OCCURRENCE AND HEALTH IMPLICATIONS OF FUNGI AND AFLATOXINS FROM CASSAVA FLAKES (GARRI) COLLECTED FROM SELECTED AGRO-ECOLOGICAL ZONES (AEZs) IN NIGERIA

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MINNA

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THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGER-STATE, NIGERIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF TECHNOLOGY IN BIOCHEMISTRY

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DECLARATION

I hereby declare that this thesis title: **"Occurrence and health implications of Fungi and Aflatoxins in Cassava Flakes(Garri) Collected from Selected Agro-Ecological Zones in Nigeria"** is collection of my original research work and has not been submitted or presented in part or full for any other diploma or degree of this or any other university. Information from other sources (published or unpublished) has been duly acknowledged.

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SIGNATURE & DATE

CERTIFICATION

This thesis titled: "Occurrence and health implications of Fungi and Aflatoxins in Cassava Flakes(Garri) Collected From Selected Agro-Ecological Zones In Nigeria" by IYIOLA, Aanuoluwa Temitayo (MTech/SLS/2018/8837) meets the regulations governing the award of MTech of the Federal University, Minna and it is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to God Almighty, my source of inspiration, wisdom and understanding and for His Benevolent mercy to complete this research.

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ABSTRACT

Garri, a pre-eminent staple diet in Nigeria, accounts for over 70 % of the total calorie intake of more than 50 % of Nigeria's population. This study investigated the fungal flora and the level of aflatoxin contamination in 68 composite samples of garri, which were randomly purchased from open markets across the five agro-ecological zones in Nigeria (AEZs): Sudan Savanna (SS), Northern Guinea Savanna (NGS), Southern Guinea Savanna (SGS), Derived Savanna (DS) and Humid Forest (HF) and were aseptically transported to the NAFDAC laboratory and cultured for fungal growth. Aflatoxin contaminations in the garri samples were identified and quantified using High Performance Liquid Chromatography (HPLC) and identification of fungal cultures was done using morphology and fungal atlas. A total of 346 fungal isolates belonging to six genera: *Aspergillus spp., Fusarium spp., Penicillium spp., Cercospora sp*, *Cladosporium sp.* and *Botrytis cinerae* were isolated from the garri samples. The population of *Aspergillus* spp. (46.53%) was higher than the population of the other fungal genera in both the (white and yellow) garri sample types from all the AEZs. *Fusarium* (25.43 %) was the second in population frequency followed by *Penicllium spp.* (16.76 %) and *Cercospora sp.* (3.76 %), and least frequency hierarchy was *Botrytis cinerae* (3.17 %). In addition, the *A. flavus* isolates were more abundant (37.5%; $p<0.05$) in the DS zone than the other zones. Of the five *Fusarium* species isolated in the garri sample, *F. verticillioides* also recorded a high occurrence (37.5%; $p<0.05$) in DS. Aflatoxin B1 (AFB1) was the most prevalent aflatoxin in the garri samples from all the AEZs. AFB1 was significantly higher ($P<0.05$) in DS zone (34.48 μg/kg) than all other zones. Aflatoxins G1 was below detection limit in the garri samples form SGS zone. The risk assessment revealed that Aflatoxin B1 was detected in the garri samples across the AEZs at concentrations exceeding the maximum allowable limits. There is a high risk of contamination of the garri samples by Nigerian consumers especially in the DS and SGS zones, resulting in a national burden of between 913 and 782 Disability Adjusted Life Years (DALY) lost.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

There are considerable evidence that human food and animal feed commodities are frequently subjected to some form of contamination and spoilage such as growth of fungi exacerbated by subsequent production of toxins as secondary metabolites (Njobeh *et al*., 2009). The safety of foods and feeds for human and animal consumption should be of topmost priority with regards to the regulation of agricultural and food industries so that markets are not compromised by the sale of low quality or unsafe foods. Unfortunately, this is not the case in many parts of Africa as the limited availability of food far outweighs other considerations such as food safety (Conway & Toenniessen, 2018).

Mycotoxins are toxic secondary fungal metabolites frequently found as contaminants in food and feed with attendant negative effects on humans and animal's health when ingested. They may develop in almost any food or feedstuff during the growing season, at harvest time, or during processing or storage, depending on the environment and method of handling (Abbas *et al*. 2017). Mycotoxins: when ingested or inhaled lead to lower performance, sickness or death in humans and other animals (Bankole and Adebanjo, 2018). Mycotoxins have attracted worldwide attention due to the significant losses associated with their impact on human and animal health, and consequent national economic implications (Bhat and Vashanti, 2019). Among the 300–400 known mycotoxins, the most important and frequently occurring ones in cassava are the aflatoxins, deoxynivalenol (DON) and fumonisins (Bankole and Adebanjo, 2018).

Aflatoxins are poisonous, carcinogenic by-products of the growth of the molds *Aspergillus flavus* and *Aspergillus parasiticus*, and are the most studied and widely known mycotoxins. There are four major groups of aflatoxins: B1, B2, G1 and G2.

Aflatoxin M1, a metabolite of Aflatoxin B1 in mammals, may be found in the milk of animals eating feeds contaminated by Aflatoxin B1 Aflatoxins B1, B2, G1 and G2 probable human carcinogen (Ioannou-Kakouri *et al*., 2019).

Aflatoxins are of economic and health importance because of their ability to contaminate human food and animal feeds, in particular cereals, nuts and oilseeds. 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 (Vincelli *et al*., 2015). Other adverse economic effects of aflatoxins include lower yields for food and fibre crops. Several methods such as the thin-layer chromatography (TLC), high performance liquid chromatography (HPLC), mass spectroscopy, enzyme-linked immune-sorbent assay (ELISA), among others, have been described for detecting and quantifying aflatoxins in food (Wacoo *et al*., 2014).

Cassava is a major root crop in the tropics and its starchy roots are significant sources of calories for more than 500 million people worldwide (FAO, 2018). The major uses of cassava in Nigeria include; livestock feeds, glues, textiles, pharmaceuticals, flour, fufu and garri, Garri is a granular starchy food prepared from cassava mash. Garri is the most popular fermented cassava product in Africa. The production process of garri involves peeling, washing, grating, fermenting and toasting fresh cassava tuber (Oluwole *et al*., 2004). Palm oil is added according to preference (to make it a yellow garri). Palm oil added to the cassava mash gives the garri an aesthetic value and the palm oil also serves as source of vitamin A. Garri is stored and marketed in a ready-to-eat form and prepared

into stiff paste or dough-like form called “eba” by adding the granules into hot water and stirring to make a paste of varied consistencies. The eba can be consumed with local soups or stews of various types by chewing or swallowing in morsels (Oghiehor *et al*., 2007). Garri can also be consumed directly (without cooking) with groundnut, smoked fish, coconut, cowpeas, moimoi, or taken as fast food when soaked in cold water (Ogugbue and Obi, 2011). However, some unhygienic practices involved in production, processing of cassava to garri and post-processing handling such as spreading on the floor and mats after frying, displaying in open bowl or buckets in the markets during sales; the use of various packaging materials to transfer finished products from rural to urban areas and the use of bare hands during handling and sales may lead to microbial contamination due to deposition of bioaerosols on exposed products and transfer of infectious agent during handling (Ogugbue *et al*., 2011). Different types of molds have been implicated to be associated with garri during storage and distribution but not all moulds are implicated in the production of aflatoxin (Ogugbue *et al*., 2011).

1.2 Statement of the Research Problem

Nigeria has also recorded the highest estimated annual global burden of HCC cases attributable to aflatoxin exposure (1,800–2,940 and 8,200– 13,400 for HBsAg-negative and HBsAg-positive population, respectively) (Li and Wu, 2010). Fungal and mycotoxin contamination of foods is an increasing issue of concern in sub-Saharan Africa (SSA). Several studies have shown that *Aspergillus, Fusarium* and *Penicillium* species and their toxic secondary metabolites are the primary contaminants of stored foods in SSA (Atanda *et al*., 2013). The FAO estimates that 25% of the world food crops are contaminated by mycotoxin, of which the most notorious are the aflatoxins (AFTs). AFTs are metabolites produced primarily by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. There are four major naturally produced AFTs, referred to as B1, B2, G1 and G2. The B1 is the

most toxic of the AFTs and potent naturally occurring liver carcinogen. Reports estimated that more than 5 billion people in developing countries world‑wide are at risk of chronic exposure to AFTs through contaminated foods (Jonathan *et al*., 2013). AFTs affect livestock and poultry causing reduced feed efficiency, subtle immunosuppression, growth rate and death of animals. Other economic adverse effects of AFTs include low yields of food and fiber crops. The etiology of liver malignancy and renal dysfunction alongside with the reproductive concerns has been linked to AFT metabolites in humans. Given their seemingly unavoidable occurrence in foods and feeds, prevention and detoxification of mycotoxins pose an enormous challenge on toxicological issues in present time. However, the implication of AFTs in cassava meal poisoning and toxicity are often not considered, poorly reported and taken for granted. Therefore, it has become imperative to study and document levels of AFT in Garri produced across the five Agro-Ecological zones (AEZs) in Nigeria, investigating the distribution and risk assessment of mycotoxins in both yellow and white garri samples purchased from the market in efforts to bring these toxic substances to innocuous levels.

1.3 Justification for the Study

One of the prominent survey on the bioload and aflatoxin content of commercial garri was carried out by Oghiehor *et al*. (2007), whom only analysed garri sample from some selected states in the southern parts of Nigeria. Till date, there is no information on the distribution and risk assessment of mycotoxins in garri samples from all the five agro-ecological zones in Nigeria. Most developed countries have mycotoxin occurrence maps that inform government, researchers and policy makers about mycotoxin prevalence and exposure risks in different regions of their country to allow for formulation of intervention strategies. Furthermore, Nigeria does not have mycotoxin occurrence maps; thus, the Mycotoxicology Society of Nigeria (MYCOTOXSON) has been a lead vanguard in the

call for the construction of these maps. This research work reports the result of surveys conducted on the distribution of some regulated mycotoxins in stored garri samples from five AEZs of Nigeria, construction of mycotoxin occurrence maps from the database and exposure risk assessment of garri consumers in the zones. In line with the foregoing, this work was designed to investigate the microbial contamination level, the presence, prevalence and distribution of aflatoxins B1, B2, G1 and G2 in both yellow and white garri sold in some selected market across the five AEZs in Nigeria, destined for consumption, with the aim of determining the distribution of the aflatoxins in these regions, as well as developing useful indices for safe handling of garri and protection of public health of the consumers in Nigeria using High Performance Liquid Chromatography. This study was also designed to elucidate the possible risk associated with the ingestion of the aflatoxins present in the garri samples with a view to assessing the health impact associated and the results as related to set food safety standards.

1.4 Aims and Objectives

Aim:

The aim of this research is to examine the occurrence of fungi and quantify the level of aflatoxins in garri consumed in the selected agro-ecological zone in Nigeria.

Objectives:

i. to isolate and identify fungi species in garri samples using their morphological characteristics.
ii. to extract and analyse the Aflatoxins presents in the samples using high performance liquid chromatography
iii. to estimate human exposure and characterize the risk in the consumption of garri from the study area

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Mycotoxin as Agent of Food Spoilage

Many pests and diseases are prevalent on food commodities such as maize, sorghum, millet, barley, yam, and cassava in the field and during storage, including a number of insects. In addition, stored and processed food products carry a wide range of microorganisms that include bacteria, yeasts and filamentous fungi. The population structure of microorganisms depends on field climatic conditions and harvesting processes. Poor postharvest management can result in rapid deterioration and reduction in food quality, including loss of nutritional value and loss of products. Fungal infestation can lead to discoloration, production of off-odors and can result in mycotoxins (Magan *et al*., 2018).

Mycotoxins are secondary metabolites produced by fungi that are toxic to humans and animals. The most important toxigenic fungi belong to the genera Aspergillus, Fusarium, and Penicillium. The most important mycotoxins worldwide include aflatoxins, fumonisins, deoxynivalenol, zearalenone, and ochratoxins (Pitt, 2020).

2.1.1 Mycotoxins and their causal agents

Mycotoxins are produced by toxigenic fungi at both field and storage levels. Fusarium and Penicillium species (Marasas, 2021) occur predominantly in the field. Fusarium species produce a variety of mycotoxins with divergent biological and toxicological effects in humans and animals (Marasas, 2021). Mycotoxins produced by Fusarium include fumonisins, deoxynivalenol and zearalenone. Penicillium and Aspergillus species are the most important storage fungi but can also be a problem in the field especially on senescent or stressed plants. Penicillium species produce ochratoxins, citrinin, patulin.

Aspergillus species produce (principally) aflatoxins, citrinin, patulin (Gokmen *et al*., 2015).

2.2 Aflatoxin (AFT)

Among the mycotoxins affecting food, aflatoxin is the major one in food that ultimately harms human and animal health (Boutrif, 2018). Aflatoxins are chemically difuranocoumarin derivatives with abifuran group attached to the coumarin nucleus and a pentanone ring (in case of AFBs) or a lactone ring (in case of AFGs) (Kumar, *et al*., 2017). The four major AFTs among the identified 20 are AFB1, AFB2, AFG1 and AFG2. The B-types are produced by A. flavus while G-types are produced by A. parasiticus (Kumar *et al*., 2017). The biosynthesis of AFTs consists of 18 enzymatic steps with at least 25 genes responsible for producing the enzymes and regulating the biosynthetic process. The level of toxicity associated with aflatoxin varies with the types present, and the order of toxicity being AFTs-B1 > AFTs-G1 > AFTs-B2 > AFTs-G2 (Yabe and Nakajima, 2019).

2.2.1 Chemistry and biosynthesis of aflatoxins

Chemically, aflatoxins (AFTs) are difuranocoumarin derivatives in which a bifuran group is attached at one side of the coumarin nucleus, while a pentanone ring is attached to the other side in the case of the AFTs and AFTs-B series, or a six-membered lactone ring is attached in the AFTs-G series (Nakai *et al*., 2018). The physical, biological and Chemical conditions of Aspergillus influence the production of toxins. Among the 20 identified AFTs, AFT-B1, and AFT-B2 are produced by A. flavus, while AFT-G1 and AFT-G2 along with AFT-B1 and AFT-B2 are produced by A. parasiticus (Kumar *et al*., 2017). AFT-B1 is highly carcinogenic, as well as heat resistant over a wide range of temperatures, including those reached during commercial processing conditions (Sirot *et al*., 2018). The biosynthetic pathway of aflatoxins consists of 18 enzymatic steps for

conversion from acetyl-CoA, and at least 25 genes encoding the enzymes and regulatory pathways have been cloned and characterized. The gene comprises 70kb of the fungal genome and is regulated by the regulatory gene, aflR (Price *et al*., 2019). The metabolic grid involved in the aflatoxin biosynthesis. Hydroxyversicolorone (HVN) is converted to versiconal hemiacetal acetate (VHA) by a cytosol monooxygenase, in which NADPH is a cofactor. Monooxygenase is encoded by the mox Y gene, which catalyzes the conversion of HVN to VHA and the accumulation of HVN and versicolorone (VONE) occurs in the absence of the mox Y gene (Wen *et al*., 2017).

2.2.2 Gene responsible for aflatoxin production

Various genes and their enzymes are involved in the production of sterigmatocystin (ST) dihydrosterigmatocystin (DHST), which are the penultimate precursors of aflatoxins. The aflatoxin biosynthesis gene nor-1, which was First cloned in A. parasiticus, is named after the product formed by the gene during biosynthesis (Chang *et al*., 1992). These genes named according to substrate and the product formed nor-1 (norsolorinic acid [NOR]), nor A, nor B, avn A (averanti [AVN]), avf A (averufin [AVF]), ver-1 (versicolorin A [VERA]), ver A and ver B while those based on enzyme functions fas-2 (FAS alpha subunit), fas-1 (FAS beta subunit), pks A (PKS), adh A (alcohol dehydrogenase), est A (esterase), vbs (VERB synthase), dmt A (mt-I; O-methyltransferase I), omt A (O-methyltransferase A), ord A (oxidoreductase A), cyp A (cytochromeP450monooxygenase), cyp X (cytochrome P450 monooxygenase), and mox Y (monooxygenase). Initially, the aflatoxin regulatory gene was named afl-2 in A. flavus and apa-2 in A. parasiticus (Chang, *et al*., 1993). However, it was subsequently referred to as aflR in A. flavus, A. parasiticus, and A. nidulans because of its role as a transcriptional activator. Previous studies have shown that aflA (fas-2), aflB (fas-1), and aflC (pksA) are responsible for the conversion of Acetate to NOR. Moreover, the uvm8

gene was shown to be essential for NOR Biosynthesis as well as aflatoxin production in A. parasiticus. The amino acid of sequence of the gene is similar to that of the beta subunit of FASs (FAS1) from Saccharomyces cerevisiae. FAS forms the polyketide backbone during aflatoxin synthesis; hence, the uvm8 gene was named fas-1. Fatty acid syntheses (FASs) is responsible for sterigmatocystin (ST) biosynthesis in A. nidulans and further identified two genes viz., stc J and stc K that encode FAS and FAS subunits (FAS-2 and FAS-1) (Brown *et al*., 2019).

2.3 Aflatoxin and Food Safety

Food safety is one of the major problems currently facing the world; accordingly, a variety of studies have been conducted to discuss methods of addressing consumer concerns with various aspects of food safety (Nielsen *et al*., 2019). Food safety and security are among the major problems in the current climate of increasing population. These are mainly determined by three key aspects viz., (i) enough food availability, (ii) access to safe food and (iii) utilization of the food in terms of quality, nutritional and cultural purposes for a healthy life (FAO, 2016). The failure of any of these aspects leads to food insecurity and malnutrition that further influences human health, in addition to the socio-economic aspect of society. In addition, food contamination by mycotoxins are one of the key factors responsible for creating food insecurity (Udomkun *et al*., 2017). As per the Food and Agriculture Organization (FAO), one-fourth of the world's crop is affected by mycotoxins (Pankaj *et al*., 2018). Since 1985, the United States Food and Drug Administration (USFDA) has restricted the amount of mycotoxins permitted in food products. The USDA Grain and Plant Inspection Service (GPIS) have implemented a service laboratory for inspection of mycotoxins in grains. Additionally, the Food and Agricultural Organization (FAO) and World Health Organization (WHO) have recognized many toxins present in agricultural products. When mycotoxins are

contaminated into foods, they cannot be destroyed by normal cooking processes. However, there have been many recent advances in food processing developed to keep final food products safe and healthy, such as hazard analysis of critical control points (HACCP) and good manufacturing practices (GMP) (Maldonado-Siman *et al*., 2019). Moreover, several physical, chemical and biological methods can be applied to partially or completely eliminate these toxins from food and guarantee the food safety and health concerns of consumers.

Food processing techniques are not sufficient to eliminate Aflatoxins (AFTs) from contaminated food due to their heat resistant nature (Medina *et al*., 2017b). The ingestion of AFTs from contaminated food has led to serious health complications in humans and animals. Therefore, different countries have implemented strict regulations for AFTs in food to maintain the health of individuals (Juan *et al*., 2017). The safe limit of AFTs lies in the range of 4–30 mg/kg for human consumption. The European Union has the strictest standard level with AFB1 and total AFTs not beyond 2 mg/kg and 4 mg/kg, respectively, in any product meant for direct consumption (EC, 2010). Similarly, the maximum acceptable limit set for AFTs in the United States is 20 mg/kg. Besides this, various innovative technologies and control strategies are applied for pre- and post-harvest management of AFTs to enhance sustainable agricultural productivity (Prietto *et al*., 2019).

2.3.1 Occurrence of aflatoxins in food

Aflatoxins are found occurring in wide varieties of food. Some of the most affected food include peanuts, nuts, figs, corn, rice, spices, oilseeds, and dried fruits (Martinez-Miranda *et al*., 2019). It has been shown that among the tested cereals, 37.6% were at least contaminated by any of the AFTs (Andrade and Caldas, 2018). Though rice is not the high-risk commodity for AFTs contamination, but AFB1 besides other mycotoxins have

been detected in rice from China, Egypt, India, Iran, Malaysia, Nepal, Pakistan, Philippines, United Kingdom and United States (Lutfullah and Hussain, 2017). Filazi and Sireli (2018) reported rice to be more prone to AFTs contamination as compared to other cereals. The fungal growth occurs due to improper drying of rice grains retaining higher moisture content (>14%). As a result, these fungi cause discoloration of grain and/or husk along with deteriorating the quality of the grains. Groundnut and beans, on the other hand, are frequently used in many African diets to supplement cereal diets (Soro-Yao *et al*., 2019). However, these are also highly prone to AFTs contamination both in field and storage conditions. The extent of fungal growth and AFTs production in cereals depends on temperature, moisture, soil type, and storage conditions (Achaglinkame *et al*., 2017). In addition, spices are susceptible to AFTs contamination and are significantly affected by storage and processing conditions.

Fungal contamination can occur in the field, or during harvest, transport and storage. Aflatoxins contamination of wheat or barley commonly happen as a result of inappropriate storage (Jacobsen, 2018). In milk, aflatoxins are generally at 1–6% of the total content in the feedstuff (Jacobsen, 2018). AFTs infect humans following consumption of aflatoxins contaminated foods such as eggs, meat and meat products, milk and milk products (Piemarini *et al*., 2017). The outbreaks due to AFTs are more prone in tropical and subtropical areas, with a few in temperate regions (like the United States Midwest). In addition, the Mediterranean zones have become prone to AFTs contamination due to shifting in traditional occurrence areas of AFTs because of climate change i.e., increase in average temperatures, CO_2 levels and rainfall patterns (Marasas *et al*., 2018). This has led to an increase in contamination of crops with fungi and AFTs worldwide.

2.3.2 Impact of climate change on aflatoxin production

Climate change significantly impacts on the quality and availability of staple foods for consumption. With the increasing population worldwide, a major emphasis has been put on the safety of food and feed that can address the increasing demand with the increase in the yields by protecting the crops from adverse climatic conditions (Medina *et al*., 2017a). The change in climate simultaneously impacts the complex communities of AFTs-producing fungi by altering the number of AFT-producers to change its fungal community's structure. Aflatoxins contamination occurs via an initial phase during crop development and a second phase during crop maturation. The contamination is greater in warm, humid, and even hot deserts and drought conditions (Cotty and Jaime-Garcia, 2017). A. flavus has highly evolved physiological mechanisms to acclimatize to adverse climatic conditions and dominates other fungal species. Climate change alters the temperature and water activity (aw) in the environment which further influences the gene expression to produce AFTs.

The conditions of temperature and aw regulate the extent of fungal growth and AFTs production (Schmidt-Heydt *et al*., 2019). The AF-producing genes are grouped on the genome and express the main regulatory genes (aflR; aflS), as well as structural genes (aflD) which are influenced by the interaction of temperature and water activity conditions. As revealed by Schmidt-Heydt *et al*. (2019), the expression proportion of aflR/aflS significantly correlates with the amount of AFB1 produced. In addition, the expression of sugar transporter genes was significantly affected by the condition of temperature and aw (Medina *et al*., 2018). Further, Bernáldez *et al*. (2017) studied the effect of interactions of temperature and aw on the biosynthetic regulatory gene (aflR) expression and production of AFB1 by A. flavus in maize. They observed the optimum growth of A. flavus at 300C/0.99 aw with no growth at 200C/0.90 aw. Both temperature

and aw influenced the relative aflR gene expression and AFB1 production, however, the trends for the production of AFB1 were not in accordance with the gene expression.

Further, the effect of temperature (20, 27, and 350C) and aw (0.82, 0.86, 0.90, 0.94, and 0.98) on the growth of A. flavus and A. parasiticus along with the production of AFB1 were investigated on ground Nyjer seeds by Gizachew *et al*. (2019). The maximum AFB1 production was observed at 270C/0.90 aw for both A. flavus and A. parasiticus. In addition to this, the fungi showed optimum growth on polished rice in the range of 28–370C/0.92–0.96 aw. The maximum AFB1 was produced at 330C/0.96 aw (Lv *et al*., 2019). Based on the investigation by Battilani *et al*. (2019) on the possible emergence of AFB1 in cereals in the European Union as a result of climate change, for every 20C increase in temperature, there is an increase in AFTs risk in the various regions of Spain, Italy, Greece, Portugal, Bulgaria, Albania, Cyprus and Turkey. The risk for AFTs contamination in maize is likely to increase in Europe due to favorable climatic conditions for A. flavus in the next 30 years (Moretti *et al*., 2019). Therefore, proper detection methods and control strategies are crucial to combat the burning issues of AFTs in food.

2.3.3 Major sources of aflatoxin

The major sources of aflatoxins are fungi such as A. flavus, A. parasiticus, and A. nomius, although they are also produced by other species of Aspergillus as well as by Emericella spp. (Reiter *et al*., 2019). There are more than 20 known aflatoxins, but the four main ones are aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2), while aflatoxin M1 (AFM1) and M2(AFM2) are the hydroxylated metabolites of AFB1 and AFB2 (Hussain and Anwar, 2018).

2.3.3.1 Aspergillus spp

The Aspergillus species are an industrially important group of microorganisms distributed worldwide. A. Niger has been given Generally Recognized as Safe (GRAS) status by the

USFDA (Schuster *et al*., 2017). However, some species have negative impacts and cause diseases in grape, onion, garlic, peanut, maize, coffee, and other fruits and vegetables (Rooney-Latham *et al*., 2018). Moreover, Aspergillus section nigari produces mycotoxins such as ochratoxins and fumonisins in peanut, maize, and grape (Mogensen *et al*., 2019). Plant–pathogen interactions have been studied using molecular markers such as green fluorescent protein (GFP) isolated from Aequorea victoria. The GFP gene has been successfully inserted into Undifilum oxytropis, Fusarium equiseti and Muscodor albus and utilised to study the expression of different proteins and production of mycotoxins (Ezra *et al*., 2018). A. flavus and A. parasiticus infect many crops in the field, during harvest, in storage, and during processing. A. flavus is dominant in corn, cotton seed, and tree nuts, whereas A. parasiticus is dominant in peanuts. A. flavus consists of mycelium, conidia, or sclerotia and can grow at temperatures ranging between 12 and 480C (Hedayati *et al*., 2017). A. flavus produces AFBI and AFB2, whereas A. parasiticus isolates produce AFGI, AFG2, AFM1, AFBI, and AFB2. A high number of propagules was reported in soil, air, and on cotton leaves during mid-to late August, while soil borne inoculum increased drastically between April and December in cotton fields in Arizona (Hedayati *et al*., 2017).

2.3.3 Mechanism of toxicity and health effects by aflatoxin

Aflatoxin are specifically target the liver organ (Abdel-Wahhab *et al*., 2017). Early symptoms of hepatotoxicity of liver caused by aflatoxins comprise fever, malaise and anorexia followed with abdominal pain, vomiting, and hepatitis; however, cases of acute poisoning are exceptional and rare. Chronic toxicity by aflatoxins comprises immunosuppressive and carcinogenic effects. Evaluation of the effects of AFT-B1 on splenic lymphocyte phenotypes and inflammatory cytokine expression in male F344 rats have been studied (Qian *et al*., 2019). AFT-B1 reduced anti-inflammatory cytokine IL-4

expression but increased the pro-inflammatory cytokine IFN-g and TNF-a expression by NK cells. These findings indicate that frequent AFT-B1 exposure accelerates inflammatory responses via regulation of cytokine gene expression. Furthermore, Mehrzad *et al*. (2014) observed that AFT-B1 interrupts the process of antigen-presenting capacity of porcine dendritic cells, suggested this perhaps one of mechanism of immunotoxicity by AFT-B1.

Aflatoxins cause reduced efficiency of immunization in children that lead to enhanced risk of infections. The hepatocarcinogenicity of aflatoxins is mainly due to the lipid peroxidation and oxidative damage to DNA (Verma, 2019). AFTs-B1in the liver is activated by cytochrome p450 enzymes, which are converted to AFTs-B1-8, 9-epoxide, which is responsible for carcinogenic effects in the kidney. Among all major mycotoxins, aflatoxins create a high risk in dairy because of the presence of their derivative, AFTs-M1, in milk, posing a potential health hazard for human consumption. AFTs-B1 is rapidly absorbed in the digestive tract and metabolized by the liver, which converts it to AFT-M1for subsequent secretion in less mutagenic and carcinogenic than AFTs-B1, it exhibits high genotoxic activity. The other effects of AFTs-M1include liver damage, decreased milk production, immunity suppression and reduced oxygen supply to tissues due to anemia, which reduces appetite and growth in dairy cattle (Akande *et al*., 2019). Several studies have shown the detrimental effects of aflatoxins exposure on the liver, epididymis, testis, kidney and heart. It has been found that aflatoxin presences in post- mortem brain tissue, suggested that its ability to cross the blood brain barrier (Qureshi *et al*., 2015). AFTs also cause abnormalities in the structure and functioning of mitochondrial DNA and brain cells. The effects of aflatoxin on brain chemistry have been reviewed in details by Bbosa *et al*. (2018). Furthermore, few reports have described the effects of AFTs-B1administration on the structure of the rodent central nervous system.

The liver toxicology of aflatoxin is a critical issue. Limited doses are not harmful to humans or animals; however, the doses that do cause-effects are diverse among Aflatoxin groups (Iqbal, 2019). The expression of aflatoxin toxicity is regulated by factors such as age, sex, species, and status of nutrition of infected animals. The symptoms of acute aflatoxicosis include oedema, haemorrhagic necrosis of the liver and profound lethargy, while the chronic effects are immune suppression, growth retardation, and cancer (Cotty and Jaime-Garcia, 2017).

2.3.4 Effects of processing on aflatoxin

Techniques to eliminate aflatoxin may be either physical or chemical methods. Removing mold-damaged kernels, seeds or nuts physically from commodities has been observed to reduce aflatoxins by 40–80% (Park, 2022). The fate of aflatoxin varies with type of heat treatment (e.g., cooking, drying, pasteurization, sterilization, and spray drying). Aflatoxins decompose at temperatures of 237–306oC; therefore, pasteurization of milk cannot protect against AFM1 contamination. Awasthi *et al*. (2017) reported that neither pasteurization nor boiling influenced the level of AFM1 in bovine milk. However, boiling corn grits reduced aflatoxins by 28% and frying after boiling reduced their levels by 34–53%. Roasting pistachio nuts at 90oC, 120oC, and 150oC for 30, 60 and 120 min was found to reduce aflatoxin levels by 17–63% (Yazdanpanah *et al*., 2018). The decrease in aflatoxin content depends on the time and temperature combination. Moreover, alkaline cooking and steeping of corn for the production of tortillas reduces aflatoxin by 52%. Hameed (2018) reported reductions in aflatoxin content of 50–80% after extrusion alone. When hydroxide (0.7 and 1.0%) or bicarbonate (0.4%) was added, the reduction was enhanced to 95%. The highest aflatoxin reduction was found to be 59% with a moisture content of 35% in peanut meal, and the extrusion variables non-significantly affected its nutritional composition (Saalia and Phillips, 2019). Saalia and Phillips (2019) reported an

84% reduction in aflatoxin of peanut meal when cooked in the presence of calcium chloride.

2.3.5 Detection methods for aflatoxin

The detection of AFTs has been performed by the Association of Official Analytical Chemists (AOAC) official method in food and feed samples (Kumar *et al*., 2017). Among the most commonly employed methods are chromatographic methods like thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and liquid chromatography mass spectroscopy (LCMS), besides the enzyme-linked immunosorbent assay (ELISA) (Sulyok *et al*., 2021). However, the drawbacks of these standard methods are that they are unsuitable for rapid and real-time applications in food and feed samples as they are tedious, time-consuming and require skilled personnel to operate. Therefore, rapid and robust methods like polymerase chain reaction (PCR) and non-destructive methods based on fluorescence/near-infrared spectroscopy (FS/NIRS) and hyperspectral imaging (HSI) have emerged for the quick and easy detection of AFTs (Tao, *et al*., 2018).

Hussain and Anwar (2018) utilized the PCR technique for the molecular detection of AF producing A. flavus from peanuts. Similarly, the avfA, omtA, and ver-1 genes encoding the major enzymes in AF-biosynthesis were used as target genes for detecting AFTs using multiplex PCR. Further, PCR was employed to detect AF-producing genes in Aspergillus species in Iranian pistachio nuts for their aflatoxigenic effect (Rahimi *et al*., 2018). In addition, Kim *et al.* (2019) utilized PCR, ELISA and HPLC for the detection of AFTs from A. oryzae isolated from different Korean foods. HSI uses the integration of both imaging and spectroscopy to record spatial and spectral characteristics of a given sample (Siche *et al*., 2019). The visible/near-infrared (VNIR) HSI has been utilized for the identification of maize kernels of different varieties. VNIR or short-wave (SWNIR) HSI techniques are feasible for the detection of AFTs as well as identification of different

fungal species in maize (Williams *et al*., 2022). Later, Kimuli *et al*. (2018b) used the VNIR-HSI system to detect AFB1 on surfaces of maize kernels from Georgia, Illinois, Indiana and Nebraska of United States. Chu *et al*. (2017) used short-wave infrared (SWIR) HIS to detect AFB1 in single maize kernels. But as the image quality could not effectively classify AFB1 level qualitatively in individual maize kernels, therefore, to improve this Kimuli *et al*. (2018a) further combined the SWIR-HSI system with chemometric data analysis for the better detection of AFB1 on the surfaces of maize kernels. Furthermore, the color-encoded lateral flow immunoassay (LFIA) technique has been used for the simultaneous detection of AFB1 as well as fumonisins in a single test line (Di-Nardo *et al*., 2019).

To further enhance the sensitivity and detection of AFTs in food and feed, nanoparticles (NPs) based on Au/Ag, carbon (CBNs), magnetic (MNPs), Quantum dots (QDs), up-conversion (UCNPs), metal-organic frameworks (MOFs) as well as hybrid nanostructures have been utilized (Xue *et al*., 2019). Rui *et al*. (2019) prepared molecular imprinted polymers (FDU- 12@MIPs) using structural analog of AFTs. This highly selective surface was used as an extraction sorbent in conjunction with HPLC for the detection of AFTs in different food and feed samples. In addition to this, the use of biosensors compared to other spectrophotometric or chromatographic methods allow for higher selectivity, direct detection with minimal sample pretreatment, minimal cost, portability and on-field analysis of mycotoxins (Rotariu *et al*., 2019). Selvolini *et al*. (2019) utilized an electrochemical enzyme-linked oligonucleotide array for easy and quick multi-detection of AFB1 in maize. Furthermore, assays based on aptamer have been developed for the rapid detection of AFB1. Wang *et al*. (2019a) successfully detected the AFB1 spiked in wine, methanol and corn flour samples using the simple aptamer molecular beacon assay, which has the potential for the rapid detection of AFTs in food and feed.

2.3.5.1 Masked mycotoxins as a major concern in aflatoxin detection

Masked mycotoxins pose a major concern in food and feed as they are not identified and detected by the usually employed detection techniques (Kamle *et al*., 2019). These are the mycotoxins produced by fungi but are modified by plant enzymes during the infection stages. They are present in vacuoles in the soluble form or bound to macromolecules, therefore, are unable to be identified by routine analysis processes and referred to as masked mycotoxins. However, the modified AFTs can hydrolyze back into the toxic forms during food processing and/or digestion process (Broekaert *et al*., 2018). Some of these modified toxins are present in different forms as complexes with matrix compounds, hence also referred to as matrix-associated mycotoxins. The masked mycotoxins have been reported to occur in Asia, Africa, America and Europe. Therefore, a high number of masked mycotoxins prevailing in various food and feed can pose serious health issues to both humans and animals (Zhang *et al*., 2019). Therefore, the detection of masked mycotoxins is an essential part to ensure food and feed safety. Masked fumonisins were determined through hydrolysis where modified forms were converted back to their free forms and subsequently analyzed and detected through LC/MS/MS. The hydrolytic process may involve either alkaline, acidic or enzymatic treatments (Vidal *et al*., 2018). However, there is less information available on the masked AFTs as most of the preference is given for the detection of free AFTs in agricultural food and feed. Therefore, methods like in vitro digestion and hydrolysis, as applied in case of masked fumonisins, can be carried out for masked AFTs in food and feed followed by detection with LC/MS/MS and confirmation by other methods like ELISA to ensure the food and feed safety (Beloglazova *et al*., 2018).

2.4 Factors Promoting Aflatoxin Contamination of Food Products

2.4.1 Drying

Drying techniques for various food crops in the country vary among different stakeholders. For instance, the majority of farmers dry maize and groundnuts on bare ground, some on polyethylene sheets or mats while others leave the crop to dry in the field (Broekaert *et al*., 2018). These drying methods are slow and may support growth and development of fungi thus increasing the potential for aflatoxin production. Besides, during the first season of maize in some production zones, harvesting takes place during the months of August – September which are relatively wet. These conditions lead to inadequate crop drying. In order to minimize aflatoxin contamination of maize, it is recommended that the grain should be dried as soon as possible, within 24 to 48 hours to moisture content no greater than 14 percent to reduce infection, growth, and toxic production by Aspergillus (Tao *et al*., 2018).. Maize kernels dried at home on bare ground have been reported to be more contaminated with aflatoxin (41.7%) than those dried on polyethylene sheets/mats (25%). Both the samples dried on bare-ground and those dried-on polyethylene sheets/mats tested positive with aflatoxin B1 only (Tao *et al*., 2018).

2.4.2 Storage

Storage systems of produce in Nigeria have also been found to encourage aflatoxin contamination. Adequate storage facilities are not available especially at farm level. It has been reported that the majority of farmers and traders in Nigeria store maize using woven polypropylene bags, which do not protect the grains against aflatoxin contamination. Grains stored or heaped on the floor (unshelled) and those stored under the verandah had 100% aflatoxin contamination. The only method that protected the grains against aflatoxin contamination was storage above fire racks but this method cannot be adopted for storage of large quantities of grains (Di-Nardo *et al*., 2019). Additionally, some

farmers use out-door storage practices for maize like granaries and silos which do not guarantee maize free from moisture pick-up, mould infection and insect infestation.

At the retail markets, produce is not properly protected from environmental influence during storage. Most of the produce is not properly packaged, always exposed, making it susceptible to infection by mycotoxigenic moulds. Maize flour, pounded/ milled groundnuts and shelled kernels are some of the produce suspected to be highly contaminated by aflatoxins due to their form (Di-Nardo *et al*., 2019).

2.4.3 Moisture content and insect damage

Moisture content and grain physical condition are major factors in moulds and mycotoxin contamination of grains (Cotty and Jaime-Garcia, 2017). Despite slow drying processes and inadequate storage methods, the moisture content and insect damage of maize and groundnuts stored for three to seven months at farm level have been found to be low, within recommended levels. Average moisture content has been reported to be seven to nine percent for groundnuts and eight to 11% for maize (Cotty and Jaime-Garcia, 2017). These grain conditions have been described as major factors in the low aflatoxin levels observed in on-farm produce compared to produce in the markets in which the majority of grains were found to have moisture content above 14% and insect damage three times that of on-farm produce. Maize stored by traders for six to seven months was reported to have mean aflatoxin levels of 107 ppb implying that these grains were not suitable for local nor export markets (Cotty and Jaime-Garcia, 2017).

2.4.5 Physical damage

No relationship between physical damage and aflatoxin content of produce has been reported in Nigeria. However, it appears physical damage of the produce may be one of the factors hastening aflatoxin contamination by promoting mould infection. The majority of farmers in Nigeria shell or thresh maize by manual beating thus, inevitably damaging

the grains and predisposing them to fungal infection. Groundnuts on the other hand, may be uprooted using hand hoes, which cause considerable damage to both the shell and kernels thus promoting fungal infection (Cotty and Jaime-Garcia, 2017).

2.5 Management and Control Strategies of Aflatoxin Exposure

The traditional approach to preventing exposure to aflatoxin has been to ensure that foods consumed have the lowest practical aflatoxin concentrations. In developed countries, this has been achieved for humans largely by regulations that have required low concentrations of the toxin in traded foods. In developed countries, where regulations allow higher aflatoxin concentrations in animals, the agricultural industries have developed alternative approaches [chemoprotection (Galvano *et al*., 2021) and enterosorption] to limit biologically effective exposure without the high cost of preventing contamination.

2.5.1 Production

Although the initial focus of research was on the prevention of contamination in storage, it was established in about 1970 that contamination, or at least invasion by the causal fungi, could start in the field before harvest. For peanuts, environmental conditions such as drought during the grain growth stage, insect damage in the field, variety, and soil characteristics have proven to be determining factors in preharvest contamination (Cole *et al*., 2015). These conditions are now sufficiently well understood for computer simulation models to describe the risk of contamination of major crops (Wright and Nageswara, 2018). The result is that management can be used to minimize contamination, and the practice of inoculating the fields with non-aflatoxigenic strains of fungi may shortly be a new tool in the battle to prevent economic loss. Because of the importance of

drought as a factor predisposing crops to contamination, irrigation is a very important means of ensuring food quality (Cole *et al*., 2015).

More recent developments have made use of biotechnology to introduce genes that either prevent the formation of aflatoxin as a result of fungal metabolism or prevent or decrease fungal action. These approaches offer considerable long-term promise, but time and sizeable investment are still needed before the research can affect human health. In developing countries, many of these preharvest opportunities to minimise contamination are not exploited by producers. Insect damage in the field is not controlled by pesticides or by cultural practices; drought is a common phenomenon, and most crops are produced without irrigation as an option. Harvesting is usually done without machinery, and drying is usually carried out very inefficiently and is dependent on the weather. Adverse weather at harvest results in slow and inadequate drying and brings attendant risks of contamination. However, models are available to aid in decisions affecting aflatoxin risks in production (Wright and Nageswara, 2018).

2.5.2 Storage

It is well understood that much of the contamination of commodities with aflatoxin occurs during storage. To preserve quality in storage, it is necessary to prevent biological activity through adequate drying (≤10% moisture), elimination of insect activity that can increase moisture content through condensation of moisture resulting from respiration, low temperatures, and inert atmospheres (Smith *et al*., 2019). In other words, the conditions needed to prevent the development of contamination are known, but it is not always easy to produce them in storage systems in developing countries. One fact that makes storage such an important issue for these countries is the subsistence nature of most farming there. Most people in rural areas grow and store their own food; in consequence, most food is stored in small, traditional granaries, and there is little investment in the management of

the conditions. Studies of grain quality in such storage structures show a steady increase in the aflatoxin content over time, which reflects the failure to maintain appropriate conditions (Hell *et al*., 2016). Achieving and preserving the conditions that prevent contamination is likely to prove a significant challenge for small-scale (household and farm level) storage and to be beyond the resources of most, even if they could be convinced of the value of making the effort.

2.5.3 Processing

Processing of commodities can be used to reduce the aflatoxin content and thereby prevent economic loss. Three main approaches exist: dilution, decontamination, and separation.

With regard to dilution, where regulations are enforced, the easiest means of satisfying the requirement is (unfortunately) to mix grain low in aflatoxin with grain exceeding the regulated limits. Thus, although the concentration is reduced, consumers are still exposed to the same overall aflatoxin burden. This approach fails when there is not enough "clean" grain to allow adequate dilution of the contaminated stock or when the infrastructure to hold stocks and achieve the desired mixing is lacking.

With regard to decontamination, considerable effort has been expended to develop methods by which contaminated commodities may be treated to denature the aflatoxin. Treatment with ammonia, alkaline substances, and ozone can denature aflatoxins, but whether this change is permanent is not clear. For instance, the processing of corn with caustic soda, as is used in traditional Mesoamerican cooking, has been shown to reduce the aflatoxin content, but there is some evidence both that the chemical change may be reversible and that, after consumption, the aflatoxin may be reformed in the acid conditions in the stomach (Bailey *et al*., 2017; Phillips *et al*., 2018).

With regard to separation, considerable success in reducing aflatoxin contamination can be achieved by separating contaminated grain from the bulk. This approach depends on the heavy contamination of only a small fraction of the seeds, so that removing those leaves a much lower overall contamination. Study of the distribution of aflatoxin in peanuts shows that a major portion (80%) of the toxin is often associated with the smaller and shriveled seed, and thus screening can lower the overall concentration in the bulk. Further removal of aflatoxin contaminated seeds may be achieved by color sorting, which, in the case of peanuts, is most effective when the seeds are blanched. A consequence of this sorting approach to aflatoxin that is a serious concern is the fate of the now highly concentrated aflatoxin in the grain removed from the bulk. The poorest producers and laborers often consume those nuts, which should have been discarded, or they feed them to their animals (Davidson *et al*., 2018).

2.5.4 Chemoprotection

The reality is that much of the grain fed to animals is contaminated, and this condition results humans' exposure to aflatoxins. Chemoprotection against aflatoxins has been demonstrated with the use of a number of compounds that either increase an animal's detoxification processes or prevent the production of the epoxide that leads to chromosomal damage (Wang *et al*., 2019a). One technical solution is drug therapy, because several compounds, such as oltipraz and chlorophyll, are able to decrease the biologically effective dose. However, sustained long-term therapy is expensive, may have side effects, and is not likely, given the health budgets of developing countries and their other pressing health problems (Wang *et al*., 2019b).

For the animal feed industries, a major focus has been on developing food additives that provide protection from the toxins. One approach has been the use of esterified

glucomanoses and other yeast extracts that provide chemoprotection by increasing the detoxification of aflatoxin (Kensler *et al*., 2018).

2.5.5 Enterosorption

Another approach has followed the discovery that certain clay minerals can selectively adsorb aflatoxin tightly enough to prevent their absorption from the gastrointestinal tract. Whereas many toxins are adsorbed to surface-active compounds, such as activated charcoal, the bonding is not often effective in preventing uptake from the digestive system. Various sorbents have different affinities for aflatoxins and therefore differ in preventing the biological exposure of the animals consuming contaminated foods. There have been several claims for different adsorption agents, but their efficiency in preventing aflatoxicosis varies with the adsorbent (Wright and Nageswara, 2018).

With enterosorption, there is also a risk that nonspecific adsorbing agents may prevent the uptake of micronutrients from the food. In vitro tests of hydrated sodium calcium aluminosilicates (HSCAS) suggest that there is little adsorption of micronutrients (Dorner *et al*., 2016). The use of HSCAS additives in contaminated feeds has proven effective in preventing aflatoxicosis in turkeys, chickens, lambs, cattle, pigs, goats, rats, and mice. The use of radiolabeled aflatoxin shows that the addition of clay in a proportion of 0.5% of the volume to a contaminated feed reduced exposure in chicks by ≤ 95% (Dorner *et al*., 2016). Selected calcium montorillonites have proven to be the most highly selective and effective of these enterosorbents. This approach is now widely used in animal production industries worldwide, and HSCAS is estimated by one manufacturer to be added to 10% of all animal feeds (Dorner *et al*., 2016).

2.6 Risk Assessment of Aflatoxins in Food.

2.6.1 Risk analysis

Risk analysis is increasingly seen as an essential component in modern science-based food safety systems and plays a growing and important role in guiding food safety authorities. In various formats, it can be applied to chemical, physical or microbiological threats to food safety. Although a relatively new concept, which still continues to evolve as a scientific tool, the risk analysis framework is generally considered to constitute three interlocking processes, namely risk assessment, risk management and risk communication (FAO/WHO, Food and Agriculture Organization of the United Nations/World Health Organization., 2016). Informed by the risk assessment process, risk management in its broadest sense involves the consideration and implementation of food policy options, while taking due cognizance of acceptable levels of risk. Risk communication involves the interchange of information concerning risk and its perception among all stakeholders in food safety, including policy makers, industry and consumers. Risk assessment has been defined as ‘‘a process of evaluation including the identification of attendant uncertainties, of the likelihood and severity of an adverse effect(s)/event(s) occurring to man or the environment following exposure under defined conditions to a risk source(s)’’ (EC, 2002).

Risk assessment is performed as an objective science-based process, distinct from the other risk analysis components which it guides and informs. It may frequently be seen as an iterative process in which risk-management requirements can formulate the problem to be addressed by risk assessment. Risk assessment is divided into four stages, namely hazard identification, hazard characterization, exposure assessment and risk characterization. In earlier literatures, the former two stages were collectively referred to as hazard assessment. It is important to distinguish between the concepts of hazard, an

inherent property of a chemical (or mycotoxin) to cause adverse health effects, and risk, which is a probability of the occurrence of this adverse effect. In applying the principles of risk assessment to mycotoxins, a number of factors need to be recognized. Unlike food additives, mycotoxins are natural contaminants formed as secondary metabolites by toxigenic fungi in the field and/or during storage. Their levels are unpredictable and can vary both temporally between seasons and spatially between different growing areas or under different storage conditions (Gordon, 2018).

As the elimination of mycotoxins is, generally, not possible, risk assessments are undertaken to guide food regulators and scientists in undertaking risk management processes, such as the setting of legislative levels or guideline targets for mycotoxins contaminations in food supplies. Although legislation can be an effective management tool in the market economies of the developed world, the situation within rural subsistence communities is more complex and less easily addressed. For the above reasons, risk assessment is an important tool in evaluating potential health implications of mycotoxin exposure (Gordon, 2018).

2.6.2 Risk analysis of aflatoxin

Aflatoxins were first discovered as a result of the deaths of thousands of turkeys in the UK in 1960 from a previously unknown condition termed Turkey X disease. Since then aflatoxins have been the focus of enormous scientific interest and innumerable investigations. Much of the research can be found summarized in the outputs of various international meetings and assessments (WHO, 2018). In brief, the main producers of aflatoxins are the toxigenic fungi Aspergillus flavus and A. parasiticus. The toxins can be produced in the field prior to harvest, or alternatively, can arise due to fungal growth under poor storage conditions. A recent survey of published research on aflatoxin contamination of foods in Africa reported data from over 20 African countries (Shephard,

2018). The commodities that are affected include staple African foods such as nuts, maize, sorghum, pulses and coconut. The effects of maize storage have been studied in Benin (Hell, *et al*., 2016). During 6 months storage, the greatest increase in contamination was observed in the Sudan savannah agroecological zone, where the percentage contamination below 5 mg/kg dropped from 90.1 to 67.8% and the percentage above 100 mg/kg increased from 2.2 to 24.2%. Studies on aflatoxin biomarkers in west Africa have indicated extensive exposure (Gordon, 2018). This exposure can occur in humans of all ages in Africa, including in utero, as aflatoxin has been detected in umbilical cord blood at birth. Evidence has been presented to indicate metabolic activation of AFB1 in fetal liver. The presence of aflatoxin in breast milk and weaning food in certain African countries indicates that exposure of human infants can begin at the earliest age and continue through life (El-Sayed *et al*., 2020).

Exposure to high levels of aflatoxin can result in acute human aflatoxicosis leading to jaundice, oedema, GI haemorrhage and, ultimately, death. There have been various reported outbreaks of human aflatoxicosis in Africa, including a recent outbreak in the eastern and central provinces of Kenya in 2004 in which over 120 people died (Probst *et al*., 2017). Apart from these acute effects, aflatoxins have a wide range of negative health consequences and have been shown in many studies to be hepatotoxic, teratogenic, mutagenic, genotoxic and hepatocarcinogenic. Of the literature detailing the adverse effects of aflatoxins, most notable is the data on hepatotoxicity and hepatocarcinogenicity in a variety of animal species and the human epidemiological evidence of an association between aflatoxin exposure and primary liver cancer. The International Agency for Research on Cancer (International Agency for Research on Cancer IARC., 1993) evaluated aflatoxins as follows: ‘‘There is sufficient evidence in humans for the carcinogenicity of aflatoxin B1’’ and ‘‘there is sufficient evidence in humans for the

carcinogenicity of naturally occurring mixtures of aflatoxins''. Hence, both AFB1 and naturally occurring mixtures were evaluated as group 1 carcinogens (Gordon, 2018).

Although the link between primary liver cancer and aflatoxin exposure has long been known, recent concerns have been expressed that the wider health implications, such as growth retardation and immune suppression, observed in veterinary studies, have until recently been ignored in human studies and could play a large role in the disease burden of affected communities (Williams *et al*., 2022). Aflatoxin exposure depresses cell-mediated immunity, which could have important consequences in the light of the burden of infectious diseases in Africa. Direct evidence for immune suppression caused by aflatoxin exposure has been found in reduced levels of secretory IgA in Gambian children who showed the presence of aflatoxin– albumin adducts in serum (Turner *et al*., 2017).

Further evidence that aflatoxins could cause impairment of human cellular immunity and decrease resistance to infection has been found in studies among Ghanaian adults in which changes in the constitution of lymphocyte subsets have been correlated with aflatoxin exposure. Recent studies in Benin and Togo among children aged 9 months to 5 years have highlighted a further health consequence of aflatoxin exposure, namely stunting in children in which a growth faltering occurs at the time of weaning due to consumption of aflatoxin-contaminated foods (Gordon, 2018). More recently, evidence has emerged that maternal aflatoxin exposure during pregnancy can also impact neonate growth during the first year of life (Turner *et al*., 2017). Although kwashiorkor is widely thought to be a form of protein energy malnutrition, some characteristic features of the disease are known to be among the pathological effects caused by aflatoxins in animals. It has been suggested that either aflatoxins could play a causal role in the disease or children suffering from the disease are at greater risk to the hazards of dietary aflatoxin (Adhikari *et al*., 2019).

A number of international risk assessments of mycotoxins have been performed. In particular, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) performed a quantitative risk assessment in 1998 on aflatoxin B1 (World Health Organization, WHO, 1998). Existing data from epidemiological studies in human populations, especially in Africa and China, and animal toxicity studies were employed. Based on published epidemiological evidence of primary liver cancer, JECFA arrived at estimates of AFB1 carcinogenic potency. These estimates can be combined with exposure assessments performed in African countries on staple foods to arrive at a risk characterization of relevance for African food safety authorities. As increasing numbers of African countries institute regulations for aflatoxin, it is important to highlight the fact that the meeting of these limits on individual foods does not of itself ensure food safety, as significant aflatoxin exposure may arise from excessive consumption of the ‘‘aflatoxin-safe’’ foodstuff (Gordon, 2018).

2.6.2.1 Risk assessment for aflatoxin B1 based on carcinogenic potency

I. Hazard identification

Hazard identification has been defined as ‘‘the identification of biological, chemical and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods’’. As briefly outlined in the above introduction, a vast literature exists describing the adverse human health effects of aflatoxins, which supports the IARC assessment of AFB1 and natural mixtures of aflatoxins as group 1 carcinogens (International Agency for Research on Cancer IARC., 1993)

II. Hazard characterization

Hazard characterization has been defined as ‘‘the qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical and physical agents, which may be present in food. For chemical risk assessments, a dose-

response assessment is performed'' (FAO/WHO, Food and Agriculture Organization of the United Nations/World Health Organization., 2016). For mycotoxin assessments, dose–response effects have been considered in two different formats. For the non-genotoxic mycotoxins, a threshold for adverse effects has been considered to exist. As a surrogate for this threshold level, a no observed adverse effect level (NOAEL) has been taken from the most relevant animal toxicity study and a tolerable daily intake (TDI) estimated by application of a safety factor approach. This approach was used by JECFA to assign provisional maximum tolerable daily intakes for fumonisins, ochratoxin A, deoxynivalenol and T-2 toxin (FAO/WHO, Food and Agriculture Organization of the United Nations/World Health Organization., 2016). In 1998, JECFA performed a quantitative risk assessment of AFB1 (World Health Organization, WHO, 1998).

As a genotoxic carcinogen, it was considered to have no threshold level for toxicity and, hence, a NOAEL approach was not considered appropriate. Based on published epidemiological evidence of primary liver cancer, JECFA arrived at estimates of AFB1 potency. In assessing AFB1, it was realized that due to the synergistic hepatocarcinogenic effects of aflatoxin B1 and hepatitis B virus infection, two potencies should be specified. In hepatitis B surface antigen positive (HBsAgþ) individuals, the potency was 0.3 cancers per year per 100,000 population per ng AFB1 kg/body weight (b.w.) per day. In hepatitis B surface antigen negative (HBsAg_) individuals, the potency was 0.01 cancers per year per 100,000 population per ng AFB1 kg/b.w. per day. There is uncertainty in these estimates of potency due to a number of factors: the epidemiological data has come from areas of both high AFB1 and high HBsAgþ levels; the reliability and precision of AFB1 exposure assessment in the study population are unknown; possible exclusion of studies showing no association between AFB1 and liver cancer; measured AFB1 exposure levels do not represent levels at the time of cancer induction; early method limitations in

detecting hepatitis B; non- confirmation of primary liver cancer by histology (European Food Safety Authority (EFSA), 2017). Although potencies estimated from animal studies fall within this same range, extrapolation to humans is difficult due to the fact that the shape of the dose– response relationship is unknown and also due to large differences that occur between animal species because of differences in rates of AFB1 activation and detoxification (World Health Organization, WHO, 1998).

III. Exposure assessment

Exposure assessment has been defined as ‘‘the qualitative and/or quantitative evaluation of the likely intake of biological, chemical or physical agents via food, as well as exposures from other sources if relevant’’ (FAO/WHO, Food and Agriculture Organization of the United Nations/World Health Organization., 2016). Whereas hazard identification and characterization relate to universal properties of the contaminant, exposure assessment is a variable across populations and subgroups of populations. It is dependent on the levels of contamination present and on the quantities of contaminated food consumed by individuals. The combination of statistical modelling, such as the Monte Carlo method, and data on food contamination levels and consumption provides a powerful tool in determining the distribution of toxin exposure in different communities. Although it is best represented by distributional data, the lack of detailed information on contamination levels and consumption patterns in African countries militates against the use of probabilistic models. Nevertheless, single-point determinations based on mean levels can provide insights into the mycotoxin exposure of African populations. As it is a function of both contamination levels and food consumption, high exposures can result from either high contamination levels of foods consumed in moderate amounts or high consumption of moderately contaminated foods. Unfortunately, in many rural African

subsistence farming communities, the staple foods consumed in high amounts are also contaminated at significant levels – a doubly adverse circumstance (Gordon, 2018).

IV. Risk characterization

Risk characterization has been defined as ‘‘the qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment’’ (FAO/WHO, Food and Agriculture Organization of the United Nations/World Health Organization., 2016). The paragraphs above have provided a case study of hazard identification and characterization for AFB1, as well as examples of exposure assessments. These processes can be combined in the final stage of risk assessment to provide a risk characterization. In many instances, the data on food contamination is restricted to total aflatoxin. Since AFB1 is the most abundant of the aflatoxin analogues and also the most biologically active, it is considered prudent, as part of a conservative and precautionary principle, to use this data for risk characterization, despite the toxicological evidence being based on the effects of AFB1 (European Food Safety Authority (EFSA), 2017).

2.6.2.2 Risk assessment for aflatoxin B1 based on growth retardation

Although risk assessments based on the above carcinogenic risk method have been used to assess the impact of various regulatory limits by the EC Scientific Panel on Contaminants in the Food Chain (2007) and by JECFA (WHO, 2018) and have been further extended by JECFA to a quantitative risk assessment of aflatoxin M1 more recent concerns have been expressed that human populations are also susceptible to the other toxicological effects of aflatoxins. In particular, evidence has recently emerged that the weaning of infants on to aflatoxin-contaminated foods is associated with growth faltering, which, in its most severe form, is manifested as stunting (Gordon, 2018). Given the

importance of stunting, which is a reflection of chronic malnutrition, on childhood development, a risk assessment based on growth retardation would appear to be essential. Even though the available published data is extremely limited, a first estimate can be made which would add value to our understanding of the health implications of aflatoxin exposure (Gordon, 2018).

The original data that demonstrated the growth faltering in young children aged 9 months to 5 years in Benin and Togo linked aflatoxin–albumin adduct biomarkers in serum with height-for-age Z-scores. For risk assessment, it is desirable to link the health outcome (growth faltering as assessed by decreased Z-scores) with direct aflatoxin exposure. For this purpose, it is first necessary to link the AFB1–albumin serum biomarker with an assessment of the actual AFB1 exposure (Gordon, 2018).

2.6.2.3 Risk assessment for aflatoxin B1 based on immunotoxicity

AFB1 has been shown to be immunomodulatory in a number of animal species (International Agency for Research on Cancer IARC., 1993). Among other effects, it suppresses cell-mediated immune response and reduces antibody formation. Only recently has evidence been gathered that this immune suppression is also observable in human populations in west Africa. Studies of children in Gambia provided strong evidence that a reduction in salivary secretory IgA may be associated with exposure to aflatoxin (Turner *et al*., 2017). However, the study failed to detect a dose–response effect when sIgA levels were separated by quintiles of aflatoxin– albumin biomarker levels. Studies in a Ghanaian population exposed to aflatoxin showed impairments in cellular immunity related to aflatoxin exposure (Jiang *et al*., 2018).

Given the known immune suppression effects in animals and the recent indications that related effects may be present in humans exposed to aflatoxin, the potential implication for human health in various communities where the burden of infectious disease is

elevated has been highlighted (Williams *et al*., 2022). A preliminary risk assessment would help clarify the potential risk to human health. As the human data is limited, it is informative to consider data obtained from animals, particularly rat and mouse studies. The immunomodulatory effects of aflatoxin have been reviewed by a number of international organizations (European Food Safety Authority (EFSA), 2017).

2.7 Cassava Plants

Cassava or manioc (*Manihot esculenta*) crop is widely grown as a staple food and animal feed in countries of tropical and sub-tropical Africa, Asia and Latin America with a total cultivated area over 13 million hectares, more than 70% of it being in Africa and Asia (El-Sharkawy, 2018). Cassava is a cheap, readily available and reliable source of carbohydrates, particularly in case of food shortage. Cassava requires less labour, water, fertilizer and pesticide input and provides more dietary energy per land unit, being one of the most efficient convertors of solar energy. The leaves and roots are the nutritionally valuable parts of the crop, and they make up 6% and 50% of the mature plant, respectively. The genus Manihot belongs to the family Euphorbiaceae and is also called Tapioca, Mandioca, Yucca, and Manioc in different languages (Burrell, 2019). Cassava (*Manihot esculenta*) is a major root crop and an important staple food for over 500 million people in the developing world (Falade and Akingbala 2019). The crop is traditionally produced in small-scale family farms and mostly processed and consumed at household level. This drought-tolerant crop has historically played an important role for famine prevention in Eastern and Southern Africa (Nweke, 2018).

2.7.1 Biological description

Cassava (Manihot esculenta) is a woody perennial shrub with tuberous roots. It is an outbreeding species possessing $2n = 36$ chromosomes and is considered to be an amphidiploid or sequential allopolyploids. Cassava is a dicotyledonous perennial plant

growing in areas with tropical climate and ranging from 1 to 5 m in height. cassava is mainly grown for its starchy tubers, producing 5 to 10 tubers per plant. The plant is drought-resistant, adaptive to harsh climatic conditions, productive in marginal soils, and flexible in planting and harvesting seasons (Haggblade *et al*., 2022). These admirable agronomic traits make it a reliable and low-cost vegetative. It is a hardy crop able to grow in dry and nutrient-depleted soils where other crops have failed. In addition, it is grown all year round and can be harvested anytime from 7 up to 18 months after planting (Balagopalan *et al*, 2018).

2.7.2 Geographic distribution

Thirty countries (18 in Africa, 4 in Latin America and 8 in Asia) are considered to be major global cassava growers, each producing from 1 million tonnes to over 50 million tonnes annually (FAOSTAT, 2014). The top five cassava producing countries are Nigeria, Thailand, Indonesia, Brazil and the Democratic Republic of the Congo. The global production of cassava exceeded 270 million tonnes in 2014, the top producers having together produced 74% of it (FAOSTAT, 2014). The species in the genus *Manihot* are native to the New World, falling into two distinct groups, one in Central America and the other in South America. Mexico and Brazil have the greatest number of *Manihot* species (Nassar, 2020). Cultivation of cassava is largely limited to the tropics, where the annual mean temperature is greater than 18°C (Kawano, 1980). Only a few *Manihot* species (e.g. *M. neusana* and *M. grahamii*) can survive in areas where frost occurs (Nassar and Ortiz, 2016). Cassava can tolerate drought but performs well at annual rainfall of 600-1 500 mm and temperatures of 25-29°C (Nassar and Ortiz, 2016). It is grown throughout all tropical regions of the world between latitudes 30°N and 30°S and at up to 2 000 m altitude, where day length is 10-12 hours (Alves, 2022). After centuries of cultivation and landrace selection, there are many varieties developed for specific landscapes, elevations,

temperatures and soil types (El-Sharkawy, 2018). *M. glaziovii* (*M. carthagenensis* ssp. *glaziovii*) was brought to Africa as a source of rubber. It is the only species within *Manihot* that is known to have naturalized in Africa.

2.7.3 Cassava root

Cassava roots develop radially around the base of the plant forming five to ten tubers per plant (Fig. 2.1). The mature tubers can be 5–10 cm in diameter and 15–30 cm long when harvested 9 – 12 months after planting (Tewe and Lutaladio, 2019). The tubers differ in weight, size and shape and are usually cylindrical and tapered. They may be white, brown or reddish in color depending on the variety. Cassava requires essential nutrients for optimum productivity. Some of the elements are required in trace amount. Some of these heavy metals that are required by cassava at trace concentration include nickel, chromium, zinc, copper, manganese, iron etc. While some others such as cadmium, lead, arsenic, mercury etc do not have any known biological function and as such its presence in biodiversity depicts contamination/ toxicity (Aloys and Hui Ming, 2016). The enlarged tuberous roots are the main carbohydrate storage locations in cassava, and they are important not just because they form the bulk weight of the plant but because they are the main part of the plant consumed (Montagnac *et al*., 2019).

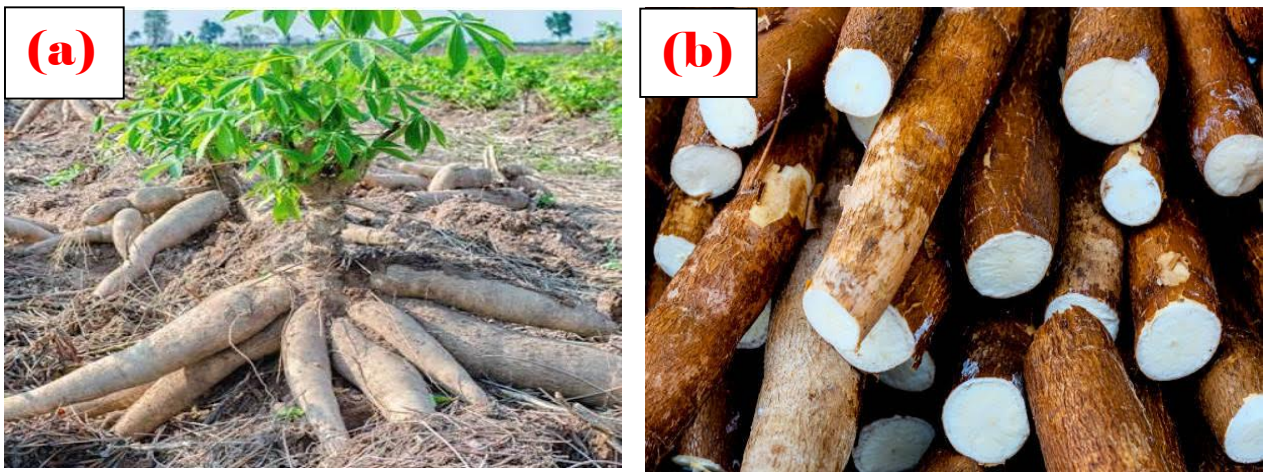

Figure 2.1: (a); Freshly harvested cassava plant with roots; (b): cassava tubers showing the inner white core (IITA, 2021).

2.7.4 Cassava propagation

Cassava is produced mainly by resource-limited small farmers, virtually without purchased inputs, by virtue of its remarkable tolerance to abiotic stresses and adverse environments, in contrast with the capital intensive and input-demanding Green Revolution cereal crops such as wheat, rice and maize. It is grown in marginal, low-fertility acidic soils under variable rain-fed conditions ranging from less than 600 mm per year in semi-arid tropics (De Tafur *et al.*, 2017) to more than 1000 mm in the sub-humid and humid tropics (Pellet and El-Sharkawy, 2017). Although cassava requires a warm climate (>20 ◦C mean day temperature) for optimum growth and production, and for maximum leaf photosynthesis (with an optimum leaf temperature of 25–35 ◦C; El-Sharkawy *et al.*, 2018), it is often cultivated in the high-altitude tropics (up to 1800 m above sea level) and in the sub-tropics with a lower mean annual temperature where crop growth is slower (Irikura *et al.*, 2019), leaf photosynthetic activities are reduced and storage roots bulking and harvesting time are much delayed compared to what occurs in the warmer climates of the lowland tropics.

The crop is vegetatively propagated by mature woody stem cuttings (or stakes, 15–30 cm long) planted horizontally, vertically, or inclined on flat or ridged soils at densities ranging from 5,000 to 20,000 cuttings per hectare, depending on the cropping system and purpose of production (Keating *et al.*, 2018). Seeds are used mainly in breeding programs, though its use in commercial cassava production is a promising option to obviate constraints, particularly diseases, associated with vegetative propagation (Iglesias *et al.*, 2019). Storage roots are generally harvested 7–24 months after planting, depending on cultivar, purpose of use and growing conditions.

2.7.5 Cassava production

Africa stands for half of the world's production of cassava. Since 1960, cassava production has tripled to 87 million milli tons (MT) per year in 1999 and the yield has doubled to around 13 tonnes per ha (Nweke, 2018). In 1999, Nigeria produced 33 million tons while a decade later it produced approximately 45 million tons, which is almost 19 % of world total production in recent time (Adekanye *et al*., 2019). As from 2000 till date, the average yield per hectare has been about 10.6 tons (IITA, 2018). With Nigeria being the highest producer of cassava in Africa, which makes it the most important agricultural. Cassava research was earlier focused on improved yields, better cultivation practices and crop protection but since 1985, it has also encouraged mechanized processing, quality control and development of new products (Adebowale *et al*, 2018). This development has transformed cassava into a commercial cash crop aimed for urban consumers (Nweke, 2018).

Utilization of cassava root cuts across various areas: food for humans, feed for livestock, and raw material for various industries, such as manufacturers of textiles, paper, biofuel, confectionary products, and adhesives (Falade and Akingbala, 2019). In Africa, which is the largest producer of cassava in the world, over 80% of the root produced is used for human consumption as a major staple item. In Latin America, about 40% of cassava produced is used for human consumption, while in Asia, most of the products from cassava are exported (Omodamiro *et al*., 2017). Cassava supplies about 200–500 cal/day for households in developing countries, and different forms of foods can be processed from the root. A major setback to the utilization of cassava is that it deteriorates rapidly and cannot be stored for more than a few days after harvesting; therefore, the roots are quickly processed into stable products such as cassava chips and flour (Udoro *et al*., 2018). The cassava root (*Manihot esculenta*) significantly contributes to food security,

incomes, and employment opportunities in the rural areas of Sub-Saharan Africa, especially in Nigeria, the world's largest cassava producer (Omodamiro *et al*., 2017). Significant post-harvest deterioration of fresh cassava roots occurs because of the natural high moisture content, which accelerates microbial deterioration and undesirable biochemical changes in the products. Processing is used to extend the shelf life, facilitate transport and, most importantly, detoxify the roots by removing the inherent cyanogens (Omodamiro *et al*., 2017).

2.7.6 Cassava as a staple food

Cassava (*Manihot esculenta* Crantz) has been used as a staple food of many nations. Its tuber—the swollen root of the plant—is the most popular form of consumption, although the leaves are also consumed at times for medicinal purposes. Cassava has the greatest conversion in terms of transforming solar energy into soluble carbohydrates per unit of area (Tonukari, 2019). Among the starchy staples, cassava gives a carbohydrate production that is about 40% higher than rice and 25% more than maize. Cassava also consists of essential micronutrients, such as vitamins A, B and C, iron and Zinc, even though it is considered not having a limited nutritional value. It is a major source of carbohydrate for many populations, and it is the third largest source of carbohydrate in the world with Africa being the largest centre of production (Adenle *et al*., 2022). Cassava is increasingly popular with African farmers because of its agricultural advantages and potential to feed rapidly increasing populations. Nigeria is the largest producer of cassava out of all the African countries. It is the third largest producer of cassava in the world after Brazil and almost double the production of Indonesia and Thailand (Oriola and Raji, 2018). It is noteworthy in this aspect that households under stress from HIV/AIDS are switching from high-input to low-input farming systems that involve cassava. With these

developments, cassava has undoubtedly been touted as one of the major crops around the world as a source of income as well as for food security purposes (FAO, 2017).

Cassava is a major source of staple food to several families in tropical Africa (Emurotu *et al*., 2022) especially in Nigeria that is the largest producing nation in the world (Izah *et al*., 2017). Cassava cultivation and processing is carried out predominantly by smallholder in Nigeria. According to Kigigha *et al*. (2017), cassava cultivation, processing and marketing of its associated products is a major source of livelihood to several families especially in rural setting in Nigeria. Cassava is a typical carbohydrate food crop. Kigigha *et al*. (2017) is with the opinion that cassava meals provide energy for over 2 billion people in the tropical regions. Cassava is typically used as food in the form of garri, *lafun* and *fufu*, livestock feeds, confectionaries, sweeteners production, additives to several pasteries such as bread cookies, biscuits and rolls, doughnut, cakes, flakes etc. especially in private house hold level (Emurotu *et al*., 2022) Beside food, cassava have also found application in several sector including glues, textiles, pharmaceuticals and bioethanol production (Ukwuru and Egbonu *et al*., 2019)

2.7.7 Nutritive and anti-nutritive properties of cassava

The nutritional composition of cassava depends on the specific tissue (root or leaf) and on several factors, such as geographic location, variety, age of the plant, and environmental conditions. The roots and leaves, which constitute 50 and 6% of the mature cassava plant, respectively, are the nutritionally valuable parts of cassava (Tewe and Lutaladio, 2019). The nutritional value of cassava roots is important because they are the main part of the plant consumed in developing countries. Cassava root is an energy-dense food. In this regard, cassava shows very efficient carbohydrate production per hectare. It produces about 250,000 calories/hectare/day (Julie *et al.*, 2019), which ranks it before maize, rice, sorghum, and wheat. The root is a physiological energy reserve with high

carbohydrate content, which ranges from 32 to 35% on a fresh weight (FW) basis, and from 80 to 90% on a dry matter (DM) basis (Julie *et al*., 2019). Eighty percent of the carbohydrates produced is starch (Gil and Buitrago, 2018); 83% is in the form of amyl pectin and 17% is amylose (Rawel and Kroll, 2018). Roots contain small quantities of sucrose, glucose, fructose, and maltose (Tewe and Lutaladio, 2019). The composition changes slightly with increasing age as the roots become more fibrous and the starch content declines. Cassava is a poor source of protein as it contains only 1-3% protein on dry matter basis (Montagnac *et al*, 2019) and is low in essential amino acids such as methionine, lysine, tryptophan, phenylalanine and tyrosine (Falade and Akingbala, 2019). A cassava-based diet therefore requires an adequate protein source of good quality to prevent nutritional deficiency symptoms (Balagopalan *et al.*, 2018).

The fibre content in cassava roots content does not exceed 1.5% in fresh root and 4% in root flour (Gil and Buitrago, 2018). The lipid content in cassava roots ranges from 0.1 to 0.3% on a FW basis. Cassava roots have calcium, iron, potassium, magnesium, copper, zinc, and manganese contents comparable to those of many legumes, with the exception of soybeans. The calcium content is relatively high compared to that of other staple crops and ranges between 15 and 35 mg/100 g edible portion. The vitamin C (ascorbic acid) content is also high and between 15 to 45 mg/100 g edible portions (Charles *et al.*, 2019). Cassava roots contain low amounts of the B vitamins, that is, thiamine, riboflavin, and niacin, and part of these nutrients is lost during processing. However, the carbohydrates, determined by the nitrogenfree extract, are more concentrated in the peeled root (central cylinder or pulp) (Gil and Buitrago, 2018). Thus, cassava roots are rich in calories but low in protein, fat, and some minerals and vitamins. Their nutritional value is, consequently, lower than those of cereals, legumes, and some other root and tuber crops

such as potato and yam. The leaves on the other hand provide protein, vitamins and minerals (Tewe and Lutaladio, 2019).

Cassava also contains its own share of anti-nutrients, which have either positive or negative effects on the health, depending upon the amount of the component being ingested (Wobeto *et al*., 2017). They basically interfere with the digestibility and uptake of some nutrients. Nevertheless, depending on the amount consumed, these substances can also bring benefits to humans. Cyanide is the most toxic factor restricting the consumption of cassava roots and leaves. Several health disorders and diseases have been reported in cassava-eating populations, owing to the presence of improperly processed cyanide (Montagnac *et al*, 2019). The consumption of lower cyanide amounts is not lethal but long-term intake could cause severe health problems such as tropical neuropathy. The nitrate content in cassava leaves ranges from 43 to 310 mg/100 g DM (dry matter) (Wobeto *et al*., 2017). Cassava-eating populations are naturally exposed to high amounts of cyanide, nitrates and nitrites—chemical compounds which are known to contribute to the risk of developing stomach cancer. Cassava-eating individuals tend to have a high amount of thiocyanate in the stomach due to cyanide detoxification by the body, which may catalyse the formation of carcinogenic nitrosamines (Ernesto *et al*., 2022).

2.7.8 Cassava for ensuring food security

Food security has become a growing concern around the world. Coupled with inadequate caloric intake, food insecurity is a major cause of death and morbidity in the world, particularly in developing countries (Sayre *et al*., 2016). The major staples of rice, wheat, maize and soybean are now recognized as not being the complete solution to world food security. Diversification of farming of agricultural crops and food production has been recognized as a need, extending towards coarse grains, roots and tubers, pulses and oilseeds (Fischer *et al*., 2022). In this aspect, cassava has been recognized as a crop that

is able to address the global food security needs around the world. It has been biotechnologically manipulated for better growth and higher crop production for this purpose. Good yield progress has been achieved for cassava crops after relatively few decades of genetic improvement compared with other staples, which are being bred and harvested for food security purposes. Adoption of new varieties of cassava has been strong in Thailand, Vietnam and Nigeria (Fermont *et al*., 2019). Given the current practice of minimal use of inputs, great scope also exists for closing the large yield gap of cassava production through better agronomy. For this purpose, commercialization of the cassava cultivation in Sub-Saharan Africa should help close the gap by providing stimulus for farmers to invest in more inputs (Okechukwu and Dixon, 2018).

2.7.9 Postharvest deterioration of cassava roots

Given the marginal environments where cassava is grown, its postharvest processing is frequently affected by large distances to the processing centres and deficient transport infrastructure, specifically roads (Morante *et al*., 2020). Cassava roots are also bulky, containing approximately 65% water, which leads extensively to the postharvest physiological deterioration (PPD). The short shelf life of the roots hinders many of the marketing options by increasing the likelihood of losses and thereby increasing the overall marketing costs. In addition, the access to urban markets and processing facilities is restricted to production sites that are relatively close to them (Pfeiffer *et al*., 2017).

Research to date concerning the study of PPD has mostly focused on biochemical signaling events several hours after harvest (Iyer *et al*., 2020). Upon examination of physiological and biochemical changes occurring after cassava root detachment, changes in the nature and type of volatile compounds emitted, secondary metabolites accumulated, and changes in the expression of key genes in reactive oxygen species (ROS) turnover had been primarily observed. Nevertheless, based on combined proteomics data,

enzymatic activities, and lipid peroxidation assays, Vanderschuren *et al*. (2019) for instance have identified glutathione peroxidase as a candidate for reducing PPD.

2.7.10 Toxic components of cassava

It is well established that cassava is not edible raw due to the presence of toxic compounds. Cassava contains two cyanogenic glucosides, namely linamarin and lotaustralin, present in all parts of the plant with the highest concentration in the root peel (Falade and Omojola 2019). Structure of some common cyanogenic glucosides (Fig 2.2). Normal levels of cyanoglucosides range from 31 to 630 ppm calculated as mg HCN/kg of fresh cassava root, although the content varies considerably depending upon variety, climate and environmental conditions. Sweet cassava varieties have often lower levels of cyanide than bitter varieties but there is no established correlation between the taste and the toxicity (Falade and Akingbala 2019). Hydrolyzing enzymes present in the plant, such as linamarase, degrade the cyanoglucosides to hydrogen cyanide (HCN) as soon as the plant tissue is wounded. If the root is ingested without previous processing, acute poisoning occurs due to the release of HCN in the body. Cyanide affects tissue respiration in mitochondria's, as it is a potent inhibitor of oxidase and other important enzymes in the respiratory chain (Balagopalan *et al.,* 2018). Chronic exposure of inadequately processed cassava can lead to diseases such as tropical ataxic neuropathy, goiter and cretinism.

The toxicity can however be reduced to safer levels during traditional processing (Falade and Akingbala 2019). Processing of cassava eliminates or reduces the level of toxic cyanogenic glucosides that result in production of more acceptable hygienic quality products and improves root palatability which enhance the shelf-life and facilitate transportation and marketing of products (Haggblade *et al.*, 2022). All parts of the cassava

plant contain cyanogens that are hydrolyzed to hydrocyanic acid (HCN) that escapes into the air during harvesting and processing (Bokanga *et al.*, 2019).

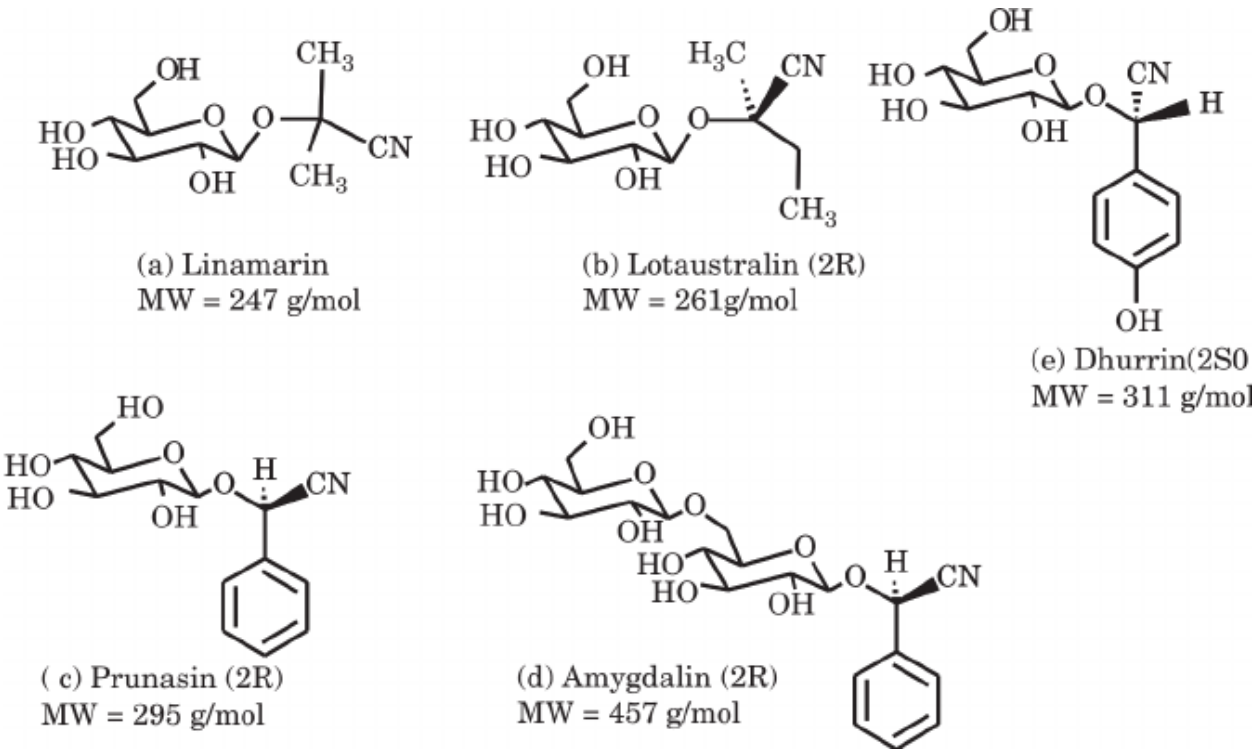

Fig. 2.2: Structure of common cyanogenicglucosides (Source: Halkier *et al*., 2018)

2.7.11 Cassava processing

Freshly harvested cassava roots start deteriorating almost immediately after harvest. This is due to its high moisture content. Thus, the best form of preservation of cassava is drying into pellets or chips or processing into flour. The traditional methods of processing cassava roots into various types of food have been adapted to suit the many attributes of the plant such as root yield, spoilage, cyanide content, nutrient content and process ability (Falade and Akingbala 2019).

2.7.11.1 *Fermentation*

Fermentation, either naturally or with selected microbial inoculums, has been extensively used to enhance the nutrient potentials of cassava and its by-products both for human and

livestock consumption (Sani and Farahni, 2021). Fermentation is an important processing technique for cassava, especially in Africa. Three major types of fermentation of cassava roots are recognized: the grated root fermentation, fermentation of roots under water and mould fermentation of roots in heaps (Westby, 2022). The grated cassava roots are allowed to ferment in sacks for 3-7 days, which encourages lactic acid fermentation. The pH after 3 days decreases from 6 to 4 and the fermentation is dominated by lactic acid bacteria. Grating is important for bringing linamarin into contact with linamarase allowing its hydrolysis to glucose and cyanohydrin and then to HCN. The hydrolysis continues during the fermentation process. Lactic acid fermented products are reported to have significant concentrations of cyanohydrins because pH decreases during fermentation and cyanohydrin is stable at low pH (Shepherd and Ilboudo, 2019).

Fermentation of cassava roots under water, followed by sun drying, is reported to be the best for cyanogens removal (Cardoso *et al*., 2015). This type of fermentation is used more in areas where there is a sufficient supply of water such as near a river or lake, and is common in countries such as Nigeria, Democratic Republic of Congo, Tanzania and Malawi (Westby, 2022). Heap fermented cassava root products are produced in Tanzania, Uganda and Mozambique (Westby, 2022). The process involves peeling of cassava roots, sun drying for 1 to 3 days, heaping and covering, fermentation, scraping off the molds, crushing into crumbs, sun drying, pounding and sieving into flour. During the fermentation of the roots, the temperature inside the heaps increases between 23 and 29℃ higher than the temperature outside the heaps (2 to 12℃). According to Sani and Farahni (2021), heap fermentation is dominated by the *Neurospora sitophila, Geotrichum candidum* and *Rhizopus oryzae.* Heap fermentation of cassava roots followed by sun drying is capable of reducing the cyanogen levels by 95%. The fermentation process has also played a significant role in the nutritional enhancement of the agro-industrial by-

products generated through the harvesting and processing of cassava roots. Apart from the food industry, cassava starch is used for textiles and the paper industry, and in the manufacture of plywood and veneer adhesives and glucose and dextrin syrups. Through fermentation, it can also be used for alcohol production, and as a waste material, it can be processed to biogas (Olukosi *et al*., 2017).

2.7.11.2 Fermented cassava products

The fermentation process for preservation of cassava root constitutes a vital body of indigenous knowledge and passed from one generation to the next. In addition to providing flavor, variety and preserving the product, the fermentation process also helps in detoxification of cassava roots. Well known fermented products of cassava are cassava bread, fermented cassava flour, fermented starch, fufu, lafun, akeyeke and garri.

2.8 Garri

Amongst the various fermented cassava products, Garri is the most commercial and useful product of cassava processing. It is creamy white, pregelatinized, granulated and dehydrated calorie rich food of cassava product with bit sour taste (Flade and Akingbala 2019). Garri is consumed raw or cooked and can be stored for several months. Garri is classified/ grouped based on texture, length of fermentation, region or place where it is produced and colour imparted by the addition/nonaddition of palm oil. It has a high swelling capability and can absorb up to four times its volume in water. (Jekayinfa and Olajide, 2017).

Garri is the most popular fermented cassava product in Africa. The production process of garri involves peeling, washing, grating, fermenting and toasting fresh cassava tuber (Manihot esculenta Crantz) (Sánchez and López, 2021). Palm oil is added according to preference (to make it a yellow garri). Palm oil added to the cassava mash gives the garri

an aesthetic value and the palm oil also serves as source of vitamin A. Yellow garri is more nutritious and preferably cherished than the white garri. Garri is stored and marketed in a ready-to-eat form and prepared into stiff paste or dough-like form called “eba” by adding the granules into hot water and stirring to make a paste of varied consistencies. The eba can be consumed with local soups or stews of various types by chewing or swallowing in morsels (Duputié *et al*., 2019). Garri can also be consumed directly (without cooking) with groundnut, smoked fish, coconut, cowpeas, moimoi, or taken as fast food when soaked in cold water. In Africa about 600 million people are dependent on cassava for their food, of which garri is the major cassava product eaten by them on a daily basis.

Only in Nigeria, garri is consumed by almost 148 million people, Garri is the most commonly consumed in Nigeria and accounts for 70% of the entire cassava production in Nigeria (IITA, 2018). The growth rate of garri has been put at least 4 to 6% per annum, primarily due to population growth and increasing urbanization, and export to the regional West African market. It has already provided up to five million farmers and producer (usually women living in poor communities) in Nigeria, as well as a large number of equipment manufacturers, retailers and suppliers a means of livelihood. In addition, small-scale garri processing business planning has been the primary source of employment in many countries (Westby, 2022).

2.8.1 Garri production

According to Oriola & Raji (2018), processing of cassava into finished or semi-finished products often involves all or some of the following operations, depending on the desired end-product: peeling, washing, grating/chipping, dewatering, fermentation, pulverizing, sieving, pelletizing, and drying/frying (Jimoh *et al*., 2019). A flow chart depicting the improved cassava processing method as provided by IITA (2018) for processing it into

garri (**Fig.2.3**). Garri is processed by women at small or medium scale with average moisture content of 12 to 14 per cent. Traditionally, the processing takes 3 to 5 days and involves peeling, washing, pulverizing and frying. It involves minimal mechanical processing and gives the product with good organoleptic quality (Kehinde *et al.*, 2001).

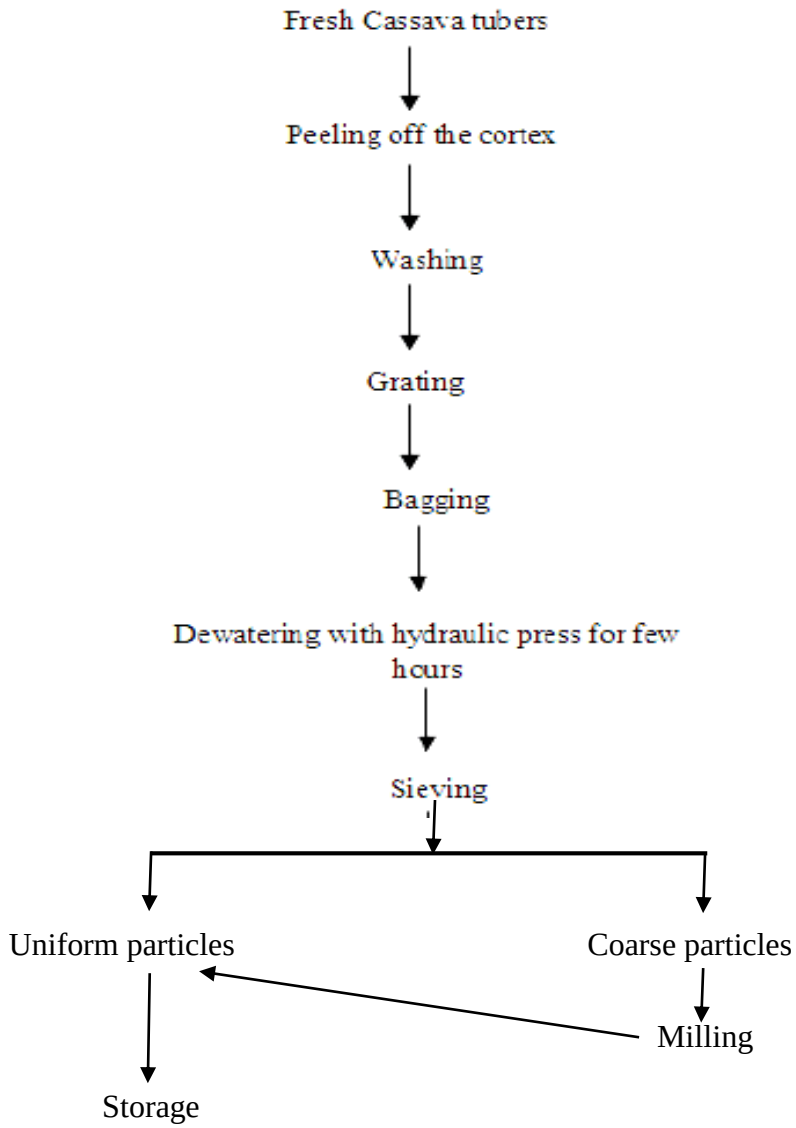

Figure 2.3: Flow Chart of garri processing operation (IITA, 2018).

2.8.2 Processing of cassava roots into Garri

The details of the processing of cassava roots into garri differ from one location to another, depending on regional preferences, resulting in a large family of different types

of garri. Producers/consumers may prefer garri with a sour, sweet or bland taste; a fine or coarse particle size; with or without palm oil added; or even garri enriched/fortified with different legumes or protein sources (Awoyale, 2018; Olaleye *et al*., 2018).

Garri processing involves various steps that include the peeling of fresh cassava roots, washing, grating, fermenting (optional), dewatering/pressing, pulverising, sifting, roasting, sieving or grading and packaging (Abass *et al*., 2022). Manual peeling of freshly harvested roots with a knife is most common, but mechanical peelers are now available in countries such as Nigeria and Ghana (Abass *et al*., 2022). The brown peel, if not removed or partly removed, might adversely affect the garri colour and increase its fibre content. Washing of the peeled roots is done to remove all extraneous materials, which could contaminate the garri. Grating of the washed cassava roots is generally done using a motorised cassava grater. However, hand graters, made by fastening a perforated grating sheet onto a wood slab, are still used in some countries. The resulting product is a wet mash. Grating increases the surface area of the root pieces so that dewatering of the mash can be done more quickly. The grated cassava mash is bagged using a polypropylene/ polyethylene woven permeable bag or basket (lined with polypropylene sack) and left for between one and five days to ferment. Fermentation time is based on location: for example, consumers in Southwest Nigeria do prefer sour garri, unlike those in the South-south and South-east. Apart from the taste, fermentation helps to reduce the cyanogenic potential of the product (Abass *et al*., 2022). The fermented mash is dewatered by pressing with a manual screw or hydraulic press (often car jacks are used) or even wood pieces tied at both ends with rope, which is still prevalent in most rural communities. Pressing is done to reduce the moisture content of the grated mash before roasting. The cake formed after dewatering is pulverized by a pulveriser/cake breaker or by hand and sieved with a standard woven sieve or rotary sieve, to remove the fibre and lumps, and to create

a grit of similar particles size. However, in some locations, after the pulverisation, generally with the grating machine, the grit is not sieved before roasting. The sieved grit is then roasted. An earthenware stove and a roasting pan made of moulded aluminium or stainless steel are used for roasting on a wood fire (Abass *et al*., 2022). In some communities, the roasting pan is smeared with a small amount of palm oil before roasting, to produce butter-coloured or yellow garri. Mechanical roasters are also now available in Nigeria and Ghana. The roasting process further develops the garri flavour, gelatinises the starch, and improves digestibility. The extent of drying determines the crispiness and storability of the product. Because starch in the grit is gelatinised during roasting, garri is a pre-cooked instant food product. In some communities, the grit is partially toasted and finally dried under the sun. Sun drying, while economical, adds risk that the product might be contaminated with dust and sand. The garri is then allowed to cool for some hours, graded (sieved) depending on the particle sizes to meet the preferences of different categories of the consumers and packaged according to the distribution outlet, from wholesale to retail. Most rural communities package in 50 kg bags for transport and distribution.

The sensory and functional properties of garri always result from the combination of raw materials (fresh cassava roots) quality and processing operations. The diversity of processing technologies and the resulting diversity of garri have been documented in the scientific literature. On the other hand, the relative influence of cassava roots characteristics and processing on the quality and consumers acceptability of the end-product remains to be investigated in details. The challenge with the consistent consumption of garri is its poor nutritional value, which is also common with all cassava products. Garri is known for its high carbohydrate (starch) content, but with low protein, fat and micronutrients contents. The regular consumption of low protein garri can

predispose consumers to protein-energy malnutrition (Alozie and Ekerette, 2017). Consequently, the enrichment of garri with protein-rich plant foods (soy beans, groundnut, seasame seed and melon seed) has been reported to improve the nutritional quality and sensory acceptability (Alozie and Ekerette, 2017). Garri enriched with palm oil and/or soybean was developed in Benin Republic, but the garri are not available on the Beninese markets, whereas they are readily available in Nigeria and their processes (Akinoso and Olatunde, 2014), physicochemical characteristics (Karim *et al*., 2016) and sensory properties are documented. In most Nigerian enriched garri, the ingredients (palm oil and/or soybean) are usually added to the fermented mash prior to roasting, whereas in Benin Republic, the palm oil/or soybean are added in the mash before fermentation (Adinsi *et al*., 2019). Awoyale *et al*. (2019) also reported that the practice of adding moringa leaf powder, groundnut paste, roasted coconut chips and milk powder to garri in Liberia caused the increase of fat and protein contents of the product compared to the unenriched white garri.

Apart from the artificial enrichment of garri, biofortified cassava varieties that contain significant levels of pro-vitamin A carotenoids have been developed by conventional plant breeding methods and released for use by the local populations for garri production among other cassava value added products. Garri produced from the biofortified varieties may help solve the issue of the additional cost of adding palm oil and the occurrence of rancidity in the use palm oil while contributing to the reduction of vitamin A deficiency (Bechoff *et al*., 2018). However, understanding how the pro-vitamin A carotenoids in the biofortified varieties degrade during storage of vitamin A-containing garri is critical because it will affect its nutritional impact (Bechoff *et al*., 2018). Onadipe (2021) studied the degradation of total carotenoids in garri from different biofortified cassava varieties and found out that 50% on average of total carotenoids were lost after 3- month storage

at 30±2 °C. Eyinla *et al*. (2019) on their own part reported that processing biofortified cassava into garri and to eba could hinder the retention of b-carotene, though some varieties have retention advantage over others irrespective of the initial concentration in the fresh roots.

2.8.3 Economic importance of garri

In Nigeria, garri processing firms occupy a substantial portion of small and medium enterprises (SMEs) that has contributed significantly to national economic growth (Ogundipe *et al*., 2018). Nigerian garri supplies Niger Republic, Chad and Cameroon (Coulibaly *et al*., 2019). Though, the annual garri production in Cameroon is around 49,000 tons, representing about 43,300 million USD (FAO, 2018). In terms of market volume and value, garri represents 45% of the Cameroonian national market of cassava products, and up to 53% in urban areas, where the demand is significant, with almost 74% of households consuming garri (Tolly Lolo, 2018). Garri consumption in Cameroon is most strongly associated with people originating from the South-West and North- West Regions (Njukwe *et al*., 2018). It may be related both to the geographical proximity of these two regions with Nigeria, which is the largest garri producer and consumer and to common colonial heritage. The extensive consumption of garri has been attributed to its relatively long shelf-life compared to other food products from cassava and its ease of preparation before consumption. The total quantity of fresh cassava roots used for the production of garri in Republic of Benin, Togo and Ghana as at 2003 is 160, 2385 and 9309 metric tons respectively. The total quantity of garri consumed by these countries as at 2003 is Republic of Benin 210.73 metric tons, Togo 159.06 metric tons and Ghana 620.66 metric tons (Africabiz online, 2020). Considering the readiness of garri to be used as diet's complement for a variety of African sauces and cooking and, the long shelf-life

under normal atmospheric conditions, it is possible to expand the consumption area of garri to covering Central, Eastern and Southern African countries.

2.8.4 Microbial safety in garri

Garri is majorly produced in artisanal units, which do not adhere to the rules of food safety (Cazumbá da Silva *et al*., 2017). The challenge of standardizing small-scale processing is that the processors have various target garri in mind, and the desired end product differs across ethnicity and regions. One constraint in the commercialization of locally produced garri is variation in the quality of the products amongst processors and processing batches of the same processor (Padonou *et al*., 2019).

Microbial growth, deterioration and spoilage of garri are major cause of food borne illnesses and threat to public health (Adetunji *et al*., 2017). However, some unhygienic practices involved in production, processing of cassava to garri and post processing handling such as spreading on the floor and mats after frying, displaying in open bowl or buckets in the markets during sales; the use of various packaging materials to transfer finished products from rural to urban areas and the use of bare hands during handling and sales may lead to microbial contamination due to deposition of bio-aerosols on exposed products and transfer of infectious agent during handling (Ogugbue *et al*., 2021). The main biological agents that contaminate and spoil garri are moulds, bacteria, insects and mites. Garri is rich in carbohydrate and therefore, suitable for fungal growth. Moulds such as *Aspergillus*, *Penicillium*, *Fusarium, Rhizopus*, *Cladosporium* and *Mucor* have been associated with garri during storage and distribution (Ogugbue *et al*., 2021). Several reports have revealed high occurrence of microorganisms in market samples of garri (Adetunji *et al*., 2017). The growth of moulds in garri results in changes in the organoleptic, microbiological and nutritive quality which lead to spoilage of the food product (Efiuvwevwere and Isaiah, 2018). Some moulds such as *Aspergillus flavus*,

Aspergillus parasiticus and *Penicillium* sp. can also produce aflatoxins (Adetunji *et al*., 2017), which can have serious effects on human health depending on the dosage consumed.

The processing conditions, retailing containers, storage containers and conditions could serve as veritable critical point of contamination of garri (Ogugbue *et al*., 2021). The aflatoxins producing microorganisms have been isolated from stored, retailed and ready-to-eat garri from some communities in Nigeria (Coulibaly *et al*., 2019). To date garri is still being consumed largely in students' communities without any form of thermal treatment which may expose them to serious health risk associated with microorganisms and their toxins (Coulibaly *et al*., 2019). Hence, the need to constantly evaluate the microbiological quality of garri sold within school environments to ascertain their safety.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Survey Sites

Surveys were conducted between November 2020 and February 2021 in five AEZs of Nigeria where Garri is predominantly produced (Adetunji *et al*., 2014) and consumed: Sudan Savanna **(SS)** (Kano, Katsina, Kebbi, Jigawa and Sokoto State); Northern Guinea Savanna (NGS) (Bauchi, Gombe and Kaduna State); Southern Guinea Savanna (SGS) (Niger and Adamawa State); Derived Savanna (DS) (Taraba, Plateau, Kwara, Ekiti, Kogi, Osun, Oyo, Benue, Ogun, Enugu, Nasarawa State and FCT-Abuja) and Humid Forest (HF) (Edo, Ondo, Cross-River, Ebonyi, Anambra, Imo, Akwa-Ibom, Abia, Rivers, Bayelsa, Delta, and Lagos State).

The Sudan Savanna (SS) zone lies between latitudes 12°2’ and 13°8’ N and longitudes 3°9’and 13°9’E with a unimodal annual rainfall averaging between 650 and 1,000 mm and maximum temperatures varying from 30 to 40 °C (Udoh *et al*., 2000). The Northern Guinea Savanna (NGS) zone lies within latitudes 9°10’and 11°59’N and longitudes 3°19’ and 13°37’E and has a unimodal rainfall distribution averaging between 900 and 1,000 mm annually and maximum temperatures varying from 28 to 40 °C (Atehnkeng, *et al*., 2008). The Southern Guinea Savannah zone lies within latitudes 8°4’and 11°3’N and longitudes 2°41’and 13°33’E, with a bimodal rainfall averaging between 1,000 and 1,300 mm per year and maximum temperatures varying from 26 to 38 °C. The Derived Savanna (DS) lies within latitudes 6°8’ and 9°30’ N and longitudes 2°40’and 12°15’ E and has a bimodal rainfall distribution averaging between 1,300 mm and 1,500 mm annually and maximum temperatures varying from 25 to 35 °C (Atehnkeng, *et al*., 2008). The humid forest (HF) zone lies within latitudes 6°4’ and 7°5’ N and longitudes 3°5’ and 8°8’E and

has a bimodal annual rainfall averaging between 1,300 and 2,000 mm and maximum temperatures ranging from 26–28 °C.

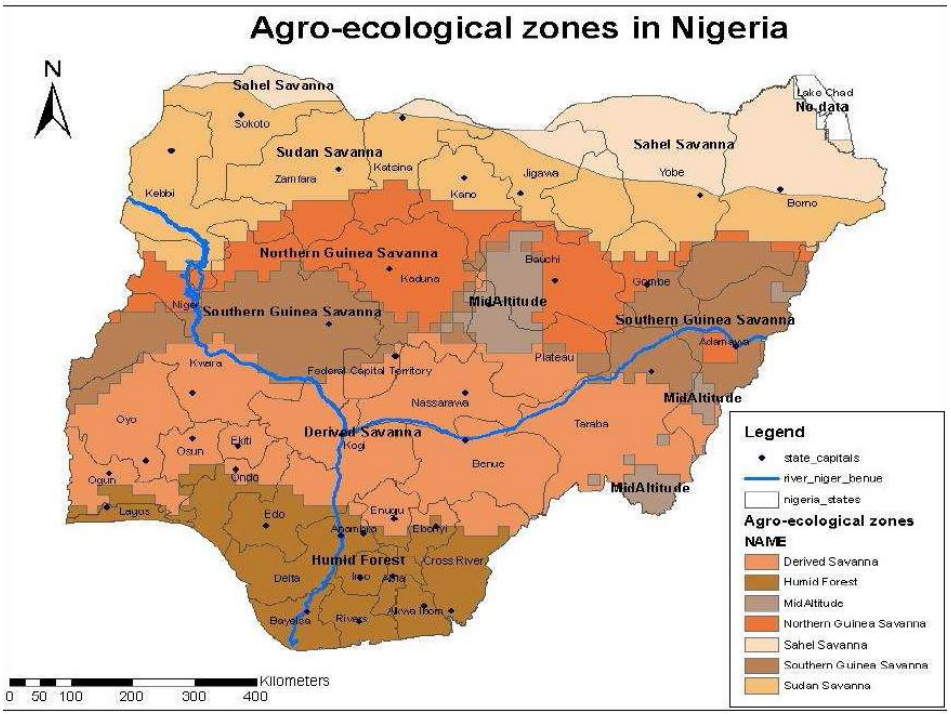

Fig. 3.1. Agro-Ecological Zones in Nigeria in relation to average rainfall

3.2 Sampling and Sample Preparation

Sampling and sample preparation prior to aflatoxin analysis were carried out as described by Adetunji *et al*. (2014) and the modified EC (2002) method, respectively in order to reduce variability. Briefly, 68 composite garri samples (2-samples from different location and 3 kg each) were collected across the five AEZs: HF–24, SS–10, NGS–6, SGS–4 and DS–24. The samples were hand-mixed, coarse grounded and allowed to pass through a No. 14 mesh screen. Sub-samples of 500 g were taken from each lot, ground with a milling machine and sieved with 1-mm mesh. Sub-samples of 50 g were further taken from the lots into zip-lock envelopes, labelled appropriately and transported to the microbiological and mycotoxin laboratory of the National Agency for Food and drug administration and control (NAFDAC) stored at −20 °C prior to analyses.

3.3 Chemicals and Reagents

Phosphate buffered saline solution (pH 7.4), Extraction solvent (Acetonitrile/water solution 8:2, v/v), Methanol (Technical grade, distilled), Water, Anhydrous $MgSO_4$, NaCl (Sodium chloride), HPLC mobile phase solvent (Water/acetonitrile/methanol HPLC grade solution 6:2:3, v/v/v), Sodium chloride, HPLC aflatoxin standard solutions LLC.

3.4 Apparatus

Laboratory balance: (Readability 0.1 g), Analytical balance: (Readability 0.1 mg), Pipettes: (10mL), Fluorescence detector: Wavelengths 360 nm excitation filter × 420 nm, HPLC column: (4.6 mm × 25 cm), Glass microfiber filter paper: (5 cm diameter, retention: 1.6 µm), 20 mL syringe, Vertical shaker: (Adjustable for max. solid-liquid agitation, Calibrated micro-litre syringe: 25 and 500 µL, Disposable filter unit: (Cellulose, 0.45 µm), Volumetric glassware: (2, 3, 10, and 20 mL (0.5% accuracy), Filter paper: (24 cm diameter, pre-folded, and 30 µm retention).

3.5 Microbiological Examination of Garri Sample

Fungi isolation was carried out using plate dilution method as described by Vanderzannt & Splittstoesser (1997) with slight modification. The media used was prepared and incubated according to the manufacturer's instructions. Three grams proportion of each sample was aseptically taken (after thorough mixing) and weighed into a beaker containing 20ml of 0.1% sterile peptone water (w/v) and allowed to soak for 2-3 minutes with occasional stirring with a sterile glass rod. The serial dilution was subsequently prepared by transferring 1ml aliquot of the supernatant into 9ml of sterile peptone water as diluent. Further serial dilution was carried out and thereafter, 1ml of appropriate dilution was aseptically plated on Rose Bengal Chloramphenicol agar (RBCA). The media was incubated at 25 ± 2 ^oC for 5-7 days. After incubation, the fungal colonies were counted using a colony counter and the number of colonies per gram of sample were

counted, recorded and expressed in colony forming unit per gram as represented in Equation 1.

$$\text{CFU/g} = \frac{Number\ of\ Colonies \times reciprocal\ of\ the\ dilution\ factor}{Volume\ (ml)\ platted} \quad (3.1)$$

The fungal isolates were identified using compound microscope based on examinations of the conider heads, phialides, conidiophores and presence and absence of foot cells or rhizoids using the appropriate identification keys and atlas in literature (Oranusi & Olarewaju, 2013). The summary of incidence rate and frequency of isolated *fungi* species from the garri samples were calculated using Equation 2 and 3, respectively.

$$\text{Incidence Rate} = \frac{Number\ of\ Isolates}{Total\ number\ of\ Isolates} \times 100 \quad (3.2)$$

$$\%\ \text{Frequency} = \frac{Number\ of\ Isolates}{Total\ number\ of\ Samples} \times 100 \quad (3.3)$$

3.6 Working Principle of High Performance Liquid Chromatography

The separation principle of HPLC is based on the distribution of the analyte (sample) between a mobile phase (eluent) and a stationary phase (packing material of the column). Depending on the chemical structure of the analyte, the molecules are retarded while passing the stationary phase. The specific intermolecular interactions between the molecules of a sample and the packing material define their time "on-column". Hence, different constituents of a sample are eluted at different times. Thereby, the separation of the sample ingredients is achieved.

A detection unit (e.g. UV detector) recognizes the analytes after leaving the column. The signals are converted and recorded by a data management system (computer software) and then shown in a chromatogram. After passing the detector unit, the mobile phase can be subjected to additional detector units, a fraction collection unit or to the waste. In

general, a HPLC system contains the following modules: a solvent reservoir, a pump, an injection valve, a column, a detector unit and a data processing unit (Fig. 1). The solvent (eluent) is delivered by the pump at high pressure and constant speed through the system. To keep the drift and noise of the detector signal as low as possible, a constant and pulseless flow from the pump is crucial. The analyte (sample) is provided to the eluent by the injection valve.

3.7 Method Validation for HPLC

The methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs lay down by (European Commission Regulation, 2010) No 401/2006 of 23 February 2006 was adopted. According to the commission, the method was validated in terms of linearity (quantification), apparent recovery (% recovery) and sensitivity (limit of detection (LOD) and limit of quantification (LOQ)) using blank matrices of the garri samples (401/2006/EC, 2006; (Abia *et al*., 2013). External calibration curves were established based on the serial dilution of the aflatoxin standard solution. Linearity was determined by injecting aflatoxin standards at three different concentrations into the HPLC column. Calibration curve between the different concentrations and correlation co-efficient (R^2) indicated good linearity with R^2 values ranging from 0.9018-0.9998 for the different aflatoxin standards.

3.8 Analysis of Garri Samples for Aflatoxin Contamination Using HPLC

A total of 68 composite of garri samples were analyzed for the presence of four prominent regulated aflatoxin: B1 (AFB1), AFB2, AFG1 and AFG2 by high performance liquid chromatography (HPLC) method. Five grams (5g) of each ground representative sample was weighed into a 50-ml polypropylene tube and extracted with 20 ml acetonitrile/water/acetic acid (79:20:1, v/v/v) for 90 min on a rotary shaker. To the extract, 10grams of Anhydrous $MgSO_4$ and 1g of NaCl was added and shaken for 1minutes. The

solution was then centrifuged for 5 minutes at 4000rpm to further separate the solid from the liquid. The method of clean up via solid-phase extraction was carried out as described by Shepherd (2003). 6ml of the supernatant was transferred into a 15ml clean up tube containing 150mg of PSA and 600mg of $MgSO_4$. The mixture was shaken vigorously using vertical vortex shaker and centrifuged for 5 minutes and 4000 rpm. 1ml of the purified extracts were then evaporated to dryness at 40°c under nitrogen and reconstituted in 1 ml of methanol/water (80/20; v/v) solution. Recovery assays for the individual samples were greater or equal to 80% and the limit of detection for aflatoxins was ≤ 0.1 µg/g.

The Aflatoxins, were quantified using HPLC Modula system (Agilent, Waldbronn, Germany) with an Ultraviolent detection. The system was accomplished with column types C-18, pressure max per flow rate of 420 pa x 1 ml/ml for the aflatoxins, for the separation and quantification of the selected Aflatoxins and standards. Twenty microlitre (20 µl) was injected as the volume used for the prepared samples with different mobile phase for individual aflatoxin: that is, water: methanol: acetonitrile (60:20:20).

Table 3.1: Calibration Parameters for HPLC Analysis

Analytes	**Calibration level (µg/kg)**	**Percentage recovery (%)**	**r^2**	**Equation of straight line**
AFB_1	2.5, 5.0, 10.0	88	0.9018	y = 2634x
AFB_2	5.0, 10.0, 20.0	92	0.9851	y = 24120x
AFG_1	2.5, 5.0, 10.0	78	0.9761	y = 62824x
AFG_2	2.5, 5.0, 10.0	92	0.9882	y = 41585x

Keys: AFG_1- Aflatoxin G_1, AFG_2- Aflatoxin G_2, AFB_1- Aflatoxin B_1, AFB_2- Aflatoxin B_2.

Percentage recoveries of the analytes were carried out by spiking three different samples (5 g of each) that were least contaminated with the analyte standard (100 µl of standard concentration). The spiked samples were left overnight in a fume cupboard at room

temperature for evaporation of the solvent to establish equilibrium between the sample matrix and the toxins. The aflatoxins from spiked samples was extracted by mycotoxin extraction method as described above. From each spiked sample, 20 µl of the extract was injected into the HPLC. The corresponding peak areas of the spiked samples were used for estimation of the apparent recovery by comparison with a standard of the same concentration prepared by dilution in pure solvent. The percentage recoveries were estimated using the formula presented in Equation 4 and the calibration parameters were presented in Table 1.

$$\% \text{ Recovery} = \frac{Peak\ Area\ of\ Spiked\ samples}{Peak\ Area\ of\ Liquid\ Standards} \times 100 \qquad (3.4)$$

The sensitivity parameters (i.e. LOD and LOQ) for mycotoxins in the garri samples were calculated from the signal to noise ratios (S/N) of the respective multiple reaction monitoring (MRM) chromatograms derived from the analysis of the spiked samples: LOD =3×S/N and LOD =10 ×S/N, respectively.

3.9 Exposure Assessment and their Potential Risk Characterization

Data Collection

A simple questionnaire was administered to address the risk characterization of human exposure to Aflatoxins present in garri sold in the various markets that are consumed in the five AEZs in Nigeria, a total of 150 respondents were required to fill the questionnaire in order to determine the exposure rate and risk associated with aflatoxin contamination. Also, the survey was age- and gender- weighted and they represent adult population groups between the ages of 20-60 years. In this regard, the adult population groups were successfully interviewed through a quota sampling and completed the dietary intake questionnaires within 15-30 minutes. A portable scale was used to determine the weight of each respondent from the studied region. The weight of forms in which garri is being

consumed was also taken in grams. The questionnaire assessed whether the respondents consumed garri or not, and explore the extent to which they consume their products (if they were consumed on a daily basis or not at all).

3.10 Aflatoxins Dietary Intake

The estimated daily intake (EDI), and percentage tolerable daily intake (% TDI) values will be estimated for the staples. The method used by Rodríguez-Carrasco *et al*. (2013) and approved by JECFA was adopted in this study. The "Estimated daily intake" which estimates the amount of toxin that can be ingested daily (µg/kg bw/day) can be obtained by using the formula presented in Equation 5.

$$\text{Estimated daily intake (EDI)} = \frac{\text{Contamination level} \times \text{Consumption rate}}{\text{Body weight (kg/persons)}} \qquad (3.5)$$

Where "Contamination level" refers to the average toxin level found in a certain foodstuff (µg/Kg/day) and "Consumption rate" is the amount of the foodstuffs ingested on daily basis (gram/day).

However, the formula presented in the equation above was implemented by multiplying the average level of each aflatoxin present in the garri samples with the average garri consumption in Nigeria: 416.7g/person/day (0.4167 kg/person/day) "as estimated from the questionnaire" and then divided by mean body weight of 63.03, 60.13 and 61.58 kg for adult male, adult female and total population groups, respectively.

3.11 Determination of Burden of Aflatoxins-Attributable to Hepatocellular Carcinoma incidence Among Garri Consumer

The JECFA estimated cancer potency values for aflatoxins were adopted in determining the annual burden and HCC incidence attributable to aflatoxins exposure in garri. The values which corresponded to 0.3 cases of cancer per 100,000 population annually, for each ng/kg bw/day, among populations infected with hepatitis B virus (HBsAg+), and 30

times lesser (0.01 cases of cancer per 100000 population per ng/kg bw/day) among people not infected (EFSA, "European Food Safety Authority", 2007) were employed for this estimation. The HBsAg+ prevalence rate used was 13.6 % in Nigeria based on previous studies, and 86.4 % was extrapolated for HBsAg- groups (Musa, *et al*., 2015). Similarly, a recent report by National HIV/AIDs Indicator and Impact Survey (NAIIS) highlighted that HBV prevalence in Nigeria was 8.1 % and affirmed Nigeria populations to be approximately 190 million (Adeyinka, *et al*., 2019). Hence, the annual HCC cases per 100,000 for $HBsAg^{+ve}$ and $HBsAg^{-ve}$ individuals, as well as that used to estimate the annual HCC cases based on populations that are $HBsAg^{+ve}$ and $HBsAg^{-ve}$ are represented in Equation 6, 7, and 8, respectively.

Annual HCC Cases/100,000 for $HBsAg^{+}$ individual = Aflatoxins EDI×Potency Factor (0.3) (3.6)

Annual HCC Cases/100,000 for $HBsAg^{-}$ individual = Aflatoxins EDI×Potency Factor (0.01) (3.7)

$$\text{Annual HCC Cases} = \frac{Aflatoxins\ EDI \times Potency\ factor\ (0.3\ or\ 0.01)}{100{,}000} \times N(\text{HBsAg}^{+} \text{ or } \text{HBsAg}^{-}) \quad (3.8)$$

Where N, represents prevalence rate multiply by the total population of the individual in each of the five AEZs.

3.12 Determination of HCC Risk

Based on the prevalence of $HBsAg^{+}$ (13.6 % or 8.1 %) of individual in Nigeria total population, the risk for liver cancer was estimated for different population groups consuming garri in each of the five AEZs using the relation presented in Equation 9 and 10, respectively.

$$\text{Cancer potency} = 0.3 \times annual\ HCC\ cases\ (\text{HBsAg}^{+}) + 0.01 \times Annual\ HCC\ cases\ (\text{HBsAg}^{-}) \quad (3.9)$$

$$\text{HCC Population Risk} = \text{EDI} \times \text{Cancer Potency} \quad (3.10)$$

3.13 Cancer Incidence Attributable to Dietary Aflatoxins from Consumption of Garri

This was computed as reported by Liu and Wu (2010) by dividing the estimated liver cancer risk per 100,000 population by the incidence rate of liver cancer in Nigeria (6.5 deaths/100,00 population) estimated for Nigeria by Global Burden of Disease Project and multiplying by 100% (WHO, World Health Organization, 2004)

3.14 Disability Adjusted Life Year (DALY) Lost

DALY is an epidemiological measure of disease burden expressed in number of healthy life years lost due to death or disability caused by a disease. It is calculated by multiplying annual HCC cases (HBsAg-positive) per 100,000 populations by sex-specific HCC DALY estimate (13.05) for both male and female population.

3.15 Statistical Analysis

All data were analyzed by SPSS 26.0 (Windows version, SPSS, IL, USA). One-way ANOVA was performed for the distribution of fungal species across the agro-ecological zones (AEZs). All means were tested for significance by the Duncan's Multiple Range Test at 95% confidence level.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Fungal Profile of Garri Samples from the AEZs in Nigeria

A total of 346 fungal isolates belonging to six genera: *Aspergillus* (n = 161), *Fusarium* (n = 88), *Penicillium* (n = 58), *Cercospora* (n = 13) *Cladosporium* (n = 15) and *Botrytis cinerae* (n = 11) were isolated from the garri samples. The population of Aspergillus (46.53%) was higher than the population of the other fungal genera in both the garri sample type (white and yellow) as well as the garri samples from all the AEZs (Figure 2). *Fusarium* (25.43%) was the second in the population frequency followed by *Penicllium* spp (16.76%) and *Cercospora* spp (3.76%%), and least frequency

hierarchy was *Botrytis cinerae* (3.17%). Among the *Aspergillus* species isolated, *A. flavus*, *A. niger* and *A. fumigatus*, as well as *Penicillium*, *and* Fusarium species were aflatoxin-producing species and were the most abundant species in all the AEZs. The incidence of *A. flavus* in the garri from the DS (30.88%) and the HF (26.47%) zones were significantly ($p<0.05$) higher than the garri from other zones. Following *A. flavus* in hierarchical succession was the *A. fumigatus*-clade whose incidence also in the DS and HF zones was significantly ($p<0.05$) higher than the SGS, SS and NGS zones. *A. niger* has the least incidences amongst the *Aspergillus* Spp in the garri samples from AEZs (≤12.0%) (Table 1). *F. verticilloides* was recorded to have the highest incidence (26.47%; $p<0.05$) present in the garri sample from the DS zone amongst the *Fusarium* isolates in AEZs (Table 2). Followed by *F. moniuforme* having incidence of (16.18%; and 13.24%;) in the DS and HF zones respectively, and *F. chlamydosporum* has the least incidence (8.82% and 5.88%; $p<0.05$) in the DS and HF zones respectively amongst the Fusarium species. The work also revealed that the white garri variety (213) were also slightly more susceptible to fungal infestation than the yellow garri variety (139). However, the

mycological analysis of the garri samples showed that none of the samples in AEZs were free from fungal infestation.

Figure 4.1: Percentage (%) occurrence of the fungal isolates belonging to six genera

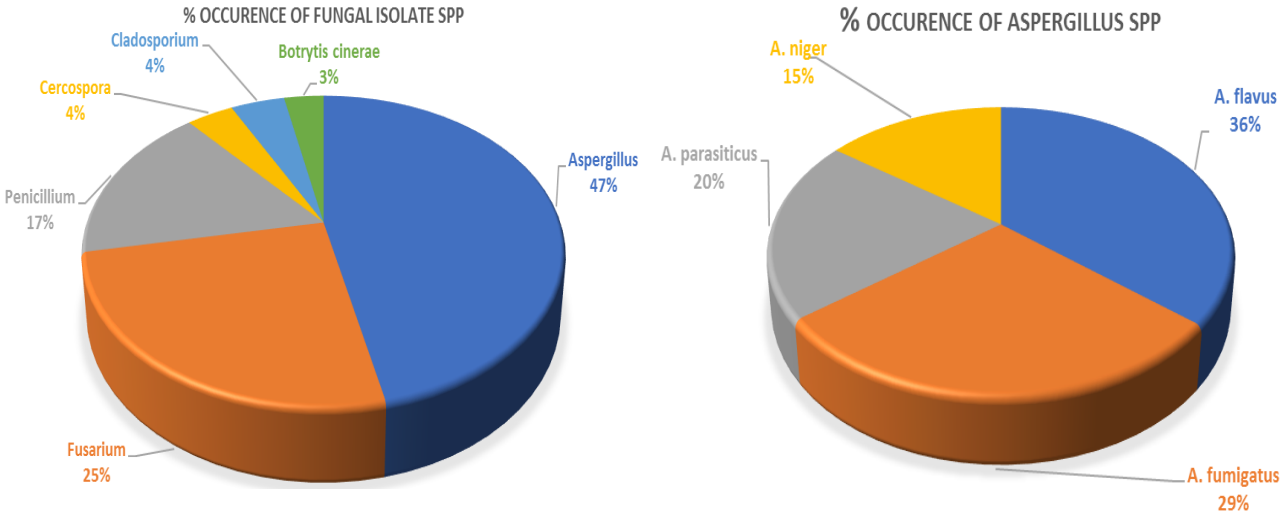

Table 4.1: Distribution of Isolated Fungal species in Garri Samples of Different Types from AEZs with Incidence Rate in Parenthesis

Fungi Species	Sample Varieties		Sample Location/Zones					Total	Sample Varieties		% Occurrence of fungal species in AEZ*				
	White (n = 34)	Yellow (n = 34)	NGS (n = 6)	SGS (n =4)	DS (n = 24)	HF (n = 24)	SS (n = 10)	Incidence (n=68)	White (n = 34)	Yellow (n = 34)	NGS (n = 6)	SGS (n =4)	DS (n = 24)	HF (n = 24)	SS (n = 10)
A. flavus	33(15.49)	27(19.42)	5(19.23)	3(12.5)	21(15.79)	18(17.30)	9(16.98)	**58**	48.53	39.71	7.35	4.41	30.88	26.47	13.24
A. fumigatus	26(12.21)	19(13.67)	4(15.39)	3(12.5)	19(14.29)	13(12.5)	8(15.09)	**46**	38.24	27.94	5.88	4.41	27.94	19.12	11.77
A. parasiticus	21(9.86)	16(11.51)	2(7.69)	1(4.17)	11(8.27)	9(8.65)	6(11.32)	**33**	30.88	23.53	2.94	1.47	16.18	13.24	8.82
A. niger	18(4.45)	8(5.75)	1(3.85)	1(4.17)	8(6.02)	7(6.73)	5(9.43)	**24**	26.47	11.77	1.47	1.47	11.77	10.29	7.35
F. verticilloides	23(10.80)	19(13.67)	5(19.23)	3(12.5)	18(13.53)	13(12.5)	9(16.98)	**45**	33.82	27.94	7.35	4.41	26.47	19.12	13.24
F. moniuforme	15(7.04)	11(7.91)	2(7.69)	3(12.5)	11(8.27)	9(8.65)	5(9.43)	**28**	22.06	16.18	2.94	4.41	16.18	13.24	7.35
F. chlamydosporum	8(3.76)	6(4.32)	1(3.85)	2(8.33)	6(4.51)	4(3.87)	3(5.66)	**15**	11.77	8.82	1.47	2.94	8.82	5.88	4.41
Botrytis cinerae	9(4.23)	4(2.88)	1(3.85)	1(4.17)	4(3.01)	3(2.89)	0(0.00)	**11**	13.24	5.88	1.47	1.47	5.88	4.41	0.00
Cercospora sp	8(3.76)	5(3.59)	1(3.85)	2(8.33)	5(3.76)	3(2.89)	2(3.77)	**13**	11.77	7.35	1.47	2.94	7.35	4.41	2.94
Cladosporium spp.	10(4.70)	5(3.59)	2(7.69)	1(4.17)	7(5.26)	4(3.87)	1(1.89)	**15**	14.71	7.35	2.94	1.47	10.29	5.88	1.47
P. scleratonium	14(6.57)	4(2.88)	1(3.85)	3(12.5)	8(6.01)	6(5.77)	2(3.77)	**19**	20.59	5.88	1.47	4.41	11.77	8.23	2.94
P. copticola	9(4.23)	6(4.32)	0(0.00)	0(0.00)	6(4.51)	8(7.69)	1(1.89)	**15**	13.24	8.82	0.00	0.00	8.82	11.77	1.47
P. citrinum	19(8.92)	9(6.47)	1(3.85)	1(4.17)	9(6.77)	7(6.73)	2(3.77)	**24**	27.94	13.24	1.47	1.47	13.24	10.29	2.94
Total	**213**	**139**	**26**	**24**	**133**	**104**	**53**	**346**	**313.26**	**204.41**	**38.22**	**35.28**	**195.59**	**152.35**	**77.94**

*****AEZ- Agro-ecological zones: (NGS)-Northern Guinea Savanna, (SGS)-Southern Guinea Savanna, (DS)-Derived Savanna, (HF)-Humid Forest and (SS)-Sudan Savanna.

4.1.2 Colony Forming Units (CFU) of Fungal Species Found in Garri Samples from the Five Agro-ecological Zones in Nigeria

The result of the average range count of microbial contamination as evaluated using the colony forming unit per gram (CFU/g) analyzed from total of 68 composite of garri samples across the five AEZs in Nigeria shows that DS has the highest fungal load (47.00±8.0 $x10^{-3}$cfu/g), followed by SGS (25.0±5.0 $x10^{-3}$cfu/g), HF (18.5±4.5$x10^{-3}$cfu/g), SS (12.75±0.75 $x10^{-3}$cfu/g) and NGS had the least (8.50±0.50 $x10^{-3}$cfu/g) fungal count in all the AEZs. Slight variations were observed amongst the groups of microorganisms within each state and from one state to another. The result shows significantly ($p<0.05$) higher colony forming units in the white garri samples (73.08±1.39$x10^{-3}$) than the yellow garri (44.62±0.46 x 10^{-3}). Based on the categorization of Gimeno (2002), samples can be categorized as good (colony count range less than 3 x 10^{-3} CFU/g), regular (count range between $3x10^{-3}$ to 7 x 10^{-3} CFU/g) and bad (greater than 7 x 10^{-3} CFU/g), the garri samples were found to be bad in terms of microbial count.

Table 4.2. Colony Forming Units (CFU) of Fungal Species Found in Garri Samples from the five Agroecological zones in Nigeria

	Average Total Fungal count CFU/g ($x10^{-3}$)		
Ecological Zones	**Sample Location/Zones**	**Sample Varieties**	
		Yellow Garri	**White Garri**
Sudan Savannah	12.75±0.75	44.62±0.46	73.08±1.39
Northern Guinea Savannah	8.50±0.50		
Derived Savannah	47.00±8.00		
Southern Guinea Savannah	25.00±5.00		
Humid Forest	18.50±4.50		

Values are in ± mean S.E. (S.E = Standard error of Mean)

Values between experimental treatments Within Groups bearing the same superscript are not significantly different at the 5% level ($P<0.05$).

4.1.3 Incidence and Level of Aflatoxins (ng/g) in Garri Samples from all the Agro-Ecological Zones in Nigeria

The concentrations of total aflatoxins in the garri samples sold in the market is in relation to average rainfall pattern/state (Nigerian Metrological Agency, 2008) are reported. In AEZs the garri samples were contaminated with high concentration of aflatoxin B1 (AFB1). In particular, Enugu state with a high mean annual rainfall of 2,000 mm (79 in) had the highest aflatoxin B1 (AFB1) contamination (87.16±0.03 ng/g) and a significantly low aflatoxin G2 (AFG2) contamination levels (1.04±0.01 ng/g), respectively. The level of aflatoxin B1 (AFB1) contamination was followed by aflatoxin G1 (AFG1) which the highest contamination was recorded in Gombe state (40.95±0.01ng/g) and Sokoto state (22.17±0.04 ng/g) in the NGS and SS parts respectively. The HF (2.34±0.01 ng/g) and SS (2.34±0.01 ng/g) regions had the least average aflatoxin B2 (AFB2) contamination levels. States in the SS; Katsina and Sokoto, DS; Plateau and Nasarawa and HF; Anambra, Abia and Akwa-Ibom had (0.00±0.00 ng/g); no record of aflatoxin G2 (AFG2)

contamination in the garri samples from these regions. The results also showed that Katsina, Plateau and River states had low levels of aflatoxin contamination (6.17±0.01, 6.75±0.03 and 10.00±0.01 ng/g) contamination, respectively, in contrast to the high incidences observed in Benue (89.48±0.04 ng/g), Enugu (94.93±3.16 ng/g) and Nasarawa (100.66±5.14 ng/g) states. The average total aflatoxin concentration (AFT) decreased from the DS to HF. The concentration of average aflatoxin increased from 23.04±0.02 ng/g in the HF zone to 36.16±0.03 ng/g in the SGS zone and snow balled to 49.18±0.02 ng/g in the DS zone (Table 4.2). Aflatoxin B1 was found in garri samples from all the AEZs, and the concentration ranged from 5.83±0.04 ng/g (HF) to 34.41±0.03 ng/g (DS). Aflatoxin B2 was also found almost in all the AEZs at concentration ranges of 2.39±0.06 ng/g (HF) to 6.49±0.03 µg/ g (DS). The highest concentration of Aflatoxin G1 was found in the NGS zone (18.61±0.01 ng/g), while it was not detected in garri sample from SGS (0.00±0.00 ng/g) zones. In addition, the highest concentration of Aflatoxin G2 was found in garri samples from NGS zone (5.58±0.04 ng/g), the least concentration of (1.82±0.01 ng/g) was recorded from DS zone. In AEZs, the highest average concentration of Aflatoxins in the garri samples was AFB1 (34.41±0.03 ng/g) and least average concentration of Aflatoxins in the garri samples was AFG2 (1.82±0.01 ng/g) both recorded in the DS region.

Table 4.3: Incidence and Level of Aflatoxins (µg/g) in Garri Samples from all the Agro-Ecological zones in Nigeria

Zone	AFB1		AFB2		AFG1		AFG2		AFT	
	N (n) % Cont.	**Mean(±)SEM (Range) EU limit (No. of Cont. sample above EU limit)**	**N (n) % Cont.**	**Mean(±)SEM (Range) EU limit (No. of Cont. sample above EU limit)**	**N (n) % Cont.**	**Mean(±)SEM (Range) EU limit (No. of Cont. sample above EU limit)**	**N (n) % Cont.**	**Mean(±)SEM (Range) EU limit (No. of Cont. sample above EU limit)**	**N (n) % Cont.**	**Mean(±)SEM (Range) EU limit (No. 0of Cont. sample above EU limit)**
NGS	6 (4) 66.7	16.04±0.12^{c} (0-124.36) 2 (5)	6 (4) 66.7	5.88±0.05^{c} (0-37.96) Na (Na)	6 (5) 83.3	18.61±0.01^{e} (0-22.97) Na (Na)	6 (5) 83.3	5.58±0.04^{e} (0-22.43) Na (Na)	6 (6) 100	46.02±0.15^{d} (22.45-58.52) 4 (3)
SGS	4 (4) 100	29.63±0.09^{d} (4.3-97.5) 2 (4)	4 (3) 75	3.74±0.11^{b} (0-9.34) Na (Na)	4 (0) 0.00	0.00±0.00^{a} (0) Na (Na)	4 (3) 75	3.12±0.10^{b} (0-10.38) Na (Na)	4 (4) 100	36.16±0.03^{c} (17.77-54.55) 4 (2)
DS	24 (20) 83.3	34.41±0.03^{e} (0-170.67) 2 (21)	24 (19) 72.9	6.49±0.03^{d} (0-84.61) Na (Na)	24 (14) 58.3	6.41±0.01^{b} (0-42.74) Na (Na)	24 (13) 54.2	1.82±0.01^{a} (0-55.77) Na (Na)	24 (24) 100	49.18±0.02^{e} (6.75-100.66) 4 (12)
HF	24 (19) 79.2	5.83±0.04^{a} (0-45.84) 2 (17)	24 (16) 66.7	2.39±0.06^{a} (0-29.16) Na (Na)	24 (16) 66.7	11.10±0.02^{d} (0-39.08) Na (Na)	24 (14) 58.3	3.81±0.06^{c} (0-20.06) Na (Na)	24 (24) 100	23.04±0.02^{a} (10.01-35.04) 4 (12)
SS	10 (5) 50	13.60±0.02^{b} (0-89.01) 2 (5)	10 (5) 50	2.44±0.10^{a} (0-17.5) Na (Na)	10 (5) 50	7.76±0.06^{c} (0-22.2) Na (Na)	10 (6) 60	4.28±0.15^{d} (0-22.96) Na (Na)	10 (10) 100	27.88±0.05^{b} (6.17-43.04) 4 (5)
Total sample	**68 (52) 76.47**	**19.90±0.06 (0-170.67) 2 (21)**	**68 (47) 69.12**	**4.19±0.07 (0-19.74) Na (Na)**	**68 (40) 58.82**	**8.78±0.02 (0-19.74) Na (Na)**	**68 (41) 60.29**	**3.72±0.07 (0-19.74) Na (Na)**	**68 (68) 100**	**36.46±0.05 (0-19.74) 4 (35)**

Values are in ± mean S.E. (S.E = Standard error of Mean)

Values between experimental treatments Within Groups bearing the same superscript are not significantly different at the 5% level (P<0.05).

KEYS: Mean = Mean concentration; SEM = Standard Error of Means; AFB1 = Aflatoxin B1; AFB2 = Aflatoxin B2; AFG1 = Aflatoxin G1; AFG2 = Aflatoxin G2; AFT = Total Aflatoxins: (NGS)-Northern Guinea Savanna, (SGS)-Southern Guinea Savanna, (DS)-Derived Savanna, (HF)-Humid Forest and (SS)-Sudan Savanna; EU limit= European Union Maximum limits; N = Number of Samples;; n = Number of Positive Samples; % Cont. = Percentage Contamination; No. of Cont. sample above EU limit= Number of contaminated samples above EU limits.

4.1.4 Exposure and Risk Assessment Garri Consumption in Agro-Ecological Zones in Nigeria

Table 4.3 summarizes the exposure and risk characterization of the aflatoxins evaluated in adults within the AEZ study areas. In all cases the average EDI of AFG2 from the staples garri was the least being (24.90 ng/kg bw/day), followed by AFB2 (28.69 ng/kg bw/day) respectively, while that of AFB1 (138.11 μg/kg bw/day) was the highest. According to American Cancer Society (2011), even EDI level as low as 0.001 μg/kg bw/day may induce liver cancer hence, the levels of AFT in food should be As Low As Reasonable Achievable (ALARA) (EFSA, "European Food Safety Authority", 2007). The risk of HCC was estimated based on two prevalence rates as presented in Table 4.3. The result shows the annual burden of HCC cases in AEZs, indicating different population groups that are susceptible to risk for cancer due to aflatoxins exposure from garri consumption. The liver cancer risk for AFB_1 (being the most potent of aflatoxins) was observed to be the highest among the overall population groups. Based on the results, an estimated annual HCC cases of 41.43 and 75.90 per 100,000 persons is anticipated due to consumption of AFB_1 and AFT respectively in staples garri in Nigeria. At 13.6% HBsAg prevalence rate, an estimated 458, 000 and 1,538, 000 new HCC cases is likely to occur annually in the over 190 million population of Nigeria due to AFB_1 and AFT in the garri, but if the HBsAg prevalence is assumed to be 8.1%, the figures will be 273, 000 and 916, 000 cases respectively. The estimated HCC cases due to aflatoxins are higher in females than in males and obviously also higher in $HBsAg^+$ than in $HBsAg^-$ populations.

Table 4.4 shows the exposure risk estimate of garri consumers to Aflatoxins across the AEZs. The mean exposure estimates of garri consumers to total aflatoxins (AFT) significantly ($p < 0.05$) increased from the SGS zone (120.25 ng/kg bw/ day) to the NGS zone (395.99 ng/kg bw/day), while the mean exposure estimate for AFG2 was highest in

the NGS zone (39.49 ng/kg bw/day) and significantly ($p < 0.05$) decreased downwards to the DS zone (12.32 ng/kg bw/ day). The mean exposure estimate for AFG1 was highest in the NGS zone (126.0 ng/kg bw/day), and the exposure risk also significantly ($p < 0.05$) decreased from the NGS to the DS zone (43.44 ng/kg bw/day). As with aflatoxins exposure, the NGS zone also had a higher mean exposure estimate for AFG1 and AFG2 than the all the zones. The table shows that consumers of garri in the NGS zone are also exposed to high risk of AFG1 and AFG2 contamination in their diets, as with consumers of the garri in the DS zone (43.71 ng/kg bw/day) and (233.32 ng/kg bw/ day) to AFB2 and AFB1 contamination respectively. As the mean national exposure estimate of AFG1 contamination was (297.33 ng/kg bw/ day), no contamination of AFG1 was detected in the SGS zone. In addition, the SS and HF zones recorded the minimum exposure estimate for AFB2 (15.83 ng/kg bw/day, while the minimum exposure limit for AFB1 was found in the HF zone (39.72 ng/kg bw/day).

The central exposure estimates of garri consumers to dietary AFB1 (Table 4.4) ranged between (39.72–233.32 ng/kg bodyweight/day) with the maximum exposure in the DS zone (233.32 ng/kg bodyweight/day) and was followed by the SGS zone (196.76 ng/kg bodyweight/ day), respectively. The range of the estimated national liver cancer risk attributable to aflatoxin AFB1 contamination of garri consumed in all the AEZs in Nigeria was between (11.29 and 70.0 cases/100,000 population/year) with a maximum case of (70.0 cases/100,000 population/year) in the DS zone and was followed by the SGS zone (59.93 cases/100,000 population/year), respectively. The cancer incidence attributable to dietary aflatoxins AFB1 was also maximum in the DS zone (1,076.9 %) and was followed by the SGS zone (922.0 %), respectively, while it was minimal in the HF zone (173.69 %). Furthermore, the national cancer incidence attributable to dietary aflatoxins AFB1 was (3,106.9 %). The range of estimated healthy life years lost due to death or disability

caused by ingestion of aflatoxins AFB1 (DALY) in the contaminated garri samples was between 147.34 and 913.5 with maximum value of 913.5 in the DS zone and was followed by SGS zone (782.09), respectively. Finally, the estimated cancer in all the AEZs cases due to aflatoxins contaminations are higher in females than in males.

Table 4.4: Risk Assessment, Estimated Annual Burden of Hepato-Cellular Carcinoma (HCC) Cases and Risk of HCC/year Attributable to Aflatoxin Exposure from Garri Consumers in Hepatitis B Virus Positive (HBsAg+) and Hepatitis B Virus Negative (HBsAg-) Populations in the Agro-Ecological Zones in Nigeria.

Mycotoxins	Mean Conc. ($x10^3$)	Estimated daily Intake (EDI)	Estimated Annual HCC (Per 100,000)		Annual HCC cases (HBsAg Prevalence = 13.6%) ($x10^3$)		HCC Risk/year (13.6%) ($x10^5$)	Annual HCC cases (HBsAg Prevalence = 8.1%) ($x10^3$)		HCC Risk/year (8.1%) ($x10^5$)
	(ng/kg)	(ng/kg. bw/day)	HBsAg +Ve	HBsAg –Ve	HBsAg +Ve	HBsAg -Ve		HBsAg +Ve	HBsAg -Ve	
		Male (TP) Female	Male (TP) Female	Male (TP) Female	Male (TP) Female	Male (TP) Female		Male (TP) Female	Male (TP) Female	
AFB_1	20.41	134.93	40.48	1.35	10.46	2.22	4.38	6.23	2.36	2.61
		(138.11)	(41.43)	(1.38)	(10.71)	(2.27)	(4.58)	(6.38)	(2.41)	(2.73)
		141.44	42.43	1.41	10.96	2.32	4.81	6.53	2.47	2.86
AFB_2	4.24	28.03	8.41	0.28	2.17	0.46	0.19	1.29	0.49	0.11
		(28.69)	(8.61)	(0.29)	(2.22)	(0.47)	(0.20)	(1.32)	(0.50)	(0.12)
		29.38	8.81	0.29	2.28	0.48	0.21	1.36	0.51	0.12
AFG_1	9.06	59.90	17.97	0.60	4.64	0.98	0.86	2.77	1.05	0.51
		(61.31)	(18.39)	(0.61)	(4.75)	(1.01)	(0.90)	(2.83)	(1.07)	(0.54)
		62.79	18.84	0.63	4.87	1.03	0.95	2.90	1.10	0.56
AFG_2	3.68	24.33	7.30	0.24	1.89	0.40	0.14	1.12	0.42	0.08
		(24.90)	(7.47)	(0.25)	(1.93)	(0.41)	(0.15)	(1.15)	(0.43)	(0.09)
		25.50	7.65	0.26	1.98	0.42	0.16	1.18	0.45	0.09
AFT	37.39	247.17	74.16	2.47	19.16	4.06	14.68	11.41	4.32	8.75
		(253.01)	(75.90)	(2.53)	(19.61)	(4.15)	(15.38)	(11.68)	(4.42)	(9.16)
		259.11	77.73	2.59	20.09	4.25	16.13	11.96	4.52	9.61

Keys: AFB_1 = Aflatoxin B_1; AFB_2 = Aflatoxin B_2; AFG_1 = Aflatoxin G_1; AFG_2 = Aflatoxin G_2; AFT = Total aflatoxins; EDI = Estimated daily intake; HCC = Hepato-Cellular Carcinoma; HBsAg = Hepatitis B virus; TP = Total populatio

Table 4.5: Risk Assessment of Aflatoxin Exposure in Nigerian Garri

	AFT		AFG2		AFG1		AFB2		AFB1				
AEZ	Mean Conc. ($x10^3$) (ng/kg)	Estimated daily Intake (EDI) (ng/kg body weight/day)	Mean Conc. ($x10^3$) (ng/kg)	Estimated daily Intake (EDI) (ng/kg body weight/day)	Mean Conc. ($x10^3$) (ng/kg)	Estimated daily Intake (EDI) (ng/kg body weight/day)	Mean Conc. ($x10^3$) (ng/kg)	Estimated daily Intake (EDI) (ng/kg body weight/day)	Mean Conc. ($x10^3$) (ng/kg)	Estimated daily Intake (EDI) (ng/kg body weight/day)	Estimated liver cancer risk (cases/100, 000 population/year)	Cancer incidence attributable to dietary aflatoxin (%)	DALY
		Male **(TP)** Female		Male **(TP)** Female		Male **(TP)** Female		Male **(TP)** Female		Male **(TP)** Female	Male **(TP)** Female	Male **(TP)** Female	Male **(TP)** Female
SS	27.88	184.32 **(188.66)** 193.21	4.13	27.30 **(27.95)** 28.62	7.82	51.70 **(52.92)** 54.19	2.34	15.47 **(15.83)** 16.22	13.59	89.85 **(91.96)** 94.18	26.95 **(27.59)** 28.25	414.62 **(424.46)** 434.62	351.69 **(360.05)** 368.66
NGS	58.52	386.88 **(395.99)** 405.54	5.54	36.63 **(39.49)** 38.39	18.62	123.10 **(126.0)** 129.04	5.83	38.54 **(39.45)** 40.40	16.02	105.91 **(108.40)** 111.02	31.77 **(35.52)** 33.31	488.76 **(546.46)** 512.46	414.60 **(463.54)** 434.70
DS	49.18	325.14 **(332.79)** 340.82	1.82	12.03 **(12.32)** 12.61	6.42	42.44 **(43.44)** 44.49	6.46	42.71 **(43.71)** 44.77	34.48	227.95 **(233.32)** 238.95	68.39 **(70.00)** 71.68	1,052.2 **(1,076.9)** 1,102.8	892.49 **(913.5)** 935.42
SGS	17.77	117.48 **(120.25)** 123.15	3.02	19.97 **(20.44)** 20.93	0.00	0.00 **(0.00)** 0.00	3.63	24.00 **(25.56)** 25.16	29.52	195.16 **(196.76)** 204.57	58.55 **(59.93)** 61.37	900.77 **(922.0)** 944.15	764.08 **(782.09)** 800.88
HF	23.04	152.32 **(155.91)** 159.67	3.76	24.86 **(25.44)** 26.06	11.08	73.25 **(74.98)** 76.78	2.34	15.47 **(15.83)** 16.22	5.87	38.81 **(39.72)** 40.68	11.64 **(11.29)** 12.20	179.08 **(173.69)** 187.69	151.90 **(147.34)** 159.21
National	176.39	1166.14 **(1193.60)** 1222.38	18.27	120.79 **(123.63)** 126.61	43.94	290.49 **(297.33)** 304.50	20.60	136.19 **(139.40)** 142.76	99.48	657.68 **(673.16)** 689.39	197.30 **(201.95)** 206.82	3,035.4 **(3,106.9)** 3,181.9	2,574.8 **(2,635.5)** 2,699.0

Keys: *SS*- Sudan Savanna, NGS- Northern Guinea Savanna, SGS- Southern Guinea Savanna, DS- derived Savanna, HF- humid forest, TP- Total population, AFB1- Aflatoxin B1; AFB2- Aflatoxin B2; AFG1- Aflatoxin G1; AFG2- Aflatoxin G2; AFT- Total aflatoxins; EDI- Estimated daily intake.

4.2 Discussion of Results

4.2.1 Fungal occurrence (load and incidence) in garri and their implications

Microbiological sources of food contamination are more preponderant and are therefore of greater concern than other sources of contamination such as chemical and physical sources because of the quantum of illnesses associated with it (Scallan *et al*., 2011). In food safety issues therefore, microbiological considerations are of paramount importance. The most palpable concern associated with fungal contamination of food is in connection with the production and deposition of mycotoxins on the food material. The most important genera of mycotoxigenic fungi are *Aspergillus, Alternaria, Claviceps, Fusarium, Penicillium* and *Stachybotrys*. (Yaling *et al*., 2008; Averkieva, 2009). The results obtained from the present study shows that, of the 2 types of garri sold in our open markets in Nigeria, the white garri sample (unpackaged food from busy open markets yielded more fungal isolates (213) than the yellow garri samples which yielded (139) fungal isolates. This data indicate that palm oil added in the processing steps of the yellow garri processing probably reduced the fungal levels in the food. The fungal contamination of the garri samples may be attributed to both post-food production practices, because it is highly unlikely that the fungal propagules can survive the frying step at approximately 100^0C for 15–20 min. To be precise, exposure of garri in the unpackaged form to the busy open markets, where human activities including constant and high human traffic and motorist influx occur for more than 10 h on a daily basis, will result in massive food contamination by diverse fungi. Thus, these open markets and the foods being sold therein (e.g., garri) become hot spots of transmission of pathogenic and/or toxigenic strains to new environments. Therefore, packaging after the frying step might limit fungal contamination and prevent the dissemination/dispersal of harmful strains (Guynot *et al*., 2003).

The load of fungal propagules in the garri samples ranged from (8.50 x10^3cfu/g) to (47.00 x10^3cfu/g). The recovered fungi comprised a total of 346 fungal isolates belonging to six genera: *Aspergillus* species; (*A. flavus, A. fumigatus, A. parasiticus* and *A. niger*), *Fusarium* species; (*F. verticilloides, F. moniuforme* and *F. chlamydosporum*), *Penicillium* species; (*P. scleratonium, P. copticola* and *P. citrinum*), *Cercospora* specie., *Cladosporium* specie., and *Botrytis cinerae* were isolated from the garri sample across the AEZs. Several of these fungal species belonging to *Aspergillus, Penicillium* and *Talaromyces* are frequent in outdoor and household/indoor air samples from different continents (Visagie *et al*., 2014a; Hernández-Restrepo, *et al*., 2016; Chen *et al*., 2017). These airborne fungi, though commonly saprophytic, have been associated with human infections. These fungal genera are known to widely contaminate garri (Oghiehor *et al*., 2007; Sanyaolu *et al*., 2019). The high incidence of A. flavus in the garri samples across the AEZs and its significantly higher occurrence in the garri sample than all other *Aspergillus* species agrees with previous reports of garri sample from some selected state in the southern Nigeria (Oghiehor *et al*., 2007). This suggests similarity of prevalent conditions such as the occurrence of *A. flavus* in the soil and plant debris (Jaime-Garcia and Peter, 2004).

The plant debris usually acts as reservoirs of inoculums for infection of cassava tubers in the field. The isolation of other aflatoxigenic members of *Aspergillus* specie, *A. parasiticus* from the garri in this study agrees with the previous reports by Ogheihor *et al*. (2007) who found this specie in Nigerian garri in addition to the widely distributed *A. flavus* at a lower frequency. This may be attributed mainly to (1) the choice of isolation and characterization medium- modified Dichloran Rose Bengal Agar (DRBA), a selective medium for isolation of *Aspergillus* and *Penicillium* species used also in our study and (2) the relatively scarce distribution of the species in garri. The occurrence of *A. fumigatus*

in the garri sample is in line with previous reports on garri in southern Nigeria (Oghiehor *et al*., 2007). However, Ogheihor *et al*. (2007) found high average occurrence of *A. fumigatus* (26%) in garri as compared to the average occurrence of (13.30%) in this study. The high incidence of some fungal species across the AEZs and low incidence of others confirm that fungi that thrive or found to be scarce in a particular area are strongly determined by the prevailing climatic conditions (Wayne, 2007). The incidence of *Aspergillus* species was higher than that of *Fusarium* species across all the AEZs except for the SGS zone. The high occurrence of *Aspergillus* species in the AEZs corroborate with the findings of Ogheihor *et al*. (2007) who reported higher incidences of *Aspergillus* species in garri samples from southern Nigeria. In spite of the relatively low occurrence of *Penicillium* species than *Fusarium* species in the garri samples in this study, its wide distribution across the AEZs of Nigeria shows that *Fusarium* species is a regular contaminant of garri (Sanyaolu *et al*., 2019). The occurrence of *F. verticillioides* as the most common *Fusarium* species isolated from the garri sample is in agreement with the work of Sanyaolu *et al*., (2019). In addition, *Cladosporium* spp. Is rarely reported in Nigerian garri as it was found in highest in garri from the DS zone in contrast to previous studies by Ogheihor *et al*., (2007) that reported only *Rhizopus stolonifer* as the rare fungal isolate in garri from South-Western Nigeria.

4.2.2 Aflatoxin contamination in garri and their implications

Aflatoxins (AFTs) have been reported as being the most common mycotoxin (Wu *et al*., 2014), affecting not less than 25% of the world´s agricultural food sources (Yard *et al*., 2013). Mycotoxins generally are produced by over 100 filamentous fungi containing some 400 secondary metabolites with toxigenic ability (Kabak *et al*., 2006). On its own, it has been reported that over 4.5 billion individuals in developing countries are at a risk of exposure to AFTs poisoning in food (Williams *et al*., 2004). The detection of AFTs in

the various Garri samples conformed to previous reports by several authors (Thoha *et al*., 2012; Jonathan *et al*., 2013) including other food crops and animals products (Beatriz and Eliana, 2000; Akande *et al*., 2019). Although there are no specific FDA standards for Garri in terms of AFT load, but the present study showed that the presence of AFT were above the minimum recommended index for general food products designed for human consumption (FDA. (Food and Drug Administration (U.S.)), 2009). AFTs in this study have been recorded to be above the permissible level of limit of 4 μg/kg set by European Commission (2006). Results from this study therefore leaves much to worry about as the mean value of 34.48 μg/kg of AFTB1 in garri samples from DS is above the permissible level in food. The far-reaching implication of AFTB1 as a group 1 carcinogen in humans (Seo *et al*., 2011) as well as their hepatotoxic and immunosuppressive nature (Mehrzad *et al*., 2014) has been documented. By implication, apart from the obvious negative effects of AFTB1 poisoning in humans, it is also expected to have a colossal adverse effect on the socio-economic matrices of the society which include loss of human and animal life, increased cost in human health care and animal care, drop and or losses in livestock productivity, loss of forage plants and animal feeds, regulatory and research costs targeted at mitigating the effects of mycotoxin poisoning.

The Aflatoxin concentrations in this study ranged between 1.82 and 34.48 μg/kg, and the highest estimated daily intake (EDI) of contaminated garri AFTB1 (233.32 ng/kg body weight/day) was found in the DS zone (Tables 5), while the lowest (39.72 ng/kg body weight/day) was in the HF zone. The low amount of rainfall (650–1,300 mm), the prevailing high temperature (26–40 °C) and long periods of dry season (6– 9 months) in the SS and NGS zones (Atehnkeng *et al*., 2008) may be responsible for the low concentration of aflatoxins found in food materials in these regions. The low concentration of aflatoxins in garri samples in the HF zone despite its high rainfall pattern

(1,300–2,000 mm) and suitable temperature (26–28 °C) for growth of mycotoxigenic fungi may be due to the fact that garri producers and sellers in the zone do not usually store their garri for long periods, as they sell their garri in the fresh state because the zone is highly urbanised. The garri are usually consumed by the populace and used by local industries either for feed production or as raw material in other industrial purposes. On the other hand, the high concentration of aflatoxin observed in the DS zone, especially in Benue and Enugu States, is probably due to the high amount (1,300–1,500 mm) of bimodal rainfall (Atehnkeng *et al*., 2008) usually recorded in this zone. The fact that the AFTs (G1) were not detected in SGS garri samples does not necessarily indicate was absent. Rather, it could be indicative of the fact that they were present below the detectable limit of 1µg/Kg. As such, an unwitting cumulative exposure to the sub lethal doses over a long time may eventually produce some undesirable effects on human health and wellbeing. In developed countries of Europe and the USA, stringent standards of evaluating and enforcing compliance to the permissible limits of mycotoxins in food have been well developed. In the developing countries of Africa however, there appears to be lax and uncoordinated regulatory and enforcement regimes by the concerned agencies of government at ensuring compliance to the locally adopted permissible mycotoxin limits adopted by these countries.

The comparative high levels of AFTs in garri samples are not unconnected to biochemical properties of fermented cassava products. Studies have shown that increase in mycotoxin production occurred at pH < 6.0 (Sood, 2011). Specifically, other mycotoxin producing fungi - Aspergillus species exhibited maximum growth phase at pH = 5.0, (Sood, 2011) and optimal mycotoxin production (Brzonkalik *et al*., 2012). From these observations, it was not unexpected that the present findings reported a high AFT (1,193.60 ng/kg body weight/day) load in garri samples consumed in Nigeria. According to the low pH

encouraged the production of AFTs (Oghiehor *et al*., 2007) in the finished cassava product, especially the samples that were allowed to ferment over a relatively longer period. Furthermore, the poorly fermented product garri samples will serve as poor media for the propagation of the causative fungi agents and production of AFTs compared with the properly fermented garri samples. Nawaz, (1989) had previously observed that palm oil did not suffer AFT contamination compared to other types of oil and its products. Therefore, the reduced levels of AFTs in palm oil treated garri samples (Yellow garri) were indications of the capacity of palm oil to retard the production of AFTs in fermented Garri. However, it is worthwhile to note that the level of AFT in food in a given geographical location is dependent on certain environmental factors such as agricultural and agronomic practices and the susceptibility of commodities to fungal invasion during pre‑harvest, storage and/or processing periods. (Kalantari *et al*., 2011). The findings from this research may bear a subtle correlation with the increasing incidence of some debilitating cancer and organ failure experienced in Akwa Ibom state (Nwafor and Nwafor, 2018).

The present work revealed high bioload and vast array of microorganisms in market garri and high rate of occurrence and prevalence of aflatoxins B1, B2, G1 and G2. respectively. These are threatening and alarming and suggest early warning signals indicating the level of safety of available garri. It also warrants renewed vigilance on the efficacies of food processing conditions, handling techniques and handlers technical know-how, hygiene practices and safety of finished products. In addition, strict application and implementation of quality control, quality assurance, good manufacturing practice and the hazard analysis critical control point principles will help to ensure the safety of garri consumed by several millions of people in Africa. The significance of this study lies in the diversity of mycotoxigenic moulds found in Nigerian garri. The author understands

that when moulds invade and colonize a suitable agricultural commodity such as garri, they utilize the available nutrients thus deteriorating the nutritional value of the garri. The moulds may further liberate mycotoxins in the commodity depending on the mycotoxigenic potential of the moulds, condition and duration of storage of the garri as well as the prevailing environmental factors. The incidence of mycotoxigenic A. flavus and Fusarium species in this study and the toxins (aflatoxins B1, B2, G1 and G2) produced by the isolates in the conditions of storage is a potential risk to the health of consumers of this food product. A major concern is the attendant health effects that could arise from consumption of this food material by Nigerians as aflatoxins are potent carcinogens, nephrotoxins and immune system toxicants (CAST (Council for Agricultural Science and Technology), 2003). Besides, multi-mycotoxin exposure has recently been reported in Nigerian population (Ezekiel *et al*., 2014; Adetunji *et al*., 2014b).

The level of AFT reported in this study should attract a great concern since the ingestion of such-contaminated food by animals and human can be of enormous public health significance, of greatest concern is the relevance of these toxins in human hepatoma and oesophageal cancer (Shephard, 2008; Shabbir, *et al*., 2013). Also, Continuous intake of small doses of aflatoxin could increase still-births and neonatal mortality, immunosuppression with increased susceptibly to infectious diseases such as pneumonia, stunted growth and HIV/AIDS (Onyedum *et al*., 2020). These unsafe levels of aflatoxin contamination will also reduce the market value of the food stuff and may render it unmarketable. People's Daily Newspaper in 2014, reported that large quantity of Nigerian foods exported to European countries are being rejected due to high presence of aflatoxin. The newspaper also reported that, World Bank has estimated that nine African countries including Nigeria will have 64% of their annual export of nuts, fruits and cereals hitherto

valued at about $64 million annually reduced as a result of rejection in overseas market due to mycotoxin contaminations. Intervention strategies such as: (1) development of cassava varieties that are resistant to a range of fungal infections and subsequent mycotoxin formation (2) reduction of wounds on the cassava tubers during harvesting or by insects (use of insecticides) and (3) harvesting of cassava tubers prior to onset of hot, dry climate conditions that may significantly increase infections by these fungi and (4) drying of garri properly to a moisture content of about 3% after production prior to storage, sales and consumption.

CHAPTER FIVE

5.0 CONCLUSON AND RECOMMENDATIONS

5.1 Conclusion

Fungi and Aflatoxins in varying amount and concentration are present in cassava flakes (garri) collected from selected agroecological zones in Nigeria. This findings confirm the occurrence of about 346 fungal solates belonging to six different genera of fungi in all the samples across the different Agro-ecological zones. This microbiological contamination of garri(sample of study) is of greater concern due to the quantum of illness associated with it; this suggests that there is a potential health risk associated with the consumption of contaminated cassava flakes(garri).

Aflatoxins are the most prevalent mycotoxins found in the cassava flakes (GARRI)samples. This aflatoxins screening from this study confirms the occurrence of four main types of Aflatoxins which are Aflatoxins B1,B2,G1 and G2 at varying proportion and level of toxicity in the garri samples across the Agro-ecological zones.The high incidence of aflatoxin load in the sixty-eight (68) composite sample of garri across the Agro-ecological zones, are very alarming and threatening and it suggest an early warning signal indicating the level of safety in cassava flakes(Garri).

The exposure estimate and risk characterisation of the aflatoxins evaluated in garri within the Agro-ecological zones study areas indicate different populations group within the study area are sucseptible to risk for cancer due to aflatoxins exposure from garri consumption. The liver cancer risk for Aflatoxins B1(the most potent and toxic aflatoxins) was observed to be highest among the overall population groups.

This research work highlights the potential health risks associated with the consumption of contaminated cassava flakes (garri) in Nigeria and underscores the need for targeted

interventions to improve food safety practices and reduce the risk of Aflatoxins contamination.

5.2 Recommendations

i. Regular monitoring of cassava flakes(garri) for fungi and mycotoxins, particularly aflatoxins, to ensure food safety and reduce the risk of exposure to harmful toxins.

ii. Adoption of good agricultural practices such as proper drying, storage, and processing methods to minimize fungal contamination and mycotoxin production in cassava flour.

iii. Implementation of appropriate storage conditions, such as using hermetic storage bags, to reduce the risk of fungal contamination and mycotoxin production during storage.

iv. Increased awareness of mycotoxin contamination and its health implications through public education and health campaigns aimed at improving food safety practices.

v. Development and evaluation of effective interventions, such as the use of biocontrol agents and post-harvest treatments, to reduce the risk of fungal contamination and mycotoxin production in cassava flour.

vi. There is a need for more research to better understand the factors that contribute to mycotoxin contamination in food products and to identify effective interventions to reduce the risk of contamination.

vii. By implementing these recommendations, it is possible to minimize the risk of mycotoxin contamination in cassava flakes(garri) and ensure the safety of this important staple food in Nigeria.

5.3 Contribution of Research to Knowledge

This study identifies and investigates the presence of fungi and productions of mycotoxins particularly aflatoxins in cassava flakes (Garri) which is a staple food in Nigeria. A total of 346 fungal isolates belonging to six genera: *Aspergillus spp., Fusarium spp., Penicillium spp., Cercospora sp, Cladosporium sp.* and *Botrytis cinerae* were isolated from the garri samples. The population of *Aspergillus* spp. (46.53%) was higher than the population of the other fungal genera in both the (white and yellow) garri sample types from all the AEZs. *Fusarium* (25.43 %) was the second in population frequency followed by *Penicllium spp.* (16.76 %) and *Cercospora sp.* (3.76 %), and least frequency hierarchy was *Botrytis cinerae* (3.17 %). In addition, the *A. flavus* isolates were more abundant (37.5%; $p<0.05$) in the DS zone than the other zones. Of the five *Fusarium* species isolated in the garri sample, *F. verticillioides* also recorded a high occurrence (37.5%; $p<0.05$) in DS.

The significance of this study lies in the diversity of the mycotoxigenic moulds found in the garri sample; this study further highlights the importance of food safety in Nigeria, especially with regards to staple foods such as cassava flakes(Garri). Nigeria is a country with a high burden of foodborne illnesses, and fungi contamination connected with the production and deposition of Aflatoxins is a significant contributor to this burden.

Aflatoxin B1 (AFB1) was the most prevalent aflatoxin in the garri samples from all the AEZs. AFB1 was significantly higher ($P<0.05$) in DS zone (34.48 μg/kg) than all other zones. Aflatoxins G1 was below detection limit in the garri samples form SGS zone. Aflatoxins G1 was significantly higher in the NGS zone compared to the low incidence of aflatoxin G2 reported in the DS zone (1.82ug/kg); Aflatoxins G1 contamination was reported to be significantly higher in Gombe state (40.95ug/kg) and sokoto State (22ug/kg) in the NGS and SS zones respectively.

This high incidence rate of Aflatoxins load in the garri samples across the agro-ecological zones exceed the minimum recommended index(4ug/kg) as set by the European union. This result indicated that the aflatoxins load is alarming and threatening and poses a significant risk to human and animal health.

The risk assessment and characterization indicated that the highest EDI (estimated daily intake) of contaminated garri with Aflatoxins B1 is higher in the DS zone and lowest EDI was found in the garri samples from the HF zones; this findings is consistent with the cancer report that an EDI level as low as 0.001ug/kg bw/day may induce liver cancer, hence the level of Aflatoxins load in food should be as low as reasonable.

Finally, This study provides a basis for further research on the occurrence and health implications of fungi and multi-mycotoxins in other food products in Nigeria and other countries with similar agricultural practices. This study further contributes to scientific knowledge by identifying potential health risks, highlighting the importance of food safety, identifying the need for improved agricultural practices, and therbey providing a basis for further research.

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