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Determination of total aflatoxin in maize and maize products sold in some retail outlets in Zaria

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Abstract
Determination of total aflatoxin in maize and maize products sampled from some retail outlets in Zaria was determined by ELISA method. A total of 300 samples was collected during both seasons, 150 samples each of maize grain and maize flour was collected for both dry and wet seasons. The data obtained was subjected to student 's T test and a one way analysis of variance to compare the statistical difference between maize grain and maize flour within the season and the aflatoxin level within the products. The predominant mycoflora of the maize grain and maize flour were; *Aspergillus flavus, Aspergillus niger, Aspergillus parasiticus, Penicillum sp, Fusarium sp and Rhizopus sp*. *Aspergillus flavus* and *Aspergillus parasiticus* were the most isolated in all the outlets. Similarly, Frequency of occurence of fungal isolates on maize grain shows that *Aspergillus parasiticus* had the highest percentage occurrence (48%) during the wet seasons compared to its frequency of occurence of 32.11% in the dry season. On the other hand, *Rhizopus spp* had lowest occurence of 1.32% on maize grain in the wet season and did not occurred at all during the dry season. Generally, *Penicillium spp* exhibited the highest frequency of occurence (44.44%) on maize flour. The number of positive samples for aflatoxin on maize grain was 33 (22%) and maize flour was 21 (14%). Quantitation of total aflatoxin in maize grain shows that Yannika recorded highest aflatoxin level of 2.2 (ppb) and 2.8 (ppb) during dry and wet seasons respectively. On the other hand, maize flour had aflatoxin levels of 0.30 (ppb) and 0.70 (ppb) during dry and wet season. Practices by local processors that involves simultaneous storage of maize grain as well as milling processes in the same area could be the major cause of the higher mould and aflatoxin contamination, therefore from this study individuals should be educated on proper hygiene and on the health problems associated with consumption of product that are contaminated with aflatoxin

Keywords: Maize aflatoxin contamination ELISA

Introduction
Aflatoxins are group of mycotoxins mainly produced by the fungus *Aspergillus flavus* and *Aspergillus parasiticus*, with *Aspergillus flavus* being the most common producer (Bradburn *et al*., 1993) [5]. The aflatoxin problem was first recognized in 1960, when there was severe outbreak of a disease referred as" Turkey 'X' Disease" in UK, in which over 100,000 turkey pouts died. The cause of the disease was shown to be due to toxins in peanut meal contaminated with *Aspergillus flavus* and the toxins were named aflatoxins. Aflatoxin contamination has been associated with abiotic factors such as draught and high temperature and biotic factor such as insects damage (McMillian *et al*., 1985, Payne, 1992) [28, 30].
Among 18 different types of aflatoxins identified, major members are aflatoxin B1, B2, G1and G2. *Aspergillus flavus* typically produce AFB1 and AFB2 whereas *Aspergillus parasiticus* produces AFG1and AFG2 as well as AFB1 andAFB2. Four other aflatoxins M1, M2, B2A, and G2A may be produced in minor amounts.
Several reports have shown that aflatoxin is a potent carcinogenic immunosuppressive agent which causes liver cancer in both animals and humans (Castegnaro and McGregor, 1988). Ingestion of higher doses of aflatoxin can result in acute aflatoxicosis which manifest in hepatoxicity (Fung and Clark 2004) [18]. It has also been implicated as the cause of Rey's syndrome and chronic hepatitis. Symptoms of toxicity in animals range from death to chronic diseases, reproductive interferences, immune suppression, decrease milk and egg production (Fung and Clark 2004) [18].
Animals are exposed to aflatoxins by consumption of feeds that are contaminated by aflatoxin producing fungal strain during growth, harvest, and storage. Exposure to aflatoxin is widespread in West Africa, with over 98% of subjects tested positive to aflatoxin markers in Gambia, Guinea, Conakry, Nigeria and Senegal, (Wild, 1996) [41].

Food products contaminated with aflatoxins include cereals (Maize, sorghum, rice, wheat) oilseeds (groundnut, soyabean, sunflower, melon seed, cotton) spices (chilles, blackpapper, turmeric) tree nuts (almond, walnuts, coconut) and milk (Reddy and Farid, 2000) [34]. Outbreaks of acute aflatoxicosis from highly contaminated food have been documented in Kenya, India and Thailand with an outbreak of acute hepatoxicity identified among people living in Kenya.Council for Agricultural Science and Technology (CAST 2003) [8]. Also Gong *et al*. (2002) [21] demonstrated that children in Togo and Benin who consume food contaminated with aflatoxins showed the kind of stunted growth, under weight and with symptoms normally associated with malnutrition. Epidemiological investigation determined that the outbreak was the result of aflatoxin poisoning from ingestion of contaminated maize with 317 cases and 125 death, making this one of the largest and most severe outbreak of acute aflatoxicosis documented worldwide.Centre for Disease Control, (CDC, 2004) [11]. Recently, in Nigeria Uriah *et al.* (2001) found that blood and semen aflatoxin levels ranged from 700 to 1393 ng/ml and 60 to 148ng/ml respectively.

With aflatoxin B1 level up to 14ug/kg and 42.5% in preharvest Maize from Benin and 33% of Maize samples from different ecological zones of Nigeria were contaminated with aflatoxins (Udoh *et al*., 2000) [37].

In the US, the Food and Drug Administration (FDA) uses an action level of 20ug/kg as the maximum residue limit allowed in food for human consumption, except for milk Food and Agricultural Organization (FAO, 1996). Also the European Union has enacted a very severe aflatoxin tolerance level to be 2ug/kg aflatoxin B1and 4 ug/kg total aflatoxins for nuts and cereals for human consumption Centre for European Commission (CEC, 1998; Dimanche 2001) [12, 13]. In Nigeria similarly, widespread aflatoxin has been reported as an important contaminant of maize and other stored grains (Amadi and Adeniyi, 2009) [4]. This is due to the fact that West African countries have tropical climate with an all year round ambient temperature and relative humidity that provide optimal condition for the growth of toxigenic mould. The sub-region also has poorly developed infrastructures such as processing facilities, storage, transportation and skilled human resources. And also several reports had demonstrated that the markets maize represents a significant source of continue exposure to aflatoxin (Lewis *et al.,* 2005) [27]. Therefore, the invasion of the cereal grain by fungi is frequent with substantial risk of contamination by mycotoxins. Maize is a staple food taken in various forms (flour or whole) with acceptability cutting across socio-economic strata in Nigeria. Maize (*Zea Mays L., poa ceae*) is the most important cereal in the world after wheat and rice with regards to cultivation areas and total production (Purseglove, 1992; Osagie and Eka, 1998) [33, 29]. The population involved is thus large and maize provides an excellent substrate for mould growth and mycotoxin contamination (Bouraima *et al*,. 1993). Therefore investigation of the food for any possible contamination by causal agents and aflatoxins is highly imperative. Maize is major staple in Nigeria and it is grown all year round with different varieties and therefore the quality and safety of maize is of public health importance;

Materials and methods

Sample collection

A total of 300 samples containing 200g each of maize grain and maize flour was collected during the wet and dry seasons from five retail outlets in Zaria.The retail outlets are Yannika, Kasuwa- mata, Sabo gari, Samaru and Tudun- wada. A period of fifteen weeks was used for the collection of samples for wet season for both maize grain and maize flour. While another fifteen weeks was used for collection of sample for dry season for both maize grain and maize flour from each outlet. Two samples each (maize grain and maize flour) was collected weekly making a total of 150 for fifteen weeks in all the outlets per season.The selection of the markets where the retail outlets are located is based on the following reasons; they are major markets where the products are sold and the geographic location of the population served by the markets is large and hence it has an increased number of maize sellers. Samples was collected in clean polythene bags and taken to the laboratory at the Department of Microbiology,Ahmadu Bello University,Zaria for analysis.

Media preparation

Preparation of Potato Dextrose Agar

Thirty nine grams (39g) of potato Dextrose Agar (PDA) was weighed and dissolved in 1000ml distilled water, boiled and autoclaved at 121^0C for 15minutes. Then 50 µg/ml ampicillin was added to the media to suppress bacterial contamination and mounted at 45^0C in water bath until used.

Microscopic identification of fungal isolates

A drop of lactophenol cotton blue was placed on a clean slide. Using a pointed needle, a portion of the mycelium from the fungal cultures was placed in the drop of the lactophenol cotton blue and teased. The cover slip was then gently placed and observed under the microscope using x10 and x40 objective lens. The fungal isolates were identified based on cultural characteristics and microscopic morphology according to the manuals of Barnett and Hunter, (1972) and Ellis, (2006).

3.4. Quantitation of aflatoxin levels

The helical total aflatoxin assay kit (model CAT NO. 941 AFL.01M-96) was utilized for the detection of alatoxin levels in maize grain and maize flour.

3.4.1 Preparation of extraction solution

Extraction solution (70% methanol) was prepared by adding 30ml of distilled water to 70ml of methanol. Then 20g of maize sample (grain and flour) each was added to 100ml of extraction solvent in the ratio of 1:5 (w/v) samples to extraction solvent. Then the sealed container was manually shaken for 2 minutes and allowed to settle. It was then filtered through Whatman 1 filter paper and the filtrate was dispensed into Micro titre wells and measured optically by a micro litre plate reader with an absorbance wave length of 450nm (OD 450). The optical densities of each micro well was read and recorded.

Result interpretation

A dose response curve was constructed following the manufactures instructions using the ummodified optical density values against the aflatoxin content of the standards (0.0, 0.2, 0.5, 1.0, 2.0 and 4.0) the unknowns were measured by interpolation from the standard curve. However since the samples were diluted at a ratio of 1:5 amounts of aflatoxin was multiplied by five in order to obtain the nanogramme of aflaloxin per gram of commodity (ppb).

Table 1: Frequency of occurrence (%) of fungal isolates on maize grain sold in some retail outlets in Zaria during wet and dry seasons.

Organisms Mean	Wet season		Dry season
Aspergillus flavus	25.5± 0.5	35.26 ±8.06	30.38 ±6.90
Aspergillus parasiticus	47.68± 4.05	32.11 ±9.25	39.89 ±11.0
Aspergillus niger	14.6± 6.27	15.26 ±3.89	14.93 ±0.47
Penicillium sp	1.99± 0.01	17.37 ±1.52	9.68 ±10.9
Fusarium sp	8.94 ±0.91	0	4.47 ±6.32
Rhizopus sp	1.32 ±0.59	0	0.66 ±0.93

Values are mean ± standard deviation of duplicate samples.

Table 2: Frequency of occurrence (%) of fungal isolates on maize flour sold in some retail outlets in Zaria

Organisms	Wet Season	Dry season	Mean
Aspergillus Flavus	33.33 ±19.5	22.22 ±10.1	27.77 ±7.86
Aspergillus parasiticus	23.8 ±10.2	22.22 ±10.1	22.22 ±10.1
Aspergillus niger	9.52 ±4.99	11.11 ±0.94	10.31 ±1.12
Penicillium sp	0	44.44 ±29.5	22.22 ±31.4
Fusarium sp	19.05 ±2.69	0	9.53 ±13.5
Rhizopus sp	14.29 ±1.69	0	7.15 ±10.1

Values are mean ± standard deviation of duplicate samples n=150.

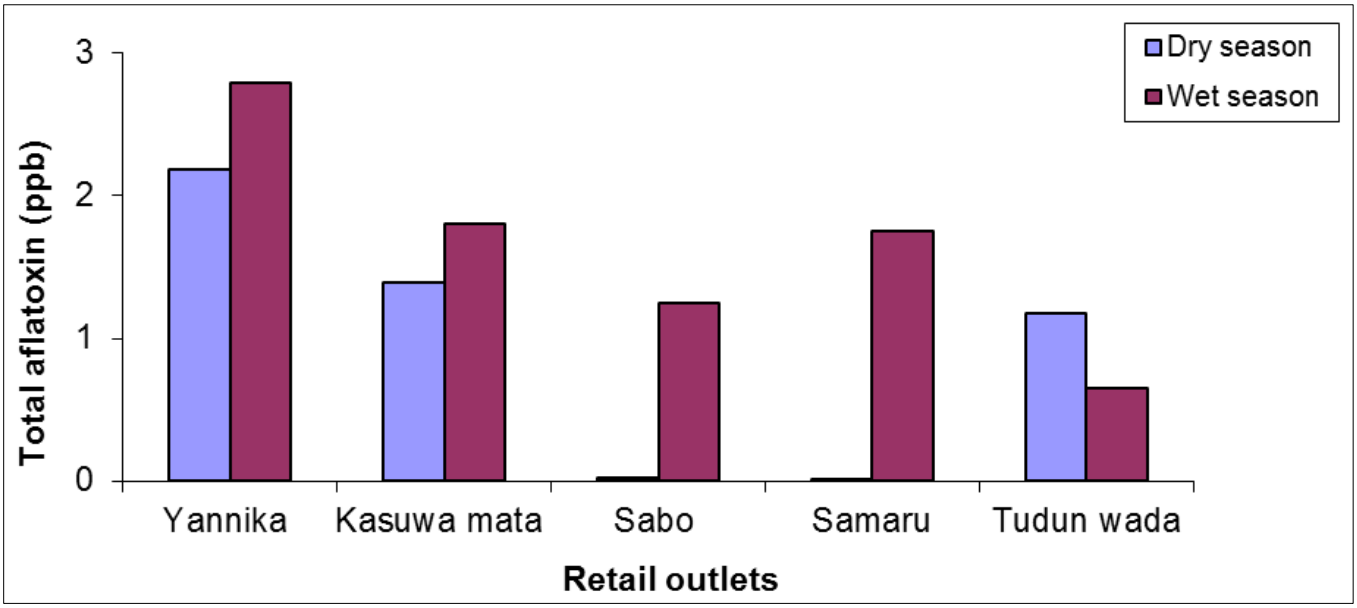

Fig 1: Mean total aflatoxin on maize grain sold in some retail outlets in Zaria during dry and wet seasons.

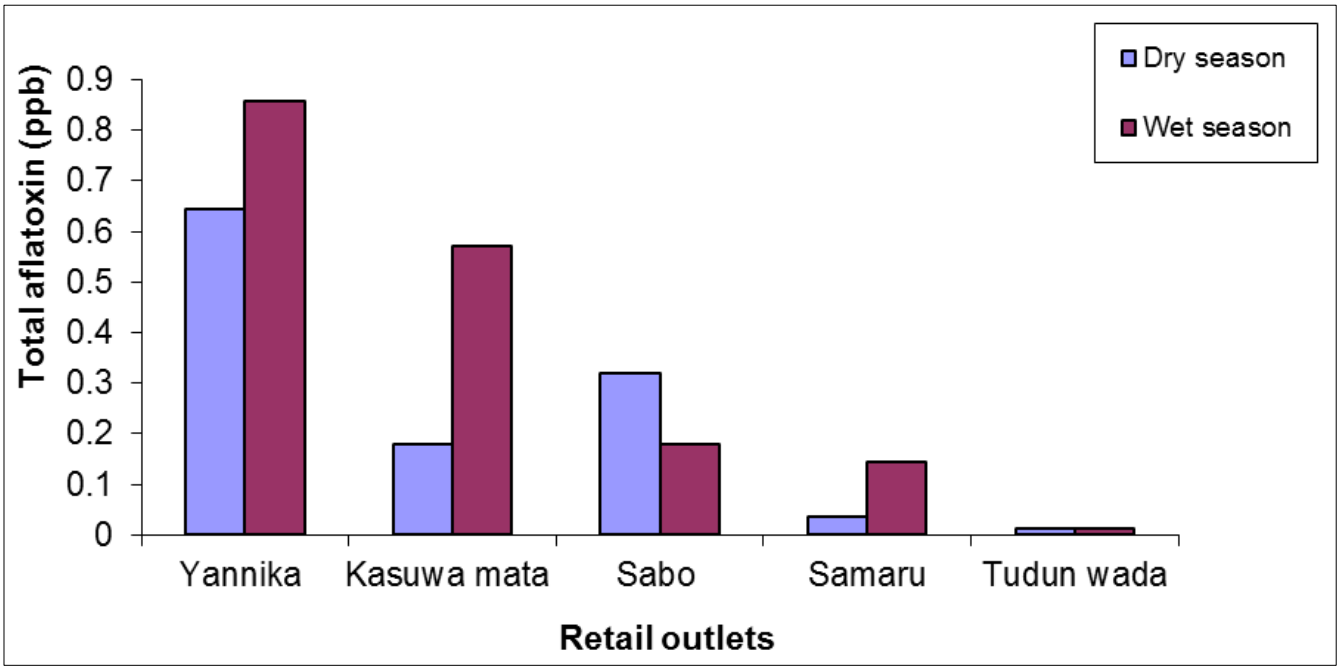

Fig 2: Mean total aflatoxin on maize flour during both season sold in some retail outlets in Zaria.

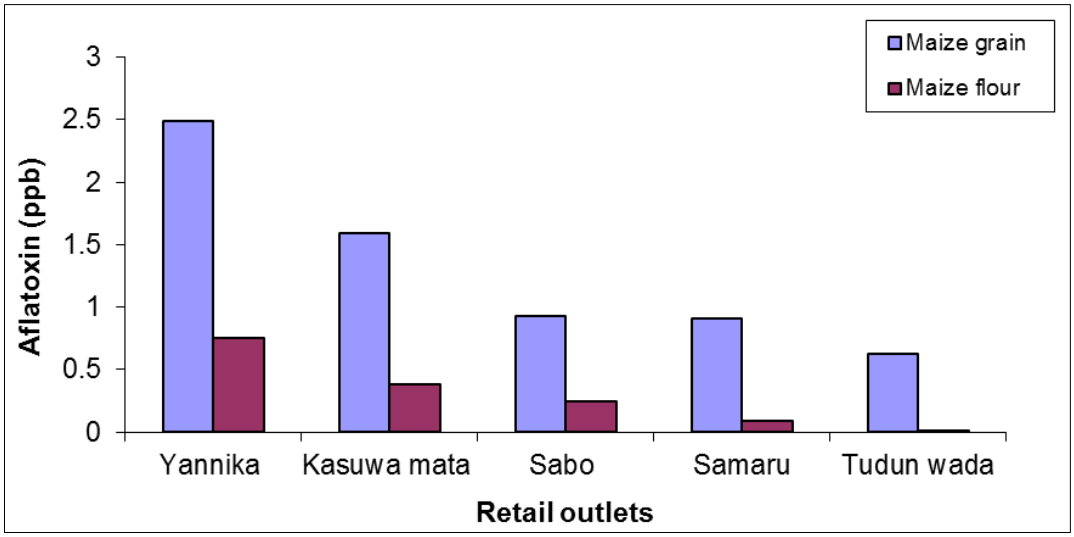

Fig 3: Total Aflatoxin on maize grain and maize flour sold in some retail outlets in Zaria.

Results

Frequency of occurence of fungi isolates on maize grains in some retail outlets in Zaria is presented in Table 1 *Aspergillus parasiticus* had the highest percentage occurrence (48%) during the wet seasons compared to its frequency of occurence (32.11%) in the dry season. *Aspergillus flavus* was found to thrived best on maize grain in the dry season (35.26%) than in the wet season (25.50%) On the other hand, *Rhizopus spp* had lowest frequency of occurence (1.32 %) on maize grain in the wet season and did not occur at all during the dry season.

Table 2 shows the frequency of occurence of fungal isolates on maize flour purchased from some retail outlets in Zaria. Generally, *Penicilliun spp* exhibited a significantly high percentage occurence (44.44%) on maize flour only in the dry season. Similarly, while varying percentage frequency of occurence of all the fungal isolates was observed in wet season, *Fusarium spp* and *Rhizopus sp* did not occur at all in the dry season.

Comparatively, the frequency of occurence of the fungal isolates on maize grain and maize flour is as presented in Table 3 The study shows that while maize grain had significantly high frequency of occurence of 30.38% and 39.89% for *Aspergillus flavus* and *Aspergillus parasiticus* respectively, *Penicilliun spp* exhibited highest frequency of 44.44% in the maize flour. It was also observed that frequency of occurence of *Fusarium sp* and *Rhizopus sp* were generally lower in both products. However, *Fusarium sp* and *Rhizopus sp* had significant lower frequency of 8.94% and 1.32% on maize grain, as compare to 19.05% and 14.29% observed on maize flour respectively.

The total aflatoxin in maize grain during dry and wet season in five retail outlets in Zaria is presented in Figure 1 The result shows that Yannika recorded significant aflatoxin level of 2.8 (ppb) and 2.2 (ppb) on maize grain during wet and dry seasons respectively. On the other hand while maize grain from Samaru outlet had the least total aflatoxin (0.001 ppb) during the dry season, an increased aflatoxin level (1.5ppb) was observed during the wet season. However, Tudun wada retail outlets recorded significant lower level of total aflatoxin during both seasons compare to their levels in Yannika and Kasuwa mata outlets.

The mean total aflatoxin on maize flour during both seasons in five retail outlets in Zaria is shown in Figure 2 The highest mean total aflatoxin values of 0.85 (ppb) and 0.062 (ppb) were recorded in Yannika and kasuwa mata outlets during the wet and dry seasons respectively. Generally, maize flour from Tudun wada retail outlets had significant low level of total aflatoxin value of 0.01ppb during both seasons compared to levels in the other outlets.Comparatively, quantification of total aflatoxin in maize grain and maize flour is presented in Figure 3. Generally, total aflatoxin on maize flour was lower than in maize grain, the maize grain from Yannika and Kasuwa mata outlets recorded the highest mean aflatoxin of 2.49(ppb) and 1.60(ppb), while a significant low total aflatoxin level of 0.70(ppb) and 0.30(ppb) was recorded for maize flour respectively.

Discussion

The study showed that *Aspergillus* are the most dominant genus on maize grains however, a significant frequency of occurence of *Aspegillus parasticus and Aspergillus flavus* during wet and dry season was observed. Although a number of these fungi had been found associated with maize grains, Abdullahi and Mohammed (2002) [1], showed that *Aspergillus flavus* and *Aspergillus parasiticus* have the highest occurence frequency on maize and cereal grains from different parts of the world (WHO 2006, Kumar *et al.*, 2008) [40, 26]. Similarly high frequency of occurence of *Aspergillus flavus* had also been reported in maize kernel (Youssef, 2009). *Penicillum sp* on the other hand, was found to have high frequency of occurence on maize flour The findings is in agreement with that of Amadi and Adeniyi, (2009) [4] who showed that storage fungi are widely distributed and almost always present, contamination can occur through small quantities of spores contaminating the grain as it is going into storage through handling and storage equiptments. During processing most of the contaminants are removed with the bran as suggested by Sekiyama *et al*., (2005) [35]. This could be responsible for the low fungal occurrence generally observed in the maize flour compared to maize grain.

Total aflatoxin in maize grain and maize flour sold in some retail outlets in Zaria.

Data from this study indicate the quantity of the maize grain that was contaminated with aflatoxin in both seasons for both products There was an obviously higher aflatoxin accumulation in Yannika and Kasuwa mata outlets with mean values of 2.7ppb and 1.6ppb respectively for maize grain. This spread of the aflatoxin contamination on maize grain, is

in agreement with previous reports on maize grain (Hennigen and Dick 1995; Bhat *et al.,* 1997; Gloria *et al.,* 1997; Ali *et al.,* 1998; Machinsky *et al.,* 2001; Vargas *et al*., 2001) [23, 6, 20, 2, 39]. In a similar study in Kenya Bourama *et al.,* (1993) found aflatoxin B_1 level up to 14ug/kg and aflatoxin G_1 level up to 58 ug/kg in stored maize.

Also, aflatoxin contamination with a total prevalence of 18% have been recorded for both maize grain and maize flour, lower than 42.5% in 1994 and 30% in 1995 as reported by Setamou *et al.,* (1997) in preharvest maize. Similarly, Udoh *et al.,* (2000) [37] reported 33% aflatoxin contamination in maize sampled from different ecological zones of Nigeria. Hell *et al.,* (2000a) [22] found that the percentage of maize samples with more than 5ug/kg aflatoxin levels was between 9.9% and 32.2% after six months of storage. All the maize samples collected from silos and ware houses in Ghana contained aflatoxins at levels ranging from 20-355ug/kg (Kpobo, 1996).

A lower aflatoxin contamination of the maize flour was observed in all the outlets comparatively to levels in maize grain. This result is similar to other findings by various authors, Furlong *et al* (1999) reported 7.7% of the 39 analysed samples. Pich, *et al* (1998) [31] found levels from 3-24ug/kg in 29 samples of corn flour. This means that processing reduces mycotoxin levels, since their aflatoxin are concentrated in the bran and the germ (Pietri *et al.,* 2009) [32]. The traditional method that involves the removal of the germ and the pericarb could imply the removal of fungi and associated mycotoxins (Fandohan *et al.,* 2005) [15]. The mean total aflatoxin in maize flour gave values of 0.85 ppb from Yannika outlets and lower concentration in other outlets. Although contamination of the flour can occur through spores contaminating the grain, handling practices and equipment which could be responsible for the variation in the fungal counts and aflatoxin levels (IRRI, 2006) [24]. The level of aflatoxin in this study for the positive samples did not exceed the regulatory limit allowed in maize and other cereals,which is below 20ug/g(20ppb). (EC Regulation, 2003; FDA, 1997; Kenya Bureau of Standard, 1998) [14].

Conclusion

They research findings show that the maize and its products have lower aflatoxin level than 20ppb recommended for food and other products by FDA. There is also need for sensitizing the commodity traders and the populace on proper hygiene in respect of food and other products.

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