work show that a section of the population in Accra is exposed to aflatoxins through their diet. The traditional steeping and fermentation procedures for maize currently used in Ghana are not effective methods for reducing aflatoxin levels of contaminated maize. Further studies are required to elucidate the role of aflatoxin precursors and fermentation products on aflatoxin levels. In the interim however, it is of utmost importance to use high quality maize with minimal aflatoxin contamination than to rely upon the processing techniques to detoxify aflatoxins.

ACKNOWLEDGEMENTS

This work was carried out with support from DANIDA (Danish International Development Agency) and the Government of Ghana. The technical assistance of Messrs. Solomon Antonio and Emmanuel Allotey both of the Food Research Institute is highly appreciated.

REFERENCES

Abdollahi, A. and Buchanan, R.L. 1981. Regulation of aflatoxin biosynthesis: characterization of glucose as an apparent inducer of aflatoxin production. J. Food Sci. 46:143 - 146.

Akinrele, L.A. 1970. Fermentation studies on maize during the preparation of a traditional African starch cake food. J. Sci Food Agric. 21:619 - 625.

Allcroft, R. and Carnaghan, R. B. A. 1962. Groundnut toxicity. Aspergillus flavus toxin (Aflatoxin) in animal products : A preliminary communication. Vet. Rec. 74:863.

AOAC, 1984 "Official Methods of Analysis, " 14th ed. Association of Official Analytical Chemits, Washington, DC.

Asplin, F.D. and Carnaghan, R.B.A. 1961. The toxicity of certain groundnut meals for poultry with special reference to their effect on ducklings and chickens. Vet. Rec. 73;46.

Banigo, E.O.I and Muller, H.G. 1972. Carboxylic acid patterns in ogi fermentation J. Sci. Food Agric 23:101-111.

Bennett, J. W. and Christensen, S.B. 1983. New perspectives on aflatoxin biosynthesis. Adv. Appl. Microbiol. 29:53-92.

Bullerman, L.B. 1979. Significance of mycotoxins to food safety and human health. J. Food Prot. 42.5.

Ciegler, A. and Vesonder, R.F. 1983. Microbial food and feed toxicants; fungal toxins, pp 57 - 166. In Handbook of food borne disease of biological origin, ed. M. Rechcigl Jr. CRC Press, Boca Raton, Florida.

Coomes, T. J., Crowther, P.C., Feuell, A. J., and Francis, B.J. 1966. Experimental detoxification of ground nut meals containing aflatoxin. Nature 209: 408-409.

Davis, N.D. 1981. Sterigmatocystin and other mycotoxins produced by Aspergillus species. J. Food Prot. 44: 711-714.

Diener, U.L. and Davis, N.D. 1969. Aflatoxin formation by Aspergillus flavus. In "Aflatoxin." Ed., Goldblatt, L.A., p. 13. Academic Press, New York.

Dovlo, F. E. 1970. Special report on local foods. Food Research Institute, Accra, Ghana.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics 11:1 - 42.

Fields, M.L., Hamed, A.M., and Smith, D.K. 1981. Natural lactic acid fermentation of corn meal. J. Food Sci. 46: 900 - 902.

Foli, A.K. and Christian, E.C. 1976. Primary liver cell carcinoma in Accra. Lancet 2: 696 - 697.

FAO, 1979. Perspective on mycotoxins. FAO Food and Nutrition Paper No. 13, Rome.

Groopman, J.D., Cain, L.G., and Kensler, T.W. 1988. Aflatoxin exposure in human populations: measurements and relationship to cancer. CRC Critical Reviews in Toxicology 19: 113 - 146.

Janssens, L., De Pooter, H.L., Schamp, N.M., and Vandamme, E.J. 1992. Production of flavours by microorganisms. Process Biochem. 27: 195 - 215.

Kpodo, K.A, and Halm, M. 1990. Fungal and aflatoxin contamination of maize stored in silos and warehouses in Ghana. Food Research Institute (FRI) Technical Report. FRI, Accra, Ghana.

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

Newberne, P.M., Wogan, G.N., and Hall, A. 1966. Effects of dietary modification on response of the duckling to aflatoxin. J. Nutr. 90: 123 - 130.

Niehaus, W.G., Jr., and Dilts, R.P., Jr. 1984. Purification and characterization of glucose - 6- phosphate dehydrogenase from Aspergillus parasiticus. Arch. Biochem. Biophys. 288: 113 - 119.

Odunfa, S.A. 1985. African fermented foods. In B.J.B. Wood (Ed.), Microbiology of fermented foods, Vol. 2, Elsevier Applied Science Publishers Ltd., Essex, England, pp 155 - 191.

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.

Plahar, W.A. and Osei-Yaw, A. 1978. Chemical and organoleptic characteristics of traditional market samples of corndough. Food Research Memo. Food Research Institute, Accra, Ghana.

Plahar, W.A. and Leung, K. 1982. Effect of moisture content on the development of carboxylic acids in traditional maize dough fermentation. J. Sci. Agric. 33:555-558.

Pons, W.A. 1979. High pressure liquid chromatographic determination of aflatoxins in corn. J. Assoc. Off. Anal. Chem. 62:586-594.

Price, R.L. and Jorgensen, K.V. 1985. Effects of processing on aflatoxin levels and on mutagenic potential of tortillas made from naturally contaminated corn. J. Food Sci. 50:347-349.

Samarajeewa, U., Sen, A.C., Cohen, M.D., and Wei, C.I. 1990. Detoxification of aflatoxins in foods and feeds by physical and chemical methods. J.Food Prot. 53:489-501.

Schroeder, H.W. and Kelton, W.H. 1975. Production of sterigmatocystin by some species of the genus Aspergillus and its toxicity to chicken embryos. Appl. Microbiol. 30:589-591.

Shank, R.C., Wogan, G.N., Gibson, J.B., and Nondasuta, A. 1972. Dietary aflatoxins and human liver cancer II. Aflatoxins in market foods and foodstuffs of Thailand and Hongkong. Food Cosmet. Toxicol. 10:61-69.

Shank, R.C. 1976. The role of aflatoxin in human disease pp.51-57. In Mycotoxins and other fungal related food problems. J.V. Rodricks (ed.), American Chemical Society, Washington, D.C.

Shepherd, M.J. and Gilbert, J. 1984. An investigation of HPLC post-column iodination conditions for the enhancement of aflatoxin B_1 fluorescence. Food Additives and Contaminants 1:325-335.

Shih, C.N. and Marth, E.H. 1974. Aflatoxin formation, lipid synthesis and glucose metabolism by Aspergillus parasiticus during incubation with and without agitation. Biochim. Biophys. Acta 338:286-296.

Snedecor, G.W. and Cochran, W.G. 1976. "Statistical Methods", Iowa State University Press, Ames, IA.

Townsend, C.A., Christensen, S.B., and Davis, S.G. 1982. Bisfuran formation in aflatoxin biosynthesis: The fate of the averufin side chain. J. Am. Chem. Soc. 104:6152-6153.

Townsend, C.A., and Christensen, S.B., 1983. Stable isotope studies of anthraquinone intermediates in the aflatoxin pathway. Tetrahedron 39:3575-3582.

WHO, 1979. Mycotoxins: Environmental Health Criteria II. World Health Organisation of the United Nations, Geneva.

Zaika, L.L. and Buchanan, R.L. 1987. Review of compounds affecting the biosynthesis or bioregulation of aflatoxins. J. Food Prot. 50:691-708.