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FOOD RESEARCH INSTITUTE

AFLATOXIN CONTAMINATION OF FOODS IN WEST AFRICAN COUNTRIES

(An EU funded Project Report)

by

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FOREWORD

This review report has been prepared under the European Union (EU) funded project Contract No: ERBIC18 CT98 0315 titled "Biological Degradation of Aflatoxins in Fermented Maize and Sorghum Products". The report has been necessitated by the popular belief that though not much published work exists on mycotoxins and aflatoxins specifically, in West African countries, a substantial amount of research work exists in unpublished forms in several universities and research institutions in West Africa.

This report therefore seeks to collate and document both published and unpublished information on aflatoxin research conducted in universities, research institutes, Standards Bureaux, and other organisations in the food and health sectors in West Africa. The report reviews aflatoxin contamination by countries with sections on agricultural research and medical research. The countries covered in this review are Benin, Burkina Faso, The Gambia, Ghana, Guinea, La Côte d'Ivoire, Mali, Niger, Nigeria, Senegal, and Sierra Leone. No information was available on Mali and Senegal and they are therefore not mentioned. It is also to be noted that though all the sections were addressed for each country, not all countries had information on aflatoxins.

1. Purpose for review report and mode of data collection

The major objective of this review report is to collect and document published and unpublished information on aflatoxin research conducted in universities, research Institutes, Standards Bureaux, and other organisations in the food and health sectors in West Africa.

Published information for inclusion in the review was obtained from all the partners involved in the project. Unpublished information was obtained through personal contacts with the various local Universities and research Institutions as well as with the help of a questionnaire (sample attached) designed in both English and French for use in Anglophone and Francophone countries respectively.

It is the hope of the authors that this review will serve as reference material for other researchers in the field and will further help to give a clearer picture of the aflatoxin problem in West Africa.

2. AFLATOXIN CONTAMINATION, CLINICAL INFORMATION AND RESEARCH BY COUNTRIES

2.1 Benin

Agricultural research into aflatoxins

Nation-wide studies for aflatoxins in stored maize in Benin have been conducted (Setamou et al., 1997). This study involved the sampling of eighty and sixty maize fields in 1994 and 1995, respectively to monitor *Aspergillus* infection and aflatoxin contamination of preharvest maize. *Aspergillus flavus* was the most prevalent of the *Aspergillus* species isolated. Mean percentage kernel infection was about 20% in the country for the two years studied. Aflatoxins were detected in at least 30% of the maize fields sampled. There was a trend towards higher aflatoxin accumulation per

percentage of *A. flavus* infection from south to the north of the country. Damage by the ear borer (*Mussidia nigrivenella*) increased aflatoxin accumulation in maize and its incidence may be related to aflatoxin contamination.

Studies by Hell et al. (1995) on fungal infection and mycotoxins in maize stores of small scale farmers from different Agro-ecological zones of Benin showed aflatoxin contamination in 25% of the maize stores in the southern zones with a mean of 100 ppb. after six to eight months storage. The northernmost zone (the Sudan Savanna) had 56% of the maize stores contaminated with aflatoxins at a mean level of 220 ppb. after six months storage.

In Benin, studies (International Institute for Tropical Agriculture, 1997) have centred around farming practices and their impact on aflatoxin contamination. The results of these studies confirmed that certain farmer's practices were linked to lower contamination of stored grains with *A. flavus* and aflatoxins. Local maize varieties had less aflatoxins than imported varieties. The use of fertiliser, drying of maize after harvest and sorting were found to reduce aflatoxin contents significantly. The use of insecticides was also found to reduce aflatoxin levels. These studies also revealed that certain farming practices were associated with higher aflatoxin levels. These were damage of maize cobs in the field, intercropping of maize with other crops, and delays in harvesting or drying of maize.

2.2 Burkina-Faso

Agricultural research into aflatoxins

In Burkina Faso, studies have been conducted on the aflatoxin contamination of two local varieties (Boanga and Wobgo) of peanuts (Nikiema et al., 1993). Results showed that aflatoxins were present in the two peanut varieties. Further studies on the physico-chemical parameters of the samples during a storage period of 12 months revealed a correlation with aflatoxin production. Water content of seeds, hygroscopic capacity, and lipid content seemed to be the most important factors in aflatoxin production in groundnut seeds during storage.

Medical research into aflatoxins

Medical research in Burkina-Faso has centered around possible interactions between human aflatoxicoses and infections due to hepatitis B virus (HBV) and hepatitis C virus (HCV) in a rural part of western Burkina-Faso (Nikiema et al., 1997). This study involved 55 HBV carriers, 47 HCV carriers and 82 non-carriers from two villages of 2000 people. Blood samples were collected during two different periods of 1996 and assessment of aflatoxin exposure was made using a competitive ELISA method on Aflatoxin-albumin adducts using an AFB1-lysine standard. Results of the study revealed that from 222 samples assessed (both cases and controls), only 2.25% had an aflatoxin level less than the detection limit of the assay (5μg AFB1-lysine equivalent/kg albumin or 5 ppb); 74.3% had a level of adduct ranging from 5 to 50 ppb, 13.1% between 50 and 100 ppb, 2.7% had between 100 and 200ppb and 3.2% had levels more than 200ppb. The maximum level of exposure recorded was 428μg AFB1-lysine equivalent/kg albumin (428 ppb).

2.3 Gambia

Agricultural research into aflatoxins

An aflatoxin-specific monoclonal antibody-based immunoaffinity chromatography method was developed and used for the rapid isolation of aflatoxins from a variety of cooked foods including maize collected in the Gambia. Nine of ten maize samples contained low levels of aflatoxins ranging from 2 to 35 ppb with a mean of 9.7 ppb. (Hudson et al., 1992).

Aflatoxin levels were measured in a variety of cooked foods including maize, rice, millet, groundnuts, sauces, and leaf sauces (Hudson et al., 1992). The highest levels of contamination were found in the groundnut sauces. Eighteen of twenty samples were positive for aflatoxins with a range of 19 to 943 ppb and a mean of 162 ppb All nine millet samples contained aflatoxins (1 – 27 ppb) whilst two of eight sorghum samples were positive with levels of 2 ppb and 16 ppb. Fourteen of twenty rice

samples contained aflatoxins (2 - 19 ppb). Three leaf sauces analysed showed aflatoxins at levels of 21 ppb, 26 ppb, and 34 ppb.

In another study conducted in the Gambia, Wild et al. (1992), a total of 87 leaf sauces, 22 flour sauces, and 47 groundnut sauces were analysed for aflatoxins; 64%, 59%, and 87%, respectively contained detectable aflatoxins. The highest level of aflatoxins found was 774 ppb in a leaf sauce that contained raw groundnuts. Fifteen samples of boiled rice examined in this study did not contain aflatoxins.

Medical research into aflatoxins

Medical research in the Gambia has centered on the use of recently developed markers of early biological effects which are able to detect genetic alterations due to aflatoxin exposure. Aflatoxin-albumin adducts in blood provide a measure of exposure to aflatoxin over the previous 2-3 months. Miele et al. (1996) determined the levels of these adducts in a group of individuals from the Gambia and these were compared to levels of various cytogenic alterations in the same individuals. Of the 35 subjects tested for aflatoxin-albumin adducts, all but 3 were positive. There were however no correlations between the adduct level and the number of cytogenic abnormalities at the individual level suggesting that more specific genetic markers of aflatoxin exposure are required to further examine the link between aflatoxin exposure and genetic alterations.

In another study conducted in Basse, Eastern Gambia, Wild et al. (1991) reported that foetal exposure could contribute to the high prevalence and early onset of primary hepatocellular carcinoma and influence susceptibility to infectious diseases, including hepatitis B and malaria in the community. Samples of sera from umbilical cord and maternal venous blood from 30 pregnant women were assayed for the aflatoxin-albumin adducts. Twenty-nine (97%) and 21 (70%) cord sera were positive for the aflatoxin-albumin adducts. This high prevalence was consistent with earlier studies by Wild et al. (1990) in the Gambia and indicates exposure of the foetal liver to the mutagenic metabolite of aflatoxin.

Zarba et al. (1992) have explored the relationship between dietary intake of

aflatoxins and a number of aflatoxin biomarkers including aflatoxin metabolite excretion into breast milk. Aflatoxin M_1 was measured in breast-milk of five subjects using a preparative monoclonal antibody immunoaffinity column/HPLC method. Aflatoxin G_1 was found in three of the five women. Estimates of the percentage aflatoxin in the diet excreted as aflatoxin M_1 ranged from 0.09 to 0.43%. The data from these studies indicated that a rapid methodology exists to assess the levels of aflatoxin M_1 excretion in human milk and to use this approach as a biomarker for exposure of children to this carcinogen.

2.4 Ghana

Agricultural research into aflatoxins

In Ghana mycotoxin studies have centered around maize, groundnuts, and their products. Kpodo et al. (1996), studied the occurrence of mycotoxins in fermented maize dough and 'Ga Kenkey' (boiled fermented dough) from markets and processing sites in Accra. Twelve fermented maize samples collected from two processing sites in Accra, over an eighteen-month period of time were analysed and found positive for aflatoxins. Total aflatoxin levels ranged from 0.7 ppb to 313 ppb. In the same study, twenty fermented dough samples collected from five major markets in Accra contained mycotoxins. Nineteen of the twenty samples contained aflatoxins at levels up to 249 ppb. All the twenty samples contained citrinin at levels up to 584 ppb. Low amounts of ochratoxin A (>0.3 to 6.4 ppb) were detected in five of the fermented dough samples. Zearalenone and α-zearalenol were not detected. These findings suggest that the mycotoxins; aflatoxins, citrinin and ochratoxin A persist during the lactic acid fermentation of maize in Ghana. Analyses of sixteen 'Ga Kenkey' (fermented dough boiled for 3 hours) samples from four production sites in Accra revealed the presence of aflatoxins in fifteen samples at levels up to 196 ppb. (Kpodo et al., 1996).

Earlier studies conducted by Kpodo and Halm (1990) on fungal and aflatoxin contamination of maize stored in some silos and warehouses in parts of Ghana

revealed that all the samples contained aflatoxins. Eighty percent of the samples contained aflatoxins above 30 ppb (the recommended maximum permissible limit set by the Protein Advisory Group, 1969). All the samples however had moisture contents below 13.5% suggesting that fungal growth and aflatoxin contamination may have occurred before storage. Dominant fungi found were *Aspergillus flavus*, *Aspergillus ochraceus*, and *Penicillium* spp.

In Ghana some work has been conducted on groundnuts and groundnut products. Awuah and Kpodo (1996) carried out a nation-wide survey on aflatoxin contamination of stored groundnuts in Ghana. Groundnut samples from 21 selected markets in all 10 regions of Ghana were screened for aflatoxins as well as their fungal flora. Total aflatoxin levels ranging from 5.7 to 22,168 ppb were identified in damaged kernels. Aflatoxins were however not detected in 50% of undamaged kernels tested and very low levels (0.1 to 12.2 ppb) were associated with the undamaged kernel samples that tested positive for aflatoxins. High levels of aflatoxigenic fungus *Aspergillus flavus* were detected. Other fungi detected from total kernels assayed were *A. niger* (34%), *A. candidus* (1.45%), *A. tamarii* (3.93), *A. ochraceus* (5.26), *Fusarium* spp. (1.7%), *Penicillium* spp. (5.19%) amongst others.

Groundnut samples from the 1994 crop season in six locations in the southern parts of Ghana had aflatoxin levels ranging from 12 to 106 ppb (Kpodo, 1995). Earlier studies conducted by Mintah and Hunter (1979), revealed levels of aflatoxins ranging from 3 to 216 ppb in groundnuts purchased from markets in and around Accra.

Kpodo (1997) screened one hundred groundnut paste (peanut butter) samples purchased from selected major markets in all ten regions of Ghana for aflatoxins. Eighty-six samples (86%) contained aflatoxins at varying levels. Sixty-five samples contained total aflatoxins above the 30ppb maximum permissible limit set by the Protein Advisory Group (1969). The highest total aflatoxin level recorded was 3,278 ppb. Regions in the Northern sector of the country showed generally low levels most likely due to the low relative humidities prevailing in those areas.

Studies on the microflora of maize kernels destined for 'Kenkey' (a Ghanaian fermented food from maize) production has been conducted by Halm et al. (1993)

and Jespersen et al. (1994). They isolated *Penicillium, Aspergillus, and Fusarium* species from raw maize kernels destined for fermentation. Detailed examination showed the most frequent species included potential mycotoxin producers, *Penicillium citrinum, Aspergillus flavus, Aspergillus parasiticus* and *Fusarium subglutinans*. Initial high counts were however reduced significantly within 24 hours of fermentation. High levels of aflatoxins were observed in the raw maize and these were not affected by the fermentation process normally used for the preparation of fermented maize dough, the basis for several foods in Ghana and other West African countries.

Detailed studies conducted by Kpodo et al. (1996) confirmed the persistence of aflatoxins and citrinin throughout the Ghanaian traditional steeping and fermentation processes for maize. Cooking of fermented maize dough for 3 hours as done for 'Kenkey' production however resulted in an 80% reduction in aflatoxins B_1 and G_1 levels, a 35% reduction in aflatoxins B_2 and G_2, and citrinin was no longer detectable.

Other research on aflatoxins in Ghana have centered around determining the effectiveness of certain plant extracts against aflatoxin synthesis (Awuah and Kpodo, 1996). Extracts from 17 plants were tested in this study. Four of them namely *Xylopia aethiopica, Monodera myristica, Cinnamomum verum* and *Piper nigrum* completely inhibited the formation of norsolorinic acid (NOR), a precursor to aflatoxin synthesis. Some fungal growth was however observed with these extracts in 1.5% potato-dextrose broth. These same extracts however failed to inhibit NOR formation in yeast extract sucrose medium.

Medical research into aflatoxins

In a study conducted by Maxwell et al. (1989) in Accra, aflatoxins were found in 32% of the breast milk and in 31% of the cord blood samples. It is suggested that aflatoxins may accumulate in the foetus. Earlier studies (Apeagyei et al., 1986) carried out on the pathological effects of aflatoxins showed the occurrence of aflatoxins in liver samples of children who died of kwashiorkor

To ascertain whether the presumed intake of dietary aflatoxins (AFB_1 and AFG_1) has

adverse effect on the liver of Ghanaians, the toxins were measured in serum, urine and faecal specimens of a group of forty apparently healthy Ghanaian adults (Ankrah et al. 1994). Aflatoxins G_1 (AFG_1), AFB_1 and AFQ_1, AFM_1 (both metabolites of AFB_1) were detected in one or more of the body specimens in 35% of the subjects. Sixty percent (26 out of 40) of the subjects had only AFG_1, in their body specimens. These results coupled with others providing information on the liver status of the subjects suggested that suspected liver inflammation may involve other factors and may not only be due to the present intake levels of aflatoxins.

Nickelsen and Jakobsen (1997) have attempted to quantify the risk posed to consumers of 'kenkey', a Ghanaian fermented maize product. The method used followed the concept of hazard identification, exposure assessment, dose-response assessment and risk characterisation. By means of exposure and dose-response assessment, they were able to calculate the risk to develop liver cancer from exposure to aflatoxin B_1 to be 1%. This calculation was based on the following: the average consumption of two balls of kenkey (750g) a day, estimate of the incidence of liver cancer due to aflatoxin to be 19 cases per 100,000 population per year, and assuming a life expectancy of 54 years for males.

It is however important to reiterate the assumptions and uncertainties inherent in the estimations: The exposure assessment was based on crude estimations about the average consumption of kenkey and the aflatoxin content on a limited number of samples showing great variations. The dose-response assessment was based on one data set from China whose accuracy of exposure data is difficult to judge in addition to the model having an inherent uncertainty as well as the various problems encountered with the transfer of Chinese data to a Ghanaian population. Finally, the risk estimation is only valid for male consumers and since the sex ratio for liver cancer is greater than two, the risk for female consumers is likely to be less than half of the risk for male consumers.

2.5 Guinea

Only one study seems to be reported in Guinea. The prevalence of exposure to

aflatoxin B_1 and the hepatitis B (HBV) and hepatitis C (HCV) viruses, three major risk factors implicated in cellular hepatocarcinoma, was examined by Diallo and Wild (1995). A total of 75 blood serum samples collected from men living in Kindia (Lower Guinea) were analysed for aflatoxin bound covalently to serum albumin as a marker for exposure to aflatoxin. More than 90% of the samples contained detectably high levels for adults. The highest level was equivalent to 358pg (picogram) aflatoxin-lysine per mg of albumin. Regarding hepatitis B, eleven patients (14.7%) were positive whilst eight patients were positive to antibodies of HCV antigen.

2.6 La Côte d'Ivoire

Only one study was reported in La Cote d'Ivoire. Studies conducted on maize and poultry feeds on three poultry farms in the Ivory Coast revealed low and moderate contamination levels of feed and maize. The highest levels found in maize was 360 ppb and in feed, 240 ppb. (Wyers et al., 1991).

2.7 Niger

Aflatoxin contamination of groundnut due to *Aspergillus flavus* is one of the main constraints to groundnut production in most West African countries. In Niger, during the 1989, 1990, and 1991 rainy seasons, 25 lines including germplasm, advanced *A. flavus* resistant breeding lines, and cultivars from West Africa, were tested at three sites in Niger namely Sadore, Bengou and Maradi (Waliyar and Hassan, 1993). Seeds collected from these sites were tested for *A. flavus* contamination and aflatoxin content. Average seed contamination varied from 5 to 37% according to the site and year. Highest seed contamination was recorded in 1991 at Sadore. All the previously known resistant lines were among the less contaminated while susceptible lines exhibited the highest seed contamination. *A. flavus* contamination was well correlated to aflatoxin content which ranged from 1 to 750 ppb. Only one line with high contamination by *A. flavus* showed a low level of aflatoxin.

2.8 Nigeria

2.8.1 Agricultural research into aflatoxins in Nigeria

Maize and maize products

Several studies have been conducted on aflatoxins in maize in various parts of Nigeria and it appears that maize could be a significant source of aflatoxins in the human and animal diet. Thirty-nine samples of maize from markets in Kano were analysed for moisture and aflatoxin contents by Opadokun et al. (1979). Thirteen samples were found to contain aflatoxins with levels ranging from 5 ppb to 400 ppb. The average aflatoxin content was 106 ppb. Eight of these samples were found to have aflatoxin levels above 30 ppb., the maximum recommended level in food destined for human consumption. Moisture content determinations however revealed that three samples had moisture contents higher than 13.5%, the moisture content of maize in equilibrium with ambient relative humidity of 70 per cent. One of these samples had sufficiently high moisture content (17%) to permit fungal growth suggesting that the toxin was produced at a much earlier stage between maturity or harvesting and when the sample was taken. Most of the maize sold in Kano is however known to come from the humid Southern parts of Nigeria and it is likely that contamination may have occurred before transporting to Kano.

Studies conducted in Nigeria have shown that maize is liable to serious microbiological deterioration during storage in southern Nigeria (Broadbent 1967a; 1967b; Oyeniran 1972). The aflatoxin content, especially in livestock feed maize were found to be often very high (< 1000 ppb). However, studies on market samples of maize (Broadbent et al. 1971) did not reveal the presence of aflatoxin to any serious level.

Studies have been conducted by Oyeniran (1973) to examine the immediate post-harvest deterioration of maize. Thirty seven samples of maize were obtained from various sources and divided into four main categories namely: unshelled maize from local farmers' stores, shelled maize from Government farms, shelled maize from

markets, and shelled, artificially dried maize from Government farms. The moisture content, discoloured grain and insect damaged grain percentages were determined for all the samples. Aflatoxin levels were determined for half the samples and mould counts conducted on seven selected samples. Results revealed generally high moisture contents (15 - 26%) in all the groups of maize samples varying only with length of storage time confirming that the moisture content of maize is high at harvest. The percentage of discoloured grain was generally low in all categories. Aflatoxin content was found to be low (<100 ppb) in unshelled samples from farms except those heaped on the floor which had aflatoxin levels between 100 and 1000 ppb. Samples from markets also contained varying levels of aflatoxins (<100 to 1000 ppb). Shelled maize used for livestock feed in a government farm contained high levels of aflatoxins (100 to over 1000 ppb). Levels in this particular group of samples were similar to those observed in another study conducted by the same author in livestock feed maize (Oyeniran, 1972).

A countrywide survey was initiated in Nigeria in 1980 to determine storage losses of food crops at the market level (Opadokun and Ikeorah, 1983). During this two-year study, 145 maize samples from the 1980/81 and 1981/82 crop seasons from markets in Kano and plateau States of Nigeria were assayed for moisture and aflatoxin contents. Results showed 7 samples with moisture content higher than the "safe" moisture content of 13% for maize. More than 20% (30 samples) had detectable aflatoxin B_1 (>5 ppb). Twenty-one samples had aflatoxin B_1 levels above 30 ppb. which is the maximum permissible level set by the Protein Advisory Group (1969) in foods destined for human consumption. A very high figure of 1250 ppb was obtained for one sample from the Kano State. It is generally known that samples with low moisture contents but with high aflatoxin contents almost certainly had high moisture contents at some stage in their post-harvest life encouraging mould growth and therefore aflatoxin formation. However, pre-harvest invasion of corn by *Aspergillus flavus* has been reported by Rambo *et al.* (1974) while Lillehoj *et al.* (1978) identified insect infestation as a possible source of contamination of maize with aflatoxin prior to harvest.

Other commodities notably cowpea, millet, rice and sorghum were also studied

(Opadokun and Ikeorah, 1983) but aflatoxins were not detected in samples of these commodities. This observation suggests that apart from groundnuts, maize is the food grain in Nigeria that is most susceptible to aflatoxin contamination. The major cause of this contamination has been identified by Oyeniran (1973, 1977) to be inadequate or too slow drying of the maize which generally has high moisture content at harvest resulting in infection and growth of *Aspergillus flavus* (among other fungi) and aflatoxin formation. Atawodi et al. (1994) screened various human foods and feeds for aflatoxins in Nigeria and found 41% of twenty-two maize samples to be positive with a mean level of 21 ppb and a range of 20 to 107 ppb.

Mouldy maize samples from the Plateau State of Nigeria have been found to contain aflatoxins, zearalenone and ochratoxin A (Gbodi et al., 1986). Aflatoxin and zearalenone levels of 960 ppb and 17,500 ppb respectively were obtained for samples from Langtang. Highest ochratoxin A levels of 150 ppb were found in samples from Jos. Six of the samples collected contained both aflatoxins and zearalenone; another six had both aflatoxins and ochratoxin A whilst five samples contained all three mycotoxins. Aja-Nwachukwu and Emejuaiwe (1994), further screened maize samples from different locations in South Eastern Nigeria for aflatoxin B_1. Eighty percent of the samples were found to be positive for aflatoxin B_1.

A survey was conducted (Udoh, 1996) to investigate farmers' maize production, harvest and storage practices which could contribute to production of aflatoxin in maize in five agroecological zones in Nigeria. No aflatoxin was detected in maize samples obtained from the Northern Guinea Savanna zone. The largest amount of 3125 ppb was obtained from the humid Forest zone while the lowest aflatoxin content of 673 ppb was obtained from the Southern Guinea Savanna zone. Storage of maize in bags and "rhumbu" (traditional clay stores) was related to lower aflatoxin levels in the Sudan Savanna region of Nigeria.

Oyelami et al., (1996) screened a total of 48 samples of maize-based gruels, used as weaning food for children on admission at the Wesley Guild Hospital, Ilesha in Nigeria. Twelve samples (25%) were positive for aflatoxins with levels up to 19.7 ppb. whilst four samples contained ochratoxin A at low levels.

Sorghum and sorghum products

Sorghum (*Sorghum vulgare*) or Guinea corn is the most widely grown cereal in Nigeria with most coming from the drier Northern States. It is probably the most nutritious of the locally eaten cereals having a protein content of about 11%. It is the major staple in Northern Nigeria but is also eaten in the South (Opadokun, 1979a). It is also widely used for the production of a local alcoholic beverage burukutu (Faparusi et al., 1973) and lager beer (Okafor and Aniche, 1980). It has also become a major constituent of feeding stuffs for many classes of animals (Opadokun, 1979a)

Studies were initiated in 1975 on the extent of exposure of the population to aflatoxins through the diet (Opadokun, 1979a). During this survey, moisture and aflatoxin contents of 89 sorghum samples collected at weekly intervals from main markets in Kano were determined. Only one sample was found to contain detectable amounts of aflatoxins (22 ppb). Detection limit of the procedure used was 5 ppb. Furthermore all the samples had moisture contents below the 'safe' level of 13.5%. This study suggested little danger of aflatoxin poisoning from the consumption of sorghum grown in the Northern states.

Studies by Opadokun and Ikeorah (1983) involving 195 sorghum samples from markets in Kano and Plateau States of Nigeria over two crop seasons namely 1980/81 and 1981/82 seasons did not show any aflatoxin contamination of sorghum samples. However, further work by Salifu (1981) to investigate possible mycotoxin contamination in short season sorghums in Northern Nigeria revealed that the short season sorghum ripened during the rains, became mouldy, and were contaminated with aflatoxins, zearalenone, and patulin. Long season sorghums ripened after the rains were not mouldy and were found not to be contaminated with mycotoxins. Infestation with species of *Aspergillus, Penicillium,* and *Fusarium* noted for the production of mycotoxins was found to be more common on the seeds of the short season varieties of sorghum than on the long season varieties. Aflatoxins were found in the ripe seeds of all the short season varieties at levels ranging from 10 ppb to 80 ppb. Zearalenone was detected at levels of 100 and 200 ppb in two short season varieties and patulin in one short season variety at a level of 150 ppb. (Salifu, 1981). This work suggests that seeds of the new short season varieties of sorghum must be

tested for resistance to fungal infection before they are released.

Investigations into the fungal and mycotoxin contamination of sorghum during storage were conducted by Elegbede (1978). This study showed a decline in number of samples showing mycotoxin contamination as storage progressed suggesting that fungal activity in the stored sorghum was minimal probably due to good storage methods allowing good aeration and drying. No aflatoxins were detected during the 10-month storage period. Other mycotoxins namely ochratoxins, zearalenone and sterigmatocystin were however detected. Samples were taken after various storage times and analysed. Eight contained ochratoxin A, 13 contained ochratoxin B, two samples had ochratoxin C. Zearalenone was detected in two samples and sterigmatocystin in six samples.

Sorghum grains from different varieties grown in four ecological zones were tested for mycotoxin contamination. Aflatoxins B_1, B_2 and zearalenone were not detected in any of the samples analysed although fungi known to produce these mycotoxins under certain conditions were associated with the grain samples (Dada, 1979).

Other commodities and their products

Investigations have been carried out in Nigeria on other commodities and products. A survey on the aflatoxin B_1 load of common foods in the savanna and forest regions of Nigeria was conducted by Nwokolo and Okonkwo in 1978. High risk foods (>200 ppb of aflatoxin B_1) were found to be groundnuts, dried fish, guinea corn (sorghum) and millet. Maize, rice, beans, and crude palm-oil contained between 30 and 200 ppb of aflatoxin B_1. Low risk foods (below 30 ppb) included cereal acha (findi), some cassava products, yams and refined vegetable oils.

Atawodi et al. (1994) screened twenty-three groundnut cake samples destined for animal feed production. The samples contained aflatoxins ranging from 20 to 1862 ppb with a mean level of 382ppb. Other foods screened were biscuits, maize, sorghum, soybeans as well as ingredients used for the preparation of animal feeds. Biscuits, sorghum, or soybeans did not contain detectable levels of aflatoxins (detection limit 20 ppb). Oyejide et al. (1986) in a survey of commercial poultry feeds

in five southern states of Nigeria reported that 57 - 62% of the chick and broiler starter, broiler grower, broiler finishes and layer rations were contaminated with aflatoxin.

A survey of sixteen Nigerian indigenous beverages and foodstuffs was conducted by Alozie et al. (1980). All the eight samples of beverages were obtained from Ugbowo in Benin City and were found to be contaminated with aflatoxins. The beverages were 'emu aran' (obtained from the fermentable sap of the Raphia palm; *R. vinifera* and *R. raphia*), 'ogoro' (fermentable sap from the immature shoot of the oil palm, *Elais guinensis*), 'brukutu' and 'pito' which are produced from guinea corn (*Sorghum sp.*) and millet (*Penissetum sp.*). Aflatoxin levels ranged from 83 ppb in 'emu aran' to 262 ppb. in 'brukutu'. Foodstuffs investigated were purchased from markets in Benin City and included 'gari' (Cassava *farina*), 'ogbono' (*Irvingia gabunesis*), 'egusi' meal (*Cucumeropsis edulis*), 'ogili-ugba' (prepared from castor bean, *Riccinus communis*), 'dawadawa' (prepared from locust bean, *Parkia filicoden*), 'ewedu' (*Coconus seratus*) and 'shoko yokoto' (*Ceropsia* sp.). All the foodstuffs except dawadawa, ewedu, and shoko yokoto contained aflatoxins (Alozie et al. 1980).

A similar study conducted in the Jos metropolis showed a high incidence of aflatoxin B_1 contamination of traditionally brewed cereal beers (Okoye and Ekpenyong, 1984). Two traditional millet based alcoholic beverages, pito and burukutu showed high aflatoxin B_1 contamination levels. Seventeen of the twenty pito samples contained aflatoxin from levels of 16 ppb to 135 ppb whilst fifteen of twenty burukutu samples had aflatoxin levels ranging from 1.7 ppb to 137.7 ppb. In this study the raw material, millet was not analysed for aflatoxins.

The potential contribution of animal products to aflatoxin load has not received as much attention as plant products. Studies by Abalaka and Eronini (1987) revealed that cow meat samples from three locations with different sanitary qualities over a 12-month period in Zaria, Nigeria, contained aflatoxins B_1, B_2, G_1 and G_2 up to levels of between 1.2 - 5.1 ppm. This implies an accumulation of the toxin within the tissues as a result of chronic exposure of the cows to aflatoxin-contaminated feeds or feeding-stuffs.

Opadokun (1979b) studied aflatoxin B_1 contamination of 50 millet samples purchased at weekly intervals from markets in Kano. All the samples had moisture contents below the 'safe' moisture content of 16% (the moisture content of millet which will be in equilibrium with ambient air of 70% relative humidity). Five (10%) of the samples contained aflatoxin B_1 at levels ranging between 20 ppb and 160 ppb. The remaining samples did not contain aflatoxins at detection levels of 5 ppb.

Gari, prepared from the roots of cassava (*Manihot utillisima* Pohl.) is commonly eaten in Nigeria and other West African countries by large sections of the population. Opadokun (1976) determined the moisture and aflatoxin contents of thirty samples each of white and yellow gari purchased from either of two main markets in Kano. None of the samples contained aflatoxins at a detection limit of 5 ppb. Average moisture contents were 13.8% and 15.3% for white and yellow gari respectively. *Aspergillus flavus* was however isolated from some of the high moisture content samples an indication that the strain was either not an aflatoxin producing strain or conditions for the production of aflatoxins were not attained in the samples.

Ibeh et al., (1991) also studied the aflatoxin contents of 100 samples of various foods from Benin City in Nigeria. The samples consisted of gari, yam flour, cassava flour, melon, onion, rice, plantain, red pepper and eggs. Aflatoxins were detected in 18 samples: fifty percent (5 samples) of yam flour, 40% (4 samples) of cassava flour, 30% (3) of gari, 20% (2) of beans and melon, and 10% (1) of rice. Extremely high concentrations of aflatoxins were recorded in yam flour (4.0 - 7.6 ppm). Cassava flour also contained high aflatoxin levels ranging from 3.5 to 5.4 ppm. Pepper, onion, plantain and eggs did not contain detectable amounts of aflatoxins.

Further studies on aflatoxin contents of commodities have been conducted in Nigeria. Opadokun and Ikeorah (1979) analysed 68 samples of rice (both locally produced and imported) from markets in Kano. No aflatoxin contamination was detected. Regarding moisture, only one sample had a moisture content above the accepted 13.0% which is the moisture content for the 'safe' storage of rice. This sample of locally produced rice had a moisture content of 15.1%. Regarding cowpeas, Opadokun and Afolabi (1979) detected aflatoxin B_1 at a low level of 6.7 ppb in one of 33 samples collected and analysed from Kano market. In addition all

the samples had low moisture contents, much below the 'safe' moisture content of 15%. Mean moisture content was 7.7% varying between 6.0% and 10.6%.

Edible oils such as groundnut and palm oils are commonly added to poultry feeds to increase the energy density and enhance feed efficiency. Studies by Kuku and Agboola (1984) on palm oil, palm kernel oil and groundnut oil obtained from Ibadan markets showed that, of the fifteen mould species isolated from such oils, six were *Aspergillus* spp. The aflatoxin levels were however not determined.

2.8.2 *Agricultural practices*

Studies in Nigeria have shown that commercially grown groundnuts are not resistant to *Aspergillus flavus* and aflatoxin contamination. Research has shown that early harvesting of mature pods controls *A. flavus* infection. In addition, broken, damaged rotten pods and mouldy seeds should be discarded as a means of reducing aflatoxin levels (Alabi et al., 1991).

Pre-harvest and stored maize produced by small-scale farmers in various agro-ecological zones of Benin and Nigeria was analysed from 1993 to 1995, to assess the fungal contamination and mycotoxin levels (Hell et al 1995). Analysis of farming systems from which the maize samples were taken indicated which practices increased and which decreased aflatoxin levels. In Nigeria, crop husbandry practices which significantly reduced aflatoxins in maize samples taken from 125 small farm stores were: use of fertiliser, pesticides, and seed protectants. Aflatoxin levels were found to increase significantly when farmers said they used "improved varieties," and when they harvested late after crop maturity. Processing steps such as drying, sorting, cleaning, dehusking, degraining, sunning, and smoking all decreased aflatoxin levels. Insect control in the field and store also decreased aflatoxin levels significantly. Furthermore, poor practices such as storage of maize on the floor in a room resulted in high aflatoxin levels (Hell et al., 1995).

Studies have been conducted by Aja-Nwachukwu and Emejuaiwe (1994) on aflatoxin-producing fungi associated with Nigerian maize. A total of 43 maize samples purchased from 5 locations in Southeast Nigeria were examined for fungal

contamination and aflatoxin-producing ability of detected fungi. Five *Aspergillus* spp. (*A. flavus*, *A. parasiticus*, *A. japonicus*, *A. ochraceus and A. niger*), three *Penicillium* spp. (*P. oxalicum*, *P. corylophilum*, *and P. citrinum*), one *Alternaria* sp., one *Fusarium*, one *Cladosporium* sp. and one *Acremonium* sp. (*Cephalosporium*) were isolated. Eighty percent of maize samples contained detectable levels of aflatoxin B_1. Eighty-five percent of the *A. flavus* isolates produced aflatoxin B_1 when cultivated on sterile maize moistened to more than 20%.

The ability of *Aspergillus flavus* strains isolated from cassava flour to produce aflatoxins was studied. One hundred and four samples of cassava flour obtained from different parts of Nigeria were analysed for the presence of aflatoxin producing strains of *Aspergillus flavus* (Kareem et al., 1990). Samples were from markets in Kaduna, Zaria, Okene, Jos, Lagos, Illorin and Port-Harcourt. Sixty *Aspergillus flavus* strains were isolated from the cassava flour samples. Thirteen of these were able to produce aflatoxins in yeast extract broth medium containing 20% sucrose. Ten of the strains produced all four aflatoxins namely aflatoxins B_1, B_2, G_1, and G_2. Three strains produced only aflatoxins B_1, and B_2. The amount of aflatoxins produced were relatively high and ranged from 3.0μg/ml to 14.2 μg/ml of B_1, 0.9 to 11.9 μg/ml of B_2, 0 to 12.5 μg/ml of G_1 and 0 to 9.2μg/ml of aflatoxin G_2.

The effects of essential oils from two Nigerian medicinal plants (*Azadirachta indica* and *Morinda lucida*) on the growth and aflatoxin B_1 production in maize grain by a toxigenic *Aspergillus flavus* were studied by Bankole (1997). The work revealed that growth of *Aspergillus flavus* decreased progressively with increasing concentration of essential oils from leaves of *Azadirachta indica* and *Morinda lucida*, and seeds of *A. indica*. The oils also significantly reduced aflatoxin synthesis in inoculated maize grains. Oils from *A. indica* seeds completely suppressed aflatoxin synthesis in inoculated maize at 500 and 1000 ppm while those of *M. lucida* also showed complete inhibition at 1000 ppm.

During their studies on artificially contaminated melon seeds during the preparation of 'ogiri', Ogunsanwo et al. (1989b) observed significant reductions in the levels of aflatoxins during *Bacillus* fermentation. At low levels of contamination (16.4 ppb),

aflatoxins were completely removed after 4 to 7 days fermentation. However at high levels, the degradation of aflatoxin during fermentation was not very effective. Earlier studies by Ogunsanwo et al. (1989a) using inoculated soybeans in the preparation of 'Soyogi' showed reductions of 55.9 and 51.4% in aflatoxins B_1 and B_2 levels respectively after 3 days of fermentation. Boiling, steaming, or frying of cowpeas to produce a porridge 'moinmoin' or 'akara' was also found to decrease initial aflatoxin B_1 levels of 0.61 to 0.73 ppm by 20 - 32%, depending on the type of cowpea (white or brown) and the type of food produced (Ogunsanwo et al., 1989c).

Akinrele (1970) investigated the surface microflora of maize kernels for the production of African starch cake and demonstrated the presence of the mould genera *Penicillium, Aspergillus,* and *Fusarium* among others. These moulds however disappeared during the maize steeping process. Fields et al. (1981) also found moulds present in unfermented maize dough but they were not detectable in the doughs after two days of fermentation.

Other researchers (Dada and Muller, 1983) showed a 12 - 16% reduction in aflatoxin B_1 content of sorghum 'ogi' while Adegoke et al. (1994) reported a reduction of more than 70% during the lactic acid fermentation of maize 'ogi' and sorghum 'ogi'.

Studies by Okoye (1986) on 'Burukutu' (an indigenous beverage produced from guinea corn or millet) to assess the efficiency of the Nigerian traditional brewing process in destroying aflatoxins in mould-infected grains showed a 41% carry-over of aflatoxin B_1 from the raw material to the final product.

2.8.3 Medical research into aflatoxins

The consequences of prenatal exposure to aflatoxins are unknown in humans though a decrease in the mean birth weight of females was recorded in Kenya and three unexplained stillbirths (two in Kenya and one in Nigeria) were recorded where aflatoxins were detected in maternal and cord blood at delivery. Furthermore, in 161 human urine samples collected in Lagos, Nigeria, aflatoxin B_{2a} was detected in 32.7% of the samples, B_1 in 3.1%, M_1 in 8.7%, G_1 in 9.9% and L in 9.3% of the samples (Bean et al., 1989). Aflatoxin G_1 was present in the highest mean

concentration at 12ng/100ml urine. Studies by Wilkinson et al. (1989) using an adapted enzyme-linked immunosorbent assay (ELISA) analysed human serum for aflatoxin and found 76% of Nigerian samples positive along with 100% of Nepalese samples. No aflatoxins were found in sera from UK subjects.

The prevalence of aflatoxins in feeds and feedingstuffs in Nigeria has been reviewed by Aletor (1990). A survey of the literature revealed an increasing wave of aflatoxin contamination of feeds and feedingstuffs and consequent poisoning of a large number of animals, especially poultry, in several parts of the country. Akinyemi et al. (1984) showed that aflatoxicosis was responsible for 93% mortality in a flock of 2977 3-week old White Peking ducklings in Ogun State following consumption of a compounded poultry ration. The ration was found to contain 3300 ppb aflatoxin B_1. Asuzu and Shetty (1986) also reported the death of over 1000 six-week old broilers belonging to the University of Nigeria poultry farm. The broilers had been fed a newly purchased commercial poultry feed which was later found to contain 265 ppb aflatoxin B_1. Day-old broilers fed the same aflatoxin contaminated diet exhibited clinical and pathological manifestations identical with naturally infected birds. An outbreak of aflatoxicoses in guinea fowls usually regarded as very resistant to aflatoxins when compared to turkey, duck, and chicken, was reported by Okaeme (1987). Examination of the feed revealed that in some cases, the rice bran and maize used in the preparation of the feeds had visible greenish-yellow plaques due to fungal discoloration. *Aspergillus flavus and Mucor* spp. were found to be the prevalent fungal isolates in the processed feed, groundnut cake, rice bran and maize used in the feed preparation. Nine feed samples analysed contained aflatoxins at levels between 49 and 72.5ppb with a mean of 59ppb.

Observations that aflatoxins can decrease the fertility in male rats (Sahay, 1993) may be established in humans by work conducted by Ibeh et al. (1994). These researchers investigated infertility in Nigeria and found aflatoxin in semen samples of infertile males more frequently than in fertile males and the defects of spermatozoa seen in infertile males resembled those seen in semen of rats exposed to aflatoxin.

2.10 Sierra Leone

Few studies seem to have been conducted in Sierra Leone. Sesame seeds from Sierra-Leone were found to contain toxigenic strains of *Aspergillus* and *Penicillium.* These were *A. flavus, A. tamarii, A. ochraceus and P. citrinum* which were isolated from 49 'ogiri' (fermented sesame seeds) samples (Jonsyn, 1990). Aflatoxin, ochratoxin and citrinin have also been detected in these seeds (Jonsyn, 1988). Further studies by Jonsyn (1991) demonstrated that early smoking (day 2) during fermentation and continuous fermentation for a week prolonged the shelf life of the product, discouraged mould growth, and inhibited mycotoxin formation.

Twenty samples of smoke-dried fish commonly called Bonga obtained from homes and markets in Njala (Sierra Leone) showed the presence of four *Aspergillus spp.* namely *A. flavus* Link ex Fries, *A. ochraceus* Wilhelm, *A. tamarii* Kita and *A. niger* van Tieghem. Examination of mould containing fish extracts revealed varying amounts of aflatoxins B_1, G_1 and G_2 and ochratoxin A (Jonsyn, 1992). Fresh and correctly preserved smoke-dried fish, however did not show signs of fungal contamination.

Recent studies have been conducted on the mycotoxin concentration of body fluids from children under 5 years of age by Jonsyn (1999). Fifty-four urine samples were all found to be contaminated with aflatoxins with 24 and 20% also contaminated with ochratoxin A and ochratoxin B respectively. Ninety-four percent of serum samples contained aflatoxins whilst 33% and 23% contained ochratoxin A and ochratoxin B respectively. Ninety-four percent of stool specimens were also found to contain aflatoxins.

3. Conclusions and recommendations

The information gathered revealed that aflatoxins appear to be a problem in most West African countries and high levels in some foods have actually been confirmed with clinical studies. It was however observed that research activities on aflatoxins in the West African sub-region are sporadic and not conducted in a systematic manner. Information on capacity as well as institutions involved in aflatoxin research was lacking as most of the questionnaires sent out were not returned.

Based on the information gathered and on three review papers (Doyle et al., 1982; Karlovsky, 1999; Galvano et al., 2001) as well as work conducted so far under the project, it is however envisaged that several approaches can be used to tackle the aflatoxin problem

- Physical approaches such as the manual or electronic removal of contaminated grains or kernels, the use of high temperatures in combination with moisture, microwave treatment at high energy levels and the combined use of elevated temperatures and pressures as in extrusion cooking.

- Nutritional or dietary approaches such as the supplementation of nutrients, food components, or the use of additives with protective properties against aflatoxin toxicity seem to be potentially capable of protecting against aflatoxins. These will involve the use of antioxidant compounds such as selenium, vitamins and provitamins, food components and additives such as ellagic acid, turmeric, garlic, medicinal herbs and plant extracts.

- Chemical approaches such as the use of ammonia, sodium bisuphite, calcium hydroxide, formaldehyde, hydrogen peroxide have been considered by several researchers and found to be effective, however, they do not fulfil all the requirements, concerning the safety of reaction products and the safeguarding of the nutritional characteristics of the treated foods. The use of chemicals in reducing aflatoxin levels in foods in

West Africa is therefore not recommended.

- The addition of sorbents such as hydrated sodium calcium aluminosilicate (HSCAS), zeolites, bentonites, clays and activated carbons to animal feeds to reduce the bioavailability and subsequent carryover of aflatoxins from contaminated feeds to animal products such as milk, meat and eggs is an important approach and could be seriously considered.

- The approach of the current project is to degrade aflatoxins with microorganisms or their cell-free enzyme preparations. In this regard, several microorganisms have been screened and some bacterial and fungal species found to bind aflatoxins rather than degrade them. This approach could be potentially useful to countries in West Africa who through spontaneous lactic acid fermentations may be consuming some of these organisms which could then offer some protection by adsorbing the toxins present in the foods preventing their uptake during passage through the intestinal tract. Currently, a high priority has been placed on the preparation of cell-free enzyme extracts from *Nocardia corynebacteroides* and *Bifidobacterium* spp. which will eventually be used to degrade aflatoxins on an industrial scale in maize and sorghum fermentation processing plants in Ghana and Nigeria respectively.

Coupled to all the above approaches,

- The most effective way to control aflatoxins is proper post-harvest management and the use of the HACCP principles in purchasing and processing of raw materials. The development of plant varieties with resistance to insect and fungal attack should also be encouraged

- There should be a co-ordination and strengthening of research efforts with the establishment of centres of excellence within the West African sub-region with a high degree of autonomy given to the local researchers and experts in the sub-region

- At the national level, maximum tolerance levels of aflatoxins in foods should be set based on sound scientific data and practical considerations. This should be supported with the needed education, inspection, and analytical services.

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5. REFERENCES

Abalaka, J. A. and Eronini, T. 1987. Incidence of Aflatoxin B_1, B_2, G_1 and G_2 residues in marketed meat in Zaria (Nigeria). *Nig. J. Sa. Res.*, **1** (1): 17 - 19.

Adegoke, G. O., Otumu, E. J. and Akanni, A. O. 1994. Influence of grain quality, heat and processing time on the reduction of aflatoxin B_1 levels in 'tuwo' and 'ogi', two cereal-based products. *Plant Foods for Human Nutrition* **45**: 113 - 117.

Aja-Nwachukwu, J. and Emejuaiwe, S. O. 1994. Aflatoxin producing fungi associated with Nigerian maize. *Environmental Toxicology and Water Quality* **9** (1): 17 - 23.

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

Akinyemi, J. O., Fatumbi, O. O. and Fasina, S. O. 1984. Aflatoxicoses on a duckling flock in Ogun State of Nigeria. *Nig. Vet. J.*, **13** (1): 36 - 38.

Alabi, O., Misari, S. M., Boye-Goni, S. R. and Olorunju, P. E. 1991. Current status of research on groundnut diseases in Nigeria. *Summary Proceedings of the Second ICRISAT Regional Groundnut Meeting for West Africa* pp 30 - 31.

Aletor, V. A. 1990. Aflatoxin contamination in some Nigerian feeds and feedingstuffs: Highlights of some nutritional physiopathological and economic implications. *Food Chemistry* **37**: 145 - 153.

Alozie, T. C., Rotimi, C. N. and Oyibo, B. B. 1980. Production of aflatoxin by *Aspergillus flavus* (UBMI) in some Nigerian indigenous beverages and foodstuffs. *Mycopathologia* **70** (2): 125 - 128.

Ankrah, N-A., Rikimaru, T. and Ekuban, F. A. 1994. Observations on aflatoxins and the liver status of Ghanaian subjects. *East African Medical Journal* **71** (11): 739 -741.

Apeagyei, F., Lamplugh, S. M., Hendrickse, R. G., Affram, K. and Lucas, S. 1986. Aflatoxins in the livers of children with kwashiorkor in Ghana. *Tropical Geographical Medicine* **38**: 273 – 276.

Asuzu, I. U. and Shetty, S. N. 1986. Acute aflatoxicosis in broiler chicken in Nsukka, Nigeria. *Trop. Vet.* **4**: 79 - 80.

Atawodi, S. E., Atiku, A. A. and Lamorde, A. G. 1994. Aflatoxin contamination of Nigerian foods and feedingstuffs. *Fd. Chem. Toxic.* **32** (1): 61 - 63.

Awuah, R. T. and Kpodo, K. A. 1996. High incidence of *Aspergillus flavus* and aflatoxins in stored groundnut in Ghana and the use of a microbial assay to assess the inhibitory effects of plant extracts on aflatoxin synthesis. *Mycopathologia* **134**: 109 - 114.

Bankole, S. A. 1997. Effect of essential oils from two Nigerian medicinal plants (*Azadirachta indica* and *Morinda lucida*) on growth and aflatoxin B_1 production in maize grain by a toxigenic *Aspergillus flavus*. *Letters in Applied Microbiology* **24** (3): 190 - 192.

Bean, T. A., Yourtee, D. M., Akanda, B. and Ogunlewe, J. 1989. Aflatoxin metabolites in the urine of Nigerians: comparison of chromatographic methods. *Toxin Review* **8**: 43 – 52.

Broadbent, J. A. 1967a. The microflora, germination and seedling vigour of some seed maize. *Rep. Nigerian stored Prod. Res. Inst.*, 1966. *Tech. Rep. No.* 15 113 - 114.

Broadbent, J. A. 1967b. Microbiological deterioration of maize used as poultry and livestock feed at farms near Ibadan during the wet season. *Rep. Nigerian stored Prod. Res. Inst.*, 1966. *Tech. Rep. No.* 16 115 - 118.

Broadbent, J. A., Oyeniran, J. O. and Kuku, F. O. 1971. The moisture and aflatoxin content survey of maize, groundnuts and cowpea on sale in Ibadan markets (Unpublished).

Dada, J. D. 1979. Studies on fungi causing grain mould of sorghum varieties in Northern Nigeria with special emphasis on species capable of producing mycotoxins. *MSc. Thesis* Submitted to the Department of Crop Protection, Ahmadu Bello University, Zaria, Nigeria.

Dada, L. O. and Muller, H. G. 1983.The fate of aflatoxin B_1 in the production of ogi, a Nigerian fermented sorghum porridge. *J. Cereal Science* **1**: 63 - 70.

Diallo, M. S. and Wild, C. P. 1995. The occurrence of exposure to aflatoxins and to hepatitis B and C viruses in Guinea. *Proceedings of the Workshop on Mycotoxins in foods in Africa.* November 6 - 10, 1995, Cotonou, Benin. Ed. Cardwell K. E.

Doyle, M. P., Applebaum, R. S., Brackett, R. E. and Marth, E. H. 1982. Physical, chemical and biological degradation of mycotoxins in foods and agricultural commodities. *J. Food Prot.* **45**: 964 – 971.

Elegbede, J. A. 1978. Fungal and mycotoxin contamination of sorghum during storage. *MSc Thesis* Submitted to the Biochemistry Department, Ahmadu Bello University, Nigeria.

Faparusi, S., Olofinboba, M. O. and Ekundayo, J. A. 1973. The microbiology of burukutu beer. *Z. F. Allg. Microbiol.* **13**: 563.

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

Galvano, F., Piva, A., Ritieni, A. and Galvano, G. 2001. Dietary strategies to counteract the effects of mycotoxins: A review. *J. Food Prot.* **64**: 120 - 131.

Gbodi, T. A., Nwude, N., Aliu, Y. O. and Ikediobi, C. O. 1986. The mycoflora and some mycotoxins found in maize (*Zea Mays*) in the Plateau State of Nigeria. *Vet. Hum. Toxicol.* **28** (1): 1 - 5.

Halm, M., Lillie, A., Sørensen, A. K. and Jakobsen, M. 1993. Microbiological and aromatic characteristics of fermented maize doughs for kenkey production in Ghana. *Int. J. Food Microbiol.* **19**: 135 - 143.

Hell, K., Udoh, J., Setamou, M., Cardwell, K. F. and Visconti, A. 1995. Fungal infection and mycotoxins in maize in different Agro-ecological zones of Benin and Nigeria, West Africa. *Proceedings of the Workshop on Mycotoxins in Food in Africa*, Cotonou, Benin, November 6 - 10.

Hudson, G. J., Wild, C. P., Zarba, A. and Groopman, J. D. 1992. Aflatoxins isolated by immunoaffinity chromatography from foods consumed in The Gambia, West Africa. *Nat. Toxins* **1**: 100 - 105.

Ibeh, I. N., Uraih, N. and Ogonor, J. I. 1991. Dietary exposure to aflatoxin in Benin City, Nigeria: a possible public health concern. *International Journal of Food Microbiology* **14**: 171 - 174.

Ibeh, I. N., Uraih, N. and Ogonor, J. I. 1994. Dietary exposure to aflatoxin in human male infertility in Benin City, Nigeria. *International Journal of Fertility* **39**: 208 - 214.

International Institute for Tropical Agriculture (IITA), 1997. Factors contributing to the distribution and incidence of aflatoxin producing fungi in stored maize in Benin. Final Report, Plant Health Management Division, International Institute for Tropical Agriculture.

Jespersen, L., Halm, M., Kpodo, K. A. and Jakobsen, M. 1994. Significance of yeasts and moulds occurring in maize dough fermentation for 'Kenkey' production. *Int. J. Food Microbiol.* **24**: 239 - 248.

Jonsyn, F. E. 1988. Seedborne Fungi of Sesame (*Sesamum indicum* L) in Sierra Leone and their potential Aflatoxin/mycotoxin production. *Mycopathologia* **104**: 123 - 127.

Jonsyn, F. E. 1990. Mycological contamination of ogiri (fermented sesame seeds) during production in Sierra Leone. *Mycopathologia* **110**: 113 - 117.

Jonsyn, F. E. 1991. Improved methods of ogiri (fermented sesame seeds) preparation to minimise and control mycotoxin contamination. In *Traditional African Foods: Quality and Nutrition* (A. Westby and P. J. A. Reilly, eds.). International Foundation for Science, Stockholm, Sweden.

Jonsyn, F. E. 1992. Mycotoxic flora and mycotoxins in smoke-dried fish from Sierra Leone. *Nahrung* **36** (5): 485 – 489.

Jonsyn, F. E. 1999. Intake of aflatoxins and ochratoxins by infants in Sierra Leone: possible effects on the general health of these children. *Journal of Nutritional and Environmental Medicine* **9** (1): 15 – 22.

Kareem, V. N. A., Okagbue, R. N. and Ogbadu, G. H. 1990. The production of aflatoxin by *Aspergillus flavus* in cassava flour. *Nigerian Food Journal* **8**: 87 – 91.

Karlovsky, P. 1999. Biological detoxification of fungal toxins and its use in plant breeding, feed and food production. *Nat. Toxins* **7**: 1 - 23.

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

Kpodo, K. A. 1995. Present status of research on the aflatoxin problem in groundnut in Ghana. *Arachide Infos.* December 1995, (6): 5.

Kpodo, K. A., Sørensen, A. K. and Jakobsen, M. 1996. The occurrence of mycotoxins in fermented maize products. *Food Chem.* **56** (2): 147 - 153.

Kpodo, K. A. 1997. Final Report of the Food Research Institute (FRI) / Texas A&M Peanut Collaborative Research Support Program (Peanut CRSP) Project titled "Mycotoxin Management in Peanut by Prevention of Contamination and Monitoring".

Kuku, F. O. and Agboola, S. D. 1984. Moulds associated with some vegetable oils and their effects on the free fatty acid (ffa) content. *Nig. J. Microbiol.*, **4** (1 - 2): 105 - 111.

Lillehøj, E. B., Fennel, D. I., Kwolek, E. F., Adams, G. L., Zuber, M. S., Horner, E. S., Widstrom, N. W., Warren, H., Guthrie, W. D., Shuer, D. B., Findley, W. R., Manwiller, A., Josephson, L. M. and Bockholt, A. J. 1978. Aflatoxin contamination of corn before harvest. *Aspergillus flavus* association with insects collected from developing ears. *Crop Science*, **18** (6): 921 - 924.

Maxwell, S. M., Apeagyei, F., de Vries, H. R., Mwanmut, D. D. and Hendrickse, R. G. 1989. Aflatoxins in breast milk, neonatal cord and sera of pregnant women. *Journal of Toxicology-Toxin Reviews* **8**: 19 - 29.

Miele, M., Donato, F., Hall, A. J., Whittle, H., Chapot, B., Bonatti, S., de Ferrari, M., Artuso, M., Gallerina, E., Abbondandolo, A., Montesano, R. and Wild, C. 1996. Aflatoxin exposure and cytogenetic alterations in individuals from the Gambia, West Africa. *Mutation Research* **349**: 209 - 217.

Mintah, S. and Hunter, R. B. 1979. The incidence of aflatoxin found in groundnuts (*Arachis hypogea* L.) purchased from markets in and around Accra, Ghana. *Peanut Sci.* **5**: 13 - 16.

Nickelsen, L. and Jakobsen, M. 1997. Quantitative risk analysis of aflatoxin toxicity for the consumers of 'Kenkey'- a fermented maize product. *Food Control* **8**(3): 149 - 159.

Nikiema, P. A., Jeannel, D. and Wild, C.P. 1997. Possible interactions between human aflatoxicosis and infections due to HBV and HCV in a rural area of Western Burkina-Faso. Presented at the 4th Alexander Hollander Training Course in Genetic Toxicology. 15 - 18 September 1997, Cairo, Egypt.

Nikiema, P. A., Traore, S. A. and Singh, B. 1993. Evolution of physical and chemical parameters and aflatoxin production in groundnut seeds during storage. In Summary Proceedings of the Third ICRSAT Regional Groundnut Meeting for West Africa. (Eds) Waliyar, F., Ntare, B. R. and Williams, J. H. 14 -17 September 1992, Ouagadougou, Burkina Faso.

Nwokolo, C. and Okonkwo, P. 1978. Aflatoxin load of common food in savanna and forest regions of Nigeria. *Trans. Roy. Soc. Trop. Med. Hyg.* **72** (4): 329 - 332.

Ogunsanwo, B. M., Faboya, O. O., Ikotun, T. and Idowu, R. 1989a. Fate of aflatoxins in soybeans during the preparation of 'Soyogi'. *Nahrung* **33** (5): 485 - 487.

Ogunsanwo, B. M., Faboya, O. O., Idowu, O. R., Ikotun, T. and Akano, D. A. 1989b. The fate of aflatoxins during the production of 'Ogiri', a West African fermented melon seed condiment from artificially contaminated seeds. *Nahrung* **33** (10): 983 - 988.

Ogunsanwo, B. M., Faboya, O. O., Idowu, O. R., Ikotun, T. 1989c. Reduction of aflatoxin content of infected cowpea seeds during processing into food. *Nahrung* **33**: 595 - 597.

Okaeme, A. N. 1987. Potential economic impact of aflatoxicosis in guineafowl production (*Numida melegris*). Proceedings 12th Annual Conf. NSAP 22 - 26. March 1987.

Okafor, N. and Anichie, G. N. 1980. Brewing lager beer from Nigerian sorghum. *Brewing and Distilling International.* **10**: 32 - 35

Okoye, Z. S. C. 1986. Carryover of aflatoxin B_1 in contaminated substrate corn into Nigerian native beer. *Bull. Environ. Contam. Toxicol.* **37**: 482 - 489.

Okoye, Z. S. C. and Ekpenyong, K. I. 1984. Aflatoxin B_1 in native millet beer brewed in Jos suburbs. *Trans. Roy. Soc. Trop. Med. Hyg.* **78**: 417 - 418.

Ong, T. M. 1975. Aflatoxin mutagenesis. *Mutat. Res.,* **32**: 35 - 53.

Opadokun, J. S. 1976. The aflatoxin contents of locally consumed foodstuffs Part I: Gari. *Rep. Nigerian Stored Prod. Res. Inst.* 1975 - 1976.Technical Report No. 7: 45 - 50.

Opadokun, J. S. 1979a. The aflatoxin contents of locally consumed foodstuffs, Part II. Sorghum. *Rep. Nigerian Stored Prod. Res. Inst.* 1976 - 1977.Technical Report No. 11: 101 - 104.

Opadokun, J. S. 1979b. The aflatoxin contents of locally consumed foodstuffs, Part IV. Millet. *Rep. Nigerian Stored Prod. Res. Inst.* 1977 - 1978. Technical Report No. 10: 83 - 85.

Opadokun, J. S. and Ikeorah, J. N. 1983. Moisture and aflatoxin contents of market grain samples in Kano and Plateau states of Nigeria. *Rep. Nigerian Stored Prod. Res. Inst.* 1983. Technical Report No. 3: 35 - 41.

Opadokun, J. S., and Ikeorah, J. N. 1979. The aflatoxin contents of locally consumed foodstuffs, Part IV. Rice. *Rep. Nigerian Stored Prod. Res. Inst.* 1978 - 1979. Technical Report No. 12: 101 - 105.

Opadokun, J. S., and Afolabi, E. 1979. The aflatoxin contents of locally consumed foodstuffs, Part VII. Cowpea. *Rep. Nigerian Stored Prod. Res. Inst.* 1978 - 1979. Technical Report No. 13: 107 - 109.

Opadokun, J. S., Ikeorah, J. N. and Afolabi, E. 1979. The aflatoxin contents of locally consumed foodstuffs, Part III. Maize. *Rep. Nigerian Stored Prod. Res. Inst.* 1976 - 1977. Technical Report No. 12: 105 - 108.

Oyejide, A., Tewe, O. O. and Okosun, S. E. 1986. Prevalence of aflatoxin B_1 in commercial poultry rations in Nigeria. *J. Institut. Fur. Tropishe Landwirt Schalt.*

Oyelami, O. A., Maxwell, S. M. and Adeoba, E. 1996. Aflatoxins and ochratoxin A in the weaning food of Nigerian children. *Ann. Trop. Paediatr.* 16: 137 - 140.

Oyeniran, J. O. 1972. Microbiological studies on maize used as poultry and livestock feed at two research farms Ibadan, Western State, Nigeria. *Rep. Nigerian Stored Prod. Res. Inst.* 1970. *Tech. Rep. No.* 6 *pp.* 47 - 56.

Oyeniran, J. O. 1973. Microbiological examination of maize from various sources soon after harvest. *Rep. Nigerian Stored Prod. Res. Inst.* 1971. *Tech. Rep. No.* 3 *pp.* 27 - 32.

Oyeniran, J. O. 1977. The deterioration of maize during storage at various moisture contents. *Rep. Nigerian Stored Prod. Res. Inst.* 1973. *Tech. Rep.* No. 7:55 - 59.

PAG FAO/WHO/UNICEF Protein Advisory Group 1969. PAG Recommendation on aflatoxin, PAG Statement No. 2. New York, United Nations, 2pp.

Rambo, G. W., Tuite, J. and Caldwell, R. W. 1974. *Aspergillus flavus* and aflatoxin in preharvest corn from Indiana in 1971 and 1972. *Cereal Chem.* **51** (5): 595, 600 - 604.

Sahay, M. 1993. Aflatoxin induced testicular degeneration and decreased meiotic index in Rattus norvegicus. *Cytobios* **75**: 191 - 195.

Salifu, A. 1981. Mycotoxins in short season sorghums in Northern Nigeria. *Samaru J. Agric. Res.*, 1 (1): 83 - 88.

Setamou, M., Cardwell, K. F., Schulthess, F. and Hell, K. 1997. *Aspergillus flavus* infection and aflatoxin contamination of preharvest maize in Benin. *Plant Disease* **81** (11): 1323 - 1327.

Udoh, J. 1996. Production, harvest and storage practices of maize in agroecological zones of Nigeria as they affect aflatoxin content of the grains. *Ph.D Thesis* submitted to The University of Ibadan, Nigeria.

Waliyar, F. and Hassan, H. 1993. Aflatoxin contamination of groundnut in Niger. In Summary Proceedings of the Third ICRSAT Regional Groundnut Meeting for West Africa. (Eds) Waliyar, F., Ntare, B. R. and Williams, J. H. 14 -17 September 1992, Ouagadougou, Burkina Faso.

Wild, C. P., Jiang, Y-Z., Allen, S. J., Hall, A. J., Jansen, L. A. M., Montesano, R. 1990. Aflatoxin-albumin adducts in human sera from different regions of the world. *Carcinogenesis* **11**: 2271 - 2274.

Wild, C. P., Rasheed, F. N., Jawla, M. F. B., Hall, A. J., Jansen, L. A. M. and Montesano, R. 1991. *In-utero* exposure to aflatoxins in West Africa. *Lancet* **337**: 1602.

Wild, C. P., Hudson, G. J., Sabbioni, G., Chapot, B., Hall, A., Wogan, G. N., Whittle, H., Montesano, R. and Groopman, J. D. 1992. Dietary intake of aflatoxins and the level of albumin-bound aflatoxin in peripheral blood in The Gambia, West Africa. *Cancer Epidemiol. Biomarkers Prevention* **1**: 229 - 234.

Wilkinson, A. P., Denning, D. W. and Morgan, M. R. A. 1989. Immunoassay of aflatoxin in food and human tissue. *Toxin Rev.* **8**: 69 – 79.

Wyers, M., Mobio, M. G., Schricke, E. and Guetta, A. N. 1991. Recherche dans trois élevages industriels de poules en Côte-d'Ivoire des lésions de l'intoxication par l'aflatoxine B_1. *Revue Élev. Mèd. vet. Pays trop.* **44** (1): 15 - 21.

Zarba, A., Wild, C. P., Hall, A. J., Montesano, R., Hudson, G. J. and Groopman, J. D. 1992. Aflatoxin M-1 in human breast milk from the Gambia, West Africa, quantified by combined monoclonal antibody immunoaffinity chromatography an HPLC. *Carcinogenesis* **13** (5): 891 – 894.

EU PROJECT ON MYCOTOXINS

Questionnaire on mycotoxin research in West Africa

1-Name: ----------

- Address (including fax, tel., e-mail):

-Name and address of Institution:

2- Information available on the occurrence of mycotoxins in foods in your country:

3- Which Institutions are involved in Agricultural Research into mycotoxins

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- Nature of work done:----------

4- Which Institutions are involved in Medical Research into mycotoxins

- Nature of work done : ----------

5- Are there any Institutions involved in other kinds of mycotoxin work

- Nature of work done :----------

6- Do you have any references for Reports, Theses etc. (unpublished) and publications on mycotoxins

7- Do you have information on on-going projects on mycotoxins in you country: ----------

Which are the Institutions involved:----------

Who are funding these projects: ----------

Are there any linkages with foreign research groups on mycotoxins and what are they:

8- Which is the most suitable Institution to contact for present and further information on mycotoxin research in your country:

Name of Institution and contact person:

-Address:

-Tel., Fax., E-mail:

Food Research Institute

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