A SURVEY OF AFLATOXIN CONTENTS IN MAIZE, SORGHUM AND TEFF SAMPLES

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ABSTRACT

Using the Thin Layer Chromatographic (TLC) methods of the AOAC procedures, the aflatoxin content of 486 samples of six cereal varieties was determined and 71 (14.6%) of these were found to be positive, anging from 9 mcg/kg to 39.0 mcg/kg. Of these, 23 (32.4%) were maize grains followed by the white sorghum, 12 16.9%) and mixed sorghum, 11 (15.5%). The positive numbers of white, mixed and red teff samples were significantly as low as 7 (9.8%),8(11.3%) and 10 (14.1%), respectively. Similarly, out of the 60 injera samples tested only 1 (5%) sample of teff injera was positive at the 7th day of storage, while 5 (25%) and 7 (35%) samples of maize and sorghum respectively, were positive after the 4th day of storage. In all cases, it was observed that some environmental factors like temperature, moisture content and relative humidity have influenced the aflatoxin formation in the examined samples. It was also confinned that poor storage conditions like open sacs in market areas and warehouses were more conducive to aflatoxin formation than the modern silo bin storage systems. Although the maximum level of aflatoxin yield (39 mcg/kg) determined in this study is not much greater than the accepted standard limit (30 ppb), it is possible to deduce that maize and sorghum are more susceptible to aflatoxin accumulation than teff grains both before and after baking. In view of these results, therefore, we consider it necessary to recommend some essential measures of controlling food materials for aflatoxin contamination.

INTRODUCTION

Fungal invasions of cereals can occur in the field before or after harvest and during storage with miscellaneous signs of deterioration. Following fungal invasion, the formation and accumulation of toxins in cereals is quite hazardous to man and other animals (I, 2). These toxins are secondary fungal metabolites (mycotoxins) whic,h cause diseases known as mycotoxicoses.

One class of mycotoxins, aflatoxins, mainly designated as BJ. B2 G1 and G2 , are produced by the unbiquitous strains of Aspergullus flavus and A. parasitious, and liable to develop on stored cereal grains like maize, sorghum, wheat, etc. (1, 3,4). The incidence of the "Turkey X" disease among one million turkey poults and ducklings, in England, Kenya, Uganda and U.S.A. from 1960 to 1961 (4-6) was the basis for our understanding of aflatoxins. Since then, it has been considered as one of the causative agents of both acute and chronic hepatotoxic effects on different species of animals, including man (7).

Following the discovery and establishment of the effects of aflatoxins, several studies have been undertaken to elucidate their occurrence and distribution in raw agricultural products under natural conditions (8). In some zones of the United States, contamination of maize with aflatoxins has been observed. For example, maize samples were found positive for aflatoxins in 30.8-35.0% in 1969-1970, while in 1973 and 1976, 17% of 214, and 16.6% of 17,245 maize , samples were found to be positive (8). It has also been reported by Kubatskaya (9) and Van Rensburg et al. (10) that in most tropical and subtropical regions of Africa and S.E. Asia, where traditional means of crop

storage, cooling and dietary habits are not yet much improved, the increased rate of primary liver cancer incidence is associated with the high rate of aflatoxin contaminated food intake. Nvokolo and Okonkwo (11) found that sorghum and maize are among the high risk foods in Nigeria with 200-350ppb and 100-200 ppb of aflatoxin, respectively.

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In Ethiopia, several investigators (12-14) have reported increased rate of primary liver diseases. Only Coady (13) has trie.d to ascribe the role of aflatoxin contamination to the incidence of liver diseases. Abraham and Petros (15) have detected aflatoxins in some selected Ethiopian food stuffs including maize, sorghum and teff injera samples. Since no more than 5 ppb of aflatoxin were obtained for most of the samples, they have tentatively concluded that aflatoxins may not be the major cause of the high incidence of liver diseases in Ethiopia. But they have suggested that more investigations on a wider scale should be conducted to make a firm conclusion. A study on the mycoflora of the Ethiopian cereals with a special emphasis on the prevalence of toxicogenic fungal groups was conducted by Dawit (16) who has also made a qualitative study of aflatoxins on maize, sorghum, teff and barley. Based on the results of his studies, Dawit assumed that maize and sorghum are more often associated with aflatoxicosis than teff and barley.

However, sufficient information is not yet available on the quantitative level of aflatoxins of poorly stored cereals, especially teff grains, the most unique and commonly consumed food product of Ethiopia. No work has also been conducted on the effects of certain environmental factors that are known to affect the formation of aflatoxins in cereals (8, 17, 18).

The main objectives of the present study, comprise determination of the aflatoxin contents of commonly used cereals like maize, sorghum and teff and the effects of temperature, moisture (content, relative humidity and other storage conditions. In comparison with the previous studies (15, 16), large number of representative samples will be analysed.

MATERIALS AND METHODS

The AOAC (19) method of chemical analysis is used to determine the qualitative aflatoxin contents of maize, sorghum and teff grains and injera samples. A total of 486 samples of cereal grains consisting of 81 samples each of maize, red teff, white sorghum, white teff, mixed teff and mixed sorghum were used for the analysis. These were collected from ware-houses and silo bins of the Agricultural Products Marketing Corporation, few stores belonging to Higher Kebeles and some grain retailers in Kolfe and Shola markets in Addis Ababa. All samples were drawn applying the standard formula of grain sampling (20), the number of samples collected depending on the size of the lot. One or two kilograms of the bulk samples was well ground and 50gm of the flour served as the analytical sample for the analysis. About 100gm of flour from each grain type was baked into injera after three days of fermentation. In addition to this, some teff injera samples were bought

from commerical sources and others collected from few households to make a total of 60 injera samples.

A mixture of 10% sodium chloride solution and Methanol in 20 : 80 (v/v) ratio was selected as an extracting solvent for maize and a mixture of water: chloroform (9 : 91, v/v) for the sorghum and teff samples. 50gm of the flour and injera samples of each grain was mixed with 250ml of these solyent systems and shaken for 30 mins in a mechanical shaker. The extracts were filtered and the filterates were purified from impurities like lipids, proteins, alkaloids, pigments and other interfering substances using the Column Chromatographic method. 50ml o f this extract was allowed to run through the column composed of 5gm anhydrous sodium sulfate, 10bm silica gel (60-200mesh) suspension in 18-20ml of chloroform and another 15gm anhydrous sodium sulfate layers. Having discarded this eluent of the samples, 150ml each of hexane and diethyl ether were subsequently passed through the same column and their eluents were again discarded. Finally 150ml. of chloroform-methanol (97 : 3) mixture was allowed to pass through and the eluent was this time collected in a suitable flask. This fmal collection was evaporated in a rotary evaporator and the residue dissolved in 0.3ml. of Benene: Acetonitirile mixture (98 : 2). The dissolved product was then stored until l ready to be used for aflatoxin determination.

Aflatoxin contents of different substrates can be determined and quantified using different methods. Scanning Fluorodensitometer, High Performance Liquid Chromatograph (HPLC) or Mass Spectrometry are highly sensitive and accurate methods with a coefficient variation of 1 to 10%, however all of them require expensive instrumentation. Another method is the screening minicolumn method which involves a relatively rapid and simple technique. But it has certain limitations such as low resolving power, low specificity, low coefficient of variation and the use of expensive equipment. The other commonly used simple technique is the Thin Layer Chromatographic (TLC) method involving visual detection and quantification of the aflatoxins. Despite its limitations such as, low reproducibility and high coefficient of variation (20-30%), the TLC method has been preferred to the other methods in this study due to its advantages for the separation of the aflatoxins in B_l B_2 G_1 and G_2 fractions; separation of these fractions from other fluorescing or fluorescence quenching impurities requiring no expensive equipment, and detection of aflatoxins at levels as low as 0.1 mcg/kgm.

Aluminium sheets (20 X 20cm), precoated with silica gel were then used to spot 3.5, 5.0 and 6.5mcl of dissolved samples side by side with similar spots of the mixtures of standard aflatoxins Bl, B2, G1 and G2. These plates were then placed in chambers of the selected a developing solvent-mixtures applying boths the Uni-and Two-dimensional principles. In the Uni-dimensional techniques, the plates were placed only in one chamber coptaining a mixture of Aceton: Chloroform (I: 9, v/v) developing solvents. Whereas, in the Two-dimensional one, each spotted plate was placed first in a chamber of Benzene: Hexane (3 : I, v/v) mixtures which is responsible for the separation of the aflatoxins from the fluorescence quenching substances. Removing the plates out of this chamber, they were later replaced in another chamber containing the actual developing solvent mixture, Acetone: Chloroform (1 : 9, v/v). The developed plate was illuminated under the UV-light of 366nm, whereby fluorescence of the detected aflatoxins in each sample spot was noted and compared with that of the standard spots. Finally the aflatoxin concentration of each sample was calculated using a standard formula (19). The whole process of aflatoxin determination was done for two replicas of each analytical sample and their average

values were then considered for the analysis. The temperature, moisture content and relative humidity of each bulk grain sample were determined and recorded in the laboratory just before grinding the grain. The analysis of the injera samples was done every day for seven consecutive days storing them at ambient conditions.

RESULTS

Table 1 indicates the overall results of aflatoxins in all of the positive grain samples. Out of the total 486 grain samples 71 (14.6%) were found to be positive for, predominantly, aflatoxin Bl ranging from 9.6 ug/kg to 39.0 ugfkg. The highest positive number of samples was obtained among the maize samples, 23 (32.4%) out of the 71 positive samples, the next was the white and mixed sorghum, in 12 (16.9%) and 11 (15.5%)of 71 positive samples, respectively. The least number of positive samples were among the teff samples, in 14.1%, 11.3% and 9.6%, respectively, for red, mixed and white teff samples. These differences between the postive proportions of the six

Table 1. Number of Grains Sample with Different Ranges of Aflatoniz Content and their Mean Values in mcg/kg

Aflatoxin in Ranges of mcg/kg	S a m p l e s, N o (Mgc/kg)						Total
	Maize Teff	White Teff	Mixed Teff	Red Teff	White Sorghum	Mixed Sorghum	
9-20	13(16.7)*	7(13.7)	8(15.8)	9(15.8)	5(17.3)	6	48(16.1
21-30	8(26.6)	- -	- -	1(21.2)	5(26.4)	4(24.4)	18(24.5)
31-39	2(35.1)	- -	- -	- -	2(35.1)	1(39.0)	5(35.9)
Total number of positive samples	23(20.7)	7(13.7)	8(15.8)	10(16.4)	12(24.0)	11(21.9)	71(19.6)
Total number of negative samples.	58	74	73	71	69	69	415
Total samples examined	81	81	81	81	81	81	486
Total amount of Aflatoxins in mcg/kg	476.25	96.0	126.05	163.58	288.0	241.2	1391.08

*All figures in parenthesis indicate the mean values of aflatoxins for the positive samples of each range.

grain types were significant at 5% level (p 0.05, X 2 = 16.5). The average level of aflatoxins among these six grain types were also significantly different at 5% level of significance (F = 4.5). The highest mean values of aflatoxins were obtained from the two sorghum grain varieties, 24.1 ug/kg and 21.9 ug/kg, respectively; followed by 20 ug/kg mean value of maize grains. Table 2 shows that the prevalence of aflatoxin contents of the grains differs significantly at different ranges of temperature, moisture content and relative humidity at 5% level of significance (p 0.05, X2 = 40.97, X2 = 47.6 and X2 = 7.72, respectively). The ranges of aflatoxin content also differs significantly at 5% level of significance (X2 = 953).

Table 2. Numbers of Grain Samples at Different Ranges of Temperature,Moisture Content and Relative Humidity of the Bulks

Grain types	AF Ranges in ug/kg	Temperature range °C			Moisture content in %			Relative humidity in %		
		Below 10	11-20	Above 21	Below 10	11-20	Above 21	40-50	51-60	61-80
Maize	9-20	1	11	3	5	8	-	11	2	1
	21-30	-	4	2	2	6	-	3	4	1
	31-40	-	2	-	1	1	-	1	1	-

White Teff	9-20 21-30 31-40	- - -	7 - -	- - -	3 - -	4 - -	- - -	1 - -	4 - -	2 - -
Mixed Teff	9-20 21-30 31-40	- - -	7 - -	1 - -	1 - -	7 - -	- - -	5 - -	2 - -	1 - -
Red Teff	9-20 21-30 31-40	- - -	6 1 -	3 - -	2 - -	7 1 -	- - -	1 1 -	3 - -	5 - -
White Sorghum	9-20 21-30 31-40	- - -	2 2 1	3 3 1	2 2 1	3 2 1	- 1 -	2 3 -	2 1 2	1 1 -
Mixed Sorghum	9-20 21-30 31-40	- - -	1 1 -	5 3 1	1 - -	4 4 1	1 - -	3 3 -	1 - 1	2 1 -
Total		1	45	25	20	49	2	34	23	15

Out of the total 60 injera samples 13 (21.7%) were found to be positive for aflatoxins {Table 3). The positive samples were 1 {5%). 5 {25%) and 7 {35%) out of 20 samples each for teff, maize and sorghum, respectively. However, the aflatoxin contents do not differ significantly between the injera samples of the three grain types at 5% level of significance, but at 10% level {p 0.1, X2 = 5.49).

DISCUSSION AND CONCLUSION

Table 1 shows that the aflatoxin contents of the two sorghum varieties were significantly higher with the means values of 24.1 mcg/kg and 21.9 mcg/kg {ppb) in 16.9% and 15.5% of positive samples, respectively. This was followed by the mean value of maize, 20.7 mcg/kg, in 32.4% of positive samples. Although these results are low in quantity, they generally agree with the results of the work of Nwokolo and Okonkwo (11), who found more mean values of aflatoxin in sorghum {200-350 ppb) than for maize grains {100-200 ppb). Significantly smaller yield of aflatoxin with the mean values of 12.3 mcg/kg, 15.8 mcg/kg and 16.6 mcg/kg were obtained from white, mixed and red teff samples, respectively. This result compares with Dawit's (16) work, where a high prevalence of A. flavus was obtained in 33% both for maize and sorghum and in 16% for the teff samples, because, toxicogenic fungal strains are indispensable for aflatoxin formation. No significant difference could be observed in the aflatoxin contents of the three teff varieties and also the two sorghum types. However, the results of this study, in general, seem to support the conclusion reached by Abraham and Petros (15), because, only small proportion, 71 (14.0%), of the examined samples were positive for aflatoxin and the larger proportion (91.5%) of the positive samples had aflatoxins below the recommended upper
tolerance limit of 30 ppb (22, 23).

Table 2 indicates that larger proportions, 63.4%, 69.0% and 47.9%, of the 71 positive samples were obtained at 11-20°C temperature, 11-20% moisture content and 40-50% relative humidity , respectively. However, at ranges below l0°C of temperature, above 21% moisture content and 61-80% relative humidity, the positive results obtained were in significantly small proportions, 1 (1.4%), 2 (2.8%) and 15 (21.1%), of the positive samples, respectively. Relatively higher amounts of aflatoxins (30-40 mcg/kg) were also obtained at 11-20°C, 11-20% moisture content and 40-60% relative humidity levels only for maize and sorghum samples. All these results seem to agree with the works of other investigators. Some have found 12-35°C, 15.5-22% moisture content as optimum ranges for aflatoxin formation and 40-45°C temperature and 22-35% moisture content,

as the maximum limits, and, 7-12°C and 13-13.5% moisture contents, as the minimum limits (16-18, 23).

In this study, more than 80% of the positive samples were from among the lots stored for more than one year, while only less than 20% of the positive samples were from those stored for less than six months. This correlates with the results of Dawit (16), where 100% recovery of A. flavas was shown for maize samples stored for more than six months while only 13% of

Table 3. Aflatoxin Content of Injera Samples at Different Storage Periods

Types of injera sample	Total No. of samples analysed	Number of positive sample		Aflatoxin in ug/kg					Total	Mea
				Storage periods in days						
		No.	%	1-3	4th	5th	6th	7th		
Teff injera	20	1	5	Nil	Nil	Nil	Nil	15	15.0	15.0
Maize injera	20	5	25	Nil	10.5	19.5	35.0	31.0	123.5	24.7
Sorghum injera	20	7	35	Nil	10.5	15.0	21.0	39.0	154.5	22.1
Total	60	13	21.7	Nil	36.0	73.0	91.0	78.0	293.0	22.5

these toxicogenic strains were obtained; in the grains stored for less than two I months. According to Mcfarlane (1) and c Gilman and Boxall (24), tranditional storage systems of most Ethiopian fanners like: ground pits, go teras, dibignits, sa1ichas, jute sacs, etc., favour the growth of storage fungi like A.. flavus. There is no doubt that samples collected for this study from the markets and warehouses must have initially been stored in some of the above storage systems. Thus, this may be one reason why significandy larger number of positive results were obtained in 47.9% and 38.0% from samples taken from markets and ware-houses, respectively, while smaller proportions (14.1%) of positive samples were from the silo bins. These results when support the fact that poorly stored grains are more susceptible to A.. flavus invasion and aflatoxin and aflatoxin formation than those freshly harvested or stored in modem storage systems (1, 2).

According to Gorelova (8) and Boltyanskaya (25), mycotoxins penetrate baked foods like injera either contaminating the initial raw material and resisting the baking heat, or, being reformed by the newly developing fungi in the longterm stored baked products. The results in Table 3 show that aflatoxing must have been reformed after the long-term storage of injera, because, even in the maize and sorghum injeras aflatoxins began to be detected only at the fourth day of storage increasing in yield upto the seventh day, from 10.5 mcg/kg to 39.0 mcg/kg. In the teff injera samples, aflatoxins were detected on the seventh day and in smaller amount (15.0 mcg/kg). This is in agreement with the fmdings of Abraham and Petros (15) that fennentation and storage conditions of teff injera are not conducive for the growth of aflatoxin producing stiains. According to the results presented here, it can be concluded that there would be no risk of acquiring aflatoxin intoxication from eating injeras stored for 3-4 days, which is the habitual storage period of injeras in Ethiopian households. Neverthless, it should be noted that a more extensive study with a larger size of samples than the present study is necessary for a finn conclusion.

In conclusion, though it is true that 30 ppb is the upper acceptable limit of aflatoxins for most foods, some worken have suggested that 15 to'30 ppb levelof aflatoxins could be considered dangerous to health (8, 22, 25). Therefore, since in this study it has been shown that most sorghum and maize samples had 16-30 mcg/kg it seems that they are at risk more than the teff grains which

showed less than 20 mcg/kg of aflatoxins in most cases. In view of the results and conclusions drown, some preventive and detoxification measures of afiatoxins from cereals adopted by the joint WHO/FAO/UNEP conference on mycotoxins (22), should be recommended.

During the cultivation, harvesting and storage periods of cereals all grains. and seeds must be protected from fungal contamination and aflatoxin fonnation by:

-obtaining grains free of insect and mechanical damage;
-researching for grain and seed varieties that are resistant to A. flavus invasion
and aflatoxin fonnation and cultivating them as widely as possible;
-timely harvesting of completely ripened grains and not allowing over ripening of grains, since overripening
can make their tissues more susceptible to fungal invasion and aflatoxin formation;
-drying the grains immediately after harvesting and hulling them as minimally as possible with the safe
moisture content maintained through out the whole storage period;
-applying forced ventilation, and,
-separating shrinken, immature, discoloured and damaged kernels,
organic admixtures and weeds from normal grains.

In addition, collaborative studies should be conducted on the aflatoxin contents of local foods with respect to the present poor storage systems and carcinogenic effects of aflatoxins and its role in the high incidence of liver cancer in Ethiopia. As suggested by Dawit (16) the mycoflora and mycotoxins of agricultural products and the possible hazards of eating molded food-stuffs and feeds should be studied in detail.

REFERENCES

1. Mcfarlane, J .A. 1969. A study of storage losses and allied problem in Ethiopia. Tropical Products lnst. Report. 40: 1-67.
2. Gilman, F.A. 1968. Storage problems in Ethiopia with special references to deterioration by fungi. Tropical Products Inst. Report. 48: 1-50.
3. Austrick, P.L.C. 1975. Mycotoxins. British Med. Bull. 31: 222-229.
4. Tutelyan, V.A. 1980. Modern concepts of mycotoxins: Characteristics of individual mycotoxins. UNEP Training Course Handout on food contamination with special reference to mycotoxin. Moscow.
5. Maggon, K.K., Gupta, S.K., and Venkitasubramania, T.A. 1979. Biosynthesis of Aflatoxin. Bact. Review. 41: 822-855.
6. Ramachandra, Pal. M., and Venkitasubramania, T.A. 1979. Aflatoxins: a review. Journal of ScientIfic and Industrial Research. 38: 73-80.
7. Alpert, M.E., Hutt, M.S.R., Wagan, G.N., and Davidson, C.S. 1971. Associated between aflatoxin content of food and hepatoma frequency in Uganda. Cancer 28: 253.
8. Goreleva, E.I. 1980. Food contamination with special reference to mycotoxin. UNEP Training Course Handout. Moscow.

9. Kubatskaya, L.A. 1980. Biological methods of aflatoxin detection in foodstuffs.UNEP Training Course Handout. Moscow.
10. Van Rensburg, S.J., et al. 1975. Circumstances associated with the contamination of food by alfatoxin in a high primary liver cancer area. S. Afric Med. Journal 49: 877.
11. Nwokolo, C., and Okonkwo, P. 1978. Aflatoxin load of common food in Savana and forest regions of Nigeria. Transactions of the Royal Society of Tropical Medicine and Hy~ene, 72 (4): 329-332.
12. Tsega, E. 1917. Current views on liver diseases in Ethiopia. Ethiop. Med. J. 15: 75.
13. Coady, A. 1965. The possibility of factors of plant (particularly fungi) origin in Ethiopian liver diseases. Ethiop. Med. J. 3: 173.
14. Editorial. 1974. The Medical diseases of Ethiopia. Ethiop. Med. J. 12: 1 15. Abraham, B., and Petros, G. 1981. A preliminary study on the aflatoxin content of selected Ethiopian foods. Ethiop. Med. J. 19: 47-51.
16. Dawit, A. 1982. A preliminary study of the fungal flora of Ethiopian cereal grains with special emphasis on the prevalence of toxicogenic groups. Thesis for M.Sc. degree requirement. Addis Ababa University, pp. 5,6,8.54.
17.Jarvis, B. 1971. Factors affecting production of mycotoxins. J. App. Bact. 34: 199-213.
18. Schroeder, H.N., and HEIN, H. 1967. Aflatoxins: Production of the toxins in vitro in relation to temperature. App. Microb. 15: 441-445.
19. AOAC. 1975. Official Method of Analysis of the Official Analytical Chemists 12th ed. Washington D.C., pp. 462-477.
20. Sandier, Zh., Ya.. 1980. Grain Sampling Methods. UNEP, Moscow. pp. 9-12, 21. FAO. 1977. Report of the joint FAO/WHO/UNEP conference on mycotoxins. FAO food and nutrition paper 2, Rome. pp.105.
22. WHO. 1983. Executive Summary of Mycotexins. Environmental Health Criteria 11. Published by WHO/UNEP. Geneva. pp. 1-4.
23. Christensen, C.M. 1972. Microflora and seed deterioration. In Dawit, A. ed. Thesis, A Preliminary study on the fungal flora of Ethiopian cereal grains with special emphasis on the prevalence of toxicogenic groups. pp. 15. 70 Ethloplon Journal of Health Development. Vol. 2 No.1. 1987.
24. Gillman, B.A., and Boxall, R.A. 1974. The storage of food grains in traditional underground pits. Trop. StoredProd. Information. 28: 19-37.
25. Boltyanskaya, E. V. 1980. Microbiological Methods of mycotoxins. UNEP Transing Handout. Moscow.