DETERMINATION OF AFLATOXIN LEVEL IN MAIZE AND MAIZE FLOUR SOLD IN SOME RETAIL OUTLETS IN ZARIA

BY

AGBOOLA, OLUWAYEMISI MULIKAT

DEPARTMENT OF MICROBIOLOGY,

FACULTY OF SCIENCE,

AHMADU BELLO UNIVERSITY,

ZARIA, NIGERIA

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MSc/SCIEN/02479/2006-07

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY,

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DECLARATION

I declare that the work in this thesis titled "DETERMINATION OF AFLATOXIN LEVEL IN MAIZE AND MAIZE FLOUR SOLD IN SOME RETAIL OUTLETS IN ZARIA" has been performed by me in the Department of Microbiology under the supervision of Prof. C.M.Z. Whong and Prof. V.J. Umoh.

The information derived from the literature has been duly acknowledged in the text and a list of references provided.No part of this thesis has been previously presented for another degree or diploma at any university

Mulikat O. Agboola		AUGUST, 2011.
Name of student	Signature	Date

CERTIFICATION

This thesis titled "**DETERMINATION OF AFLATOXIN LEVEL IN MAIZE AND MAIZE FLOUR SOLD IN SOME RETAIL OUTLETS IN ZARIA** by Mulikat Oluwayemisi Agboola meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

Prof. C M. Z. Whong.

Chairman, Supervisory Committee

Date

Prof. V.J.Umoh

Member, Supervisory Committee

Date

Prof. O.S. Olonitola.

Head of Department

Date

Prof. A.A Joshua

Dean, Postgraduate School

Date .

DEDICATION

I dedicate this work to my babies Aishat and Mardiyya Abdulrahman.

ACKNOWLEDGEMENTS

I thank almighty God for his infinite mercy, protection and strength given to me during the course of this study. I am particularly grateful to my able supervisors, Prof.C. M. Z. Whong, and Prof V.J.Umoh for their keen interest and constant supervision,. their inputs eased the process of revision considerably. My sincere appreciation goes to my husband Mr M. B. Abdulrahman for his encouragement, support, patience and understanding,and also to my siblings Hajia Seidat, Idowu Mutiat Mr Abdulrasaq Agboola and to the memory of my late brother Kola Agboola and their families for their constant concern, help and encouragement.I would also like to express my everlasting appreciation to my mum 'sweet mother''for taking good care of my baby during my study.and also to my dad Alh R.A. Agboola for his encouragement and concern.My sincere appreciation goes to Prof A.A Ahmad and other staff of Microbiology Department A.B.U Zaria My course mates Rueben Wartu, John Idakwoji,Tasalla Debo ,and Dapiya Hyncith for their help, I thank Theresa Tafida, Aunty Lara and other students of Microbiology Department for their constant encouragement. I also thank Haj.Hadiza Yero,Bola Aluko Zainab Sodangi who accomodated me at various times during my stay in Zaria.I wish to acknowledge my colleagues at college of education Azare and my numerous friends who have help me with prayers for the successful completion of my Master programme,my sincere appreciation also goes to my in laws Mama Agba and Alfa Dayo for their constant prayers, last but not the least i am indeed grateful to Mal. D .M. Adamu who gave me the use of his laptop which eased my work and also to Ahmad Lamido and others too numerous to mention.

ABSTRACT

A seasonal study on the prevalence of aflatoxin in maize and maize flour sampled from some retail outlets in Zaria was undertaken using the enzyme linked immunosorbent assay(ELISA) method. A total of 300 samples were collected during dry and wet seasons, (150 sampleseach of maize and maize flour).Fungal countof maize was 4.58±0.24 (log_{10} $cfug^{-1}$) in the dry seasonwhile that of maize flour was4.2±0.10 ($log_{10}cfug^{-1}$). In the wet season the counts were 5.6±0.36 ($log_{10}cfug^{-1}$) for maize and 5.0± 0.72 ($log_{10}cfug^{-1}$) for maize flour. The predominant mycoflora on maize grain and maize flour were; *Aspergillus flavus, Aspergillus niger, Aspergillus parasiticus*, *Penicillum* sp, *Fusarium* sp *and Rhizopus* sp.The aflatoxin level obtained showed that Yannika recorded the 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 respectively. Proximate composition of maize grain showed a mean moisture content of 14.05±1.38% in wet season while it was 13.4±0.19 for maize flour in the same season. An average ash content of 1.05±0.45% was recorded for maize grain in wet season, and 1.15±0.45% in dry season. Average ash content for maize flour was 0.88±0.01 and 0.84±0.03.Crudefat content in maize grain was 1.075±0.45% in wet seasons while formaize flour it was 0.84±0.03% in the same season. The crude protein content wasalso different in maize and maize flour. Handling practices by local processors that involved simultaneous storage and milling processes of maize grains in the same area could be the major cause of the higher mould and aflatoxin contamination. Therefore from this study it can be deduced that consumers subsisting on this product could be at risk of aflatoxin poisoning (The permissible level of aflatoxin in food is less than 20ug/kg).

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CHAPTER ONE

1.1Introduction

Aflatoxins are groups of mycotoxins mainly produced by the fungal*Aspergillus flavus* and *Aspergillus parasiticus*,with*Aspergillus flavus*being the most common producer (Bradburn *et al*., 1993). The aflatoxin problem was first recognized in 1960, when there was a severe outbreak of a disease referred as" Turkey 'X' Disease" in UK, in which over 100,000 turkey poulets 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,).

Among 18 different types of aflatoxins identified, the major ones are aflatoxin B1, B2, G1and G2. *Aspergillus flavus* typically produces aflatoxin blue flourescence (AFB1andAFB2) whereas*Aspergillus parasiticus* produces aflatoxin green fluorescence (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 manifests in hepatoxicity (Fung and Clark 2004). 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, decreased milk and egg production (Fung and Clark, 2004).

Animals are exposed to aflatoxins by consumption of feeds that are contaminated by aflatoxin producing fungal strains during growth, harvest, and storage. Exposure to aflatoxin

is widespread in West Africa, with over 98% of subjects testing positive to aflatoxin markers in Gambia, Guinea, Conakry, Nigeria and Senegal, (Wild, 1996).

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). Outbreaks of acute aflatoxicosis from highly contaminated food have been documented in Kenya, India and Thailand with outbreak of acute hepatoxicity identified among people living in Kenya.(CAST 2003). Also Gong *et al*,. (2002) demonstrated that children in Togo and Benin who consumed food contaminated with aflatoxins showed the kind of stunted growth, under weight and symptoms normally associated with malnutrition. Epidemiological investigation showed that the outbreak was as a result of aflatoxin poisoning from ingestion of contaminated maize with 317 cases and 125 deaths, this became one of the largest and most severe outbreak of acute aflatoxicosis documented worldwide (CDC, 2004). Recently, in Nigeria Uriah *et al.* (2001) found that blood and semen aflatoxin levels range from 700 to 1393 ng/ml and 60 to 148ng/ml respectively. Aflatoxin B1 level was found to be 42.5% in preharvest maize from Benin and 33% of maize samples from different ecological zones in Nigeria (Udoh *et al*., 2000) .

In the US, the Food and Drug Administration (FDA) uses an action level of 20µg/kg as the maximum residue limit allowed in food for human consumption, except for milk. (FAO,1996).The European Union has also enacted a very severe aflatoxin tolerance level to be2µg/kg aflatoxin B1 and 4µg/kg total aflatoxins for nuts and cereals for human consumption (CEC, 1998, Dimanche 2001).

Maize is a staple food taken in various forms (flour or whole) with acceptability cutting across socio-economic strata in Nigeria. The population involved is thus large.Maize serves as 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.

1.2 Statement of the problem

Aflatoxin is a highly potent toxigenic, carcinogenic, mutagenic and immunosuppressive agent which causes liver cancer in both animals and humans. This is the only toxin which has gained prominence in scientific literatures in food products from West Africa sub-region (Fung and Clark 2004; CDC, 2004). There is ample evidence that the inhabitants of sub-Saharan Africa are experiencing heavy dietary exposure to food-borne mycotoxins particularly aflatoxins (CAST, 2004; Lewis *et al.,* 2005). Findings on the outbreaks of maize aflatoxin and acute aflatoxicosis from highly contaminated food had been documented world wide.(Betran *et al.,* 2002; Lewis *et al.,* 2005; Muthomi *et al.,* 2009). According to Miller (1996), 40% of the productivity loss to diseases in developing countries is due to diseases exacerbated by aflatoxins. In Nigeria similarly, widespread aflatoxin has been reported as an important contaminant of maize and other stored grains (Amadi and Adeniyi, 2009). 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 conditions for the growth of toxigenic mould. The sub-region also has poorly developed infrastructures such as processing facilities, storage, transportation and unskilled human resources.Several reports have demonstrated that maize represents a significant source of continued exposure to aflatoxin (Lewis *et al.,* 2005). Therefore, the invasion of the cereal grain by fungi is frequent with substantial risk of contamination by mycotoxins.

1.3 Justification

The safety of food for human consumption should be of topmost priority with regards to the regulation of agricultural and food industries. Maize is a major staple food in Nigeria and it is grown all year round with different varieties and therefore the quality and safety of maize should be of public health importance;

- Aflatoxin causes deterioration in maize quality making it unfit for consumption.
- Aflatoxin causes liver cancer in both animals and humans.
- Aflatoxin affects marketability of maize internationally and hence has implication on the economy.

Therefore, efforts to successfully interrupt exposure to producing moulds will require considerable survey of the market system (Lewis *et al.*, 2009). This will aim at assessing the extent of market maize contamination, replacement of contaminated market products and interventions through comprehensive food safety programmes targeted at the maize vendors and the farmers in order to prevent/ minimize future exposures.

1.4 Aim

The aim of this study is to determine the presence of aflatoxin in maize and maize flour sold in some retail outlets in Zaria.

1.4.1 Specific objectives

1 To enumerate the total fungal counts in maizeand maizeflour.

2 To identify *Aspergillus flavus* and *Aspergillusparasiticus* presentin maize and maize flour.

3 To determine and quantify aflatoxin level in maize and maize flour.

4 To determine the physicochemical properties of maizeand maize flour.

CHAPTER TWO

2.1 Review of Related Literature

2.2 Origin of maize

Maize (*Zea may)* 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). Maize cultivation date back as far as 12000 years ago.(Kochhar, 1986). The name maize is derive from South American Indian Arawak–Carib word MAHIZ, it is also known as Indian corn or corn in America (Purseglove, 1992).

During a period in Europe when the cultivation of maize was unknown among the majority of agriculturalist, maize was introduced into Spain by Columbus, while the Portuguese introduced its use to Guinea and Congo, from where it has become the staple grain crop for much of sub Saharan African. (FAO, 1992).

2.3 Cultivation and distribution of maize

Maize thrives best in a warm climate and its growth depends more on high summer temperature. It withstands extreme heat with a relatively short average maturing period and this makes it possible to grow at fairly high latitudes. In the United Statesof America, maize growing area stretches from approximately 40 degrees south to 45 degrees north.

The global production of maize is estimated to about 300 million tonnes per year, and the world harvests almost as much maize as wheat and this may vary from 2.5 to 7tons per acre according to the soil and its cultivation (C B N, 1992). In Nigeria however, its production is quite common in all parts of the country, from the north to the south with an annual production of about 5.6 million tonnes (FAO, 1992).Table 2.1 shows the world maize production.

2.3.1 Starch content of maize

The major chemical component of maize kernel is starch, which provides up to 72 to 73percent of the kernel weight (Boyer and Shannon, 1987). Other carbohydrates are simple sugar present as glucose, while sucrose and fructose vary in amount from 1-3 percent of the kernel. The starch in maize is made of two glucose polymers:-amylase, an essentially linear molecule, and amylopectin a branched form. In commonmaize with either the dent or flint type of endosperm, amylose makes up 25 to 30percent of the starch and amylopectin makes up 70 to 75percent. Waxy maize contains a starch that is 100 percent amylopectin. An endosperm mutant called amylase-extender (ae) induces an increase in the amylose proportion of the starch to 50percent and higher. Other genes, alone or in combination, may also modify the amylose-to-amylopectin ratio in maize starch (Boyer and Shannon, 1987).

2.3.2 Protein content of maize

The next largest chemical component of maize kernel is protein, which varies in common varieties from 8 to 11percent of the kernel weight. Most of it is found in the endosperm and it is made up of at least five different fractions (Landry and Moureaux, 1982).In their scheme albumins, globulins and non-protein nitrogen amount to about 18percent of total nitrogen, in a distribution of 7,5 and 6 percent respectively (Landry and Moureaux, 1982). These protein fraction increases as the grains mature (Patterson *et al*; 1980).Quality protein maize (QPM) differs from common maize in weight distribution.The five proteins fractions is affected by gene type and cultural conditions; therefore the nutritional quality of maize as a food is determined by the amino acid make up of its protein (Prasanna *et al*.,2001).

2.3.3 Oil and fatty acidscontent of maize

The oil content of the maize kernel comes mainly from the germ with values ranging from 3 to 18 percent. Maize oil has a low level of saturated fatty acids with on average, 11percent palmitic and 2 percent strearic acid.It also contains a high level of polyunsaturated fatty acids,

mainly linoleic and arachidonic acids (Bressani, 1990).Also,maize oil is relatively stable since it contains only small amount of linoleic acids (0.7percent) and a high level of natural antioxidants.The oil is highly regarded because of its fatty acid distribution which is mainly oleic and linoleic acids.(Bressani,1990).

2.3.4 Dietry fibre

Dietary fibre is the chemical component found in the greatest amounts after carbohydrate fat andprotein.This complex carbohydrate content of maize kernel comes from the pericarp and the tip cap. It is also provided by the endosperm cell walls and, to a smaller extent, the germ cell walls.Notably only a small difference occurs between soluble and insoluble dietary fibre, even though QPM Nutrict has higher level of total dietary fibre than common maize, mainly due to ithigher insoluble fibre (Sandstead et *al*., 1978).

2.3.5 Other carbohydrates

When mature, the maize kernel contains carbohydrates other than starch in small amounts, with total sugars in the kernel between 1 and 3 percent by weight, and sucrose is mostly found in the germ. Higher levels of monosaccharide and trisaccharides are present in the maturing kernels and as the kernel mature the sugar declines. Research shows that sugar content could reach 94 percent of kernel dry weight in 16 days old kernels, but the level decreased significantly with age. Similarly sucrose concentration at 15 to 18 days after pollination was between 4 and 8 percent of kernel dry weight (Bressani *et al.*, 1989). The relatively high levels of reducing sugar and sucrose are possibly the reason why immature common maize and, even more, sweet maize are so well liked by people.(FAO,1992).

2.3.6 Mineral content of maize

The concentration of ash in the maize kernel is about 1.3 percent by weight, whichis slightly lower than the crude fibre content.The germ is relatively rich in minerals.Environmental factors can influence the mineral content (FAO, 1992). The most abundant mineral is phosphorus found as phytate of potassium, and magnesium.

2.3.7 Fat - Soluble vitamins

The maize kernel contains two fat soluble vitamins: provitamin A, or carotenoids, and vitamin E.Carotenoids are found mainly in yellow maize in amount that may be genetically controlled, while white maize has little or no carotenoids content (FAO, 1992). Most of the carotenoids are found in the hard endosperm of the kernel and only small amounts are in the germs. The beta carotene content is an important source of vitamin A, but unfortunately yellow maize is not consumed by humans as much as white maize (FAO, 1992).

2.3.8 Water soluble vitamins

Water soluble vitamins are found mainly in the aleurone layer of the maize kernel, followed by the germ and endosperm.This pattern of distribution is important because it reduces significant losses of the vitamins (Gopalan and Rao, 1975). The water soluble vitamin, nicotinic acid, has attracted much research because of its association with niacin deficiency or pellagra, which is prevalent in populations consuming high amount of maize (Christianson *et al*., 1968).

As with other vitamins, niacin content varies among varieties with average values of about 20µgper gram,Afeature peculiar to niacin is that it is bound and therefore not available to the animal organism. The association of maize intake and pellagra is a result of the low level of niacin in the grain, although experimental evidence has shown that amino acid imbalances, such as the ratio of leucine to isoleucine, and availability of tryprophan are also important (Patterson *et al.,* 1980). Variable amounts of thiamine and riboflavin have been reported, (FAO 1992) with only small amounts of ascorbic acid and no vitamin B12 present in the mature kernel. About 2.6mg per kg of available Pyridoxine and other vitamins such as choline, folic acid and pantothenic acid are found in very low concentrations (FAO, 1992).

The importance of cereal grain to the nutrition of millions of people around the world is widely recognized. Even though maize is chiefly a carbohydrate rich food, it contains an appreciable amount of proteins (Table 2.2), minerals (Table 2.3) and vitamins (Table 2.4), it therefore means that, it is a complete food for low income people (Osagie and Eka,1998).

Table 2.2. Amino acid composition of maize grown in Nigeria

Amino acid	Amount (gm per 100kg protein)
Argentine	3.20
Cystine	1.70
Histidine	2.60
Isoleucine	4.40
Leucine	18.90
Lysine	1.50
Methromine	2.10
Phenyl nine	6.60
Threonine	3.50
Tryptophan	0.40
Tyrosine	5.20
Valine	5.50
Alanine	10.10
Aspartic acid	7.20
Glutamine acid	26.40
Glysine	3.10

Source: Epenyoung *et al.* (1977).

Table 2.3: Mineral composition of maize grain in Nigeria

Mineral	Composition dry weight basis (mg/100g)
Calcium	2.20
potassium	757.00
Magnesium	377.00
Iron	5.30
Manganese	2.10
Copper	0.80
Zinc	0.38

Source: Olaofe (1988); Osagie and Eka (1998).

Table 2.4: Vitamin composition of maize grain in Nigeria

Vitamins	Composition (dry weight basis)
Vitamin A (i.u.)	510.00
Thiamin(mg/100g)	0.38
Riboflavin(mg/ 100g)	0.11
Pantothenic acid (ppm)	8.00
Niacin (mg/100g)	2.00

Source; Oyenuga, (1988)

2.3.9 Methods of improving nutritive value of maize

Processing of food stuff stabilizes nutrients in food,but losses may take place when optimum conditions are exceeded.As a result of the great importance of maize as a basic staple food for large groups of people, particularly in developing countries,and its low nutritional value,mainly with respect to protein content, many ideas have been tried to improve on the nutritive value of maize, e.g genetic manipulation,processing and fortification.(Ortega,*et al.*, 1986).Lime –cooking a process of improving nutrient in maize,but it causes some losses in nutrient (Bressani,1990).The use of calcium hydroxide in converting maize into tortillas,the calcium content of the product increases up to about 400 percent (Bressani, 1990). Losses in thiamine, riboflavin, niacin and carotene occurred during processing of maize into tortillas by lime cooking. Other processes include natural fermentation of cooked maize which results in higher vitamin-B concentration and protein quality (Bressani,*et a*l;1989).Fortification is another approach, by which the nutritional limitation in maize particularly that of its protein, can be ameliorated through addition of amino acids or protein sources rich in the limiting amino acids.

2.4 Types of maize

There are about 50 different species of maize which differ in characteristic features such as kernel sizes, colour and structure of the kernel. White, red and yellow are the most common basic colours of maize, but it is possible to find a wide range of shades, from red-brown to high red and from a pale yellow to orange(FAO,1996).The shape of the kernels can be divided into two main groups:flint maize (round shape) dent maize (tooth shape).Based on the kernel characteristic,maize can be categorized into:dent corn, sweet corn (which is mostly grown by farmers in the U.S.A), Indian corn, and other popular varieties of maize such as flour corn (FAO,1992).

Research found that two amino acids: lysine and tryptophan are deficient in maize and this prompted the development of another variety Q.P.M, in an attempt to provide 18% more good

protein and balance the amino acids (FAO,1992). However, this variety has one draw back, it is more susceptible to insects attack. (FAO, 1992).

2.5 Pest anddiseases of maize

Corn ear worm is actually a moth whose larvae feed on various crops that have been cultivated by humans. Similarly, European corn borer is native to Europe and originally began infesting various forms of millet and broom corn.It damages the ears and stalks of the corn by basically chewing through the stalk, causing the corn to fall over. Of all the pests that pose a danger to maize, the western corn root worm is the worst and if left untreated had been reported to destroy an estimated 30 million acres of maize crop (FAO, 1996).

2.6 Maize as an important staple food

Maize constitutes a fundamental ingredient in many part of the world's cuisines, ranging from Mexican enchiladas and Chinese baby–corn, to African-American grits, cornflakes, pop-corns, Italian polenta or gruel, cornmeal,maize-based alcoholic beverages (such as whiskey and bourbon), mayonnaise and corn oil (Coe,1994). In Romania, mamaliga is prepared from sweet corn meal and consist of a food akin to polenta that is sometimes referred to as "corn mean mush". Corn meal remains a primary staple of Romanians and Hungarians alike, with Puliszka being the staple food of Hungarians (Johnson, 1998). In the sixteen century, maize meal called posho was among the most popular food of eastern Africa; Johnson (1998) noted that the primary African use of maize as a food is in mush or porridge. Maize porridge is known as "kpelple" in Ghana and "bicha" in Zaire. In Zimbabwe, people consume "sazda", whereas East Africans eat "posho" or "ugali," Zulu speaking people consumed putu as a primary source of nutrition.(Etijere and Bhat, 1985).

In Nigeria is known and called by different vanacular names depending on the locality. Like "agbado" "igbado" or "yangan" (Yoruba);"masara" in (Hausa) "ogbado" or "oka" (Ibo) "apaapa" (Ibira) "oka" (Benin and Esan),"Ibok pot" and "Igumapa" (Yala). In Nigeria maize is cooked, roasted, fried, ground, pounded or crushed to prepare various food

items(Abdulrahaman and Kolawole, 2006). Tuwo is a very important and popular stable food among various ethnics group in Nigeria.

2.6.1 Medicinaluses of maize

Maize is highly edible and nutritious. It is also believed to have also has some medicinal uses among the local people of Nigeria. Water filtered through charcoal obtained from maize stalk can be as a treatment to cure gonorrhea (Abdulrahaman, 1997). An infusion obtained from the stigma of maize can be used for treatment of diseases of the urinary tract or passage (Abdulraman, 1997). Water "omi-eko"or `"omikan" or "omidun" obtained during the preparation of pap is use to soak bark or root of some plants (eg"dokita igbo") for the treatment of fever and malaria (Abdulraman and Kolawole, 2006).Holes are made in some maize grains to make rosary which is put on the wrist of a child believing it to prevent him or her from becoming slim (Abulrahaman and Kolawole, 2006).Whole dried maize fruit and dried yam with some charms are planted together or buried together.This preparation is done to unite or bind couple together with effect that either cannot remarry to another person. It means that they will remain husband and wife forever (Abulrahaman and Kolawole, 2006).

2.6.2 Industrialapplication of maize

In the pharmaceutical industry, maize starch can be used as dusting materials on various types of coating as well as binders. It also finds application as filler for capsules and tablets and provides stiffness and colour in the textile industry. Maize starch in the food industry is invaluable as a viscosity and opaque paste and applied as thickeners in sauces, gravies and pie.It has numerous applications in the baking industry,(Coe,1994).It is also used in the Paper industry for sizing as well as to increase paper strength.

In the United States and Canada, maize is primarily used as a feed for livestock, forage, and silage and fish bait called "dough balls" for coarse fishing.The maize grain can also be hydrolyzed to produce syrups particularly high fructose corn syrup(a sweetener).It is also

fermented to produce grain alcohol, increase biomass fuel source such as ethanol and tolower pollutants.

2.6.3 Field and storage fungi of maize

Fungi that produce toxins in food are classified into field fungi and storage fungi, based on their ecological requirement for growth (Bankole, 1994). Field fungi require grain moisture above 20% in cereals and often cause ear rot diseases and toxin production before harvest, when the crop is still in the field. The important genera of field fungi include *Fusarium*, *Cladosporium* and *Alternaria species.*The storage fungi usually grow in grain with moisture content in equilibrium with 70 – 90 percent relative humidity which corresponds to less than 18% moisture content in cereals. The most important genera are *Aspergillus* and *Pencillium*; they are frequently associated with crops in the field but are associated with plant debris, plant surfaces, atmosphere and other surfaces where the water activity is relatively low. The most important field fungi of maize in Africa and worldwide are *Fusarium spp* and they are known to produce over 100 secondary metabolites that can adversely affect human and animal health (Visconti, 2001). *Fusariumverticillioides* (syn *Fusariummoniliforme*) has been found to be the most widespread. Many studies in Nigeria have found it to be the most frequent in preharvest and stored maize (Ekpo and Banjoko, 1994; Essien, 2000). This fungus is so intimately associated with maize that it was frequently observed in symptomless maize kernels in Nigeria (Thomas and Buddenhagen, 1980).

2.6.4 Distinguishing differences between *Aspergillus flavus* and *Aspergillus parasiticus*.

The two fungi,*Aspergillus flavus* and *Aspergillus parasitcus,* are closely related and grow as saprophytes on plant debris of many crop plants left on the soil (Reddy and Farid, 2000). They are distributed worldwide, with a tendency to be more common in countries with tropical climates that have extreme ranges of rainfall, temperature and humidity. (Bhat and Vassanthi, 2003). *Aspergillus flavus* is green-yellow when grown on Cyber yeast extract agar.Conidia are finely roughened,variable in size, and oval to spherical in shape. They have

biseriate sterigmata, and reddish-brown sclerotia are often present(Pitt and Hocking 1997, Samson *et al.,* 2000).

Aspergillus parasiticus is dark green on Cyber yeast extract agar, and remains green with age. Sterigmata are unseriate; while sclerotia are usually absent (Domsche *et a.,l* 1980, Klich, 2002).The habitat of both *Aspergillus flavus and Aspergillus parasiticus* is found in the soil,decaying vegetation,hay and grains undergoing microbiological deterioration.The invade all types of organic substrate whenever conditions are favourable for their growth.Favourable conditions include high moisture content, high temperature and high relative humidity (Samson *et al;*2000).

2.7 Structure and types of aflatoxins

Aflatoxins are groups of difuranocoumarins,and are classified according to their chemical structure into: difurocoumorocyclopentenone series (AFB1, AFB2, AFB2A, AFM1, AFM2, AFM2A and aflatoxicol) and the –difurocoumarolactone series (AFG1, AFG2, AFG2A, AFGM1, AFGM2A, and AFB3)Klich, 2002 The aflatoxins display potency of toxicity, carcinogenicity, mutagencity in the order of; AFB1>AFG1> AFB2>AFG2 as illustrated by their LD 50 values for day old ducklings. Structurally the hydrofuran moiety, containing double bond, and the constituents liked to the coumarin moiety are of importance in producing biological effects. The aflatoxins fluorescence strongly in ultraviolet light (ca. 365 nm); B1 and B2 produced a blue fluorescense whereas G1 and G2 produce green fluorescence (Reddy and Farid,2000).

2.7.1 Physical and chemical properties of aflatoxins

The reactions of aflatoxins to various physical condition and chemical reagents have been studied extensively, because of the possible application of such reactions to the detoxification of aflatoxins

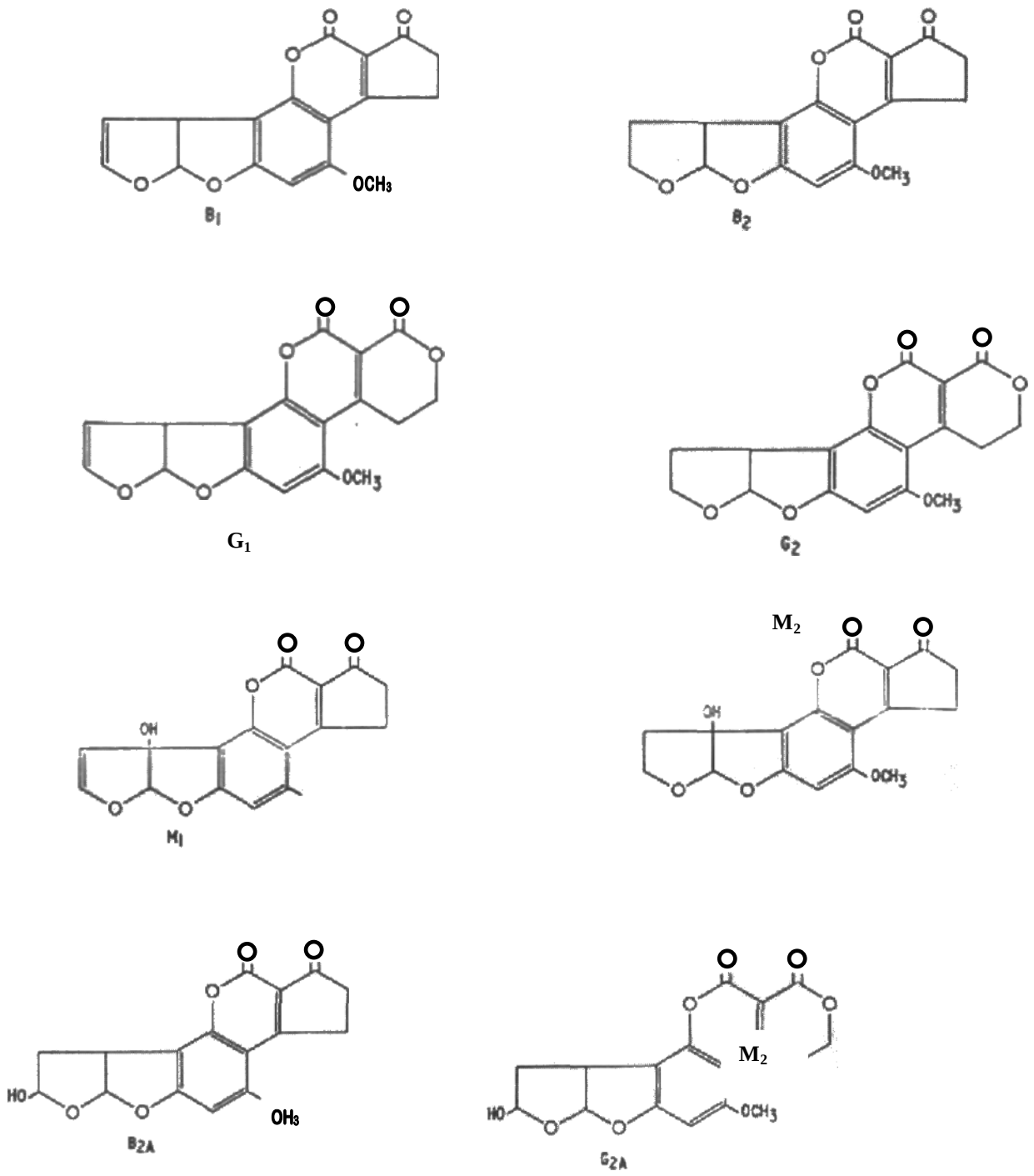

Fig. 2.1 Sturctures of aflatoxins B_1, B_2, G_1, G_2, M_1, M_2, B_{2A} and G_{2A}

2.7.1.1 Effect of heat on aflatoxin

Aflatoxins in dry state are very stable to heat up to melting point however, in the presence of moisture and at elevated temperatures; there is destruction of aflatoxin over a period of time (Reddy and Farid, 2000).

2.7.1.2 Effect of Alkali on aflatoxin

In alkali solution, hydrolysis of the lactone moiety occurs which appears to be reversible, since it has been shown that recyclization occurs following acidification of a basic solution containing aflatoxin (Reddy and Farid, 2000). At a higher temperature(100^0C) ring opening followed by decarboxylaton occurs and reaction may proceed leading to the loss of the methoxy group from the aromatic ring. Similar series of reactions also seems to occur with ammonia and various amines (Reddy and Farid,2000).

2.7.1.3 Effect of Acid on aflatoxin

In the presence of mineral acids, aflatoxins B1 and G1 are converted into aflatoxins B2A and G2A respectively, due to acid –catalysed addition of water across the double bond in the furan ring. In the presence of acetic anhydride and acitoxy derivative, similar products of aflatoxin B1 and G1 are formed with formic acid thionyl, chloride,acetic acid-thionyl chloride and trifluoroacetic acid.

2.7.1.4 Oxidizing agent and reduction reaction of aflatoxin

Many oxidizing agents such as sodium hypochlorite, potassium permanganate, chlorine, hydrogen peroxide, ozone and sodium perborate react with aflatoxin and change the aflatoxin molecule in some ways as indicated by the loss of fluorescence (Reddy and Farid,2000). The mechanisms of these reactions are uncertain and the reaction products remain unidentified in most cases.Hydrogenations of aflatoxin B1and G1 yield aflatoxins B2 and G2 respectively.Reduction of aflatoxin B1 by 3 moles of hydrogen yields tetrahydroxyaflatoxin. These arise as a result of

opening of the lactone ring followed by reduction of the acid group and reduction of the keto group in ring (Reddy and Farid (2000).

2.8 Methods of aflatoxin control in cereals

2.8.1Adoption of good agronomic practices

Agronomic practices have been shown to have profound effect in reducing aflatoxin contamination of crops in the field. Avantaggio*et al.* (2002) found extremely high fumonisin contamination levels in maize ears visibly damaged by insects and recommended field control of insects to reduce fumonisin contamination.

2.8.2 Early harvesting and rapid drying

Early harvesting has been advocated as a means of reducing the risk of aflatoxin contamination. Though many farmers are aware of the need for early harvesting, however labour constrains, unpredictable weather, the need for cash and the threat of thieves, rats and other animals often compel farmers to harvest at inappropriate time (Amyot, 1983). Ruchaputi *et al,.*(2002), had demonstrated the importance of assessing aflatoxin risk on a site-by-site basis to appropriate decisions on the timing of harvest to minimize aflatoxin levels and obtain maximum returns. Early harvesting and threshing under high aflatoxin risk conditions could result in lower aflatoxin levels and higher gross returns.

Among the recommendations for solving aflatoxin problem, rapid drying of agricultural products to low moisture is often emphasized. This is to reduce all scenarios leading to mycotoxin contamination related to non-maintenance of stored products at safe moisture content. Dry grains keep longer safe from insects and mould because the water activity required for their growth is not met. Drying harvested maize to 15.5 percent moisture content or lower within twenty four to twenty eight hours will reduce the risk of fungal growth and consequent aflatoxin production (Hamilton,

2000). Most African farmers spread their harvests to dry under the sun, which often require longer durations for the products to attain“safe” moisture levels. The grain are spread out on rock surfaces or on nylon sheets spread on the floor, and the stirring or turning is done manually till the product is dry (Begum, 1991).The efficacy of drying was demonstrated in the report of Awuah and Ellis (2002) when groundnutkernels with 6.6 percent moisture were free of fungi regardless of the storage protectant used for six (6) months, whereas at 12 percent moisture, only jute bags with *S. aromaticum* effectively suppressed the cross infection of healthykernels.

However, when the moisture content was increased to 18.5percent, the latter treatment was not as effective. In addition to ensuring that grains going into store have “safe” moisture content, effort should be made to prevent moisture migration into grains through leaking roofs and condensation resulting from inadequate ventilation.Since sun drying may be a difficult task due to the high rainfall at the time of harvest, a lot of work has been done on the design of solar and mechanical dryers for use by farmers in the tropics (Axtell and Bush, 1991, Carruthers and Rodriquez, 1992). However, these dryers are not in use by farmers because large capital investment is involved.

The UK-Thai Aflatoxin in maize project (1) has identified a set of criteria, called the UK-Thai project (UTP) system, which have been shown to reliably produce low aflatoxin content maize during the rainy season. With the UTP system, maize is first field dried on the stack for one to two weeks before harvesting to reduce moisture content to 18 to 22 percent; it is next shelled within twenty four to forty eight hours of harvest, and loaded into a drier within twelve hours of shelling. Thus, within forty eight hours, it is dried to 14% moisture content, with no part exceeding 15 percent. Aflatoxin content is monitored rapidly by a special adaptation of the bright greenish-yellow fluorescence (BGYF) test.Maize dried to 14 percent by the UTP system can be safely stored for a minimum of two months. The system is now being used commercially for about 50,000 tons of

maize (Boonma *et al.,* 1980; Tanboon-ek*et al.,* 1986).Other methods of drying which are equally effective and rapid,and may be applicable include microwave or sonic drying. However,these could not be implemented in the sub-region because farmers do not have the requisite facilities.(Tanboon-ek,*et al* 1986). Mechanical dryers could also be set up in the strategic locations which farmers can utilize if sun drying is proven difficult.

2.8.3 Physical separation

Sorting out physically damaged and infected grains (known from colorations) from the apparently healthy ones is an efficient and feasible method of reducing aflatoxin contamination. This could be done manually and also by using electronic sorter. Infection of seeds or grain often imparts colour changesand some other physical characteristics. Udoh (1997) identified sorting of damaged maize cob as a practice that led to a significant reduction in aflatoxin contamination. This was corroborated by the report of Hell *et al*. (2000a) who advocated the removal of insect damaged cobs at harvest to reduce aflatoxin contamination. Martin *et al*. (1999) recommended the final sorting either by hand or by colorimetry as the only possible remedial technique against aflatoxin contamination of groundnut in Senegal, and that the efficacy of this depends on the extent of seed contamination.

Schatzki and Haddon (2002) asserted that presently, there is no sorting mechanism that meets commercial needs of adequate reduction and product preservation. They suggested a rapidnondestructive selection of peanuts for high aflatoxin content by soaking and tandem mass spectroscopy.

2.8.4Sanitation and the use of improved storage structures

Clearing the remains of previous harvests and destroying infested crop residues are basic sanitary measures against storage deteriorations. Cleaning stores before loading in the new harvest was

correlated to reduction in aflatoxin levels (Hell *et al;* 2000a). Disposing heavily damaged ears having greater than 10% ear damage also reduced aflatoxin levels in maize (Setamou *et al.,* 1998). Wild hosts which constitute a major source of infestation for storage pests should be removed from the vicinity of stores.Traditional storage structures used by farmers for on –the- farm storage include containers made of plant materials (wood, bamboo, thatch) or mud placed on raised platforms and covered with thatch or metal roofing sheet (Hell *et al*; 2000a). Essentially the stores are constructed to prevent insect and rodent attack, and to prevent moisture from getting in to the grains. The adoption of high yielding varieties (mostly with poor storability) by farmers has made the traditional storage systems to become inadequate. However it is very difficult to promote the new storage technologies such as the use of metal bins to small – scale farmers due to their high cost (Hell *etal;* 2000a) Research is needed to develop and refine suitable storage systems that are not capital intensive.

2.8.5 Smoking

Smoking is an efficient method of protecting maize against infestation by fungi. However, if not carefully applied, it may discolour the product and change the taste (Hell *et al.,* 2000a). The efficacy of smoking in protecting against insect infestation was found to be comparable to that of the chemical Actellics (Primiphos- methyl) (Daramola, 1986).Some farmers in various ecological zones in Nigeria used smoke to preserve their grains and this practice was found .to decrease aflatoxin levels in farmer's stores (Hell *et al* .,2000a).

2.8.6 Use of plant products and synthetic chemicals

Some traditionally useful plants have been shown to exhibit antifungal properties: *Occimum gratissimum, Cymbopogon citratus, Xylopia aethiopica, Monodera myristica, Syzigium aromaticum, Cinnamum verum* and *Piper nigrum* (Awuah, 1996). These plants are effective in

inhibiting formation of nonsorbic acid, a precursor in aflatoxin synthetic pathway. The essential powder extracts of *Cymbopogon citratus* inhibited the growth of fungi including toxigenic species such as *Aspergillus flavus* and *Aspergillus fumigatus* (Adegoke and Odelusola, 1996). Workers have shown that the minimum inhibitory concentration of the essential oil monoterpenes of the spice *Aframomun, danielli* for the aflatoxingenic mould *Aspergillus parasiticus* was 78µg/ml (Adegoke *et al*., 2000). It was found that the terpenoid had a damaging effect on biological membranes of susceptible organism. Essential oils from *Azadirachta indica* was found to inhibit the growth of a toxigenic *Aspergillusflavus* and significantly reduced aflatoxin synthesis in inoculated maize grains (Bankole, 1997). Bouda *et al*., (2001) reported that the essential oils of some weed species such as *Ageratum conyzoides, Chromolaena odorata* and *Lantana camara* effectively controlled *Sitophilus zeamais*, and suggested that they could be exploited for insect control in stored products. It should be noted that despite the vast literature on the efficacy of plant material in controlling mycotoxingenic moulds, there has not been any concerted effort at a large – scale trial of plants on the farmers' field. Udoh *et al*., (2000) were of the view that caution must be exercised in using plant material to control mycotoxins because some of these materials are natural media for *Aspergillus flavus* growth (Efuntuye, 1996; 1999). Hell *et al*., (2000a) had found that the use of the bark of *Khaya senegalense's* stem bark to protect maize against insects increased the risk of aflatoxin development and that even the farmers in Benin were aware of the low efficiency of the indigenous products. Most of the plants being screened for ability to control storage fungi are used in traditional medicine.

Chemicals such as insecticides or fungicides can result in highly economic gains when used in the right quantity (Giga and Biscoe, 1989). However, the poor educational background of the farmers often led to misuse of these pesticides. Also farmers across the sub-region are still using pesticides

such as gammalin that have for long been banned. Thus hundreds of people died in Nigeria recently as a result of consumption of cowpea treated with in appropriate pesticides. (Udoh *et al*., 2000).

2.8.7 Use of resistant varieties and biological control

Differences exist in the storability of different crop varieties and it is preferable for farmers to grow crop varieties that have long durability in store. In most cases this is not practicable, as most of the high yielding varieties increasingly being introduced to farmers are more susceptible to storage deterioration than the traditional varieties (Hamilton,2000). Reducing contamination by variety screening is one possible way, but the multiplicity of variety resistant parameters means that totally resistance varieties will not be available for some time to come (Martin *et al*; 1999).Scientist at USDA have identified two maize lines that are resistant to *A. flavus* and *F.moniliforme* infection (Hamilton,2000).Biological control by introducing atoxigenic strains of *A. flavus* and *A. parasiticus* to soil of developing crop is one strategy that has recently gained prominence in literature.IITA, 2003a). The application of non- aflatoxigenic strain of *A. flavus* around developing cotton plant led to a 68-87% reduction in aflatoxin contamination (Cotty, 1994). Field application of non toxigenic strains of *A. flavus* and *A.parasiticus* had a carry over effect and reduced post harvest aflatoxin contamination with about 95.9% reduction (Dorner and Cole, 2002).

Under the German development agency (BMZ) funded programme, scientist at IITA are spear heading a new offensive to minimize the formation of aflatoxins by exploiting a strategy called competitive exclusion (IITA, 2003b). The principle is to introduce and establish a benign strain to replace the toxigenic strain. The next challenge of the researchers is to test the atoxigenic stain in different parts of Africa and in different agricultural products where aflatoxins are a threat.

2.8.8Fumigation and detoxificationmethods

Seed fumigation with ethylene oxide and methyl formate was found to significantly reduce the incidence of fungi including toxigenic species (Bankole, 1996). There are research reports that sodium chloride (2.5, 5.0 and 10.0 %), propionic acid (1.0, 2.5 and 5.0 %), acetic acid (1.0, 2.5 and 5.0 %) inhabited aflatoxin B production in *A. flavus* inoculated groundnut and maize kept in gunny bags (Kavita and Reddy, 2000). However, all treatments except sodium chloride had adverse effects on seeds' germination and viability. A novel detoxification technique that is currently under investigation is the possibility of introducing harmless phyllosilicate clay, (hydrated sodium calcium aluminosilicate (HASCAS), which is widely used as an anti caking agent in animal feed (Phillips, 1997).

CHAPTER THREE

3.1 Materials and methods

3.1.1 Sample collection

A total of 300 samples each containing 200g of maize and maize flour were collected during the wet and dry seasons from five retail outlets in Zaria.The retail outlets were Yannika, Kasuwa- mata, Sabo gari, Samaru and Tudun- wada. A period of fifteen (15) weeks was used for the collection of samples for wet season,and another fifteen weeks was used for collection of samples for dry season for both maize and maize flour from various outlets.Two samples each (maize and maize flour) were collected weekly making a total of 150 for fifteen weeks in all the outlets per season.The selection of the markets outlets were based on availability of samples,the number of retailers,the population served and market size.Samples were collected in clean polythene bags and taken to the laboratory at the Department of Microbiology,Ahmadu Bello University,Zaria, for analysis.

3.1.2Media preparation

3.1.3. Preparation of Potato Dextrose Agar

Thirty nine grams (39g) of potato Dextrose Agar (PDA) were 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 held at 45^0C in water bath until used.

3.2. Determination of fungal counts in maize grain and maize flour

Ten gram (10g) of maize grains were weighed and transferred into conical flask containing 90ml of 0.1%w/v buffered peptone water to give the stock solution.One mililitre(1ml) was transferred from the stock solution into a test tube containing 9ml of 0.1% peptone water to give 1:10 dillution.This process was repeated until 1:1000 dilution was achieved.Then 0.1ml from the 1:1000 was transferred aseptically to the surface of solidified Potato Dextrose Agar plate. A sterile L-shaped rod was used to spread it on the medium and then incubated at room temperature (30^0C$\pm 2^0$) for 3-5 days.The fungal colonies formed were counted and the counts were expressed as colony forming units per gram (cfu/g) using this formula:

Fungal count (Colony forming unit/gram) = Average/mean colony counts × dillution factor / Inoculum volume

The distinct fungal colonies formed were further subcultured on Potato Dextrose Agar.All pure cultures were thereafter maintained on PDA slants and stored in a refrigerator at 4^0C.The same procedure was done for maize flour.

3.3 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. A 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 Germany) was utilized for the detection of aflatoxin 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.

3.4.2 Result interpretation

A dose response curve was constructed following the manufacturers instructions.This was carried out 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 ratioof 1:5amounts of aflatoxin was multiplied by five in order to obtain the nanogramme of aflaloxin per gram of commodity (ppb).

3.5 Determination of physico-chemical content of maize grain and maize flour

3.5.1 Moisture content

The moisture content of each sample was determined using standard methods (AOAC, 1990). A clean crucible was dried to a constant weight at 110^0C for one hour in a hot air oven, cooled in a desiccator and weighed (W_1). Two grams of maize grain were accurately weighed into the crucible and reweighed (W_2).

The crucible containing the sample was dried in an oven to a constant weight (W_3). The percentage moisture content was calculated using the formular:

$$\% \text{ moisture content} = \frac{(W1 - W3) - (W2 - W3)}{W1 - W3} \times 100$$

3.5.2 Ash content

A porcelain crucible was dried in a hot air oven at 100^0C to a constant weight, cooled in a desiccator and weighed.Two grams of maize grainand maize flour at each determination were placed in the crucible and reweighed.The sample was first ignited and transferred into a muffle furnace at 550^0C,and left in the furnace for eight hours.The crucible containing the ash was then

removed, cooled in the desiccator and weighed again. The percentage ash content was calculated thus:

$$\% \text{ Ash Content} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

3.5.3Crude fat content

Five (5) grams of maize grain at each determination were put into a clean dry 500ml round bottom flask, containing anti bumping granules and weighed. Three hundred milliliters of petroleum ether (boiling range, 40-60^{0}C) was poured into the flask fitted with soxlet extraction unit. The round bottom flask and a condenser were connected to the soxlet extractor and cold water circulation was put on. The heating mantle was switched on and the heating rate adjusted until the solvent was refluxing at a steady rate. Extraction was carried out for six hours. The solvent was recovered and the oil was dried in an oven at 100^{0}C for one hour. The round bottom flask and oil were cooled and weighed.

$$\% \text{ crude fat content} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100$$

3.5.4 Crude Protein

The microkjedahl method as described by AOAC (1990) was adopted. This method determines the total nitrogen which include non-protein as well as true protein nitrogen. This procedure consists of three steps namely, digestion, distillation and titration.

3.5.4.1 Digestion of protein

Maize, weighing 1.5g, was wrapped into an ashless filter paper and dropped into 300ml Kjedahl flask. Twenty five milliliters of concentrated H_2SO_4 and 13g of mixed catalyst copper sulphate and

anhydrous sodium chloride (1:1 w/w) weighed sepaerately into an ashless filter) were also dropped in the Kjedahl flask. The flask was then transferred to the Kjedahl digestion apparatus. The sample was digested until a clear green colour was obtained after two hours.

3.5.4.2 Distillation of the digest

The digest was transferred into 500ml Kjedahl flask containing anti bumping clips. Two hundred and fifty milliliters of distilled water was added (rinsing the flask into the 500ml flask with part of the 250ml water). Slowly, 70-120ml of 40% NaOH was added by the side of the flask and followed by three drops of 1% phenolphthelaeinindicator.Five hundred milliliters Erlenmeyer flask containing a mixture of 125ml 4% boric acid and four drops of mixed indicator was used to trap the ammonia liberated. The distillation was carried out until 125ml of the distillate was trapped in the boric acid solution (to make a total volume of 250ml).

3.5.4.3Titration

The distillate was then titrated with 0.5M H_2SO_4 and the percentage nitrogen was calculated thus.

$$\% N_2 = \frac{\text{Volume of acid x molarity of standard x } 0.014}{\text{Weight of test sample}} \times 100$$

% Crude Protein = % Nitrogen (N_2) x 6.25

3.4.4.4 Total carbohydrate

The total carbohydrate content was calculated thus.

% Carbohydrate = 100 – (% moisture + % ash + % crude fat + % crude protein) (McClement, 2005)

3.7Statistical analysis

The results of the physicochemical and microbiological analysis obtained for maize grain and maize flour, were subjected to student's t- test and a one-way analysis of variance to compare the

statistical difference between maize and maize flour within the season and the aflatoxin level between the products (Stat Box Logiciel, Gummersoft; version 6.4, France) with $P<0.05$ considered statistically significant.

CHAPTER FOUR

4.1 Results

The total fungal counts of maize grain andflour in some retail outlets in Zaria are aspresented in Tables 4.1, 4.2 and 4.3.

4.2. Total fungal counts of maize grain and flour

The results showed that a mean fungal count of 5.59±0.36($\log_{10}$ $cfug^{-1}$)was recorded on maize grain in wet season, while the lowest mean fungal count of 5.47±0.05 ($\log_{10}cfug^{-1}$) was also recorded on maize grain in the same season.Samples from Yannika outlet had highest fungal counts for both seasons followed by maize from Samaru and Tudun wada outlets with a range of $\log_{10}$ 4.41-$\log_{10}$ 4.58 during dry season on maize grain(Table 4.1).

The result for maize flour showed thata mean fungal count of4.17±0.05 ($\log_{10}$ $cfug^{-1}$) was observed in the wet season at Yannika and a count of4.14±0.05 ($\log_{10}$ $cfug^{-1}$) wasobtained for flour in Tudun wada.In the dry season a mean fungal count of 4.16±0.10($\log_{10}$ $cfug^{-1}$) was obtained compared

to4.11±0.05 (log_{10} $cfug^{-1}$) obtained for Tudun wada outlet.A count range of log_{10} 4.11-log_{10} 4.16 was obtained for maize flour during the dry season (Table 4.2).

The comparison of the mean total fungal count on maize grain and maize flour is as presented in Table 4.3..Maize grain recorded the highest fungal count of 5.04±0.72(log_{10} $cfug^{-1}$),while fungal count on maize flour had highest count of 4.16±0.01 (log_{10} $cfug^{-1}$) Acount rangeof 4.99 to 5.04(log_{10} $cfug^{-1}$), on maize grain,and 4.13to 4.16(log_{10} $cfug^{-1}$), on maize flour, was obtained.

*Aspergillus parasiticus*was the most isolated fungus (80) followed closely by *Aspergillus flavus* (62) and then *Aspergillus niger* (30). They were all evenly distributed across the outlets,while the least distributed isolates in all the outlets were *Fusarium* sp(9) and *Rhizopus* sp (3)Table 4.4

Table 4.1Fungal counts (log_{10} $cfug^{-1}$) of maize sold in some retail outlets in Zaria.

Outlet n=150	Dry season	Wet season
Yannika	4.58±0.24[a]	5.59±0.36[b]
Kasuwa mata	4.51±0.25[a]	5.58 ±0.38[b]
Sabo	4.45±0.08[a]	5.58±0.05[b]

Samaru	4.42± 0.25[a]	5.57 ±0.05[b]
Tudun wada	4.41±2.78[a]	5.47±0.05[b]

Values are $\log_{10}$ of mean ± Standard deviation of duplicate samples Means from the same row with varying superscript differ significantly ($P < 0.05$) n=Total number tested

Table 4.2 Fungal counts ($\log_{10}$ cfug^{-1}) of maize flour sold in some retail outlets in Zaria.

Outlet n=150	Dry season n	Wet season
Yannika	4.16±0.10[a]	4.17±0.05[b]

Kasuwa mata	4.13 ±0.13[a]	4.16±0.09[b]
Sabo	4.12±0.11[a]	4.16±0.10[b]
Samaru	4.12±0.06[a]	4.14±0.06[b]
Tudun wada	4.11±0.05[a]	4.14±0.11[b]

Values are $\log_{10}$ of mean ± Standard deviation of duplicate samples.Means from the same row with varying superscript differ significantly ($P < 0.05$) n=Total number tested

Table 4.3 Total fungal counts($\log_{10}$ cfug^{-1}) of maize and maize flour sold in some retail outlets in Zaria

Outlet n=300	Maize grain	Maize flour
Yannika	5.04±0.72[a]	4.16±0.01[b]
Kasuwa mata	5.02±0.76[a]	4.15±0.02[b]
Sabo	5.02±0.79[a]	4.14±0.03[b]
Samaru	5.00±0.81[a]	4.13±0.02[b]
Tudun wada	4.99±0.71[a]	4.13±0.02[b]

Values are $\log_{10}$ of mean ± Standard deviation of duplicate samples.Means from the same row with varying superscript differ significantly ($P < 0.05$) n= Total number tested)

Table: 4.4 Distribution of isolates on maize grain and flour during wet and dry seasons sold in some retail outlets in Zaria.

Outlets	***A****spergillus flavus*	*Aspergillus parasiticus*	*Aspergillus niger*	*Penicillium* sp	*Fusarium* sp	*Rhizopus* sp
Yannika	14	19	10	4	3	0
Kasuwa mata	17	20	7	1	3	0
Sabo	13	18	3	7	2	1
Samaru	11	11	1	3	1	0
Tudun wada	7	12	9	4	0	1
Total	62	80	30	19	9	3

4 .3Frequency of occurrenceof fungal isolates on maize grain and maize flour

Thefrequency of occurrence of fungi isolated from maize grains and maize flour in some retail outlets in Zaria areas presented in Tables 4.5, 4.6 and 4.7 respectively.*Aspergillus parasiticus* had the highest percentage occurrence (48%) during the wet seasoncompared to its frequency of occurrence (32.11%) in the dry season. *Aspergillus flavus* was found to thrive best on maize in the dry season (35.26%) than in the wet season (25.50%) On the other hand, *Rhizopus* sphad lowest frequency of occurrence(1.32 %) on maize grain in the wet season and did not occur at all during the dry season.

Table 4.6 shows the frequency of occurrence of fungal isolates on maize flour purchased from some retail outlets in Zaria. Generally, *Penicilliun* sp exhibited a significantly high percentage occurrence (44.44%) on maize flour only in the dry season. Similarly, while varying percentagefrequency of occurrence of all the fungal isolates was observed in wet season, *Fusarium*sp and *Rhizopus sp* did not occur at all in the dry season.

Comparatively, the frequency of occurrence of the fungal isolates on maize grain and maize flour is as presented in Table 4.7. The study shows that while maize grain had asignificantly high frequency of occurrence of 30.38% and 39.89% for *Aspergillus flavus* and *Aspergillus parasiticus* respectively, *Penicilliun* spexhibited highest frequency of 44.44% in the maize flour. It was also observed that frequency of occurrence of *Fusarium*sp*and Rhizopus* spwere generally lower in both products. However, *Fusarium* spand *Rhizopus*sp had significantlylower frequency of 8.94% and 1.32% on maize grain, than values 19.05% and 14.29% observed on maize flour respectively.

T able: 4.5 Frequency of occurrence (%) of fungal isolates on maize grain sold in some retail outlets in Zaria during wet and dry seasons

Species	Wet season	Dry season	Mean n=150
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.

n= Total number tested 0(no growth of specie)

Table: 4.6 Frequency of occurrence (%) of fungal isolates on maize flour sold in some retail outlets in Zaria (at different season of the year)

Species	Wet Season	Dry season	Mean n=150
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=Total number tested 0(no growth of specie)

Table: 4.7 Frequency of occurrence (%) of fungal isolates on maize grain and maize flour sold in some retail outlets in Zaria

Organisms	Maize grain	Maize flour n=300
Aspergillus flavus	30-38±6.90	22.77±7.86
Aspergillus parasiticus	39.89 ±11.0	23.01 ±1.12
Aspergillus niger	14.93 ±0.45	10.31 ±1.12
Penicillium sp	9.68 ± 10.9	22.22 ±31.4
Fusarium sp	4.47 ±6.32	9.53 ±13.5
Rhizopus sp	0.66 ±0.93	7.15 ±10.1

Values are mean ±standard deviation of duplicate samples
n=Total number tested

4.4 Quantitation of total aflatoxin in maize grain and maize flour

The total aflatoxin in maize grain during dry and wet seasons infive retail outlets in Zaria is presented in Figure 4. 1 The result shows that Yannika recordedsignificantaflatoxin level of 2.8 (ppb) and 2.2 (ppb) on maize grain during wet and dry seasons respectively. On the other hand maize grain from Samaru outlet had the least total aflatoxin (0.001 ppb) during the dry season but an increased aflatoxin level (1.5ppb) during the wet season. However, Tudun wada retail outlets recorded significantly lower levels of total aflatoxin during both seasons comparedto their levels in Yannika and Kasuwa mata outlets.However the aflatoxin content of food products including maize is regulated in many countries to be below 20ppb allowable limits, (EC Regulation 2003; FDA, 1997).The mean total aflatoxin on maize flour during both seasons infive retail outlets in Zaria is shown in Figure 4. 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 significantly low levels of total aflatoxin value at 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 4. 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 significantlylow total aflatoxin levelsof 0.70(ppb) and 0.30(ppb) were recorded for maize flour from both outlets respectively.

Table 4.8 shows the distribution of the number of aflatoxin positive samples for both maize grain and maize flour from five retail outlets in Zaria. Maize grain had a totalof 33 positive samples with aflatoxin (22%) while maize flour had a total of 21 positive samples for aflatoxin (14%).Out of a total of 300 samples, 15% was positive for total aflatoxin.

Table 4.8 Total numbers of positive samples with aflatoxin in maize grain and maize flour from five retail outlets in Zaria.

Samples	Yannika	Kasuwa mata	Sabo	Samaru	Tudun wada	Total number of positve samples/ %
Maize grain n=150	10	7	5	8	9	33 (22%)

Maize flour n=150	6	4	4	5	2	21(14%)

n =Total number of samples

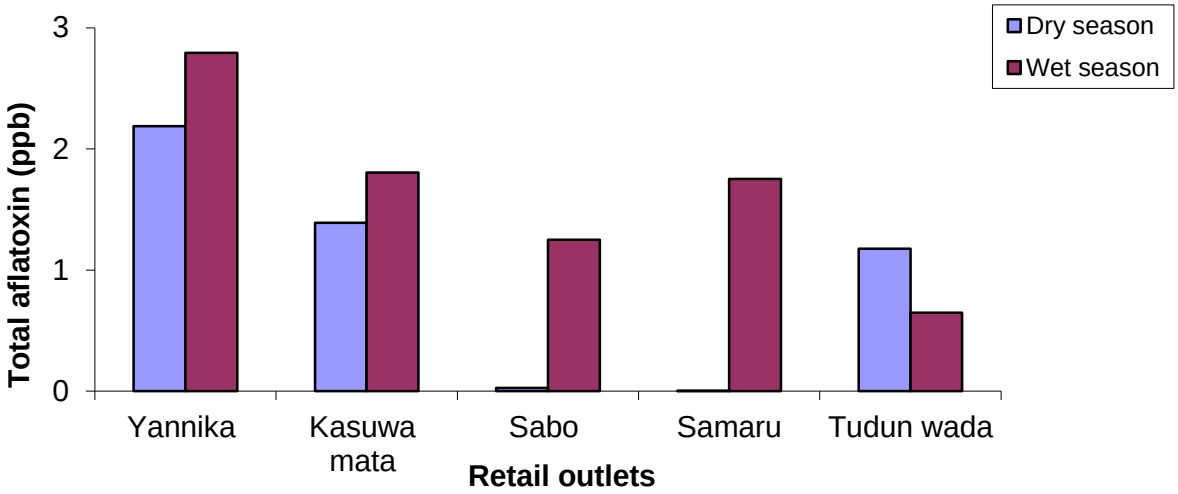

Figure 4.1 Mean total aflatoxin on maize grain sold in some retail outlets in Zaria during dry and wet seasons

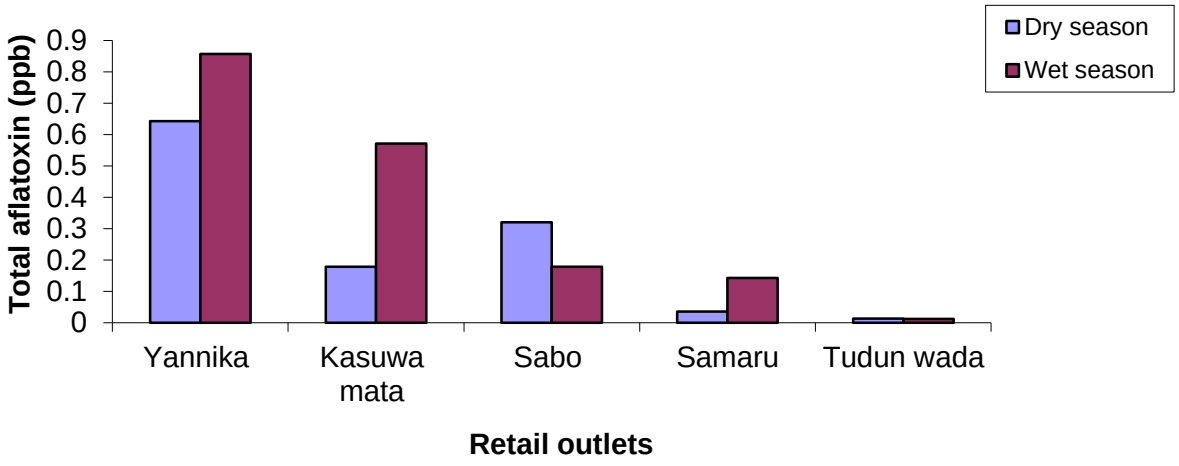

Figure 4. 2 Mean total aflatoxin on maize flour sold in some retail outlets in Zaria during dry and wet seasons

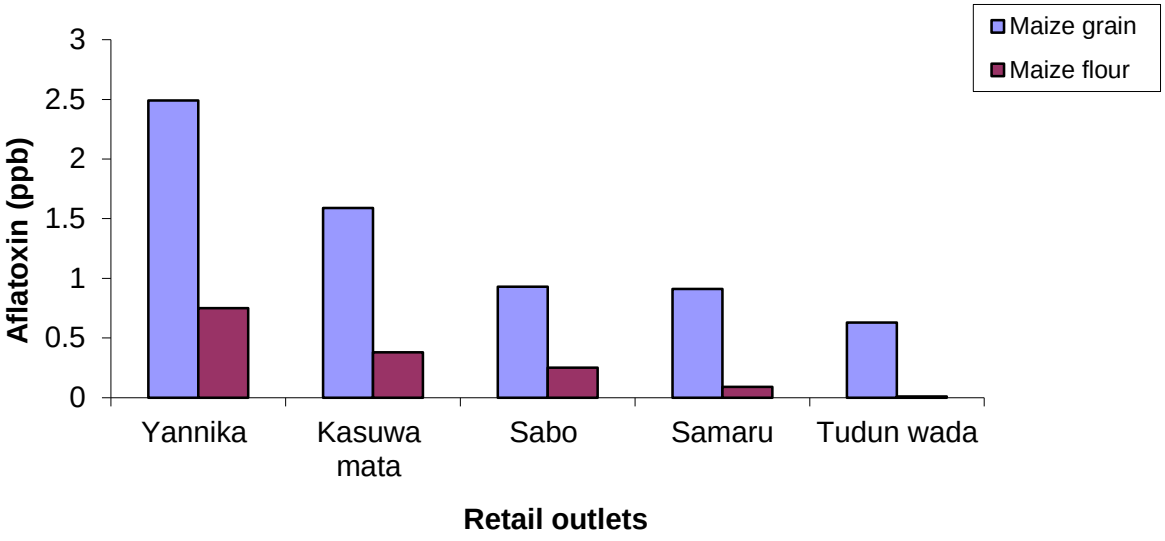

Figure 4. 3 Total Aflatoxin on maize grain and maize flour sold in some retail outlets in Zaria during dry and wet seasons

4.5Proximate composition of maize grain and maize flour

The proximate composition of maize in some retail outlets is as shown in Table 4.9 Mean moisture content of 13.10±1.38% to 14.05±1.38% was recorded for both dry and wet seasons respectively. The study showed an average ash content of 1.15±0.45% on maize in dry season, higher than mean ash content of 1.05±0.45 in wet season. Also analysis of crude fat in maize grain had 1.30±0.45 in dry season, while 1.18±0.45% was recorded for maize grain in the wet season. Crude protein values were also different for the two seasons with 2.43±0.40% in dry season and 3.05±0.40% in wet season. Similarly, carbohydrate content of maize grain increased from 71.5±1.09% to 72.5±1.09% for wet and dry season respectively.

The mean moisture contents of maize flour were 12.9±0.11% and 13.4±0.19% for both dry and wet season respectively, while the mean ash content of 0.88±0.01 was recorded in the dry season and 0.84±0.03 in the wet season.The crude fat value was between 0.75±0.01 in dry season and 0.81±0.03 in the wet season.The crude protein values were 1.86±0.08 and 1.93±0.04 for dry and wet season respectively.The carbohydrate value was 71.0±0.71 in the dry season and 71.5±0.71 in the wet season(Table 4.10).

Table 4.9 Proximate composition of maize grain sold in some retail outlets in Zaria

Composition	Dry season	Wet season n=150
Moisture content	13.10±1.38[a]	14.05±1.38 [b]
Ash	1.15±0.45 [a]	1.05±0.45 [b]
Crude fat	1.08±0.47[a]	1.30±0.47 [b]
Crude protein	2.43±0.40[a]	3.05±0.40 [b]
Carbohydrate	72.50±1.09 [a]	71.50±1.09 [b]

Values are mean ± standard deviation of samples and means from the same row with varying superscript differ significantly ($P < 0.05$) n=Total number tested

Table 4.10 Proximate composition of maize flour sold in some retail outlets in Zaria

Composition	Dry season	Wet season n=150
Moisture content	12.9±0.11[a]	13.4±0.19[b]
Ash	0.88±0.01[a]	0.84±0.03[b]
Crude fat	0.75±0.01[a]	0.81±0.03[b]
Crude protein	1.86±0.08[a]	1.93±0.04[b]
Carbohydrate	71.0±0.71[a]	71.5±0.71[b]

Values are mean ± Standard deviation of duplicate samples and means from the same row with varying superscript differ significantly (P< 0.05) n=Total number tested

CHAPTER FIVE

5.1 Discussion

5.2Total fungal counts on maize and maize flour

A significant high total fungal count was observed in all the maize sampled in the wet season compared to samples in the dry season The mould contamination level of the samples exceeded the standard regulatory limits of (10^3–10^4 cfug-^{1}) set by Food and Agricultural Organization(FAO) for cereals (FAO,1991). Fungal contamination was observed in all the maize grains sampled. However, the high total fungal count observed in the wet season had been reported previously (Rocha *et al*., 2009). The finding was attributedto relatively high humidity in wet season, which encouraged the germination and colonization by these fungi (Tanboon-ek*et al.,* 1986). Similar studies had also reported higher total fungal counts than values in this study. Muthomi *et al.,* (2009) had also shown

variation in fungi contamination base on agro-ecological zones.In this study, significantly higher ($P< 0.05$) total fungal count was recorded on maize and maize flour from Yannika and Kasuwa mata outlets compared to those samples from Samaru and Tudun wada. Apart from having more retailers, milling of these grains was also simultaneously carried out in higher number in the same outlet. It could therefore, be assumed that cross contamination between these grains and their flour, could be responsible for the high fungal count observed in this retail outlets.

5.3 Seasonal distribution of mycoflora on maize grain and maize flour

The study revealed that four fungal genera comprising six fungal species were isolated on maize grains and flour. Seed infestation by species of fungi had been reported by many researchers (Almeida *et al.,* 2000; Kpobo and Bankole 2005). Work by Muthomi*et al.* (2009) had also reported *Aspergillus* and *Penicillum* species as common storage fungi. Similarly, *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifer* and *Penicillium italicum* had been isolated in stored maize (Amadi and Adeniyi, 2009).

Predominantly, *Aspergillus* sp were seen throughout the seasons and this agrees with the report that *Aspergillus flavus* invades and infects maize in the field before harvest, during harvest and in storage (Ranajit *et al.,* 2005). These characteristics said to be peculiar to *Aspergillus* sp could be responsible for their all season abundance (WHO, 2006; Richard, 2007; Kumar *et al.,* 2008).

5.4 Frequency of occurence of fungal isolates on maize grain and flour

The study showed that *Aspergillus* was the most dominant genus on maize grains and a significantfrequency of occurrence of *Aspegillus parasticus and Aspergillus flavus* during wet and dry season was observed.This is in agreement with Abdullahi and Mohammed (2002),who showed that *Aspergillus flavus* and *Aspergillus parasiticus* have the highest occurrence frequency on maize and cereal grains from different parts of the world (WHO 2006, Kumar *et al.,* 2008). Similarly high

frequency of occurrence of *Aspergillus flavus* had also been reported in maize kernel (Youssef, 2009).

Also*Penicillum* sp was found to have high frequency of occurrence on maize flour. This finding is in agreement with that of Amadi and Adeniyi,(2009)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 reported by Sekiyama *et al.*, (2005). This probably could be responsible for the low fungaloccurrence generally observed in the maize flour compared to maize grain.

5.5 Total aflatoxin in maize grainand flour

Data from this study indicated that a significant quantity (15%) of the maize grain was contaminated with aflatoxin in both seasons. There was an obviously higher aflatoxin levels 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). In a similar study in Kenya Bourama *et al.*,(1993) found aflatoxin B_1 level up to 14µg/kg and aflatoxin G_1 level up to 58 µg/kg in stored maize.Aflatoxin contamination with a total prevalence of 18% have been recorded for both maize grain and flour, lower than 42.5% in 1994 and 30% in 1995(Setamou *et al.*,1997) in preharvest maize. Similarly, Udoh *et al.*, (2000) reported 33% aflatoxin contamination in maize sampled from different ecological zones of Nigeria. Hell *et al.*, (2000a) 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 20-355µg/kg (Kpobo, 1996).

A lower aflatoxin contamination of the maize flour was observed in all the outlets compared to the levels in maize grain.This result is similar to findings by various authors. Furlong *et al*(1999) reported 7.7% of the 39 analysed samples.Pich,*et al* (1998) found levels from 3-24µg/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 (Vargas, *et al.,* 2009).The traditional processing method that involves the removal of the germ and the pericarb could imply the removal of fungi and associated mycotoxins (Fandohan *et al.*, 2005).

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). The level of aflatoxin in this studydid not exceed the regulatory limit allowed in maize and other cereals,which is below 20µg/g(20ppb). (EC Regulation,2003;FDA,1997;Kenya Bureau of Standard,1998).

5.6 Proximate composition of maize grain and maize flour

The data obtained for the moisture content of maize grain showed a significance difference between the seasons ($p<0.05$).Samples from wet season was within the 14% Standard Organization

of Nigeria recommended value.it has been reported previously that fungal contamination of cereals is due to many factor which include high temperature,and insects infestation and not only moisture (Bhat *et al.*,1997).while the lower moisture obtained for maize flour could be due to good manufacturing practice The slight variation in ash content between maize grain and maize flour ranging from 0.88-1.15 could be due to certain processing procedure.Protein quality in food is determined by the amino acid pattern and each type of cereal has different amino acid composition and amino acid pattern (Prasanna *et al.,* 2001).

5.7 Summary and Conclusion

The results of this study indicate that *Aspergillus flavus, Aspergillus parasiticus, Aspergillus niger Penicillum*sp *Fusarium* spand *Rhizopus* sp are the mycoflora of the selected maize grain and maize flour. The finding demonstrate widespread aflatoxin contamination of maize grain and maize flour with a total prevalence of 15%.Aflatoxin.These 22% aflatoxin contamination in maize grain and 14% for maize flour recorded for all the retail outlets. However seasonal variation in the incidence and severity of aflatoxin contamination was observed withsignificantly ($P.<0.05$) higher levels recorded in the wet seasons. Comparatively, maizeflour contained reduced aflatoxin level below maximum permissible levels

Simultaneous milling/dehulling process as well as milling of the flour could be the major cause of the higher mould contaminant and aflatoxin levels observed in Yannika and Kasuwa mata.

5.8Recommendations

The study indicates that there is a need to effective management strategies to reduce aflatoxin levels below regulatory limits to protect the consumers from the harmful effects of these mycotoxins. Therefore continuous nationwide surveillance and increased food and feed inspection by NAFDAC and other regulatory bodies could be an intervention strategy that will ensure food safety.

- With this NAFDAC should set up more inspection places in every state where products are monitored before they are taken into the open markets.

- General cleanliness of retail outlets is very important to avoid cross contaminations between the products, since some of the products (grain and flour) are usually kept at the same place.

- There is the need to avoid storage and milling of cereal simultaneously in the same environment.

- Laws should be enacted in each state and local government area to ensure that farmers properlydry their products to verylow moisture level before they are being brought to the open market.

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APPENDIX 1

MAIZE GRAIN ANALYSIS FOR DRY SEASON

SOURCE	**DF**	**SUM OF SQUARES**	**MEAN SQUARE**	**F – VALUE**	**Pr > F**
Model	4	0.28670667	0.07167667	1.28	0.2848
Error	70	3.90869333	0.05583848		
Corrected total	74	4. 19540000			

APPENDIX 2

MAIZE GRAIN ANALYSIS FOR WET SEASON

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F – VALUE	Pr > F
Model	4	0.14916800	0.03729200	1.33	0.2680
Error	70	1.96506667	0.02807238		
Corrected total	74	2.11423467			

APPENDIX 3

MAIZE FLOUR ANALYSIS FOR DRY SEASON

SOURCE	**DF**	**SUM OF SQUARES**	**MEAN SQUARE**	**F – VALUE**	**Pr > F**
Model	4	0.01667467	0.00416867	0.45	0.7724
Error	70	0.64922533	0.00927505		
Corrected total	74	0.66592800			

APPENDIX 4

FLOUR ANALYSIS FOR WET SEASON

SOURCE	**DF**	**SUM OF SQUARES**	**MEAN SQUARE**	**F – VALUE**	**Pr > F**
Model	4	0.01165333	0.00291333	0.41	0.7991
Error	70	0.49441333	0.00706305		
Corrected total	74	0.50606607			

APPENDIX 5

Student-Newman-Keuls Test for maize grain dry season

MEAN	N	WEEK
4.6960	5	1
4.5380	5	8
4.5380	5	11
4.5260	5	10
4.5180	5	9
4.5180	5	3
4.5120	5	6
4.5100	5	12
4,4900	5	2
4.4780	5	7
4.4580	5	4
4.3720	5	15
4.3440	5	5
4.3220	5	13
4.2940	5	14

N=number of outlets

APPENDIX 6

Student-Newman-Keuls Test for maize grain wet season

MEAN	N	WEEK
5.6160	5	8
5.6120	5	6
5.6100	5	3
5.6040	5	7
5.6020	5	13
5.5920	5	10
5.5840	5	4
5.5840	5	15
5.5740	5	5
5.5720	5	9
5.5680	5	11
5.5600	5	12

5.5420	5	14
5.4060	5	1
5.3620	5	2

N=number of outlets

APPENDIX 7

Student-Newman-Keuls Test for maize flour dry season

MEAN	N	WEEK
4.1940	5	12
4.1900	5	10
4.1720	5	2
4.1500	5	4
4.1240	5	5
4.1240	5	7
4.1200	5	1
4.1160	5	6

4,1120	5	3
4.1120	5	9
4.1080	5	15
4.1000	5	14
4.1000	5	11
4.0960	5	8
4.0780	5	13

N=number of outlets

APPENDIX 8

Student-Newman-Keuls Test for maize flourwet season

MEAN	N	WEEK
4.2380	5	5
4.2240	5	3
4.1900	5	6
4.1720	5	8
4.1700	5	14
4.1660	5	15

4.1600	5	10
4.1500	5	1
4,1360	5	1 3
4.1280	5	11
4.1220	5	2
4.1180	5	4
4.1120	5	7
4.1060	5	9
4.0980	5	12

N=number of outlets

APPENDIX 9

Table 2.1: World maize production

Region and year	Area harvested (1000 ha)	Yield (kg/ha)	Production (1000MT)
Africa			
1979 – 81	18193	1554	28268
1985	19099	1522	29069
1986	19580	1575	30840
1987	19512	1395	27 225
North and Central America			
1979 – 81	39 399	5 393	212384
1985	40915	6092	249258
1986	37688	6116	230511

1987	35187	5690	200211
South America			
1979 – 81	16751	1928	32369
1985	17813	2182	38859
1986	18799	2021	38001
1987	19413	2143	41595
Asia			
1979-81	36815	2296	84431
1985	35246	2628	92629
1986	37474	2729	102274
1987	37399	2788	104269
Europe			
1979 – 81	11738	4668	54792
1985	11556	5423	62673
1986	11539	6207	71621
1987	11405	6039	68901
Oceania			
1979-81	76	4359	332
1985	124	3804	471
1986	107	4402	471
1987	84	4302	363
USSR			
1979-81	3063	2989	9076
1985	4482	3214	14406
1986	4223	2955	12479
1987	4600	3217	14800
World			
1979 – 81	126 035	3345	421751
1985	129411	3757	487198
1987	127605	3584	457365

Source: FAO, 1992