Microbial and Aflatoxins Analysis of Selected Cereal Flours Processed and Sold in Abakaliki Metropolis

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Abstract

Aflatoxins are very potent mycotoxins produce by molds. Molds are very common pre-harvest and post harvest contaminant of cereals/cereal products. Despite improved handling, processing and storage of cereals, aflatoxins still remain a major problem in the cereal processing industry causing both health hazards and economic losses. Therefore, some researchers have suggested that in order to avoid the toxicity, the levels of aflatoxins and similar toxic compounds in foodstuffs have to be monitored closely, and to be kept under control continuously. Otherwise, related health effects like acute and chronic intoxications, and even deaths, will still be an issue. Therefore, in this research the microbial and total aflatoxins analysis of selected cereal flours processed and sold in Abakaliki metropolis was carried out. A total of four cereal samples (maize, millet, sorghum and wheat) were studied. The total aflatoxins were analyzed using highly sensitive competitive Enzyme Link Immunosorbent Assay (ELISA) machine. The cereal samples were also analyzed for total fungal count using digital colony counting machine(CCM China). The result showed that all the cereal flours (wheat, sorghum, millet and maize) analyzed were heavily contaminated with fungal cells. The flours also contain unacceptable levels of aflatoxins. The total aflatoxins were above the minimum acceptable limits (10ppm) according to National Agency for Food and Drug Administration and Control (NAFDAC). The millet and sorghum have the highest fungal and total aflatoxins concentrations while the wheat flour has the lowest fungal and total aflatoxins concentrations. There were significant difference ($p<0.05$) among the total aflatoxins level of the different cereal flours. The research also revealed that flours have high moisture content. It is therefore recommended that a more improved process line be put in place to ensure that all cereal flours sold in Abakaliki are produced using Standard Operating Procedure (SOP).

Key words: Cereal Aflatoxins, Mycotoxins, ELISA, NAFDAC

INTRODUCTION

Aflatoxins are potent toxic, carcinogenic, mutagenic, immunosuppressive and teratogenic agents produced as secondary metabolites by *Aspergillus flavus* and *Aspergillus parasiticus* (Krishnamurthy and Shashikala, 2006; Jackson, and Al-Taher, 2008; Williams et al 2015). These fungi can invade and produce toxins in cereals before harvest, during drying, and in storage (Hom et al., 1995). The aflatoxin problem in cereals is not restricted to any geographic or climatic region. Toxins are produced on cereals, both in the field and in storage; they involve both the grain and the whole plant (Williams et al 2004; Filaz et al., 2010). Cereals and its products are the main foods for human consumption throughout the world. The cereal grains belong to corn, rice, barley, wheat and sorghum are found susceptible to aflatoxins accumulation by aflatoxigenic fungus (Filaz et al., 2010). Aflatoxins are highly stable molecules that do not decompose easily by high temperatures as could be assumed by industrial processes.

There are four major groups of aflatoxins: B1, B2, G1 and G2. These Aflatoxins occur naturally in most food commodities, including wheat, corn, soybean and peanut and other grains which are consumed by human and animal (Juan et al 2008). Aflatoxins are of economic and health importance because of their ability to contaminate human food and animal feeds, in particular cereals, nuts and oil seeds. Cheese, almonds, figs and spices have been also associated with aflatoxins contamination (Kaaya and Warren, 2005).

These naturally occurring toxins have been characterized by the World Health Organization (WHO, 2002) as significant sources of food borne illnesses (Williams et al 2015). Humans can be exposed to aflatoxins by the periodic consumption of contaminated food, contributing to an

increase in nutritional deficiencies, immunosuppression and hepatocellular carcinoma (Williams et al 2015; Filazi et al 2010). High moisture and temperature are two main factors that cause the occurrence of aflatoxins at pre-harvest and post harvest stages (Ayciceket et al 2005). Aflatoxins are among the most carcinogenic substances known (Azi et al 2015). Aflatoxins interact with the basic metabolic pathways of the cell disrupting key enzyme processes including carbohydrate and lipid metabolism and protein synthesis (Beyhan et al 2016; Quist et al 2000; Williams et al., 2015). After entering the body, aflatoxins may be metabolized by the liver to a reactive epoxide intermediate or hydroxylated to become the less harmful (Hussein, and Brassel, 2001). Aflatoxins are most commonly ingested, but the most toxic type of aflatoxin, is aflatoxin B1, which can permeate through the skin (Williams et al 2004). The United States Food and Drug Administration (FDA) action levels for aflatoxin present in food or feed is 20 to 300 ppb. The FDA has had at some occasion declared both human and pet food recalls as a precautionary measure to prevent exposure.

The economic impact of aflatoxins is derived directly from crop and livestock losses due to aflatoxins and directly from the cost of regulatory programs designed to reduce risks to human and animal health. The Food and Agricultural Organisation (FAO) estimates that 25% of the world's crops are affected by mycotoxins, of which the most notorious are aflatoxins.

Aflatoxin losses to livestock and poultry producers from aflatoxin-contaminated feeds include death and more subtle effects of immune system suppression, reduced growth rates, and losses in feed efficiency (Kaaya and Warren, 2005). Other adverse economic effects of aflatoxins include lower yields for food and fibre crops. The aflatoxin problem has been reported to be more serious in tropical and subtropical regions of the world where climatic conditions of temperature and relative humidity favour the growth of *Aspergillus flavus and A.parisiticus*. Beside human

consumption, maize, wheat and sorghum are also a major ingredient in animal feeds. Therefore, contamination of the produce by aflatoxins puts consumers at high health risk and the hazards reduces the export potential of the country. Signs of acute aflatoxicosis include depression, nervousness, abdominal pain, diarrhea and death (Herrman, 2002). Since these toxins have been considered unavoidable contaminants in food chain, the Food and Drug Administration (FDA) of USA has established an action 732 level for total aflatoxins which is at 20 ppb for all foods, including animal feeds (Munkvold et al 2005).

As of today aver 5 billion people, mostly in developing countries, are at risk of chronic exposure to aflatoxins from contaminated food (Shuaib et al 2010). Therefore, in order to avoid the toxicity, the levels of aflatoxins and similar toxic compounds in foodstuffs have to be monitored closely, and to be kept under control continuously. Otherwise, related health effects like acute and chronic intoxications, and even deaths, will still be an issue (Becer, U. K., and Filazi, A. (2010). Of the currently identified many types of aflatoxins, aflatoxin B1, B2, G1 and G2 occur naturally and are the most significant contaminants of a wide variety of foods and feeds (Juan et al 2008). Thus, despite improved handling, processing and storage of cereals, aflatoxins still remain a major problem in the cereal processing industry causing both health hazards and economic losses. Therefore the objective of this work is to determine the microbial and total aflatoxin levels of the selected cereal flours (wheat, sorghum, millets and corn) milled and consumed in Abakaliki metropolis. Findings of this study will serve the purpose of alerting consumers on the dangers of consuming flours (wheat, sorghum, millets and corn) on sale in selected market within Abakaliki Ebonyi State, Nigeria.

Materials and Methods

Sources of Raw Materials:

All the samples (maize, wheat, millet and sorghum) were sourced at different milling locations at meat market , Kpirikpiri and eke Aba, Abakaliki Ebonyi state.

pH value

The pH of the samples were determined using highly sensitive digital pH meter (Montini 095, Romania).Two grams of each of the samples (milled cereals) were measured into a Cylindrical glass container containing 20ml of distilled water. The mixture was stirred and allowed to stand for about 1h. The pH was determined at temperature of about $29^{O}C$ by dipping the pH meter tip into the sample solution and the pH of the solution read off.

Proximate Analysis

Determination of Moisture Content

Moisture content was determined by the Gravimetric method. A measured weight of each sample (5g) was weighed into a cleaned, dried Petri dish. The dish and samples were dried in an oven at $105^{O}C$ for 3 h at the first instance. It was then cooled in a desiccator and reweighed. The weight was recorded while the samples were returned to the oven for further drying. The drying, cooling and weighing continued repeatedly until a constant weight was obtained. By the difference, the weight of the moisture loss was determined and expressed as a percentage.

It was calculated as shown below;

$$\% \text{ Moisture Content} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

Where; W_1 = Weight of the empty Petri dish

W_2 = Weight of the dish and sample before drying

W_3 = Weight of the dish and sample after drying to a constant weight

Determination of crude Protein

The protein content of the sample was determined using the Kjeldahl method. The total Nitrogen was determined and multiplied by the factor 6.25 to obtain the protein content. Five grams (5g) of the grounded cereal floor was weighed into the Kjeldahl digestion flask. A tablet of Selenium catalyst was added to it. Concentrated H_2SO_4 (10 ml) was then added to the flask and digested by heating it under a fume cupboard until a clear solution was obtained. Then it was carefully transferred to a 100ml volumetric flask and made up to mark. A 100ml of the digest was mixed with equal volume of 45% NaOH solution in Kjeldahl distillation apparatus. The mixture was distilled and the distillate collected into 10ml of 4% boric acid solution containing mixed indicator methyl red bromocressol. A total of 50ml distillate was collected and titrated against 0.02N H_2SO_4. The crude protein was obtained by multiplying the nitrogen value by a factor of 6.25.

$$\% \text{ Crude Protein} = \frac{\text{Titre value} \times 50 \times 5.46}{\text{Weight of the sample}} \times \frac{100}{1}$$

Determination of Ash and crude fibre

The method of AOAC, (1995) was used to determine the ash and crude fibre contents of the sample.

Determination of Carbohydrate

The carbohydrate content of sample was determined by estimation using the arithmetic difference method. The carbohydrate content was calculated and expressed as the Nitrogen free extract as shown below:

% CHO = 100 - % (a + b + c + d + e)

Where; a = Protein

b = Ash

c = Fat

d = Crude fibre

e = Moisture content

Total viable fungal count

Ten- fold serial dilution and pour plate method were used for the fungal count. The medium used (Saboraud Dextrose Agar) were prepared according to manufacturer‘s instruction (BIOTECH India) and autoclave for 15minutes at 121^{o}C and 15psi. The prepared medium was allowed to cool to about 40°C in a water bath and was then poured into sterile petri- dishes containing 1 ml aliquot of the appropriate dilutions (normal saline as diluents) prepared from the samples. The samples solutions were prepared by adding 1 g of the sample into 10 ml of normal saline. The plates were incubated for 3 days at room temperature and colonies formed were counted using digital colony counter and expressed in colony forming unit per gram CFU/g.

Total Aflatoxin analysis

Determination of total aflatoxin on the cereal flours samples were done by the use of Enzyme link immunosorbent assay (ELISA) Method. Extraction of the aflatoxin was done with Tween-ethanol. Twenty five mililitre of Tween- ethanol was added to 5 g of the sample and mixed properly. The sample solution was then centrifuged at 250 rpm for 3 mins. The centrifuged sample was filtered with Watman1 filter paper. Aflatoxin conjugate (200 micro liter) was dropped in a clean mixing wall and 100 microliter of the sample analyte was added. The mixture of the aflatoxin conjugate and the sample was then transferred into antibody incubated micro-walls and incubated under dark cover at room temperature for 15 mins. This process was allowed for the antibody/antigen reaction to take place. After the incubation the solution was then washed

off 5 times using deionized water and then 100 microliter of the substrate was added and allowed to stand for 5mins. Finally a stop solution was added and the result read with ELISA machine.

214 *Results*

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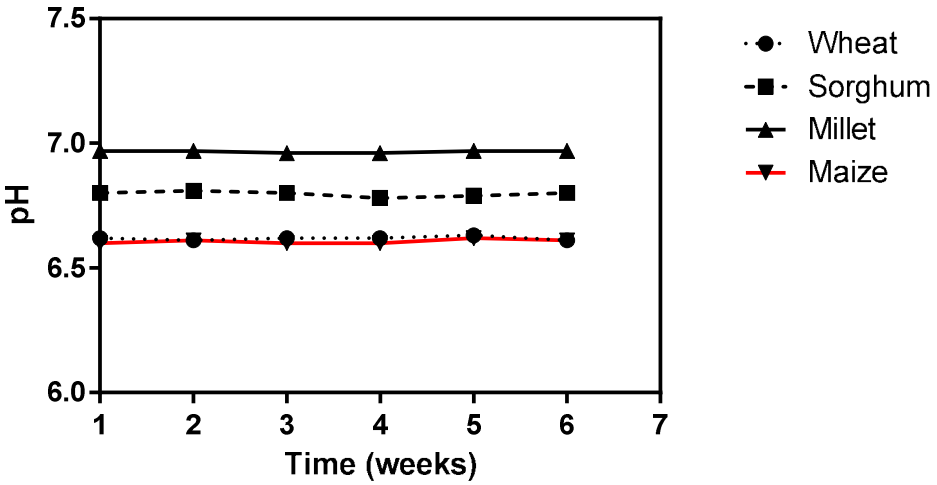

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218 Fig 1: The pH of the different cereal flour

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Table 1: Shows the proximate composition of the different cereal flour

	Samples(%)			
	Wheat	Sorghum	Millet	Maize
Protein	11.2 ± 0.14 [a]	9.9 ± 0.22 [b]	12.1 ± 0.13[c]	11.1± 0.21[d]
CHO	68.9 ± 0.12[a]	63.4 ± 1.61[b]	64.3 ± 0.21[c]	63.8± 0.01[d]
Moisture	13.8 ± 0.21[a]	13.0 ± 0.17[b]	12.3 ± 0.50 [c]	12.9± 0.31[d]
Ash	2.6 ± 1.03[a]	3.1 ± 0.25[b]	2.7 ± 0.71[c]	2.8± 0.12[c]
Fat	2.0 ± 0.41[a]	5.0± 0.17[b]	5.8 ± 1.09[c]	6.2± 0.13[d]
Fibre	2.5 ± 0.15[a]	5.6± 0.12 [b]	2.8 ± 0.14 [c]	3.2 ± 0.21[d]

Values are mean of triplicate determination and standard deviation (±SD). Means with different superscript along the row are significantly different ($p<0.05$)

Table 2: Moisture Content of the cereal flour

Weeks	Samples (%)			
	Wheat	Sorghum	Millet	Maize
1	13.8 ± 0.13[a]	13.0 ± 0.12 [b]	12.3 ± 0.13[c]	12.9 ± 0.71[d]
2	13.5 ± 0.15 [a]	13.2 ± 1.61 [b]	12.5 ± 0.21 [c]	12.8 ± 0.60 [d]
3	13.7 ± 0.91 [a]	13.1 ± 0.17 [b]	12.3 ± 0.50 [c]	12.9 ± 0.41 [d]
4	13.8 ± 1.01 [a]	13.0 ± 0.25 [b]	12.2 ± 0.71 [c]	12.7± 0.11 [d]
5	13.5 ± 0.3 [a]	13.1 ± 0.17 [b]	12.9 ± 1.09 [c]	12.9± 0.14 [d]

Values are mean of triplicate determination and standard deviation (±SD). Means with different superscript along the row are significantly different ($p<0.05$)

Table 3: Total fungi counts of the cereal flour

Weeks	Samples (CFU/g)			
	Wheat	Sorghum	Millet	Maize
1.	$4.8 \times 10^6 \pm 0.12^a$	$6.3 \times 10^7 \pm 0.12^b$	$6.2 \times 10^7 \pm 0.11^b$	$3.6 \times 10^6 \pm 0.0^c$
2.	$4.9 \times 10^6 \pm 0.12^a$	$6.4 \times 10^7 \pm 1.02^b$	$6.2 \times 10^7 \pm 1.21^b$	$3.8 \times 10^6 \pm 0.0^c$
3.	$4.6 \times 10^6 \pm 0.29^a$	$6.0 \times 10^7 \pm 0.19^b$	$6.3 \times 10^7 \pm 0.5^b$	$3.9 \times 10^6 \pm 0.6^c$
4.	$4.3 \times 10^6 \pm 1.02^a$	$6.5 \times 10^7 \pm 0.23^b$	$6.5 \times 10^7 \pm 0.7^b$	$3.6 \times 10^6 \pm 0.2^c$
5.	$3.5 \times 10^6 \pm 3.10^a$	$6.4 \times 10^7 \pm 0.17^b$	$6.2 \times 10^7 \pm 1.9^b$	$3.6 \times 10^6 \pm 0.4^c$
6.	$4.6 \times 10^6 \pm 0.14^a$	$6.1 \times 10^7 \pm 0.12^b$	$6.6 \times 10^7 \pm 0.14^b$	$3.8 \times 10^6 \pm 0.3^c$

Values are mean of triplicate determination and standard deviation (±SD). Means with different superscript along the row are significantly different ($p<0.05$)

Table 4: Total aflatoxins content of the cereal flour as analyzed

Weeks	Samples (ppb)			
	Wheat	Sorghum	Millet	Maize
1	8.4 ± 0.14[a]	17.3 ± 0.22[b]	18.4 ± 0.13[a]	23.3 ± 0.33[d]
2	8.3 ± 0.12 [a]	18.0 ± 1.61 [b]	19.4 ± 0.21 [c]	24.6 ± 1.00 [d]
3	8.0 ± 0.21 [a]	17.2 ± 0.17 [b]	17.3 ± 0.50 [c]	23.3 ± 0.61 [d]
4	7.3 ± 1.03 [a]	17.2± 0.25 [b]	17.3 ± 0.71 [c]	23.6± 0.32 [d]
5	9.0 ± 0.41 [a]	17.0 ± 0.17 [b]	18.4± 1.09 [c]	23.3± 0.14 [d]
6	8.2 ± 0.15 [a]	19.3± 0.12 [b]	18.4 ±0.14 [c]	23.3 ± 0.35 [d]

Values are mean of triplicate determination and standard deviation (±SD). Means with different superscript along the row are significantly different ($p<0.05$)

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339 *Discussion*

340 Cereal flours are prone to both pre-harvest and post harvest fungal contamination and spoilage.
341 This study revealed that the severity of the postharvest fungal contamination and spoilage varied
342 among the different cereal flour studied. Thus the total fungal population and the concentrations
343 of total aflatoxins varied among the cereal flours.

344 The pH analysis showed that the different cereal flour has different pH that ranges from 6.55 to
345 6.89 (Fig.1). The variation in the pH value of the cereal could be as a result of the varied
346 chemical composition of the cereal flours. According to Peter Koehler and Herbert Wieser
347 (2013), the pH of any cereal grain is significantly affected by the chemical composition of the
348 cereal grain. The variety of the cereal flour studied could also be factor that might have
349 influenced the pH as genetic composition of cereal grains have been found to determine the
350 chemical composition of the cereal and thus it pH (Shibanuma et al 1994).

351 The cereal flours studied consist of mainly carbohydrate making up between 63 to 68% of the
352 total proximate composition of the cereal flours (Table 1). This is consistent with the findings of
353 other researchers. (Franz, M. and Sampson, L. 2006; Goesaert, et al 2005). The protein content
354 of the cereals ranged from 9.9 – 12.1 (Table 1). The significant differences at ($p<0.05$) in the
355 protein content of the cereal flours studied could be as a result of the cereal genotype (species
356 and variety) and growing conditions (soil, climate and fertilization) as stated by Peter Koehler
357 and Herbert Wieser, (2013), in a similar study. The moisture content of the cereals flours were
358 found to be between 12.3 and 13.8%. This suggest that the cereal grains were not sufficiently
359 dried before milling and this affected the overall chemical composition of the cereal flours.This
360 result is similar to the finding of Fox et al (1992) in which the variation in moisture content of
361 maize flour significantly affected the overall chemical composition of the maize flour studied.

362 The result also further showed that the cereals were low in ash, fat and fibre (Table 1). Cereals
363 are generally known to be low in ash, fat and fibre by their genetic nature (Steadman et al 2001;
364 Fox et al 1992).

365 The finding of this research shows that the different cereal flours studied were heavily
366 contaminated by fungi (Table 3). The wheat flour has the lowest average fungal populations

while millet flour had the highest fungal cells during the study period. There was significant difference among the fungal populations of the cereal flours. The level of fungal load in cereal flours is used to determine the extent of storage stability of cereal flour. Flours with high fungal populations tend to spoil faster than those with lower fungal populations. This is because with higher fungal population the rate of metabolic activities of the fungal cells on the flour becomes faster resulting in production in high proportion of certain undesirable metabolites which subsequently lead to off-flavors and general change in the chemical composition of the flour. This high fungal population seen in these flours could be due to poor processing resulting in cross-contaminations of the flours from both the milling machines and the personnel. These high fungal populations may be as a result of high moisture content of the cereal flours. Cereal grains with high moisture content have been reported to witness high fungal invasion according to a research conducted by Reddy et al (2013). According to their research cereal grains (rice) with a moisture content higher than the desired level (>14%) that entered the storage system witnessed high fungal invasion. The harmful effects of such fungal invasion are discoloration of the grain, loss in viability, loss of quality, and toxin contamination (Ayhan Filazi and Ufuk Tansel Sireli, (2013); Reddy et al 2009). The finding of this research is also similar to that of Adriana et a*l.* (2006) and Enyisi et al *(*2015) in which they reported post- harvest fungal contamination of millet grains stored for over six month before processing. They further explained that some of the fungal contaminant is potential aflatoxins producer thus represent significant public health risk. It is therefore imperative from the finding of this study that an urgent and comprehensive review of the storage and processing methods of cereal flours in milled and sold in Abakaliki metropolis in order to avert aflatoxins epidemic which often characterized by jaundice, rapidly developing ascites, and portal hypertension (Ayhan Filazi and Ufuk Tansel Sireli, 2013).

The finding of this research showed that were also contaminated with different concentrations of aflatoxins with maize flours having the highest aflatoxins concentration while wheat flour has the lowest aflatoxins level Table4. The level of aflatoxins is one of the key safety and quality indicator parameter in cereal products. Cereal products with total aflatotins level beyond 10ppm are designated as unfit for human consumption according to National agency for Food and Drug Administration and Control. This is because consumption of foods with such high levels of aflatoxins contaminations has been linked with both acute and chronic heart diseases including cancer (Azi et al 2016). It has been established that over 5 billion people, mostly in developing

countries, are at risk of chronic exposure to aflatoxins from contaminated foods; cereal and cereal based products inclusive (Shuaib *et al* 2010). This high concentration of aflatoxins revealed in this study could be as results of high fungal contamination of the cereal flours. The high fungal populations and subsequent aflatoxins production could be as a result of high moisture content together with storage temperature. High moisture and temperature are two main factors that have been established to be responsible for occurrence of aflatoxins at pre-harvest and post harvest stages cereal products (Ayciceket et al 2005). While According to Goldblatt, (1969), At moisture content <12 per cent, aflatoxin synthesis can commence with 80 per cent relative humidity. The finding of this research is similar to the investigative report of FAO and FDA risk assessment report on aflatoxins in foods in which they discovered different concentrations of aflatoxins in the different foods assessed including cereals and cereal products.

Conclusion

The finding of this research showed that wheat, sorghum, millet and maize flour processed in Abakaliki is heavily contaminated with molds with potential for aflatoxins production. The aflatoxins analysis also revealed unacceptable levels of aflatoxins in the cereal flours. It is therefore recommended that urgent review of the entire process line for cereal flours sold in Abakaliki metropolis be carried out to ensure that all flours sold in Abakaliki are produced following standard operating procedures (SOP). Also the cereal grain should be stored at dry and cool environment (temperature preferably below 20°C and relative humidity below 80%), to reduce the chance of fungal contamination and proliferation during storage.

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