FUNGAL AND AFLATOXIN OCCURRENCE IN SMALL-SCALE PROCESSED DRY FOODSTUFFS SOLD AT INFORMAL RETAIL OUTLETS IN THE JOHANNESBURG METROPOLIS, SOUTH AFRICA

by

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DECLARATION

I, **CHINENYE KATE OKAEKWU,** declare that the above dissertation is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references. I further declare that I have not previously submitted this work, or part of it, for any degree or examination in any other higher education institution.

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SIGNATURE:_____ **DATE:** 30 January 2019

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DEDICATION

This dissertation is dedicated to Almighty God for His divine inspiration and knowledge and my entire family.

ABSTRACT

Fungal species and their mycotoxins are the most predorminant contaminants of dried agricultural products in sub-Saharan Africa (SSA) and the main species of fungi that can synthesize mycotoxins are *Aspergillus, Fusarium and Penicillium.* In Africa, aflatoxin is labelled as a great threat to human and animal health due to its high contamination levels reported of aflatoxins in foods. The aim of this study was to survey fungi and aflatoxin contamination of small-scale processed foodstuffs sold at informal retail outlets in the Johannesburg metropolis, South Africa. A total of 270 food samples (10 starch and legume based foods, 11 meat and fish based foods, 22 spices and local condiments, 14 dried fruits and vegetables) were collected from retailers; and analysed four (4) times in different seasons of spring, summer, autumn and winter. Out of the 270 samples analysed, only 27.8% were contaminated with fungal. Of all the six categories of foods analysed, roots and tubers (60.0%), nuts and seeds (40.0%), dried vegetables (37.1%), and the Meat and Insect foods (33.3%) respectively, had the most contaminated samples with fungal respectively. The least contaminated food groups were the fish foods (10.0%) and spices and local condiments (16.7%) respectively. Twenty percent of the 270 dried food analysed were contaminated by *Aspergillus* species out of which 61.1% of the contaminated samples had fungal counts above 10^3 cfu/g. *Aspergillus niger* was the most predominant *Aspergillus* species identified in all the categories of food samples analysed. Fruits and vegetables (24.4%) and the nuts and seeds (20.0%) food groups had the highest number of samples contaminated with aflatoxin. Peanut flour and Cardamom had the most incidence of aflatoxin. AFB_1, AFB_2 & AFG_1 were the most prominent aflatoxin types recovered from the food samples. Almost all the food samples in which aflatoxin were identified had aflatoxin values above 10µg/ml.

Keywords: Aflatoxins, fungi, *Aspergillus*, food contamination and food safety

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LIST OF ACRONYMS

AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AFG1	Aflatoxin G1
AFG2	Aflatoxin G2
ANOVA	Analysis of Variance
DAD	Diodide Array Detector
DON	Deoxynivalenol
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization
FHB	Fusarium Head Blight
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Point
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
LC MS/MS	Liquid Chromatograhy Mass Spectrometry
OTA	Ochratoxin-A

PACA	Partnership for Aflatoxin Control in Africa
PIC	Purdue Improved Crop Storage
RTE	Ready-to-eat
SSA	Sub-Saharan Africa
TLC	Thin Layer chromatography
UHPLC	Ultra High-Performance Liquid Chromatography
USFDA	United States Food and Drug Administration
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Food safety involves ensuring that food along the supply chain is safe and void of contamination by microbial and chemical agents (Uyttendaele et al., 2016). The agents of food contamination are mainly bacteria, fungi, viruses, parasites and chemicals. The contamination of food with these agents occurs at different levels during food production, processing and storage (Hanson et al., 2012). Around the world, both in the developed and developing countries, the risk of foodborne diseases is inevitable. Globally, fungal are the main microorganism reponsible for food spoilage (Njobeh et al 2009). Fungal contamination of food is recognised as a serious threat to food safety and security. This is so because, filamentous fungal are able to produce highly toxic secondary metabolites so of which are called mycotoxins . Mycotoxins are the most predorminant contaminants of agricultural products in sub-Saharan Africa (SSA) and contribute a significant fraction of the estimated annual 1.3 billion metric tons of food lost globally (Salem & Ahmad 2010; Udomkun et al 2017). Mycotoxins can be found along the food chain due to fungal contamination that occurs during cultivation and further infestation can continue when food is stored and processed (Temba et al., 2017).

The significant species of fungi that can synthesize mycotoxins are those belonging mainly to the *Aspergillus, Fusarium* and *Penicillium* genera (Udomkun et al., 2017). *A. flavi* is the main species of *Aspergillus* that contaminate a wide array of crops such as maize, peanut and spices and this species has aflatoxin producing abilities (Singh & Cotty 2017). In Africa, aflatoxin is

labelled as a great threat to human and animal health due to the high contamination levels reported in foods (Nishimwe et al 2017). The ignorance of the occurence of aflatoxins in food chain has been linked to the possible cause for the high infestation rate of aflatoxins in agricultural commodities in Africa (Nishimwe et al., 2017).

Severe aflatoxicosis (aflatoxin poison) cases have been recounted in different continents (Mwalwayo & Thole 2016). In Africa, numerous authors have reported the incidence of aflatoxins in various agricultural commodities such nuts, grains, fermented foods, spices, fruits and vegetables (Bumbangi et al., 2016; Chala et al., 2013; Ezekiel et al., 2016; Gnonlonfin et al. Gnonlonfin et al., 2012; Gnonlonfin et al., 2013; Hell et al., 2009; Kamika et al., 2016; Makun et al., 2013; Matumba et al., 2015; Mutungi et al., 2008; Mwalwayo & Thole, 2016; Probst et al., 2014; Riba et al., 2010; Udomkun et al., 2017; Waliya et al., 2015).

In Mali, Waliyar et al., (2015); reported the incidence of aflatoxin contamination in groundnut, at high levels (101–500 μg/kg) that are unsafe for human and animal consumption. Probst et al., 2014, reported severe contamination of all the maize samples analysed across sub-Saharan Africa (SSA). The study indicated that maize samples from Kenya, Uganda, Sierra-Leaone, Somalia, DR Congo and Cameroon were all contaminated with aglatoxins at levels above the set EU limits of 4 μg/kg for ready-to-eat maize.

Kamika et al., (2016) also reported high aflatoxin contamination of maizes samples from DR Congo. The report states that the levels of aflatoxin contaminations exceeded the permissible limit set by EU and WHO. In a study in Malawi, Matumba et al., (2015) reported way over 50% of all the samples (maize- based baby food and peanut butter) analysed contained aflatoxin.

An estimated 250,000 deaths among humans occur yearly in (SSA) caused by aflatoxicosis (Mwalwayo & Thole, 2016). Kenya is at the top of the list in SSA with several cases of aflatoxin poisoning reported. The most notorious of these outbreaks was recorded in 2004 with 317 cases and 125 deaths reported (Kimanya 2015). Between 2005-2010, approximately 104 cases of aflatoxicosis was reported of which 46 deaths were recorded. Consequently in 2014 , 10 cases of aflatoxicosis were recorded according to the Ministry of Health (Kimanya 2015). According to Nishimwe et al., (2017), the eastern regions of Africa are at high risk of aflatoxicosis due to the constant consumption of aflatoxin contaminated foods.

According to the United States Food and Drug Administration (USFDA) aflatoxins recognized as an inevitable contaminant of foods. According to Mwalwayo & Thole (2016), aflatoxins are a common contaminants of food. Some studies have linked the contamination of food with aflatoxins in developing countries to environmental conditions like high temperature and relative humidity (Alakonya & Monda, 2012), as well as poor preharvest and postharvest practices especially during storage and transportation (Jonathan et al., 2013; Bbosa et al., 2013; Magnussen & Parsi, 2013).

Mycotoxins, particularly aflatoxins affect staple foods including cereals like maize, wheat, rice and their derivates, oilseeds like cotton, peanut, rapeseed, coconut, sunflowers, groundnuts and other nuts, dry fruits, delicatessen products, spices, legumes, cassava, fruits, milk and milk derivates (Gimeno, 2004; Wild & Gong 2010). The main food sources that expose humans to aflatoxin are maize and peanut because these crops are most susceptible to contamination by aflatoxins and are highly consumed worldwide (Wu and Khlangwiset, 2010; Chala et al., 2013; Matumba et al., 2015; Kamika et al., 2016). Larger grains like maize are more susceptible to

fungal infection and mycotoxin formation compared to small compact grains like wheat, rice, oat, and sorghum as well as other grains like beans and soybeans, which are encapsulated in hard seed coats (Makun et al., 2012).

Studying the microbial and aflatoxin quality of small-scale processed foods of African origin is of importance because aflatoxin contamination of various food commodities in Africa has been a frequent problem (Shephard, 2008). It has been reported that more than 5 billion people in developing countries around the world are exposed to the health risk associated with aflatoxin from consumption of contaminated foods (Wu et al., 2011). The purpose of this study was to determine the microbial and aflatoxin contamination levels of processed foods of African origin being sold in formal and informal retail outlets in Gauteng.

1.2 PROBLEM STATEMENT

Across the globe, contamination of food with aflatoxins is considered a huge problem because of the severe health consequence aflatoxins have on humans and animals. Hence this research is targeted at addressing some of these issues by analyzing the occurrence of aflatoxins in arstianal processed dried foods in Johannesburg metropolis. In the developing countries, about 40% of agricultural produce is attributed to aflatoxin contamination (Adegoke & Letuma, 2013). Aflatoxin contamination of crops has a direct effect on the economic development as well as the well being of the population (Singh and Cotty, 2017). The economic consequence of aflatoxin contamination of agricultural produce include; food loss and low market value of product (Bhatnagar-Mathur et al., 2015) and reduces the availability of quality and safe foods (Bumbangi

et al., 2016). Other consequences it has on the economy are healthcare cost and loss of farm animals (Bhatnagar-Mathur et al., 2015).

The implementation of aflatoxin regulations on crops in Africa is minimal which further increases the risk of human exposure to this toxic group of mycotoxins (Singh and Cotty, 2017). According to Hell and Mutegi (2011), Africans are exposed to aflatoxin from infancy and throughout their entire life span. The health-related risk factors associated with mycotoxin exposure in humans includes: oesophageal and liver cancer, childhood stunting, neural tube defects and gastrointestinal disorders (Lombard et al., 2012). Evidence has shown that during pregnancy, maternal aflatoxin exposure can have a negative impact on the growth of an infants during the first year of life (Turner et al., 2007). Studies from West Africa reveals that the level of aflatoxin exposure among weaned infants is at an alarming level, which is comparable to levels observed in adults (Mwalwayo & Thole, 2016).

Although aflatoxins are ubiquitous in nature, the prevailing climatic conditions (temperature and relative humidity) in Africa make it favourable for aflatoxin biosynthesis (Hell & Mutegi 2011; Waliyar et al., 2015). Hell & Mutegi et al.,(2011) suggested controlled environmental conditions as one of the strategies to control aflatoxin contamination of food in Africa. This hold true because fungal growth and proliferation occurs at temperatures above 28 °C.

Quality control issue has been a major challenge for small-scale processing industries due to lack of stringent control (Battcock & Azim-Ali, 1998). The hygienic quality of artisanal processed food is dependent on some factors such as the quality of ingredient and raw materials; the hygiene conditions during harvest, processing, packaging and storage (Benkerroum, 2013) as

well as the methods adopted during food processing and packaging. Such methods contribute to increased level of fungal contamination as well as the degree of aflatoxin invasion (Hell et al., 2009).

The aflatoxin contamination of food undermines the efforts towards food and economic security and constitutes a significant threat to poverty eradication efforts in Africa considering that aflatoxin contamination is the major cause of post-harvest loss of crops (PACA, 2013).

1.3 IMPORTANCE OF THE STUDY

This research will provide relevant information on the current *Aspergillus* and aflatoxin contamination levels in small-scale processed foods of African origin sold in the formal and informal retail outlets in the Johannesburg metropolis, South Africa. Findings from this research will be presented to relavant authorities responsible for making decisions regarding the provision of safe foods to consumers. This study will also create awareness about the aflatoxin safety concerns of certain food products sold to consumers in Gauteng with respect to aflatoxin contamination.

1.4 AIMS AND OBJECTIVES

The aim of this study was to analyse the fungi and aflatoxin profile of small-scale processed foodstuffs sold at informal retail outlets in the Johannesburg metropolis, South Africa.

1.5 OBJECTIVES

- To determine the occurrence of fungi as well as strains of *Aspergillus* species in small-scale processed foodstuffs sold at informal retail outlets; and

- To investigate the occurrence of aflatoxins in small-scale processed foodstuffs sold at informal retail outlets in Johannesburg metropolis.

1.6 RESEARCH QUESTION

The research questions listed below were formulated based on the severe nature of aflatoxin contamination of food in Africa and also because of the potential health hazards aflatoxin contamination of food pose when consumed.

1. Is there a high probability for the occurrence of toxic species of fungi and *aspergillus* from food in this investigation?
2. Will the levels of aflatoxin contamination be within or above the acceptable set limits regulatory bodies like the EU and WHO?

1.7 DISSERTATION LAYOUT

This study is made up of six (6) chapters, organised as follows:

Chapter 1: Introduction

This is an introductory chapter to the study, which gives the background and overview of the research.

Included in this chapter are the background, problem statement, importance of the study, aim and objectives, research questions and dissertation layout.

Chapter 2: Literature Review

This chapter gives an overview of existing literatures on microbiology of some locally processed foods of African origin, on mycotoxins and methods of detection of mycotoxins in food.

Chapter 3: Research Methodology

In this chapter, details of the area of study, method of sampling, data collection method and instruments used were outlined.

Chapter 4: Results

The chapter clearly outlines the results of this research study with respect to the fungi, *aspergillus* and aflatoxin profile of artisanally processed foods sold at small retail stores in Johannesburg metropolis.

Chapter 5: Discussion

This chapter discusses the results obtained in terms of the occurrence of different species of fungi and *aspergillus* species. With details of the type of aflatoxin synthesized by certain strains of *aspergillus* species isolated.

Chapter 6: Conclusions and Recommendations

This chapter concludes the findings, discussions, and makes recommendations for future studies.

CHAPTER TWO

LITERATURE REVIEW

2.1 MICROBIOLOGY OF FERMENTED FOODS

Fermentation is a form of food processing that involves the use of yeast or bacteria under anaerobic conditions to convert carbohydrates to alcohol and carbondioxide or organic acids (Oyewole & Isah, 2012). Fermentation methods are native to African cultures and have been adopted in many countries across Africa for centuries (Anihouvi et al., 2012). Cassava *(Manihot Esculenta)* is a principal food crop for the people living in the continent of Africa, Asia and South America. It is the third major source of calories in the world, after rice and maize (Adetunji et al., 2016). Processing of cassava by lactic acid fermentation results in the of production food products such as garri, fufu, lafun or koknte mostly eaten in West Africa, and kivunde and cingwada common in Eastern Africa (Franz et al., 2014). Garri is a type of fermented dehydrated food product from cassava (*Manihot esculenta poir*) and is a staple food in most West African countries (Omonigho and Ikenebomeh, 2002). The *Lactobacillus plantarum* and *Lactobacillus fermentum, Lactobacillus brevis* and *Leuconostoc mesenteroides* are microrganisms used in the fermentation of cassava (Penido et al., 2018; Freire et al., 2015).

The diverse microbial population contaminating garri usually occur during fermentation by mixed microbial cultures. Correspondingly, further contamination can occur during the post-harvest phase. Post-harvest practices like sieving, air drying and marketing of products in open markets contribute to the diverse microbial population in garri (Olopade et al., 2014). The use of specific starter culture, effective HACCP application and good manufacturing practice help

reduce microbial contamination of this product (Olopade et al., 2014). Chikezie & Ojiakor (2013) reported that aflatoxin contamination was reduced in garri samples treated with palm oil because palm oil has the capacity to retard the growth of fungi and the production of aflatoxins in fermented garri.

2.2 MICROBIOLOGY OF DRIED/SMOKED MEAT

Biltong is an uncooked air-dried ready-to-eat (RTE) meat product often consumed in South Africa as a snack. A vareity of lean meat cuts from beef or game are used in the production of biltong (Burfoot et al., 2010; Petit et al., 2014). The manufacturing of biltong involves three processing steps; meat preparation, marinating, and drying under low temperature (Naidoo and Lindsay, 2010). Petit et al., (2014) reported on the safety profile of in relation to the microbial load of commercially processed "dry" biltong. The report suggests that the dry biltong analysed is safe as it meet the sanitatory requirement. This was attributed to the characteristic low water content, low pH and high salt content observed in biltong (Montahan et al., 2018). High microbial counts (6 to 7 log cfu/g) have been recorded in chicken and venison biltong products, while low counts were noted in the spiced varieties (Mhlambi et al., 2010). Furthermore, microorganisms that cause food poisoning and spoilage may be present in commerical biltong at high levels of (≥4 log cfu/g) (Burfoot et al., 2010).

2.3 MICROBIOLOGY OF DRIED/SMOKED FISH

Fish is a major source of high quality protein for humans worldwide. It constitutes 40-70% of protein intake in West Africa (Ikutegbe and Sikoki, 2014). Fish can be preserved using methods like smoking, drying, salting, freezing and fermentation. Fish processed using these methods can

be consumed as brined, fermented, frozen, smoked, or dried depending on the consumer's choice (Ikutegbe and Sikoki, 2014). Obtaining high quality fish product and still maintaining the nutritional value is notably influenced by the "*processing type"* and "*processing parameters"* used (Sampel, 2015). During canning, cooking, or hot smoking of fish, the heat applied has the ability to disrupt the cell membrane which further encourages lipid oxidation. This has a significant effect on the nutritional as well as the safety value of the product (Sampel, 2015). Smoking/drying of fish gives it a characteristic taste. According to Ikutegbe and Sikoko (2014) in a study in Nigeria, contamination of smoked-dried fish by dust and insects can occur in open markets where the fish is displayed uncovered, unhygienically handled.

2.4 MYOCOTOXINS

Mycotoxins are toxic secondary metabolites that are produced by most fungi mainly of the genera *Aspergillus*, *Penicillium* and *Fusarium*, which contaminate various agricultural products during pre-harvest or under post-harvest conditions (Brankov et al., 2013). Aflatoxins, citrinin and ochratoxins of the *Aspergillus* spp. and fumonisins, deoxynivalenol and zearalenone of the *Fusarium* spp. are the most significant mycotoxins occurring in sub-Saharan Africa. Mycotoxins occur in nature in a free or modified form. Human exposure can occur either directly through consumption of contaminated food or indirectly through consumption of animals (meat) contaminated with mycotoxins (Hove et al., 2016).

The number of known mycotoxins that are significant in food safety are more than 400 and they have the ability to cause several acute and chronic illnesses (Quiles et al., 2016). These naturally occuring fungal toxins pose profound challenges to food safety as result of their proliferation and

production under tropical conditions such as high temperatures and humidity, monsoon, unseasonal rains during harvest and flash floods (Adegoke and Letuma, 2013). Other conditions that contribute to fungal growth and mycotoxin production include: Poor harvesting practices and improper storage, transportation, marketing and processing (Atanda et al., 2011). According to the US Food and Drug Administration (USFDA), aflatoxins are considered to be inevitable contaminants of food and are of great importance in Africa (WHO, 2006). From a health and trade perpective, the mycotoxins of significance are aflatoxins, ochratoxins, deoxynivalenol, zearalenone, fumonisins, T-2 and T-2 like toxins (Adegoke & Letuma, 2013).

2.5 TYPES OF MYCOTOXINS

2.5.1 Fumonisins

Fumonisins are a group of mycotoxins produced by the *Fusarium* genus (Coronel et al., 2016). They are non-flourescent, water soluble and polar, (Roohi et al., 2012). Fumonisin B1 is the most abundantly produced of toxin among the different fumonisins species (Reddy et al., 2010). Six different fumonisins; FA_1, FA_2, FB_1, FB_2, FB_3 and FB_4 have been identified (Zaki et al., 2012). The most potent toxin among this group is FB1 and it contaminates about 70% of world's food (Martins et al., 2012). The *Fusarium* species; *F. verticillioides, F. proliferatum* and *F. nygamai,* as well as the species including *Alternaria alternata* f. sp. *lycopersici* and *A. niger,* produce fumonisins (Reddy et al., 2010). Unlike other mycotoxins, fumonisins are highly water-soluble because they do not have an aromatic structure (Murphy et al., 2006). Fuminisin is the main contaminant of maize and maize-based foods, and this is a major concern for the food industry considering that maize is a staple food in many countries (Boutigny et al., 2012). Around the

world, the fungus, *Fusarium verticillioides* often infects maize and causes several diseases in humans and animals (Mwalwayo and Thole, 2016). In China and South Africa (Eastern Cape province), it has been reported that there is an association between the consumption of fumonisin contaminated maize and risk of human esophageal cancer (Mwalwayo and Thole, 2016).

During food processing fumonisins are stable and are not degraded during corn fermentation. They are also heat stable and resistant to canning and baking processes. However, maize nixtamalization process also reduces fumonisin B1 levels in the final product (Zaki et al., 2012). Nixtamalization is a food processing step which involves cooking maize in large water bath with the addition of calcium hydroxide. This process has been reported to be effective in reducing mycotoxin in maize (James & Zikankuba, 2018).

The maximum daily intake limit set for fumonisin B_1, B_2 and B_3 by World Health Organization (WHO) and the Food and Agriculture Organization (FAO) is 2 µg/kg body weight/day. While in the United States, food intended to be consumed directly by humans has the limit set at 2-4 mg/kg (2 to 4 ppm) and in the European Union, the limit is 1 mg/kg (1 ppm) for food to be consumed directly by humans and 4 mg/kg (4 ppm) for food intented to be processed further (Mwalwayo and Thole, 2016).

5.2.1 Ochratoxin

Ochratoxin is a fungal toxin that occurs naturally and is insoluble in water, moderately soluble in organic solvents such as methanol, chloroform, and ethanol, and fairly heat stable (National Toxicology Program, 2011). Ochratoxin A (OTA) is the second most important mycotoxin because it has been classified by the International Agency for Research on Cancer (IARC), as a

class 2B human carcinogen (Hope J and Hope B 2012; Copetti et al., 2012; Kara et al., 2015). Various studies conducted revealed that OTA has a high range of toxicity in humans and poses a serious threat to public health (Darouj et al., 2016). It is mostly produced by *Aspergillus ochraceus* and *Penicillium verrucosum* as well as *Aspergillus niger* and *A. carbonarius* which have been reported to be capable of producing OTA (Bhat et al., 2010). Ochratoxins A, discovered by South African scientists in 1965, is a toxic secondary metabolite of *Aspergillus ochraceus*. It contaminates crops like-cereal grains (e.g corn, barely, wheat and oats), beans (e.g soybeans, coffee, cocoa), peanuts and meat in some countries (Zaki et al., 2012). Although this toxin has been reported to occur in food commodities around the world, with Africa and Europe being the main regions of concern (Atanda et al., 2013). In Nigeria, there are reports of OTA contamination of maize, sorghum, rice cocoa beans, kolanut and tiger nuts as well as in weaning foods produced from maize for children (Makun et al., 2013).

5.2.2 Deoxynivalenol (DON)

Deoxynivalenol (DON) also known as vomitoxin, is produced principally by *F. graminearum* and *F. culmorum* and is grouped in the Type B class of mycotoxins which are referred collectively as the trichothecenes (Reddy et al., 2010). 3-acetyl DON, 15-acetyl DON and 3-15-acetyl-DON are the acetyl derivatives of DON, which can be present in lower concentrations together with DON in cereal grains and cereal-based products (European Food Safety Authority, EFSA, 2013). Deoxynivalenol (DON) has a polar organic structure, a molecular weight of 296.36 g/mol and a melting point of ranging from 151 to 153 °C (Bonnet et al., 2012). It is stable because of its high melting point, which allows it to survive milling, heat and chemical processing, hence finding way from contaminated maize and wheat grains in processed animal

feed and food products leading to economic loss (Bhat et al., 2010). Furthermore, low amounts of DON and its metabolite, de-epoxy-DON have been detected in eggs and beer (Bhat et al., 2010). In 1987, DON was responsible for the greatest outbreak of mycotoxin poisoning in India with over 50,000 people affected (Probst et al., 2014).

5.2.3 Aflatoxins

Aflatoxins (AF) are secondary metabolites produced by a large number of *Aspergillus* species such as: *A. flavus, A. parasiticus* and *A. nominus,* the main producers of the four major aflatoxins (AFB_1, B_2, G_1, G_2) *(*Lizarraga-Paulín et al., 2011*).* Aflatoxins B_1 and B_2 found in cattle and other animals that have consumed aflatoxin contaminated feed are hydrolysed to aflatoxins M_1 and M_2, respectively, found in their milk (Dhanasekaran et al., 2011). Among all the aflatoxins, aflatoxin B_1 (AFB_1) has the highest toxicity and is classified by the World Health Organisation (WHO) as a human carcinogen with no acceptable safe dose (Saini and Kaur, 2012)**.** With regards to various epidemiological studies, the International Agency for Research on Cancer (IARC) has also classified AFB_1 as Group 1 carcinogen to humans (Azaman et al., 2016; Oplatowska-Stachowiak et al., 2016). Chronic exposure to AFB_1 can lead to the development of liver cancer, particularly in individuals with hepatitis B antigen (Saini and Kaur, 2012)**.** Aflatoxins mostly occur in latitudes between 40° N and 40° S of the equator and developing countries in the tropics are the most affected *(*Lizárraga-Paulín et al., 2011). In Africa and Asia, aflatoxin contamination of food commodities has rapidly increased over time (Azaman et al 2016). While in sub-Saharan Africa, it has been reported that aflatoxin awareness among the general public is minimal (Matumba et al., 2015). Figure 2-1 shows the chemical structures of aflatoxins.

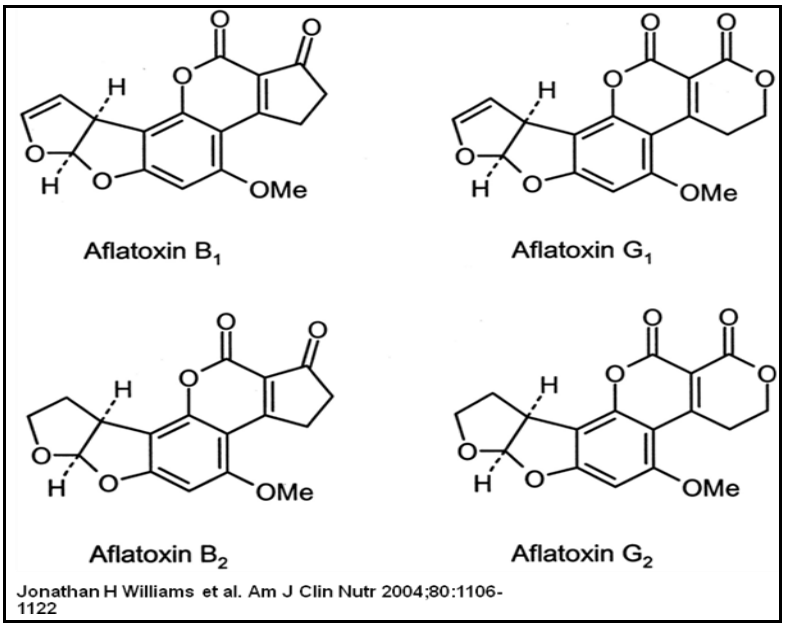

Figure 2 - 1: Chemical structures of aflatoxins

2.5 OCCURRENCE OF AFLATOXINS IN FOODS

Aflatoxin, particularly *A. flavus* are most prevalent in nature and are prevalent in some crops like maize growing under stressful conditions such as drought. They also occur in soil, microbiological decaying, hay, grains and vegetation (Makun et al., 2012). Principal sources of aflatoxin contamination are "*cereals (maize, sorghum, pearl millet, rice, wheat), oilseeds (peanut, soybean, sunflower, cotton), spices (chili peppers, black pepper, coriander, turmeric and ginger), and tree nuts (almond, pistachio, walnut, coconut and Brazil nuts)"*. Other means of mycotoxin contamination in human are "*fruits, vegetables, animal tissues, animal products and fermented products"* (Kabak 2016; Makun et al., 2012; Oplatowska-Stachowiak et al., 2016; Waliya et al 2015).

2.6 MYCOTOXIN CONTAMINATION OF WHEAT, FLOUR AND BREAD

Approximately 25% of world cereals are contaminated by mycotoxins (Škrbić et al., 2012). *Fusarium* species are common contaminants of cereals such as maize, wheat, and barley (De Angelis et al., 2013). *Fusarium* species mainly F. *graminearum* and F. *culmorum* cause *Fusarium* Head Blight (FHB) disease in wheat, barley, oats, rye and maize (Simsek et al., 2012). They are widely distributed in the temperate zones across worldwide and they can grow both in the field and during the storage of agricultural products. Trichothecenes are produced by the genus *Fusarium*, and this class of mycotoxins is a highly toxic and is sub divided into four groups (A–D). Within this group, the most common are trichothecenes A and B (De Angelis et al., 2013). Contamination of wheat with DON is directly related to the incidence of FHB. The occurrence of FHB is greatly linked with moisture present at the time of flowering (anthesis). *F. graminearum* grows best at a temperature of 25 ^{o}C and at water activity above 0.88 while *Fusarium culmorum* grows best at a temperature of 21 ^{o}C and at water activity above 0.87 (Simsek et al., 2012). The study by Iqbal et al., (2014), in Pakistan, demonstrated that a notable percentage of wheat-based products analysed contained AFs and ZEN at levels that are above the acceptable limits.

2.7 MYCOTOXIN CONTAMINATION OF MAIZE FLOURS AND POPCORN KERNELS

Maize (*Zea mays)* is one of the commonly cultivated cereals worldwide and it serves as food and feed for humans and animals. In the developing countries and Africa in particular, maize is the major staple food (Matumba et al., 2009). Maize and maize products are prone to contamination

by mycotoxins such as aflatoxins (Karami-Osboo et al., 2012). Recently in Africa, cases of aflatoxin poisoning (*aflatoxicosis*) have been reported as a result of consumption of contaminated maize (Wu & Guclu 2012). Several outbreaks of aflatoxicosis have occurred in Kenya in years 2001, 2004, 2005, 2006 and 2010 due to consumption of contaminated maize grains (Gachara et al., 2013).

The main source of aflatoxin exposure to human is maize, because it is widely consumed around the world and also because of its susceptibility to aflatoxin contamination (Wu and Guclu, 2012). Mycotoxins contaminate 25- 40% of cereals around the world (Riba et al., 2010). Mycotoxins contamination of maize can occur in the field through the exposure of the crop to fungi from the environment, during storage of crops as well as improper transportation and food processing (Van Asselt et al., 2012; Wu & Guclu 2012). *Fusarium* infection of maize and toxin formation is influenced by climatic factors during cultivation. As climate changes, the dominant species in maize may also change accordingly (Van Asselt et al., 2012).

Probst et al., (2014), carried out a study in 18 countries in sub-Saharan Africa to examine aflatoxins in maize meant to be consumed by humans. The study revealed high contamination levels of aflatoxin in maize. Blankson & Mill-Robertson (2016), reported in their study in Ghana, that locally produced cereal-based baby foods contained high levels ($4\ ng\ g^{-1}$) for total aflatoxins that were beyond the aflatoxin limits set ($2\ ng\ g^{-1}$) by the European Union (EU).

Due to the toxicity of mycotoxins, regulations on the maximum limits of mycotoxins in maize intended commercial purposes have been set. The maximum limits set by the European Union (EU) regulation for different mycotoxins with the exception of unprocessed maize intended to be

processed by wet milling maize is, 1750 μg kg^{-1} for DON; 350 μg kg^{-1} for ZEA and 4000 μg kg^{-1} for the sum of FB_1 and FB_2 and 5 μg kg^{-1} for AFB_1.The maximum limit of 10 μg kg^{-1} for total AF (sum of B_1, B_2, G_1 and G_2) was set for maize that is to be subjected to sorting or other physical treatment before human consumption or use as an ingredient in foodstuffs (Garrido et al., 2012). In South Africa, the maximum limits for total aflatoxin is set at 4 ppb for AFB1 and 10 ppb for total aflatoxin (Kachapulula, et al 2017).

2.8 MYCOTOXIN CONTAMINATION OF HERBS AND SPICES

Spices are plant substance from exotic or indigenous origin, with strong taste and aroma and are used worldwide for flavour, colour, and to preserve food or beverages (Kulshrestha et al., 2014). Climatic conditions, like temperature and humidity, as well as inadequate manufacturing environments during production favours fungal growth and development on spices. Aflatoxins (AFs) and ochratoxin A (OTA) are the two main groups of mycotoxins of significance in spices (Ozbey & Kabak 2012; Prelle et al., 2014; Kulshrestha et al., 2014). Post-harvest practices like drying and storage can encourage the growth of mould especially when good storage practises are not observed (Prelle et al., 2014). Furthermore, more spoilage can occur, during transportation and handling (Gnonlonfin et al., 2013). The co-occurrence of AFs and OTA and other mycotoxins has been reported in spices in several studies reported in Poland (Waśkiewicz et al., 2013), India (Kulshrestha et al., 2014), Malaysia (Jalili & Jinap 2012), West Africa (Gnonlonfin et al., 2013), Morocco (El Mahgubi et al., 2013), and Turkey (Golge et al., 2013).

Studies have demonstrated that contaminated spices can be a direct source of mycotoxin exposure to humans when consumed. Thus the regulatory limit are set to control the presence of

mycotoxins in spices (El Mahgubi et al., 2013). The maximum limit set by the European Union "*for chili, chili powder, paprika, white and black pepper, nutmeg, turmeric, ginger and spice mixtures containing one or more of the above-mentioned spices"* is 5 ng g^{-1} for AFB_1, and 10 ng g^{-1} for total AFs (AFB_1, AFB_2, AFG_1 and AFG_2). For OTA, the tolerable limits in spices has been reduced from 30 to 15 μg kg^{-1} in 2012 (Prelle et al., 2014).

2.9 MYCOTOXIN CONTAMINATION OF NUTS

Nuts and oilseeds are the most studied of all foods and feeds with reference to aflatoxin contamination because they are most susceptible to aflatoxin contamination and are the main components of many human and animal diets (Makun et al., 2012). There are many kinds of nuts consumed around the world, which include groundnut, almond, hazelnut, Brazil nut, cashew, pine nut, pistachios, pecan, walnut, and other tree nuts. Oilseed crops such as soybeans, sunflower seed, canola, rapeseed, safflower, flaxseed, mustard seed, peanuts and cottonseed, have a variety of uses like in the production of cooking oil, livestock feeds, and other industrial uses. Oilseeds and their derivatives are primarily eaten as snacks and serve as ingredients to prepare certain dishes (Filazi & Sireli, 2013).

Matumba et al., (2015), in a study in Malawi, reported high levels of aflatoxins in peanut used for the local production of peanut butter. The report further stated that the peanut butter produced were not suitable for human consumption based on the global regulatory limits set. Ali et al., (2013) in a study in Arak, in Iran, reported high levels of aflatoxins in various nuts analysed (fig, walnut, almond, hazelnut, pistachio and sunflower) Ndung'u et al., (2013), studied the prevalence of aflatoxins in groundnut and peanut butter, in the Nairobi market and reported high

levels of aflatoxins and other mycotoxins. Riba et al., (2013) in Algeria, reported that Pistachios, shelled almonds, shelled peanut and unshelled walnuts were highly contaminated by fungal. The most recurrent fungi species isolated were *Aspergillus, Penicillium* and *Mucor*.

Factors such as season, humidity, temperature and drought in the field coupled with prevailing climatic conditions during storage have significant effect on the production of aflatoxins in crops (Ali et al., 2013). Poor hygiene and handling practices during processing of nuts can further contribute to contamination of the finished product. The Codex Alimentarius has set the maximum limit for aflatoxins in shelled, ready-to-eat nuts at 10 μg/kg and 20 μg/kg for in-shell nuts (Andersson 2012) while the *European Union set aflatoxin tolerance standards of 2 μg* kg^{-1} *AFB1 and 4 μg* kg^{-1} *total aflatoxins in nuts* (Filazi & Sireli 2013).

2.10 AFLATOXIN LIMITS IN FOODS

The maximum permittable limits for aflatoxins in food and feeds have been set to ensure the safety of food products. Several countries, particularly developed countries have in place specific regulations for aflatoxins. In industrialized countries, the limits for aflatoxin B_1 in foodstuffs is 0 to 30 μg/kg, while total aflatoxins range from 0 to 50 μg/kg (WHO, 2006). In Africa, in 2003, fifteen countries including South Africa, owing to approximately 59% of Africa's population, implemented mycotoxin regulations (FAO, 2004).

2.11 DETECTION OF MYCOTOXINS IN FOOD STUFFS

2.12.1 Thin Layer Chromatography (TLC)

Thin layer chromatography is a traditional and the most widely used method for mycotoxins analysis because little time is required to analyze large number of samples. The popular matrix

used for TLC is silica gel layer. The principle of TLC involves separation of compounds based on their migration rate on specific matrix with the aid of particular solvents (Bhat et al., 2010). Some of the advantages of TLC analysis are that it involves both quantitative and semi quantitative tests for mycotoxins, it is inexpensive, and UV-Vis spectral analysis is used for easy identification of targeted compounds (Rai et al., 2012).

2.12.2 High Performance Liquid Chromatography (HPLC)

In recent times, the most commonly used technique for analysis of mycotoxins prevalent in cereals is the HPLC coupled with UV, diode array detector (DAD) or fluorescence detector (FD) (Pascale, 2009). The principle of HPLC is based on adsorption, separation and ion exchange, used id dependent on the kind of stationary phase used (The International Pharmacopoeia). High and ultra-high-performance liquid chromatography (HPLC/U-HPLC) combined with different mass spectrometric techniques provides a powerful analytical tool for multi-toxin analyses (Hajslova et al., 2011).

HPLC is based on mechanisms of adsorption, partition and ion exchange, depending on the type of stationary phase used.

2.12.3 Liquid Chromatography/ Mass Spectrometry (LC-MS)

To screen, identify and measure a large number of mycotoxins simutaneously, LC-MS and LC-MS/MS are the techniques mostly used. LC-MS has been used to examine of list of mycotoxins like aflatoxins, patulin, fumonisins, ochratoxin A, trichothecenes and zearalenone and its metabolites (Pascale, 2009).

The principle of LC-MS involves a combination of the resolving power of liquid chromatography (LC) with the specific detection technique of mass spectronetry. Samples are seperated with and then introduce to mass spectrometer (MS) (Hewlett-Packard 1998). Mass spectrometer works by creating charged ionised state from the analyte molecules. The principle of MS is based on the analysis of ions and any fragment ions produced during ionisation process, on the basis of their mass to charge ration (*m/z*) (Pitt 2009).

2.13 INTERVENTION STRATEGIES FOR MYCOTOXIN MANAGEMENT

Various diverse biological, physical and chemical techniques have been established to control mycotoxigenic fungi and to preclude hazard associated with mycotoxin contamination, both at pre- and post- harvest stages, or to decontaminate or detoxify mycotoxins accumulated in food and feeds (Tsitsigiannis et al., 2012). In Africa, mycotoxin problem can be managed primarily through the following ways: Preventing mycotoxin exposure, decontamination and continual surveillance and monitoring of food/feed contamination with moulds (Rachaputi et al., 2002).

2.13.1 Preventing mycotoxin exposure

The adherence to good agricultural practices (pre and post harvest) is effective in the reduction of mycotoxins contamination in crops. Fungal infections of crops during cultivation can be reduced when crops are harvested early (Rachaputi et al., 2002). Furthermore, post-harvest practices like drying of agricultural commodities reduces the moisture content thus, preventing fungal growth and proliferation and prolong the shelf-life. The degree of mycotoxin contamination is influenced by the level of insect damage giving their ability through their

feeding habits to impair the kernel of grains and carry spores of mycotoxigenic fungi from the plant superficial to the internal (Zain, 2011).

2.13.2 Decontamination of mycotoxin contaminated foods

The various means to remove, inactivate or detoxify mycotoxins contamination in foods and feeds include physical, chemical and biological based on the conditions.

Physical methods involve processing of foods with heat like cooking, boiling, baking, frying, roasting, microwave heating, extrusion and irradiation (Zaki et al., 2012). Instruments such as photoelectric detector can be used to separate fungi-contaminated seeds from the good seeds, however, this method is expensive to use. A less expensive but labour intensive method of removal of contaminated seeds is by hand picking (Atanda et al., 2013). Almost 70% of aflatoxins in rice is destoryed when cooked or heated under pressure and aflatoxin B_1 is reduced by 50-70% in oilseeds during drying and oil roasting. Heating at 100 ^{o}C can inactivate mycotoxins while organic solvent can be used to extract aflatoxins from other agricultural products (Atanda et al., 2013).

Treatment with chemoprotection or enterosorption chemicals such as Oltipraz and Chlorophylin are the most effective method applied for removal of mycotoxins in agricultural commodities (Kensler et al., 2004). Entersorption involves the use of certain clay minerals, like Novasil, which has the ability to selectively adsorb mycotoxins tightly to prevent their absorption in the gastrointestinal tract of animals (Zain, 2011). Similarly, dietary intenvention using broccoli sprouts and green tea have been used for chemoprotection from aflatoxin because they both increase detoxification processes in animal (Kensler et al., 2004). Broccoli sprouts and green tea

prevent the production of epoxide that can lead to damage of chromosomes (Wagacha and Muthumi, 2008). From this literature review, it is evident that aflatoxin is ubiquitious in nature and there must be continuous studies and regulatory monitoring of aflatoxins in agricultural produces and in processed foods. Thus, this study is targeted at filling part of that gap, by evaluating the safety profile in terms of fungi and aflatoxin of artisanal processed food sold at small-scale retail stores in Johannesburg Metropolis.

CHAPTER THREE:

MATERIALS AND METHODS

3.1 BRIEF DESCRIPTION OF STUDY AREA

The growth and development of fungal is favoured at a high humidity (above 85%) and high temperature (36 - 38°C). The climate of Africa is well suited for the growth of toxigenic fungi. Aflatoxin contamination of crops occurs in most regions in Africa. Thus, aflatoxin exposure in African communities is inevitable posing serious threat to the health of the people of the continent (Hell & Mutegi, 2011). This study is focused on the city of Johannesburg with attention on locally processed foods from different African countries, sold at small retail shops. Johannesburg is local municipality located in the province of Gauteng, South Africa. Figure 3-1 shows the different regions in Johannesburg. It is the country's main industrial and financial metropolis (Encyclopaedia Britannica Inc. 2016). It spans an area of 1645 km^2 (Statistics South Africa 2011). It hosts over 500 suburbs and townships. A large percentage of the population in Johannesburg are black, with the remaining population made up of whites, coloured and Indians (Encyclopaedia Britannica Inc., 2016). The city of Johannesburg has a temperate climate. During summer the temperature averages about 75 ^oF (24 ^oC); winter 55 ^oF (13 ^oC) and sometimes temperatures get to below freezing. During summer and winter seasons, the city enjoys about 8 hours of sunshine daily (Encyclopaedia Britannica Inc., 2016).

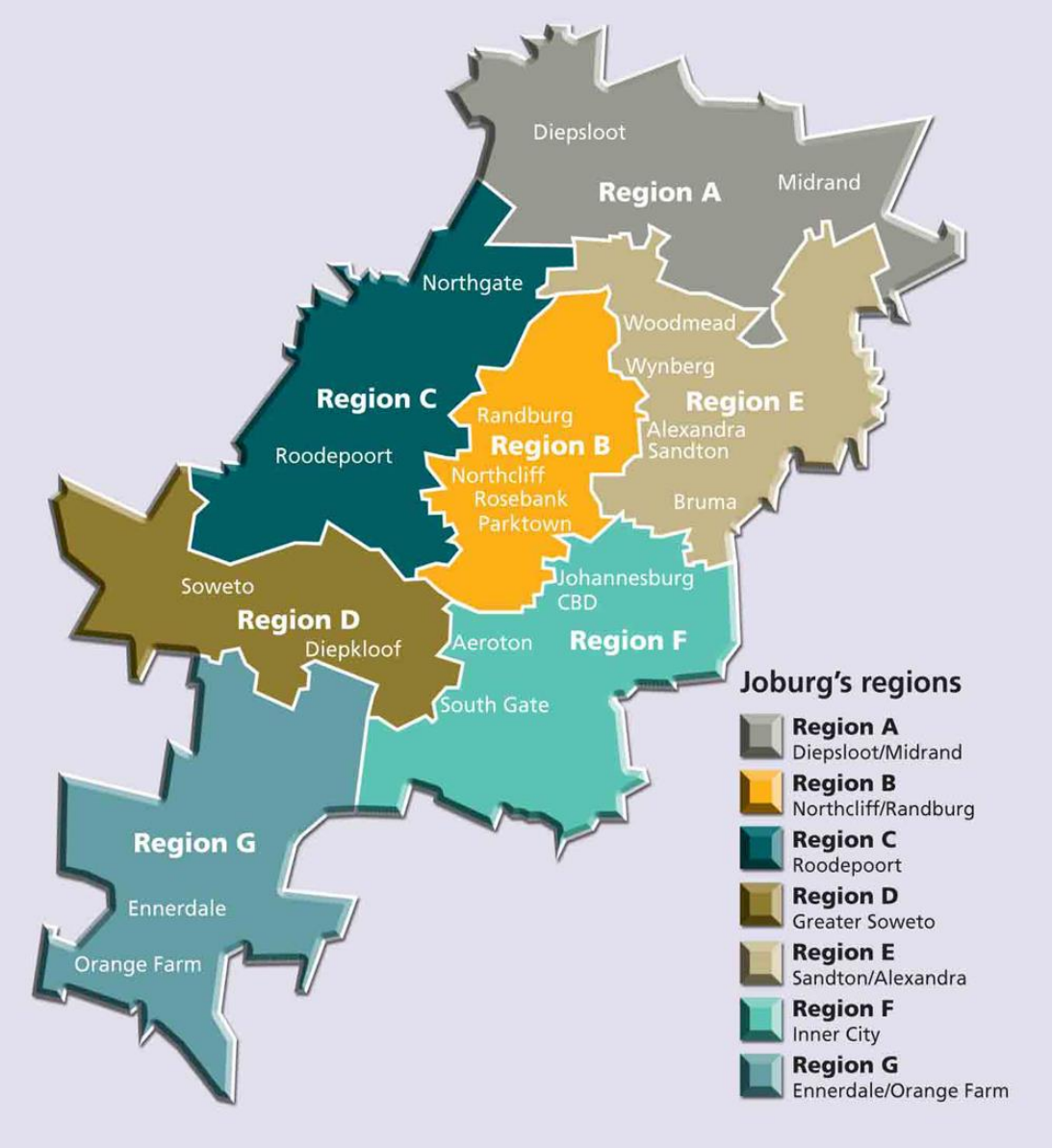

Figure 3 - 1: Map of Johannesburg showing the different regions (Courtesy: google 2017)

3.2 RESEARCH DESIGN AND SAMPLING

This research was based on a cross-sectional analysis of processed foods sold at various retail outlets in Johannesburg municipality between Feburary 2015 to March 2016.

3.3 SAMPLE COLLECTION

Food samples were purchased from formal and informal retail outlets in Gauteng. A total of 270 food samples (10 starch and legume based foods, 11 meat and fish based foods, 22 spices and local condiments, 14 dried fruits and vegetables) were collected from same retailers; and analysed four (4) times in different seasons of spring, summer, autumn and winter. Samples where all dried locally processed foods from different African countries (Nigeria, Zimbabwe, Congo, Zambia, South Africa) and India. The samples shown in Tables 3.1 and 3.2 where purchased from small retail outlets in Randburg, Yeoville and New Town. Sample collection started from the end of summer 2015 to the end of summer 2016. The samples were collected in packages used by vendors and transported in child cooler box to the laboratory in University of South Africa (UNISA), Florida Campus for analysis.

Table 3.1: Dried root, tuber, nut, seed and fishery foodstuffs sold in the Johannesburg metropolis and their mode of utilization

Food groups	**Mode of utilisation**
Root & Tuber (n=15)	
Gari	Granular flour of cassava (*Manihot esculenta*) produced by subjecting cassava tubers in a machine grater. Hot or cold water can be added to it to form a thick dough like paste that can be eaten with soups or stews.
Cassava Flour	Prepared by milling raw or cooked and dried cassava tubers in flour. Used to prepared a thick form of porridge and eaten with soups
Nuts & Seeds (n=40)	
Roasted peanut (*Arachis hypogaea)*	Peanut is roasted and eaten as a snack
Peanut flour	Prepared by milling dried peanuts into flour. It is used in preparing soup in some regions in West Africa.
Soya bean flour (*Glycine max)*	It prepared by milling soya bean into flour. It is used as soup thickeners in some African countries. In Nigeria, it is added to locally processed baby porridge (pap or ogi).
Mustard seed (*Brassica nigra*)	Spice for cooking Indian currie soups.
Sesame seed (*Sesamum indicum*)	Garnish for food used primarily on top of bread bun.
Sesame seed flour (*Sesamum indicum*)	It prepared by milling dried sesame seed. It is used as a soup thickener in many African dishes.
Egusi (*Citrullus colocynthis*) seed	It is usually blended into paste and used as ingredient/thickener in soup dishes in West Africa.
Ogbono (*Irvingia gabonensis*) seed	It is usually blended into paste and used as ingredient/thickener in soup dishes in West Africa.
Fisheries (n=40)	
Cray fish (*Cambarus bartonii*)	A source of protein and an ingredient to prepare various soups in West Africa.
Prawns (*Penaeus monodon*)	A source of protein and an ingredient to prepare various soups in West Africa.
African sharptooth catfish (*Clarias*	Used in preparing fish stews and soups in most countries in West Africa.

gariepinus)	
Biyoto/Spade fish) (*Ephippus orbis*)	Used in preparing fish stews and soups in most countries in West Africa.
Cod (Stock fish) (*Gadus morhua*)	Used in preparing fish stews and soups in most countries in West Africa.
Dakala/Lake Victoria sardine (*Rastrineobola argentea*)	Used in preparing fish stews and soups in most countries in West Africa.

Table 3.2: Dried meat, insect, vegetable, spices & condiments food stuffs sold in the Johannesburg metropolis and their mode of utilisation

Food groups	**Mode of utilisation**
Dried meat and insect (n=	
Emperor moth caterpillar (Maponya worm) (*Tenebrio Molitor*) *Tenebrio Molitor*	Source of protein and ingredient used in the preparation of various dishes in countries in sub-Saharan Africa. They can also be roasted and eaten as snacks.
Dried Sausage (Droëwors)	Can be eaten as snack, used in preparing soup and bread sandwiches
Biltong (dried cured meat)	Can be eaten as a snack
Vegetables (n=	
Bitter leaf (*Vernonia amygdalina*)	It is used to prepare vegetable soups in many West African countries.
Okazi leaf (*Gnetum africanum*)	It is used to prepare vegetable soups in many West African countries.
African Basil leaf (*Ocimum gratissimum*)	It is used to prepare vegetable soup in many African countries.
Dilombolombo leaf (*Piper umbellatum*)	It is used to prepare vegetable soup in many sub saharan African countries.
Okra (*Abelmoschus esculentus (L.) Moench)* leaf	It is used to prepare vegetable soup in many African countries.
Spider flower leaves (*Cleome hassleriana*)	It is used to prepare vegetable soup in many SouthernAfrican countries.
Bean leaf (*Phaseolus vulgaris*)	It is used to prepare vegetable soup in many African countries.
Roselle plant/Zobo flower) (*Hibiscus*	Use for making local beverages in many countries in West Africa.

sabdariffa)	
Golden raisin (*Vitis Vinifera*)	Ingredient used in cooking and baking of ready to eat snack such as cakes, buns and bread.
Dried Apricot (*Prunus armeniaca*)	Ingredient used in cooking and baking of ready to eat snack such as cakes and Morrocan stews.
Dried Figs (*Ficus carica*)	Ingredient used in cooking and baking of ready to eat snack such as cakes and pies.
Spices & condiments (n=90)	
Pepper soup spice blend consisting of : African nutmeg (*Monodora myristica),* West African pepper (*Piper guineense*) and negro pepper (*Xylopia aethiopica).*	It is made from a variety of spicy seeds (such as African orchid nutmeg, piper guineense, native to the west Afrian regions. It is used in making spicy soup without oil or fat many countries in West Africa popularily refered to as pepper soup.
Chilli (*Capsicum annuum)* fruit and powder, ginger powder (*Zingiber officinale*), mustard seed powder (*Brassica nigra L*), pepper corn (*Piper nigrum*)	Spice used in cooking various dishes

3.3 SAMPLE PREPARATION FOR FUNGAL ANALYSIS

Five to ten grams of food samples was weighed out on sterile foil paper using a scale (Uni Bloc) and tranferred to a sterile Waring blender. Fifty-hundred-millimeter 50-100 ml of freshly prepared buffered peptone water (Biolab, South Africa) was measured and added to the blender. The blender was used to homogenize the food samples. The glass container of the blender was washed properly with a dishwasher liquid and a brush and treated with 70% alcohol before reuse to prevent cross-contamination. The homogenised food sample was transfered to a sterile tube with a lid, labelled and stored in the refrigerator at 4^{o}C and used for further analysis within 24 hours.

3.4 MICROBIAL ANALYSIS

3.4.1 Total fungal count

For each sample, serial dilutions in tenfold was done, up to (10^{-2}), an aliquot of 1 ml of the sample was spread on Rose Bengal Chloramphenicol agar plates. The plates were labelled and incubated at 25°C for 5-7 days. Plates containing 30-100 visible fungal colonies with darker pink colour were identified and counted with a hemocytometer

3.4.2 DETECTION AND ENUMERATION OF *ASPERGILLUS* SPECIES

For each sample, serial dilution to tenfold was done, up to 10^{-2}, an aliquot of 1 ml of the sample was spread on *Aspergillus* differenciation agar plates. The plates were labelled and incubated at 30 °C for 3-5 days. Visible fungal colonies with orange/yellow colour were counted and identified as *Aspegillus* species. Individual fungal mycelia were carefully harvested using a sterile inoculation loop and transfered in sterile Eppendorf tubes containing freshly prepared nutrient broth (Biolab, South Africa) and incubated for 7 days and later used for DNA extraction.

3.4.3 DNA EXTRACTION ON ASPERGILLUS SPECIES

Fungal genomic DNA was extracted from the culture using ZR Fungal/Bacterial DNA Miniprep™ kit (Zymo Research Corporation, USA). Extraction was done following the manufacturer's protocol. The DNA was quantified using the nanophotometer (IMPLEN) following the manufacturer's protocol.

3.4.4 AMPLIFCATION OF THE ITS GENE OF *ASPEGILLUS* SPECIES

The amplification of the ITS gene was done using the universal primers ITS1 (5'- TCC GTA GGT GAA CCT GCG G-3') that causes hybridization at the end of 18s rDNA and ITS4 (5'- TCC TCC GCT TAT TGA TAT GC-3') that causes hybridization at the beginning of the 28s rDNA (Ferrer et al., 2001). The primers used were synthesized by Inqaba Biotechnology Company (South Africa). PCR amplication was done in a 25 µl reaction volume, which contained 1.5 µl of template DNA, 0.5 µl of forward primer, 0.5 µl of reverse primer, 10 µl of nuclease free water, and 12.5 µl 2x PCR Master Mix [50 units/ml of Taq DNA polymerase in a buffer (pH 8.5), 400µM dATP, 400 µM dGTP, 400 µM dCTP, 400 µM dTTP, 3 mM $MgCl_2$] (Promega, Madison, USA).

Amplification was carried out in a thermocycler (BIO RAD T100) under the following PCR cycler conditions: initial denaturation at 95^{o}C for 2 minutes, 6 cycles of denaturation at 95 ^{o}C for 30 seconds, annealing at 55 ^{o}C for 30 seconds, extension at 72 ^{o}C for 1 minute and the denaturation step was repeated for another 30 cycles, then annealing at 55 ^{o}C for 30 seconds, extension at 72 ^{o}C for 1 minute, and the final enlongation at 72 ^{o}C for 10 minutes. For gel electrophoresis, the wide Mini-sub R cell GT was used (Bio- Rad Laboratories CA, USA). 1% agarose (Sigma) gel was prepared with 2 drops of ethidium bromide used for staining. 5-10µl of individual PCR products and 3 µl of loading buffer was pipetted in each agarose gel well submerged in 0.5 x TAE buffer (Sigma) and the gel was run at 100V for 35 minutes. GelDoc-It ^{TM} 310 Imaging system (California, USA) was used to visualise the gel. Distinctive bands were observed that correspond with the molecular ladder used. For samples with very faint bands, the

PCR was repeated using the PCR products of the sample as template DNA for the reaction. This produced very visible band when the gel was visualised under UV light.

3.4.5 IDENTIFICATION OF ASPERGILLUS ISOLATES BY DNA SEQUENCING

MinElute PCR Purification Kit (Qiagen) was used to purify amplified DNA from PCR following the manufacturer's protocol. The DNA products were sent to Inqaba Biotechnology Company (South Africa) for sequencing. The resulting sequences were aligned using ClustalW in BioEdit (vision 7.0.9.1) and manually edited. The edited sequences obtained were then blasted in the EMBL nucleotide sequence database (Maidak et al., 1999). Species identification was considered at sequence similarity ≥ 97% (Stackebrandt and Goebel, 1994).

3.5 ANALYSIS OF AFLATOXIN

3.5.1 Extraction of aflatoxin from food sample

Two grams of each food samples was weighed, blended with a 20 mL of methanol/water (80/20; v/v) solution for 2 minutes A Whatman No 2 filter paper was used to filter the sample solutions and the filtrates were collected in airtight vials. The filtrate of each sample was purified by pasing 4 ml into a SupelMIP® SPE Cartridges immunoaffinity column (Sigma) following the manufacturer's protocol. The purified samples were then evaporated to dryness 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.

3.5.2 Analytical procedure

The detection and quantification of aflatoxins was conducted using the UHPLC system which is coupled to a triple quadrupole mass spectrometer (LCMS-8050, Shimadzu, Japan). Various

concentration (0.029 to 5 µg/ml) of aflatoxin standards were used to obtain calibration curves for quantification. Both standard and samples were analysed using the LC and MS/MS conditions describe below. The LC conditions consisted of the following: The analytical column was Shimadzu GLC Mastro™ C18 150 x 2.1 mm 3µm (Shimadzu, Japan), the mobile phase A consisted of an aqueous solution of 2 mM ammonium acetate and 0.5% acetic acid while mobile Phase B consisted of a methanol solution of 2 mM ammonium acetate and 0.5% acetic acid. The reaction gradient consisted of 2% B (0.0 min), 10% B (0.01 min), 55% B (3.0 min), 80 % B (7.0 -8.0 min) and 2% B (8.01 min), Stop (11.0 min). The column temperature was 50 ºC, while the injection volume was 10 ul and the flow rate was 0.4 mL/min. The MS/MS conditions was as follows: inonisation mode (Heated ESI (+/-), temperatures (HESI: 400 ºC, desolvation line: 250

ºC and heat block: 300 ºC,), gas flows (Heating gas): 15 L/min and drying gas (N2): 5 L/min). The CID gas pressure was 270 kPa while the polarity switching time was /s and the pause time 1 m/s. The dwel time was 6 to 62 ms depending on the number of concomitant transitions to ensure a minimum of 30 points per peak in a maximum loop time of 200 ms (including pause time and polarity switching).

3.6 STATISTICAL ANALYSIS

Statistical analysis of the data on *aspergillus* analysis was done using SPSS software (Statistical Package for the Social Sciences, version 11.0, SPSS Inc., Chiago, III, USA). Colony forming units data were transformed [log10(x + 1)] before performing descriptive statistics. Maximum acceptable levels in processed foods were considered to be 20 mg kg^{-1} (Kuhumba et al., 2018).

3.7 ETHICAL CONSIDERATION

The Johannesburg municipality provided the permission to conduct this food safety study and ethics clearance was granted by the Ethics Committee of the College of Agriculture and Environmental Sciences, University of South Africa.

3.8 LIMITATION OF THE RESEARCH

This research involved randam sampling and analysis of locally processed foods of African origin sold at small retail shops in Johannesburg. During the sampling process, retailers indicated that they were not consistent with a particular supplier of the food products. Thus, this could make traceability of products somewhat difficult because the food samples used in this study were mostly imported from different African countries.

CHAPTER FOUR

RESULTS

4.1 FUNGI CONTAMINATION DRIED FOOD STUFFS

Out of the 270 samples analysed, only 27.8% were positive of fungal growth. Of all the 6 categories of foods analysed, Root and Tuber (60.0%), Nuts & Seeds (40.0%), Dried Vegetables (37.1%), and the Meat and Insect foods (33.3%), respectively, had the most contaminated samples with fungal. The least contaminated food groups were fish foods (10.0%) and the Spices and condiments (16.7%). The food groups with the most fungal contaminated samples with counts above 3 log cfu/g were the spice and condiments (100.0%), followed by vegetables foods (92.3%), root and tuber foods (77.8%) and lastly the nut and seeds foodstuffs (68.8%). On the other hand, the majority of the the fisheries and meat & insect food groups had fungal counts below 3 log cfu/g as in Table 4.1.

Table 4.1: The occurrence of fungal in dried foodstuffs sold at informal retail outlets in the Johannesburg metropolis

RTE SVF category¥	**Positive samples**	**Average counts (Log cfu/g)**	**Samples with Counts ≤ 3 log cfu/g**	**Samples with Counts® >3 log cfu/g**
Root & Tuber (n=15)	**9(60%)**		**2(22.22%)**	**7(77.78)**
White Gari (n=5)	2	2.65(±1.48)	1	1
Cassava tubers Pieces (n= 5)	3	4.34(±2.38)	1	3
Cassava Flour (n= 5)	3	4.52(±1.47)		3

NB Fungi was not detected in the following dried root and tuber food stuff: Yellow Gari n=5)				
Nuts & Seeds (n=40)	**16(40%)**		**5(31.25)**	**11(68.75%)**
Roasted peanut (n=5)	1	1.96	1	
Peanut flour (n= 5)	2	4.67(±1.23)		2
Soya bean flour(n=5)	1	4.91		1
Mustard seed (n=5)	2	3.70(±2.15)	1	1
Sesame seed (n=5)	1	3.96		
Sesame seed flour (n=5)	3	3.30(±1.37)	1	2
Citrullus colocynthis seed (Egusi)(n=5)	3	4.07(±1.76)	1	3
Irvingia gabonensis seed (Ogbono) (n=5)	3	3.28(±1.34)	1	2
Fisheries (n=40)	**4(10%)**		**3(75%)**	**1(25%)**
Catfish (n=5)	2	2.96(±0.20)	2	
Biyoto(n=5)	1	2.80	1	
Dakala/Lake Victoria sardine (*Rastrineobola argentea*) (n=5)	1	3.72		1
N:B Fungi was not detected in the following dried fisheries food stuff: Cray fish (n=5), Prawns (n=5) & Cod (Stock fish) (n=5).				
Dried meat and insect (n=15)	**5(33.33%)**		**3(60%)**	**2(40%)**
Emperor moth caterpillar (Maponya worm) (n=5)	3	3.02(±0.96)	1	2
Dried Sausage (Droëwors) (n=5)	1	2.42	1	
Biltong(n=5)	1	2.27	1	
Vegetables (n=70)	**26(37.14%)**		**2(7.69%)**	**24(92.31%)**
Vernonia amygdalina (Bitter) leaf (n=5)	3	5.04(±0.24)		3

Gnetum africanum (Okazi) leaf (n=5)	3	5.03(±1.15)		3
Basil (Scent) leaf (n=5)	2	4.43(±0.53)		2
Piper umbellatum (Dilombolombo leaf (n=5)	2	6.13(±1.15)		2
Delela (okra leaf) (n=5)	4	4.39(±0.20)		4
Spider flower leaves (Lude) (n=5)	3	5.23(±1.42)		3
Phaseolus vulgaris (bean leaf) (n=5)	4	5.03(±0.62)		4
Zobo flower (n=5)	3	2.99(±0.77)		3
Golden raisin (n=5)	2	2.38(±0.61)	2	
NB Fungi was not detected in the following dried fisheries food stuff: Dried Apricot (n=5) & Dried Figs (n=5)				
Spices & condiments (n=90)	**15(16.67%)**		**0(0%)**	**15(100%)**
Chilli powder (n=5)	4	4.49((±1.11)		4
Chilli fruits	4	4.26(±1.56)		4
Ginger powder (n=5)	3	3.79(±0.42)		3
Mustard seed powder (n=5)	2	3.11(±0.19)		2
Pepper corn (n=5)	2	3.68(±1.74)		2
Total: 270	**75 (27.78%)**		**15(20%)**	**60(80%)**

¥ = Only RTE SVFs that were made essential of a particular food category were considered.

Ø = Kota is a south African based street food in which bread is filled with chips, egg, ham sausage and cheese

® = 3 log cfu/g maximum limit set by European Commission Health & Consumer Protection Directorate-General

4.2 CONTAMINATION OF DRIED ARTISANAL FOOD STUFFS BY ASPERGILLUS SPECIES

Twenty (20) percent of the 270 dried foodstuffs analysed were contaminated with *Aspergillus* species and 61.1% of the contaminated samples had counts above 3 log cfu/g. The dried vegetables, (25.7%) and the nuts & seeds (30.0%) foodstuffs, respectively, had the greatest number of samples contaminated by *Aspergillus* species. This was followed by dried meat and insect (20.0%) (only the Emperor moth caterpillar had incidence of Aspergillus contamination) and the Spices & condiments (17.8%) food stuffs. The Root & Tuber (13.3%) and the fisheries (7.5%) foodstuffs respectively, had the least number of samples contaminated by *Aspergillus* species. Vegetables (83.3%) and the nuts & seeds (66.7%) foodstuffs had the highest number of samples with *Aspergillus* count was above 10^3 cfu/g as shown in Table 4.2.

Table 4 .2: Incidence and colony count of *Aspergillus* species in dried foodstuffs sold in informal retail outlets in the Johannesburg metropolis

Dried foodstuff categories	**Number of Positive samples (%)**	**Average counts (Log cfu/g)**	**Count range (Log cfu/g)**	**Counts ≤103 cfu/g (%)**	**Counts >10^3 cfu/g (%)**
Root & Tuber (n=15)	**2 (13.33%)**	**3.20 (±0.69)**	**2.92-3.36**	**1**	**1**
Gari (n=5)	2	3.20 (±0.69)	2.92-3.36	1	1
NB *Aspergillus* species were not detected for the following roots and vegetables foodstuff: Cassava tubers Pieces (n= 5) & Cassava Flour (n= 5)					
Nuts & Seeds (n=40)	**12 (30%)**			**5**	**7**
Peanut flour (n= 5)	4	3.68 (±0.68)	2.41-4.4.1	1	3

Sesame seed flour (n=5)	1				
Citrullus colocynthis seed (Egusi)(n=5)	4	2.67 (±0.45)	2.27-3.31	3	1
Irvingia gabonensis seed (Ogbono) (n=5)	3	3.29 (±0.46)		1 (33.33)	2 (66.67)
NB *Aspergillus* species were not detected for the following nuts and seeds food stuff: Roasted peanut (n=5), Soya bean flour(n=5), Mustard seed (n=5) & Sesame seed (n=5)					
Fisheries (n=40)	**3 (7.5%)**			**2**	**1**
Catfish (n=5)	2	2.98 (±0.02)		2	-
Biyoto (king kong fish) (n=5)	1	2.80		1	-
Dakala (n=5)	1	3.72			1
NB *Aspergillus* species were not detected for the following fisheries food stuff: Cray fish (n=5), Prawns (n=5) & Cod (Stock fish) (n=5)					
Dried meat and insect (n=15)	**1 (6.67%)**				**1**
Emperor moth caterpillar (Maponya worm) (n=5)	1	3.32			1
N:B *Aspergillus* species were not detected for the following dried meat and insect food stuff: Dried Sausage (Droëwors) (n=5) & Biltong(n=5)					
Vegetables (n=70)	**18 (25.7%)**			**3**	**15**
Vernonia amygdalina (Bitter) leaf (n=5)	2	3.86 (±0.56)	3.82-3.90		*2*
Gnetum africanum (Okazi) leaf (n=5)	2	3.78 (±0.47)	3.10-4.46	-	2
Basil (Scent) leaf (n=5)	3	4.28 (±0.47)	3.78-4.73	-	3
Piper umbellatum (Dilombolombo leaf (n=5)	5	3.46 (±0.48)	2.90-3.85	2	3
Delela (okra leaf) (n=5)	1	3.79		-	1
spider flower leaves (Lude) (n=5)	2	3.88 (±0.48)	3.13-4.63	-	2
Phaseolus vulgaris (bean) leaf) (n=5)	1	3.87		-	1
NB *Aspergillus* species were not detected in the following dried fruit and vegetable food stuff: Zobo flower (n=5), Dried lemon (n=5), Golden raisen (n=5), Dried Apricot (n=5), Dried Figs (n=5)					
Spices & condiments (n=90)	**16(17.78%)**			**10**	**6**

Chilli (n=5)	2	3.76 (±0.56)	3.12-4.40	1	1
Ginger powder (n=5)	5	2.96 (±0.31)	*2.58-3.22*	4	1
Mustard seed powder (n=5)	3	3.12 (±0.31)	*2.81-3.44*	1)	2
Black pepper corn (n=10)	1	2.83		*1*	-
NB *Aspergillus* species were not detected in the following spices & condiments food stuff: Pepper soup spice (n=5), Njansa (n=5), Mustard seed (n=5) & White pepper corn (n=5)					
Total = 270	**54 (20%)**			**21 (38.89)**	**33 (61.11)**

Table 4.3: Pearson's correlation between total fungal colony counts and *Aspergillus* counts in foodstuffs sold in informal retail outlets in the Johannesburg metropolis (n=53)

		***Aspergillus* count in food groups**					
		RootTuber A	NutSeed A	Fish A	Meat Insec	Fruits Vegetable A	Spices A
Total fungi count in food groups	RootTuber	0.821*					
	NutSeed		0.910**				
	Fish			0.510*			
	MeatInsec				0.685*		
	Fruit Vegetables					0.776**	
	Spices						0.623*
	** Correlation is significant at the 0.01 level of probability (2-tailed).						
	* Correlation is significant at the 0.05 level of probability (2-tailed).						

The total fungal count and the total aspergillus count in the respective food group correlates significantly ($p \leq 0.05$) for all food groups. The correlation between the total fungi counts and the total *Aspergillus* counts was highest for root and tubers ($r = 0.821$) nuts and seed and the fisheries ($r = 0.910$) food groups. This was followed by fruits and vegetable ($r = 0.776$) food groups as shown in Table 4.3.

4.3 ASPERGILLUS SPECIES IDENTIFIED IN DRIED ARTISANAL FOODS

Aspergillus niger was the most predominant *Aspergillus* species, identified in 64.2% of the dried foodstuffs which were contaminated by *Aspergillus*. This was closely followed by *Aspergillus flavus* and *Aspergillus tubingensis*, which were identified in 32.1% and 13.2% of the dried foods stuffs, respectively. The Nuts & seed foods groups had the highest number of samples contaminated with *Aspergillus* specie, in which *Aspergillus niger* and *Aspergillus flavus* were each identified in 83.3% of all the samples analysed. All three *Aspergillus* species (*A. niger, A. flavus* and *A. tubingensis*) were identified in the meat & insect, vegetables, and spices & condiment food groups as in Table 4.4.

Table 4.4: Identification of *Aspergillus* species isolated from contaminated dried foodstuffs sold in informal retail outlets in the Johannesburg metropolis (n=53)

Dried food stuff category	**Frequency of samples contaminated with each *Aspergillus* species (cfu/g) (%)**			
	A. tubingensis **7(13.21)**	***A. niger*** **34(64.15)**	***A. flavus*** **17(32.08)**	**Others *Aspergillus sp* 13(24.53)**
Root & Tuber (n=1)	-	-	-	1(100)
Nuts & Seed (n=12)	-	10 (83.33)	10(83.33)	4(33.33)
Dried Fish (n=3)	-	-	-	3(100)
meat and insect (n=3)	1(33.33)	2(66.67)	1(33.33)	-
Vegetables (n=18)	2(11.11)	12(66.67)	4(22.22)	2(11.11)
Spices and condiment(n=16)	4(25)	10(62.5)	2(12.5)	4(25)

4.4 AFLATOXIN CONTAMINATION OF DRIED FOODSTUFFS

Out of 270 dried foods samples analysed only 16.0% were found to be contaminated by aflatoxins. The fruits and vegetables (24.4%) and the nuts and seeds (20.0%) food group had the highest number of samples contaminated with aflatoxin. Peanut flour and Cardamom the most contaminated samples with aflatoxins. AFB_1, AFB_2 & AFG_1 were the most prominent aflatoxin types in the food samples. Almost all the food samples in which aflatoxin was identified had aflatoxin values above 10 ug/ml. Dried meat and insect had the lowest incidence of aflatoxin (6.67%) and this was closely followed by fisheries foodstuffs (10.0%). Aflatoxin was detected in some food samples in which *Aspergillus* was not detected.

The Root and Tuber food category had AFB_1 & AFG_2 identified in two samples while the Meat & Insect food category had the least incidence of aflatoxin in the food samples with only AFG_2 identified in one food sample as shown in table 4-5.

Table 4 - 5: Occurrence of aflatoxin in in dried foodstuffs sold in informal retail outlets in the Johannesburg metropolis

DRIED FOODSTUFFS [¥]	**NUMBER OF SAMPLES WITH AFLATOXIN AND TYPE OF AFLATOXIN IDENTIFIED (µg/kg)**	
Root & Tuber (n=15)	**2(13.33%)**	
Cassava (*Manihot esculenta*) flakes (Gari) (n=5)	1	AFB1 (27.5), AFG1 (17.5)
Cassava (*Manihot esculenta*) tubers Pieces (n= 5)	1	AFB1 (18.7) [a]
NB Aflatoxin was not detected in the following root and tubers foodstuffs: Cassava (*Manihot esculent*) tuber flour (n= 5)		
Nuts & Seeds (n=40)	**8(20%)**	
Roasted peanut (n=5)	2	AFB1 (100.5), AFB2 (57.8), AFG1 (43.5)
Peanut flour (n= 5)	4	AFB1 (110.5, 100, 172.3 & 107.8)
Egusi *(Citrullus colocynthis)* seed (n=5)	2	AFB1 (106 & 112)
NB Aflatoxin was not detected in the following nuts and seed foodstuffs: Soya bean flour(n=5) Mustard seed (n=5), Sesame seed (n=5), Sesame seed flour (n=5) & Ogbono (*Irvingia gabonensis*) seed (n=5)		
Fisheries (n=40)	**4(10%)**	
Stock (Cod) fish (n=5)	3	AFB1 (34.4 & 22.7) [a], AFB2 (71.5) [a]
Dakala (n=5)	1	AFB1(43), AFB2 (16.5)
NB Aflatoxin was not detected in the following fisheries foodstuffs: Cray fish (n=5), Prawns (n=5), Catfish (n=5) & Biyoto (n=5)		
Dried meat and insect (n=15)	**1(6.67%)**	
Emperor moth caterpillar (Maponya worm) (n=5)	1	AFG2 (69.6)
NB Aflatoxin was not detected in the following dried meat and insect foodstuffs: Dried Sausage (Droëwors) (n=5) & Biltong(n=5).n.d		

Vegetables (n=70)	**15(24.43 %)**	
Vernonia amygdalina (Bitter) leaf (n=5)	1	AFG1(71*)*
Basil (Scent) leaf (n=5)	3	AFB1 (135.2), AFB2 (185.2), AFG1 (84.4 & 57.6)
Piper umbellatum (Dilombolombo leaf (n=5)	3	AFG1 (35.5), AFG2 (16.6),
Delela (okra leaf) (n=5)	3	AFG1 (132.4), AFG2 (67.9 & 70.3)
Phaseolus vulgaris (bean) leaf) (n=5)	2	AFG1 (74.6 & 82.4), AFG2 (82.4)
Dried lemon (n=5)	2	AFB2 (55.2), AFG2 (43.5)
Golden raisin (n=5)	1	AFG2 (63.2)
NB Aflatoxin was not detected for the following fruits and vegetables food stuff: Okazi (*Gnetum africanum*) leaf (n=5), spider flower leaves (Lude) (n=5), Zobo flower (n=5), Dried Apricot (n=5) & Dried Figs (n=5)		
Spices & condiments (n=90)	**11(12.22 %)**	
Pepper soup spice (n=5)	2	AFB1 (219.4 & 123.7), AFB2 (41.4)
Ginger powder (n=5)	1	AFG2 (56.7), AFG2 (72.7)
Achu spice (n=5)	1	AFB1 (102.6)
Cardamom (n=5)	4	AFB1 (129.4 & 122.6), AFG_1 (74.6), AFG2 (58.9)
Dawadawa (n=5)	2	AFG1 (41.2g), AFG2 (32.4g)
Bongo spice (n=5)	1	AFB1 (119.8), AFG2 (42.3) [a]
Chilli (n=5), Njansa (n=5), Mustard seed powder (n=5), Mustard seed (n=5), White pepper corn (n=5), Bonga spice & Black pepper corn (n=10)		
Total: 270	**41(15.19 %)**	

ND = no detection

a= Aspergillus was not detected but aflatoxin was detected

Table 4.6: Aflatoxin database showing observed retention time (min) and mass (m/Z)

Aflatoxin type with Standard Retention times (min)	Retention time (min) Recorded	Observed mass (m/Z)
AFB1 4.665	4.672	312.90
AFB2 4.58	4.564	314.80
AFG1 4.313	4.302	328.70
AFG2 4.205	4.203	330.80

The standard retention time for AFB_1 and AFB_2 was 4.665 and 4.58 min, respectively. For our samples, we observed a retention time of 4.672 min with a mass of 312.90 m/z for AFB_1 and for AFB_2, the rentention time was 4.564 min at a mass of 314.80 m/z. The standard retention time for AFG_1 and AFG_2 was 4.313 and 4.205 min respectively. For samples, a retention of 4.302 min and a mass of 328.70 m/z for AFG_1 were noted for AFG_2, the rentention time was 4.203 min with a mass of 330.80 m/z (Table 4-6).

The chromagram depicts the peaks of different concentrations of AFB_1, AFB_2, AFG_1 and AFG_2. AFB_1 and AFB_2 had the highest peaks in which the the retention time for AFB_1 was 4.672 min and the calculated mass was 312.90 m/z. Similarly, the rentention time for AFB2 was observed at 4.564 (min) and the calculated mass was 314.80 m/z. AFG_1 and AFG_2 had the lowest peaks and their retention time was 4.302 and 4.203 min respectively, while their calculated masses were 328.70 and 330.80 m/z respectively as shown in figure 4.1.

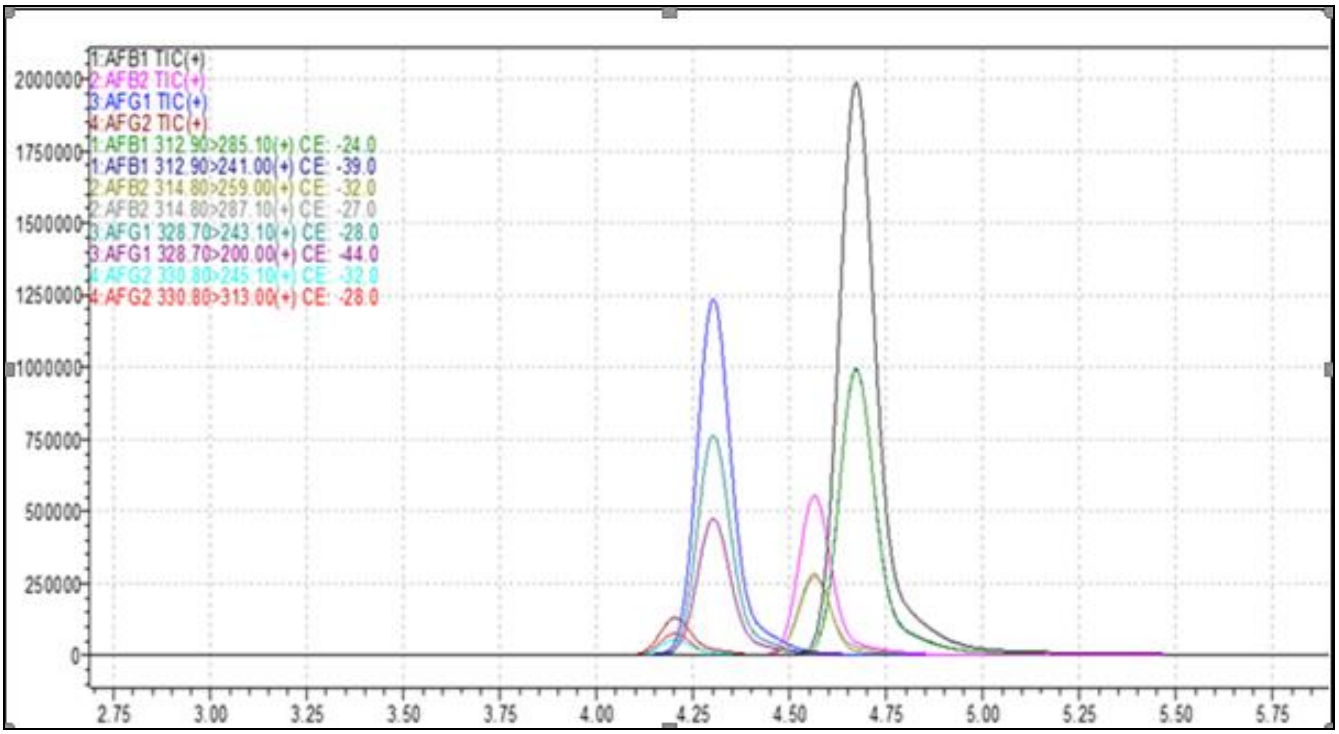

Figure 4.1: LCMS chromatograph of aflatoxin standards showing peaks and retention times of different concentrations of Aflatxin G_2, G_1, B_2 and B_1 from left to right respectively.

CHAPTER FIVE: DISCUSSION

5.0 FUNGAL CONTAMINATION OF DRIED FOODSTUFFS

Out of the 270 samples analysed, 27.78% contained fungal. This can be attributed to the fact that some dried foods were susceptible to contamination due to improper post harvest handling and storage (Gnonlonfin et al., 2013). Contamination usually begins in the farm during pre-harvest and proceed to the post-harvest phases during inadequate drying, packaging, transportation and storage of food commodities (Adekoya et al., 2018; Wang et al 2018; Neme & Mohammed, 2017). Fungal proliferation can be encouraged in poorly packed foods due to changes in moisture content and aeration (Hammami et al., 2014). The study by Gnonlonfin et al., (2013), they compared the effectiveness of packaging materials in reducing aflatoxin contamination in food. Citing that spices packed in bags and placed on uncovered floor had potentially greater risk of fungi contamination when compared to foods packed in wooden boxes, glass or metal vessels.

Inadequate drying using sun energy can also facilitate the contamination and growth of fungi in foodstuffs due to occurrence favourable moisture condition for fungi growth, caused by probably low light intensity from the sun. Alternatively, conventional drying method like inverted windrow technique has been demonstrated to reduce fungal contamination in peanut (Matumba et al., 2018).

Foodstuffs cultivators in developing countries should be enlightened on the importance of implementing pre and post harvest management system like good agricultural practices (GAP) good manufacturing practices (GMP), as the first tool to combating fungal contamination of agricultural food stuffs (Udomkun et al., 2017).

In this current study, the Root & Tubers food group had the highest incidence (60%) of fungal contamination and this can be attributed to the use of contaminated tubers for food production. In this case, in the production of cassava flakes, flour and large chips (Gnonlonfin et al., 2008). Fungal can grow in tuber products during the slow drying period and during storage under humid conditions (Chilaka et al 2018). Fungal can also contaminate produce from bruises on tubers or roots caused by poor agricultural handling practices and because these bruises are directly exposed, the chances of fungal contamination are high (Gnonlonfin et al., 2008).

The Nuts and Seeds foods category had the second highest incidence (40%) of fungal contamination. Probably attributed to fungal contamination that occur in the field before or during harvest when the nut kernels are very susceptible to moldy growth because of their high moisture content and relatively long drying time (Hepsag et al., 2014). Furthermore infestation by insects can create wounds in the kernel of nuts and seeds making them more susceptible to fungal contamination (Hepsag et al., 2014; Iqbal et al., 2013; Lamboni et al., 2016).

The vegetables, dried meat and insect food category had a minimal incidence of fungal contamination. This may be attributed to poor agricultural and food processing practices (Juan et al 2008), such as improper drying of agricultural commodities. Sun drying is often a preferred way of preserving vegetables on an artisanal scale. This method often favours fungal contamination and proliferation because this technique is not effective in reducing the moisture content to safe levels that discourage fungal growth (Gnonlonfin et al., 2013; Juan et al., 2008). The occurrence of mycotoxigenic fungi in meat products (33.33%) can be as a result of contamination originating from the animal feed or fungal growth directly on the surface of the meat product or the use of already contaminated additives and spices. The fish food category as

well as the spice and condiment food category had low incidence of 10% and 16.66 % of fungal contamination respectively. This is due to several factors such as the composition of the substrate in term of moisture and the method employed during processing (Gonkowski et al 2018). Increased moisture content in food caused by inadequate drying is a major cause of fungal growth in stored foods. Poor application of food drying techniques is accountable for the high prevalence of mycotoxigenic fungal along the food chain across Africa (Bradford et al., 2018). Further contamination can also occur from the environment, during storage and transportation (Gonkowski et al 2018).

5.1 CONTAMINATION OF DRIED ARTISANAL FOODSTUFFS BY *ASPERGILLUS* SPECIES

The reason why only 20% of the dried foodstuffs analyzed were contaminated by *Aspergillus* species can be attributed to the presence of other competing fungi. Aflatoxins occurrence can be influenced by the presence of other competing fungi as well as the nature of the substrate (Gnonlonfin et al.,2008). The varied mycoflora present in these foodstuffs is significant as it creates competition among these genera for accessible nutrients present. This point has been confirmed by previous studies (Gnonlonfin et al.,2008). Out of the samples contaminated with *Aspergillu*s species, more than 60% had contaminated level of *Aspergillus* species above 10^3 cfu/g. This high contamination level *Aspergillus* could be because *Aspergillus* species flourish under tropical conditions (high relative humidity and temperature), with increased contamination occurring during the pre-harvest and post-harvest stages (Gnonlonfin et al 2013). An investigation by Ezekiel et al., (2013b), in Nigeria, reported the presence of different *Aspergillus* species like *A. flavus, A. nigerclade* and *A. tamarii; Fusarium*, *Penicillium* and *Talaromyces* spp

in dried mushroom samples. They further reported the occurrence of *Aspergillus* strains with toxin producing potentials being higher than those strains without toxin producing ability.

The Nuts & Seeds food category had the highest of samples (30%) contaminated with *Aspergillus* species and this can be attributed to the fact that moldy fungi like the *Aspergillus species,* flourish in food with comparatively low moisture content making the nut and seed food group a good substrate. This is also aggravated by inadequate conditions during storage and handling, which can promote insect infestation and increase moisture content in the seed (Mendoza et al., 2017). Dried vegetables also had high number of samples (25.7%), contaminated with *Aspergillus* species. Vegetables are predisposed to aflatoxin contamination across the food chain from farm to fork mainly because of their characteristic high moisture content, which is a known pre-requisite for toxin production (Prelle et al., 2014).

Within the Dried Meat & Insect food group only one Emperor moth caterpillar had *Aspergillus* contamination, and this can be attributed to the fact that dry cured proteins normally have characteristic high salt content and low water activity. These conditions are natural habitat of xerophilic mycotoxins of *Aspergillus* and *Penicillum*. Also due to the fermentation process in the production of dry-cured meat, fungi are natural components of these products and they confer important characteristic flavours to the products. The mycofloral population present in these products compete with the growth and biosynthesis of toxin producing fungi like *Aspergillus*, thus reducing their occurrence in dry-cured meat (Montanha et al., 2018). Thus this explains the low incidence of *Aspergillus* specie in this category of food group. Furthermore, contamination can exacerbate from either the spices or other additives used during preparation or growth of

aflatoxigenic fungi on the surface of the meat prior to processing (Abd-Elghany and sallam 2015; Montanha et al., 2018).

The spices & condiments foodstuffs also had a fairly high incidence of *Aspergillus* contamination (17.78%) and this could be attributed to poor agricultural practices before or during harvest that provide favourable conditions for mycotoxin production (Hammami et al., 2014). This statement is concurrent with the study of El Maghubi et al., 2013 on marketed spices sold in Morocco. They reported 85% of mycotoxins in the spice samples were mainly *Aspergillus* specie (*Nigiri and Flavus*). They further explained how the level of contamination in individual spice (paprika, cumin, white and black pepper) sample can be affected by the composition of the substrate. Paprika recorded the highest incidence of *Aspergillus* contamination, and this is attributable to its high moist content during harvest. White pepper had the least incidence of *Aspergillus* because of the outer pericarp is removed during processing.

Furthermore, the level of *Aspergillus* contamination in individual spices have been found to be determined by the quality of ingredients from which a particular spice was made (El Maghubi et al., 2013).

The Root & Tuber food category had a low (13.33%) incidence of *Aspergillus* contamination. This is likely because, the proliferation of aflatoxigenic fungi in processed cassava products normally occurs during the process of slow drying, storage at high humidity and during the fermentation process when fermentation is not carried out adequately (Nicolau et al., 2016).

According to Essono et al., (2009), the duration of storage of cassava chips could be the most significant contributing factor in the formation of aflatoxin in stored cassava chips. The study

linked storage time to increase in moisture content, which encourages the growth of aflatoxin producing fungi. The Fisheries category also had least samples (7.5%) contaminated by *Aspergillus* species and this can be due to the low water content of the fish samples (Mohammed et al., 2012). Other contributing factors could be the quality of fish during smoke drying, the temperature and duration during smoke drying, the salt content during curing and the storage conditions of the smoke dry fish (Antonia da Silva et al., 2008).

5.3 *ASPERGILLUS* SPECIES IDENTIFIED IN DRIED ARTISANAL FOODS

5.3.1 Aspergillus niger

In this study, *Aspergillus niger* was the most predominant *Aspergillus* species identified (64.15%) in the foodstuffs. *A. niger* belongs to the section *Aspergillus nigri*, known as the black *Aspergillus* (Park et al., 2017; Plascencia-Jatomea et al 2014). Black *Aspergillus* is found primarily in the soil (Varga et al., 2011), thus their occurrence in the food chain can be traced back to contamination that occurred in the field. Some strains of this *Aspergillus nigri sect.* are associated with food spoilage and toxin production, which can lead to food poisoning that could be considered a public health hazard (Park et al., 2017).

A.niger is considered as the most significant fungal species that contaminates a wide variety of agricultural produce mainly at the post harvest phase. *A.niger* is associated with sun dried foods, processed fish (either smoked, dried or cured), spices, vegetables and most importantly nuts (Plascencia-Jatomea et al 2014). *Aspergillus niger* is able to deteriorate food on account of their ability to grow fast over a wide pH range and their ubiquituous in the environment (Nielsen et al., 2009). This occurrence of (64.15%) of *Aspergillus niger* in this present study is significant in

terms of food safety because *A. niger* has universally been recognised as a safe organism. It is deemed as a non-pathogenic *Aspergillus*. *Aspergillus niger* are ubiquitous in nature, humans are constantly exposed to their spores with no significant health threat arising. Although in rare cases, *A.niger* has been reported to be harmful to human especially when it colonises an individual with certain pre-existing disease conditions such as HIV (Schuster et al., 2002; Plascencia-Jatomea et al 2014). According to United States Food and Drug Administration (USFDA), a majority of the strains of *A. niger* are credited as "*generally recognized as safe (GRAS")(* Park et al., 2017; Susca et al., 2014) owing to its use in biotechnology in the synthesis of proteins and in the fermentation industry (Varga et al., 2011). Although, current research reports that *A. niger* strains are capable of producing toxins such as ochratoxins (Park et al., 2017).

5.3.2 Aspergillus Flavus

Aspergillus flavus was the second most predominant *Aspergillus* species identified in the foodstuffs (32.08%). This can be due to the fact that *A. Flavus* often occurs and grow rapidly during storage rather than in the fields (Njobeh et al., 2009). Sanzania et al., (2016), postulated that the first contamination of crops with *A. flavus* occurs in the field where its spores come in contact with plant and remain dormant until during the post harvest phase during rapid growth, proliferation and toxin production. Plascencia-Jatomea et al., (2014) further explained the versatility of. *Aspergillus flavus*, citing that *A. flavus* has no specific host, which means, a wide range of agricultural crops can serve as good substrates suitable for its growth and proliferation.

The significance of the result obtained in this study in terms of food safety is worrisome because *A. flavus* is known to produce aflatoxin B1, a toxin that seriously compromise human and animal health. AFB1, is classified as a human carcinogen by the International Agency for Research on Cancer (IARC) (Iqbal et al 2014; Hepsag et al 2014; Sanzani et al., 2016).

5.3.3 The co-occurrence of *Aspergillus niger* and *flavus* in some food category

Aspergillus niger and *A. flavus* were the most predominant *Aspergillus* species identified in all category of food group. The Nuts & Seed food category had the highest incidence (83.3%) of *A. flavus and A. niger*. This is so due to their long duration of storage, nuts and seed are highly susceptible to contamination by these two species of *Aspergillus*. Monda and Alakonya (2016) proposed that effective drying of agricultural commodities reduces the moisture content in grains, which inversely improves the shelf life and quality of grains. This point is further supported by Bradford et al., (2017), who reported that reducing the moisture content in maize grain to approximately 12-14% was effective in mitigating the growth and biosynthesis of mycotoxic fungal during storage. *Aspergillus niger* and *A. flavus* have been reported to infect foods at post harvest stage, thus they are regarded as post-harvest fungi. In a study by Lamboni et al., (2016), in Benin, *A. flavi* and *A. nigri* were reported to be present in raw cashew nuts but at levels that did not exceed the maximum tolerable limits of 2 and 4 mg/kg for processed nuts suitable for immediate human consumption.

In terms of food safety, these findings are significant demonstrating that nuts and seeds sold in some informal retail outlets in Johannesburg can regularly be contaminated with aflatoxigenic fungal, which is known to have detrimental effect on human and animal health. The presence of

aflatoxins in crops has been reported to be difficult to completely eliminate along the food chain due to their ability to survive during food processing (Ruadrew et al 2013; Park et al.,2017). Hell et al., (2009), suggested that improvement in technologies applied during food processing, packaging, storage and handling can considerably reduce toxin contamination of agricultural commodities. One of such technologies is extrusion cooking. This technology thermally inactivates aflatoxins by aggressively reducing aflatoxins (50%-80%) in processed foods. This innovative technology is applied in the manufacturing of ready-to-eat space cereals, snack etc (Temba et al., 2016; Ismail et al; 2018).

5.3.4 Aspergillus tubingensis

Aspergillus tubingensis was identified only in meat & insect (33.3%), vegetables (11.1%) as well as spices & condiment foodstuffs (25.0%). *Aspergillus tubingensis* belongs to the *Aspergillus sect. nigri.* These strains of *Aspergillus* can contaminate a variety foods either before harvest (pre-harvest stage) or after harvest amid storage (post-harvest stage) (Susca et al., 2014). Implementation of preventive measure during drying of food, ensuring that food is dried to an ideal water activity as well as storing processed food at conditions (appropriate temperature and humidity) that discourages the biosynthesis of mycotoxins, is a strategic way of preventing or reducing toxigenic fungal in dried processed foods (Sardinas et al., 2011).

5.4 OCCURRENCE OF AFLATOXIN IN DRIED FOODSTUFFS

15.99% were found to be contaminated by aflatoxin out of 270 dried food samples analysed. This is significant in terms of food safety because the less occurrence of aflatoxigenic fungi in this study reveals the organoleptic and nutritional quality of artisanal food sold in small retail

stores in Johannesburg. Aflatoxin have been reported to be very prevalent in the tropical region of Africa, mainly due to favourable climatic conditions for fungal growth, socio-ecomonic status in terms of poverty and technology deficiency prevalent in the region (Ismail et al., 2018). According to Mwalwayo and Thole (2016), a majority of African countries still use less mechanised pre and post-harvest practices that are ineffective in mitigating mycotoxin contamination when compared to those used in the developed countries. Ismail et al (2018), further emphasizes the use of innovative farming technologies at post-harvest phase as one of the contributing factors in limiting aflatoxin contamination in western countries compared to the high level of aflatoxins experienced in African countries. One of such technologies is the use of Purdue Improved Crop Storage (PICS) hermitic bags. Lane & Woloshuk, (2017) illustrated the effectiveness of Purdue Improved Crop Storage (PICS) hermitic bags in negating the effect of humidity, pest infestation and microfloral contamination on the quality of stored grains.

Wang et al., (2018) illustrated the effect of poverty on the rapid rate of aflatoxin contamination of food in the tropical region. Using the Democratic Republic of Congo (DRC) and Malaysia as a case study. Although they are both located in the tropical region, they have different economic status. Malaysia's economy is consideredxg7 to be thriving compared to that of DRC and based on that study, higher aflatoxin contamination levels were reported in agricultural commodities in DRC than for samples from Malaysia. That study further demonstrates that the economic status of a country influences the quality of agricultural commodities with respect to aflatoxin contamination of food.

The reason why dried Fruits, Vegetables, Nuts and Seeds foodsstuffs had high levels of aflatoxin incidence can be attributed to their characteristic low moisture content, their nutritional content,

long shelf life, and storage at high temperature and humidity, that provide favourable condition for aflatoxin biosynthesis (Yu-jiao et al., 2018; Asghar et al., 2017; Sanzani et al., 2016). Asghar et al., (2017) provided meteorological data to support the hypothesis that temperature and relative humidity affect aflatoxin contamination by studying dried fruits and nuts. They reported varied aflatoxin contamination levels at different months of the year witvery high aflatoxin levels recorded in the months of July, August and September with higher temperatures and relative humidity of 33 °C/50%, 31 °C/58%and 30 °C/43% respectively.

The high aflatoxin levels reported in the fruits and nuts foodstuffs is significant in terms of food safety because this category of food groups are popular choices for ready-to-eat snacks (roasted peanuts & dried raisin) and the risk for food borne breakout is inevitable since aflatoxins are toxic food contaminants. Within the Nuts and Seeds foodstuffs, peanut flour had the highest incidence of aflatoxins probably because it is the most susceptible oil seed to aflatoxin contamination. This result is also consistent with the study by Waliyar et al., (2013). They also reported high levels of aflatoxigenic fungi in groundnut sampled in Mali. They observed that the level of aflatoxin (AFB1) in stored groundnut increased with longer storage time. That study concluded that the occurrence of AFB1 in groundnut samples was as a result of poor storage that was favourable for fungal growth.

Mutumba et al., (2018) attributed inadequate drying of harvested groundnut pods as the reason for aflatoxin contamination of groundnut. In their study, they stated the use of less mechanized method of drying groundnut pods as the sole cause of increased moisture content during storage that encouraged further aflatoxin contamination during storage. The study further illustrated the use of inverted windrow technique as an effective way of drying groundnut pods whilst

decreasing the risk of aflatoxigenic fungal growth during storage. In Africa, peanut is one of the important cash crops cultivated for income generation. But due to the recurrent incidence of aflatoxins in peanut, contaminated products are deemed not suitable for trade (Kachapulula et al., 2017; Waliyar et al., 2015). According to Waliyar et al., (2015), over 50% of peanuts exported from Malawi to Europe were not acceptable for trade because of their high aflatoxin content.

AFB1, AFB2 and AFG1 were most prominent in the food samples analyzed. *Aspergillus flavus* is responsible for the production of aflatoxin B1 & B2, while other *Aspergillus* species such as *A. paraciticus* are responsible for the production of the other aflatoxins (AFG1 & AFG2) (Udomkun et al., 2017). The co-occurrence of AFB1, AFB2 and AFG1 in foodstuffs reported in this study, is similar with reports presented by several authors, and these findings are listed below. In a study by Kuhumba et al., (2018), in Tanzania, 71% of the food samples (peanut enriched flours) contained high incidence of total aflatoxins (152.09 $\mu g\,kg^{-1}$) and AFB_1 at (87.43 $\mu g\,kg^{-1}$) levels above the set limit of (5 $\mu g\,kg^{-1}$) for total aflatoxins and (10 $\mu g\,kg^{-1}$) for AFB_1. This incidence was attributed to manufacturers not adopting Good Manufacturing Practices (GMP).

The report of aflatoxins incidents in foodstuffs analysed in this present study, is concurrent with results from previous studies. Kachapulula et al., (2017), reported the incidence of AFB_1, AFB_2, AFG_1 & AFG_2 in groundnut samples analysed in Zambia, this is similar with the report from this study, AFB1, AFB2 and AFG2 were also detected in groundnut samples analysed. While Ezekiel at al., (2016), reported the incidence of AFB_1, AFB_2, AFG_1 & AFG_2 (37.5 μg/kg) in melon seed analyzed in Nigeria, this is in accordance with the results from this present study as AFB_1 was also detected in melon seeds analysed. Our results, (AFB_2 and AFG_2) in dried fruits is concurrent

with the report by Heshmati et al., (2017), this study reported the incidence of AFB_1, AFB_2, AFG_1 & AFG_2 (6μg kg^{-1}) in dried fruit analyzed in Hamadan city in Iran. And lastly, for the dried meat foodstuffs, this present study reported the incidence of AFG_2, this is in accordance with the findings of Markov et al., (2013), they reported the incidence of AFB_1 (2.7 – 3.0 μg kg^{-1}) in dried cured meat samples.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

In this study, the safety profile in terms of fungi and aflatoxins quality of small-scale processed foodstuff sold at informal retail outlets in the Johannesburg metropolis was analysed.

The findings of this study revealed the occurrence of fungi and strains of *Aspergillus* specie and aflatoxin. Five different category of food groups were analysed (the root & tuber, fruits & vegetable, nut & seeds, spices & condiments, insect & meat food group). The root and tuber food group had the most incidence of fungi, while the fish food group had the least incidence of fungi. *Aspergillus niger* was the most predominant *Aspergillus* species identified in all the categories of food samples analysed. The Nuts & Seed foods groups had the highest number of samples contaminated with *Aspergillus* specie, in which *Aspergillus* niger and *Aspergillus* Flavus were the most *Aspergillus* specie identified. The Fruits and Vegetables (24.43%) and the Nuts and Seeds (20%) food group had the highest number of samples contaminated with aflatoxin. Peanut flour and Cardamom had the most incidence of aflatoxin. AFB1, AFB2 & AFG1 were the most prominent aflatoxin types in the food samples. Almost all the food samples in which aflatoxin were identified had aflatoxin values above 10µg/ml.

6.2 RECOMMENDATIONS

Based on the findings from this study, we recommend that there should be a consistent monitoring for the occurrence of aflatoxin in the food chain especially in artisanal processed foods. Aflatoxin contamination can be reduced from the food chain by a combination of pre and

post harvest measures. Therefore, I recommend the implementation of post-harvest strageties like good manufacturing practices (GMP), good agricultural practices (GAP) that involves; adequate drying, storage and transportation of agricultural commodities to help mitigate aflatoxin contamination in the food chain.

REFERENCES

Abd-Elghany, S. M. and Sallam, K. I. (2015). Rapid determination of total aflatoxins and ochratoxins A in meat products by immuno-affinity fluorimetry. *Food Chemistry*; 179 : 253-256.

Achi, O. K. (2005). The potential for upgrading traditional fermented foods through biotechnology. *African Journal of Biotechnology*, 4 (5): 375-380.

Adegoke, G. O., and Letuma, P. (2013). Strategies for the prevention and reduction of mycotoxins in developing countries. Available from: http://www.intechopen.com/download/pdf/44089. [Accessed 2 April 2014].

Adekoya, I., Obadina, A., Phoku, J., De Boevre, M., De Saeger, S. and Njobeh, P. (2018). Fungal and mycotoxin contamination of fermented foods from selected South African markets. *Food Control*, 90: 295-303.

Adetunji, A. I., Clou, H., Walford, S. N., and Taylor, J. R. N. (2016). Complementary effects of cell wall degrading enzymes together with lactic acid fermentation on cassava tuber cell wall breakdown. *Industrial Crops and Products*, 90: 110-117.

Alakonya, A. E., and Monda, E. O. (2012). A new approach in Aflatoxin management in Africa: Targeting Aflatoxin/Sterigmatocyst in biosynthesis in Aspergillus species by RNA silencing technique. Available from: http://www.intechopen.com/download/pdf/38211. [Accessed 15 June 2014].

Ali, F., Mohammad, R., Abdolatif, M., Parisa, F., Parisa, M., Akbar, M. A., Mohammad, R., and Nasrin, A. (2013). Aflatoxin occurrence in nuts consumed in Arak, Iran. *International Journal of Food Nutrition and Safety*, 4 (2): 91-97.

Andersson, E. (2012). A small growth study of *Aspergillus* section flavi, and potentially aflatoxigenic fungi and aflatoxin occurrence in Brazil nuts from local markets in Manaus, Brazil. Available from http://stud.epsilon.slu.se/4967/1/andersson_e_121015.pdf [Accessed 14 June 2014].

Anihouvi, V. B., Kindossi, J. M., and Hounhouigan, J. D. (2012). Processing and quality characteristics of some major fermented fish products from Africa: A critical review. *International Research Journal of Biological Sciences*, 1 (7): 72-84.

Antonia da Silva, L. V., Prinyawiwatkul, W., King, J. M., No, H. K., Bankston Jr., J. D., Ge, B. (2008). Effect of preservatives on microbial safety and quality of smoked bluecatfish (Ictalurusfurcatus) steaksduringroom-temperaturestorage. *Food Microbiology*, 25; 958–963

Asghar, M. As., Ahmed, A., Zahir, E., Asghar, M. A., Iqbal, J. and Walker, G. (2017). Incidence of aflatoxins contamination in dry fruits and edible nuts collected from Pakistan. *Food Control*. 78: 169 -175.

Atanda, S. A., Pessu, P. O., Agoda, S., Isong, I. U., Adekalu, O. A., Echendu, M. A. and Falade, T. C. (2011). Fungi and mycotoxins in stored foods. *African Journal of Microbiology Research*, 5 (25): 4373-4382.

Atanda, S. A., Pessu, P. O., Aina, J. A., Agoda, S., Adekalu, O. A., and Ihionu, G. C. (2013). Mycotoxin management in agriculture. *Greener Journal of Agricultural Sciences*, 3 (2): 176-184.

Azaman, N. N. M., Kamarulzaman, N. M., Shamsudin, M.N., and Selamat, J. (2016). Stakeholders' knowledge, attitude, and practices (KAP) towards aflatoxins contamination in peanut-based products. *Food Control*, 70: 249-256.

Battcock, M., and Azam-Ali, S. (1998). Fermented fruits and vegetables: A global perspective. FAO Agricultural Services Bulletin No.134. Avaliable from www.fao.org/docrep/x0560e/x0560e13.htm [Accessed 30 October, 2016].

Bbosa, G. S., Kitya, D., Lubega, A., Ogwal-Okeng, J., Anokbonggo, W. W., and Kyegombe, D. B. (2013). Review of the biological and health effects of aflatoxins on body organs and body systems. InTech. Avaliable from: http://www.intechopen.com/ download/pdf/38731. [Accessed 10 May, 2016].

Benkerroum, K. (2013). Traditional fermented foods of North African countries: Technology and food safety challenges with regards to microbiological risk. *Comprehensive Reviews in Food Science and Food Safety*, 12 (1): 54-89.

Bhat, R., Rai, R. V., and Karim, A. A. (2010). Mycotoxins in food and feed: Present status and future concerns. *Comprehensive Reviews in Food Science and Food Safety*, 9 (1): 57–81.

Bhatnagar-Mathur, P., Sunkara, S., Bhatnagar-Panwar, M., Waliyar, F., and Sharma, K. K. (2015). Biotechnological advances for combating *Aspergillus* flavus and aflatoxin contamination in crops. *Plant Science,* 234: 119–132

Blankson, G. K. and Mill-Robertson, F. C. (2016). Aflatoxin contamination and exposure in processed cereal-based complementary foods for infants and young children in greater Accra, Ghana. *Food Control,* 64: 212-217.

Bonnet, M. S., Roux, J., Mounien, L., Dallaporta, M., and Troadec, J. (2012). Advances in deoxynivalenol toxicity mechanisms: The brain as a target. *Toxins*; 4: 1120-1138. doi:10.3390/toxins4111120

Boutigny, A. L., Small, B. I., Zühlke, S., Spiteller, M., Van Rensburg, B. J., Flett, B. and Viljoen, A. (2012). Quantitative detection of *Fusarium* pathogens and their mycotoxins in South African maize. *Plant Pathology*, 61 (3): 522–531.

Bradford, J. Dahal, P., Asbrouck, J. V., Kunusoth, K., Thompson, J., Wu, F. (2018). The dry chain: Reducing postharvest losses and improving food safety in humid climates. *Trends in Food Science & Technology;* 71: 84-93.

Bradford, K. J., Dahal, P., Van Asbrouck, J., Kunusoth, K., Bello, P., and Thompson, J., et al. (2017). The dry chain: Reducing postharvest losses and improving food safety in humid climates. *Trends in Food Science & Technology*, 71: 84-93. https://doi.org/10.1016/J.TIFS. 2017.11.002.

Brankov, T. P., Jovanović, M., and Grujić, B. (2013). Aflatoxin standards and maize trade. *Economics of Agriculture*, 60 (3): 595-607.

Bumbangi, N. F., Muma, J. B., Choongo, K., Mukanga, M., Velu, M.R., Veldman, F., Hatloy, A., and Mapatano, M. A. (2016). Occurrence and factors associated with aflatoxin contamination of raw peanuts from Lusaka district's markets, Zambia. *Food Control*, 68: 291-296.

Burfoot, D., Everis, L., Mulvey, L., Wood, A., and Betts, R. (2010). Literature review on microbiological hazards associated with biltong and similar dried meat products. [Accessed 15 April, 2014]. Available at https://www.food.gov.uk/sites/default/files/mnt/drupal_data/sources/

files/multimedia/pdfs/committee/acm982rep.pdf

Chala, A., Mohammed, A., Ayalew, A., and Skinnes, H. (2013). Natural occurrence of aflatoxins in groundnut (*Arachis hypogaea L.*) from eastern Ethiopia. *Food Control*, 30: 602-605.

Chikezie, P. C., and Ojiako, O. A. (2013). Cyanide and aflatoxin loads of processed cassava (*Manihot esculenta*) tubers (Garri) in Njaba, Imo state, Nigeria. *Toxicology International*, 2 (3): 261-267.

Chilaka, C. A., De Boevre, M., Atanda, O. O., and De Saeger, S. (2018). Prevalence of *Fusarium* mycotoxins in cassava and yam products from some selected Nigerian markets. Food Control: 84 : 226 - 231

Copetti, M. V., Iamanaka, B. T., Pereira, L. J., Lemes, D. P., Nakano, F., and Taniwaki, M. H. (2012). Co-occurrence of ochratoxin and aflatoxins in chocolate marketed in Brazil. *Food Control*, 26: 36 – 41.

Coronel, M. B., Vicente, S., Resnik, S. L., Alzamora, S. M., and Pacin, A. (2016). Fumonisins in maize and gluten meal analysed in Argentinean wet milling industrial plants by ELISA compared with HPLC-FLD method. *Food Control*, 67: 285-291.

Darouj, E., Massouha, L., Ghanem, I. (2016). Investigation of ochratoxin A in Syrian consumed baby foods. *Food Control*, (62):97–103.

De Angelis, E., Monaci, L., Pascale, M., and Visconti, A. (2013). Fate of deoxynivalenol, T-2 and HT-2 toxins and their glucoside conjugates from flour to bread: An investigation by high-performance liquid chromatography high-resolution mass spectrometry. *Food Additives & Contaminants Part A*, 30 (2): 345-355.

Dhanasekaran, D., Shanmugapriya, S., Thajuddin, N., and Panneerselvam, A. (2011). Aflatoxins and aflatoxicosis in human and animals, aflatoxins. (Ed.), ISBN: 978-953-307-395-8, InTech. Available from: http://www.intechopen.com/download/pdf/20393

El Mahgubi, A., Puel, O., Bailly, S., Tadrist, S., Querin, A., Ouadia, A., Oswald, I. P., and Bailly, J. D. (2013). Distribution and toxigenicity of Aspergillus section Flavi in spices marketed in Morocco. *Food Control*, 32 (1): 143–148.

Encyclopaedia Britannica Inc. (2016). "Johannesburg". Available from: https://global.britannica.com/place/Johannesburg-South-Africa. [Accessed 27 September 2016].

Essono, G., Ayodele, M., Akoa, A., Foko, J., Filtenborg, O., Olembo, S. (2009). Aflatoxin-producing *Aspergillus* spp. and aflatoxin levels in stored cassava chips as affected by processing practices. *Food Control,* 20: 648–654.

European Food Safety Authority (EFSA). (2013). Deoxynivalenol in food and feed: Occurrence and exposure. *EFSA Journal*, 11 (10): 3379. [Accessed 22 August, 2014]. Available from: http://www.efsa.europa.eu/en/efsajournal/doc/3379.pdf

Ezekiel, C. N., Sulyok, M., Babalola, D. A., Warth, B. Ezekiel, V. C. and Krska, R. (2013a). Incidence and consumer awareness of toxigenic *Aspergillus* section *Flavi* and aflatoxin B_1 in peanut cake from Nigeria. *Food Control*, 30 (2): 596-601.

Ezekiel, C. N., Sulyok, M., Frisvad, J.C., Somorin, Y., Warth, B., Houbraken, J., Samson, R. A., Krska, R., and Odebode, A.C. (2013b). Fungal and mycotoxin assessment of dried edible mushroom in Nigeria. *International Journal of Food Microbiology*, 162: 231–236.

Ezekiel, C. N., Sulyok, M., Somorin, Y., Odutayo, F. I., Nwabekee, S. U., Balogun, A. T., and Krska, R. (2016). Mould and mycotoxin exposure assessment of melon and bush mango seeds, two common soup thickeners consumed in Nigeria. *International Journal of Food Microbiology*, 237: 83-91.

Ferrer, C., Colom, F., Frases, S., Mulet, E. Abad, J. L., and Alio, J. L. (2001). Detection and identification of fungal pathogens by PCR and by ITS2 and 5.8S ribosomal DNA typing in ocular infections. *Journal of Clinical Mirobiology*, 39 (8): 2873-2879.

Filazi, A., and Sireli, U.T. (2013). Occurrence of Aflatoxins in food. Available from http://www.intechopen.com/download/pdf/38172 [Accessed 4 July 2016].

Food and Agriculture Organisation (FAO). (2004). Worldwide Regulations for mycotoxins in food and feed in 2003. Available from http://www.fao.org/3/a-y5499e.pdf [Accessed 14 May, 2014].

Franz, C. M. A. P., Huch, M., Mathara, J. M., Abriouel, H., Benomar, N., Reid, G., Galvez, A., and Holzapfel, W. H. (2014). African fermented foods and probiotics. *International Journal of Food Microbiology*, 90: 84-96.

Freire, A. L., Ramos, C. L., Schwan, R. F. (2015). Microbiological and chemical parameters during cassava based-substrate fermentation using potential starter cultures of lactic acid bacteria and yeast. *Food Research International,* 76 :787–795.

Gachara, G., Gnonlonfin, G. J. B., Harvey, J., Wainaina, J., Wanjuki, I., Skilton, R., and Nyamache, A. K. (2013). Post-harvest fungi diversity and aflatoxin contamination in stored maize: Case studies of Kitui, Kitale and Nakuru in Kenya. Available from

http://www.esriea.co.ke/seminars/wp-content/uploads/2013/09/ILRI-poster-pdf [Accessed 15 August, 2016].

Garrido, C. E., Hernández Pezzani, C., and Pacin, A. (2012). Mycotoxins occurrence in Argentina's maize (Zea mays L.), from 1999 to 2010. *Food Control*, 25 (2): 660-665.

Gimeno, A. (2004). Aflatoxina M_1 no leite. Riscos para a saúde pública, prevenção e controlo. *Alimentação Animal (Revista de la Associação Portuguesa dos Industriais de Alimentos Compostos para Animais (IACA)*, 49 : 32-44.

Gnonlonfin, G. J. B., Adjovi, C. S. Y., Katerere, D. R., Shephard, G. S., Sanni, A., and Brimer, L. (2012). Mycoflora and absence of aflatoxin contamination of commercialized cassavachips in Benin, West Africa. *Food Control,* 23: 333-337.

Gnonlonfin, G. J. B., Adjovi, C. S. Y., Tokpo, A.F., Agbekponou, E. D., Ameyapoh, Y., de Souza, C., and Brimer, L., Sanni, A. (2013). Mycobiota and identification of aflatoxin gene cluster in marketed spices in West Africa. *Food Control*, 34 (1): 115–120.

Gnonlonfin, G. J. B., Hell, K., Fandohan, P., and Siame, A. B. (2008). Mycoflora and natural occurrence of aflatoxins and *fumonisin* B_1 in cassava and yam chips from Benin, West Africa. *International Journal of Food Microbiology*, 122:140–147.

Golge, O., Hepsag, F., and Kabak, B. (2013). Incidence and level of aflatoxin contamination in chilli commercialised in Turkey. *Food Control*, 33 (2): 514–520.

Gonkowski, S., Obremski, K., Makowska, K., Rytel, L., and Mwaanga, E. S. (2018). Levels of zearalenone and its metabolites in sun-dried kapenta fish and water of Lake Kariba in Zambia — A preliminary study. *Science of the Total Environment*, 637-638: 1046-1050.

Hajslova, J., Zachariasova, M., and Cajka, T. (2011). Analysis of multiple mycotoxins in food. *Methods in Molecular Biology*, 747: 233-258

Hammami, W., Fiori, S., Al Thani, R., Ali Kali, Balmas, V., Migheli, Q., and Jaoua. S. (2014). Fungal and aflatoxin contamination of marketed spices. *Food Control*, 37: 177-181.

Hanson, L. A., Zahn, E. A., Wild, S. W., Döpfer, D., Scott, J., and Stein, C. (2012). Estimating global mortality from potentially foodborne diseases: An analysis using vital registration data. Population Health Metrics, 10 (5). Available from: http://www.pop healthmetrics.com/content/pdf/1478-7954-10-5.pdf [Accessed 14 July 2014].

Hell, K., Cardwell, K.F., Setamou, M., and Poehling, H.M. (2009). The influence of storage practices on aflatoxin contamination in maize in four agroecological zones of Benin, West Africa. *Journal of Stored Products Research*, 36: 365–382

Hell, K., and Mutegi, C. (2011). Aflatoxin control and prevention strategies in key crops of Sub-Saharan Africa. *African Journal of Microbiology Research*, 5 (5): 459-466.

Heshmati, A., Zohrevand, T., Khaneghah, A. M., Nejad, A. S. M., Sant'Ana, A. S. (2017). Co-occurrence of aflatoxins and ochratoxin A in dried fruits in Iran: Dietary exposure risk assessment. *Food and Chemical Toxicology*, 106: 202-208.

Hepsag, F., Golge, O., and Kabak, B. (2014). Quantitation of aflatoxins in pistachios and groundnuts using HPLC-FLD method. *Food Control*, 38: 75-81.

Hope, J., H., and Hope, B., E. (2012). A review of the diagnosis and treatment of Ochratoxin A inhalational exposure associated with human illness and kidney disease including focal

segmental glomerulosclerosis. *Journal of Environmental and Public Health*, 2012 (835059): 1-10. doi:10.1155/2012/835059

Hove, M., De Boevre, M., Lachat, C., Jacxsens, L., Nyanga, L.K., and De Saeger, S. (2016). Occurrence and risk assessment of mycotoxins in subsistence farmed maize from Zimbabwe. *Food Control,* 69: 36-44.

Ikutegbe, V., and Sikoki, F. (2014). Microbiological and biochemical spoilage of smoke-dried fishes sold in West African open markets. *Food Chemistry,* 161: 332–336.

Ismail, A., Gonçalves, B. L., De Neeff, D. V., Ponzilacqua, B., Coppa, C. F. S. C., Hintzschec, H., Sajid, M. Cruz, A. G., Corassin, C. H. and Oliveira, C. A. F. (2018). Aflatoxin in foodstuffs: Occurrence and recent advances in decontamination. *Food Research International,* 113:74–85.

Iqbal, S. Z., Asi, M. R., Zuber, M., Akrama, N., and Batool, N. (2013). Aflatoxins contamination in peanut and peanut products commercially available in retail markets of Punjab, Pakistan. *Food Control*, 32: 83-86.

Iqbal, S. Z., Asi, M. R., Jinap, S., Rashid, U. (2014). Detection of aflatoxins and zearalenone contamination in wheat derived products. *Food Control*, 35: 223-226.

Jalili M., and Jinap S. (2012). Natural occurrence of aflatoxins and ochratoxin A in commercial dried chili. *Food Control*, 24: 160–164.

James, A., and Zikankuba, V. L. (2018). Mycotoxins contamination in maize alarms food safety in sub-Sahara Africa. *Food Control*, 90: 372-381.

Jonathan, S. G., Abdul-Lateef, M. B., and Ayansina, A. D. V. (2013). Fungal and aflatoxin detection in fresh and stored 'garri ijebu' (locally processed food). *Report and Opinion*, 5 (2): 13-19.

Kachapulula, P. W., Akello, J., Bandyopadhyay, R., and Cotty, P. J. (2017). *Aspergillus* section Flavi community structure in Zambia influences aflatoxin contamination of maize and groundnut. *International Journal of Food Microbiology*, 261: 49–56.

Kamika, K., and Ngbolua, M.T. (2016). Occurrence of aflatoxin contamination in maize throughout the supply chain in the Democratic Republic of Congo. *Food Control*, 69: 292-296.

Karami-Osboo, R., Mirabolfathy, M., Kamran, R., Shetab-Boushehri, M., and Sarkari, S. (2012). Aflatoxin B1 in maize harvested over 3 years in Iran. *Food Control*, 23 (1): 271–274.

Karlovsky, P., Suman, M., Berthiller, F., De Meester, J., Eisenbrand, G., and Perrin, I., et al. (2016). Impact of food processing and detoxification treatments on mycotoxin contamination. *Mycotoxin Research*, 32(4): 179-205.

Kensler, T. W., Egner, P. A., Wang, J. B., Zhu, Y. R., Zhang, B. C., Lu, P. X., Chen, J. G., Qian, G. S., Kuang, S. Y., Jackson, P. E., Gange, S. J., Jacobson, L. P., Munoz, A., and Groopman J. D. (2004). Chemoprevention of hepatocellular carcinoma in aflatoxin endemic areas. *Gastroenterology*, 127 (5): 310–318.

Kimanya, M. E. (2015). The health impacts of mycotoxins in the eastern Africa region. *Current Opinion in Food Science*, 6: 7–11.

Kuhumba, G. D., Simonne, A. H., and Mugula, J. K. (2018). Evaluation of aflatoxins in peanut-enriched complementary flours from selected urban markets in Tanzania. *Food Control,* 89: 196-202.

Kulshrestha, P., Singh, C., Gupta, A., Mahajan, S., and Sharma, R. (2014). Mycoflora associated with spices. *International Journal of Current Microbiology and Applied Sciences*, 3 (5): 741-746.

Kumar, G. D. S. and Popat, M. N. (2010). Farmers' perceptions, knowledge and management of aflatoxins in groundnuts (*Arachis hypogaea L.)* in India. *Crop Protection*, 29: 1534-1541.

Lamboni, Y., Frisvad, J. C., Hell, K., Linnemann, A. R., Nout, R. M. J., Tamo, M., Nielsen, K. F., Boekel, M. A. J. S., and Smid, E. J. (2016). Occurrence of *Aspergillus* section Flavi and section Nigri and aflatoxins in raw cashew kernels (*Anacardium occidentale L.*) from Benin. LWT - *Food Science and Technology,* 70:71-77.

Lane, B., and Woloshuk, C. (2017). Impact of storage environment on the efficacy of hermetic storage bags. *Journal of Stored Products Research*, 72: 83-89. https://doi.org/10.1016/J.JSPR. 2017.03.008.

Lizarraga-Paulin, E. G., Moreno-Martinez, E., and Miranda-Castro, S. P. (2011). Aflatoxins and their Impact on human and animal health: An emerging problem. *Aflatoxins - Biochemistry and Molecular Biology,* 255–284. doi: 10.5772/26196

Lombard, M., Steyn, N., Burger, H., Charlton, K., and Gelderblom, W. (2012). A proposed method to determine fumonisin exposure from maize consumption in a rural South African population using a culturally appropriate FFQ. *Public Health Nutrition*, 17 (1): 131–138.

Magnussen, A., and Parsi, M. A. (2013). Aflatoxins, hepatocellular carcinoma and public health. *World Journal of Gastroenterology*, 19 (10): 1508-1512.

Maidak, B. L., Cole, J. R., Parker, C. T., Jr and 11 other authors (1999). A new version of the RDP (Ribosomal Database Project). *Nucleic Acids Research*, 27: 171–173.

Makun, H. A., Adeniran, A. L., Mailafiya, S. C., Ayanda, I. S., Mudashiru, A. T., Ojukwu, U. J., Jagaba, A. S., Usman, Z. and Salihu, D. A. (2013) 'Natural occurrence of ochratoxin A in some marketed Nigerian foods', *Food Control*, 31 (2): 566–571.

Makun, H. A., Dutton, M. F., Njobeh, P. B., Gbodi, T. A., and Ogbadu, G. H. (2012). Aflatoxin contamination in foods and feeds: A special focus on Africa. Available from: http://www.intechopen.com/download/pdf/. [Accessed 15 July 2014].

Markov, K., Pleadin, J., Bevardi, M., Vacic, N., Sokolic-Mihalak, D., Frece, J. (2013). Natural occurrence of aflatoxin B_1, ochratoxin A and citrinin in Croatian fermented meat products. *Food Control*, (34): 312-317.

Martins, F. A., Ferreira, F. M. D., Ferreira, F. D., Bando, E., Nerilo, S. B., Hirooka, E. Y., and Machinski Jr., M. (2012). Daily intake estimates of fumonisins in corn-based food products in the population of Parana, Brazil. *Food Control*, 26: 614-618

Matumba, L., Monjerezi, M., Biswick, T., Mwatseteza, J., Makumba, W., Kamangira, D., and Mtukuso, A. (2015). A survey of the incidence and level of aflatoxin contamination in a range of locally and imported processed foods on Malawian retail market. *Food Control*, 39: 87-91.

Matumba, L., Monjerezi, M., Chirwa, E., Lakudzala, D. and Mumba, P. (2009). Natural occurrence of AFB_1 in maize and effect of traditional maize flour production on AFB_1 reduction in Malawi. *African Journal of Food Science*, 3 (12): 413-425.

Matumba, L., Poucke, C. V., Monjerezi, M., Ediage, E. N., and De Saeger, S. (2015). Concentrating aflatoxins on the domestic market through groundnut export: A focus on Malawian groundnut value and supply chain. *Food Control*, 51: 236-239.

Matumba, L., Singanob, L., Tranc, B., Mukangad, M., Makwendae, B., Kumwendae, W., Mgwiraf, S., Phirie, S., Matayae, F., Mthunzig, T., Alfredg, S., Madzivhandilag, T., Mugabeh, J., Bennett, B., and Chancellor, T. (2018). Managing aflatoxin in smallholder groundnut production in Southern Africa: Paired comparison of the windrow and Mandela cock techniques. *Crop Protection*, (112): 8–23.

Mendoza, J.R., Sabillon, L., Martinez, W., Campabadal, C., Hallen-Adams, H. E., Bianchini, A. (2017). Traditional maize post-harvest management practices amongst smallholder farmers in Guatemala. *Journal of Stored Products Research*, 71: 14-21.

Mhlambi, S. G., Naidoo, K., and Lindsay, D. (2010). Enterotoxin-producing Staphylococcus strains associated with South African biltong at point of sale. *Journal of Food Safety*, 30: 307–317.

Mohammed, S., Mo, L., Flint, S., Palmer, J., and Fletcher, G. C. (2012). Effect of water activity and temperature on the germination and growth of *Aspergillus tamarii* isolated from “Maldive fish”. *International Journal of Food Microbiology*, 160: 119–123.

Mohammed, S., Khalid, A., and Sallam, I. (2015). Rapid determination of total aflatoxins and ochratoxins A in meat products by immuno-affinity fluorimetry. *Food Chemistry*, 179(15): 253-256.

Monda, E., and Alakonya, A. (2016). A review of agricultural aflatoxin management strategies and emerging innovations in sub-Saharan Africa. *African Journal of Food, Agriculture, Nutrition and Development.* 16(3): 11126-11138. https://doi.org/10.18697/ajfand.75.ILRI11.

Montanha, F. P., Anatera, A., Burcharda, J. F., Lucianoa, F. B., Mecab, G., Manyesb, L., and Pimpão, C. T. (2018). Mycotoxins in dry-cured meats: A review. *Food and Chemical Toxicology*, 111: 494–502.

Murphy, P. A., Henderick, S., and Landgren, C. (2006). Food Mycotoxins- An Update. *Journal of Food Science.* Available from:http://www.ift.org/~/media/Knowledge%20Center/Science%20Reports/Scientific%20Statu s%20Summaries/Editorial/editorial_0606mycotoxins.pdf [Accessed 28 may 2014].

Mutungi, C., Lamuka, P., Arimi, S., Gathumbi, J., and Onyango, C. (2008). The fate of aflatoxins during processing of maize into muthokoi – A traditional Kenyan food. *Food Control,* 19: 714–721.

Mwalwayo, D. S., and Thole, B. (2016). Prevalence of aflatoxin and fumonisins (B_1 + B_2) in maize consumed in rural Malawi. *Toxicology Reports*, 3: 173–179.

Naidoo, K. and Lindsay, D. (2010). Pathogens associated with biltong product and their in vitro survival of hurdles used during production. *Food Protection Trends*, 3 (9): 532–538.

National Toxicology Program, *Department of Health and Human Services*. (2011). Report on Carcinogens, Thirteenth Edition. Avaliable from: http://ntp.niehs.nih.gov.ntp.roc/content/profiles/octratoxina.pdf [Accessed 14 August 2014].

Ndung'u, J. W., Makokha, A. O., Onyango, C. A., Mutegi, C. K., Wagacha, J. M., Christie, M. E., and Wanjoya, A. K. (2013). Prevalence and potential for aflatoxin contamination in groundnuts and peanut butter from farmers and traders in Nairobi and Nyanza provinces of Kenya. *Journal of Applied Biosciences*, 65: 4922 – 4934.

Neme, K., and Mohammed, A. (2017). Mycotoxin occurrence in grains and the role of postharvest management as a mitigation strategy: A review. *Food Control*, (78): 412-425.

Nicolau, A. I. (2016). Safety of Fermented Cassava Products. Regulating Safety of Traditional and Ethnic Foods. 6: 319-333. http://dx.doi.org/10.1016/B978-0-12-800605-4.00016-5.

Nielsen, K. F., Mogensen, J. M., Johansen, M., Larsen, T. O., Frisvad, J. C., 2009. Review of secondary metabolites and mycotoxins from the *Aspergillus niger* group. Anal. Bioanal. Chem. 395, 1225–1242.

Nishimwe, K., Wanjuki, I., Karangwa, C., Darnell, R., and Harvey, J. (2017). An initial characterization of aflatoxin B1 contamination of maize sold in the principal retail markets of Kigali, Rwanda. *Food Control*, 73: 574-580.

Njobeh, B. P., Dutton, M. F., Koch, S. H. and Chuturgoon, A. (2009) Contamination with storage fungi of human foods from Cameroon. *International Journal of Food Microbiology*, 135: 193-198.

Olopade, B. K., Oranusi, S., Ajala, R., and Olorunsola, S. J. (2014). Microbiological quality of fermented Cassava (Gari) sold in Ota Ogun State Nigeria. *Internatinal Journal of Current Microbiology and Applied Sciences*, 3 (3): 888-895.

Omonigho, S. E., and Ikenebomeh, M. J. (2002). Microbiological quality assessment of garri with reference to thermonuclease activity. *Food Control*, 13: 535–541.

Oplatowska-Stachowiak, M., Sajic, N., Xu, Y., Haughey, S.A., Mooney, M.H., Gong, Y.Y., Verheijen, R., and Elliott, C.T. (2016). *Food Control*, 63: 239-245.

Oyewole O. A., and Isah P. (2012). Locally fermented foods in Nigeria and their significance to national economy: A review. *Journal of Recent Advances in Agriculture*, 1 (4): 92-102.

Ozbey, F., and Kabak, B. (2012). Natural co-occurrence of aflatoxins and ochratoxin A in spices. *Food control*, 28 (2): 354–361.

Partnership for Aflatoxin Control in Africa (PACA). (2013). Aflatoxin Impacts and Potential Solutions in Agriculture, Trade, and Health. Aflatoxin Effects in Agriculture, Trade, Health. pp. 1-11. Avaliable from: http://www.aflatoxinpartnership.org/ uploads/ files/Aflatoxin%20Impacts%20Paper_0.pdf [Accessed 13 May 2014].

Pascale, M. (2009). Dectection methods for mycotoxins in cereal grains and cereal products. doi:10.2298/zmspn0917015p. Avaliable from: http://www.doiserbia. nb.rs/ img/doi/0352-4906/2009/0352-49060917015P.pdf [Accessed 15 July 2014].

Penido, F. C. L., Piló, F.B., Sandesc, S. H. C., Nunesc, A. C., Colena, G., Oliveira, E. S., Rosab, C. A., Lacerda, I. C. A. (2018). Selection of starter cultures for the production of sour cassava

starch in a pilot-scale fermentation process. *Biotechnology and Industrial Microbiology*, 49 (4): 823-831.

Plascencia-Jatomea, M., Yepiz-Gomez, M. S. and Velez-Haro, J. M. (2014). *Aspergillus* spp. (Black Mold), Chapter 8, Postharvest Decay, *Academic Press*, 267-286.

Petit, T., Caro, Y., Petit, A. S., Santchurn, S. J. and Collignan, A. (2014). Physicochemical and microbiological characteristics of biltong, a traditional salted dried meat of South Africa. *Meat Science*, 96: 1313–1317.

Pitt, J. J. (2009). Principles and applications of liquid chromatography-mass spectrometry in clinical biochemistry. *Clinical Biochemist Review*, 30 (1): 19–34.

Prelle, A., Spadaro, D., Garibaldi, A., and Gullino, M. L. (2014). Co-occurrence of aflatoxins and ochratoxin A in spices commercialized in Italy. *Food control*, 39: 192–197.

Probst, C., Bandyopadhyay, R., and Cotty, P. J. (2014). Diversity of aflatoxin-producing fungi and their impact on food safety in sub-Saharan Africa. *International Journal of Food Microbiology*, 174: 113–122.

Quiles, J. M., Saladino, F., Manes, J., Fernandez-Franzon, M., and Meca, G. (2016). Occurrence of mycotoxins in refrigerated pizza dough and risk assessment of exposure for the Spanish population. *Food and Chemical Toxicology*, 94: 19-24.

Rachaputi, N. R., Wright, G. C., and Kroschi S. (2002). Management practices to minimise pre-harvest aflatoxin contamination in Australian groundnuts. *Austrian Journal of Experimental Agriculture*, 42: 595–605.

Rai, M. K., Bonde, S. R., Ingle, A. P., and Gade, A. K. (2012). Mycotoxin: Rapid detection, differentiation and safety. *Journal of Pharmaceutical Education and Research*, 3(1): 22-34

Reddy, K. R. N., Nurdijati, S. B., and Salleh, B. (2010). An overview of plant-derived products on control of mycotoxigenic fungi and mycotoxins. *Asian Journal of Plant Science*, 9: 126-133.

Riba, A., Bouras, N., Mokrane, S., Mathieu, F., Lebrihi, A., and Sabaou, N. (2010). *Aspergillus* section Flavi and aflatoxins in Algerian wheat and derived products. *Food and Chemical Toxicology*, 48: 2772–2777.

Riba, A., Matmoura, A., Mokrane, S., Mathieu, F., and Sabaou, N. (2013). Investigations on aflatoxigenic fungi and aflatoxins contamination in some nuts sampled in Algeria. *African Journal of Microbiology Research*, 7 (42): 4974-4980.

Roohi, S., Gholampour, I., Azizi, A., and Hashemi, M. (2012). Fumonisin contamination based on flour quality used in bakeries and confectioneries in Qaemshahr (city of the Northern Iran). *African Journal of Microbiology Research*, 6 (8): 1815-1818.

Ruadrew, S., Craft, J. and Aidoo, K. (2013). Occurrence of toxigenic *Aspergillus* spp. andaflatoxins in selected food commodities of Asian origin sourced in the West of Scotland. *Food Chem. Toxicol.* 55, 653–658.

Sanzania, S. M., Reverberib, M., and Geisen, R. (2016). Mycotoxins in harvested fruits and vegetables: Insights in producing fungi, biological role, conducive conditions, and tools to manage postharvest contamination. *Postharvest Biology and Technology,* 122: 95–105.

Saini, S. S., and Kaur, A. (2012). Aflatoxin B1: Toxicity, characteristics and analysis: Mini review. *Global Advance Research Journal of Chemistry and Material Science*, 1 (4): 063-070.

Sampels, S. (2015). The effects of processing technologies and preparation on the final quality of fish products. *Trends in Food Science and Technology*. 44 (2): 131-146.

Sardinas, N., Gil-Serna, J., Santos, L., Ramos, A. J., González-Jaén, M. T., Patiño, B., and Vázquez, C. (2011). Detection of potentially mycotoxigenic *Aspergillus* species in Capsicum powder by a highly sensitive PCR-based detection method. *Food Control,* 22 : 1363-1366.

Schuster, E., Dunn-Coleman, N., Frisvad, J.C., van Dijck, P.W.M., 2002. On the safety of *Aspergillus niger* – a review. *Applied Microbiology and Biotechnology,* 59: 426–435.

Shephard, G., S. (2008). Risk assessment of aflatoxins in food in Africa. *Food Additives and Contaminants: Part A,* 25 (10): 1246-1256

Simsek, S., Burgess, K., Whitney, K.L., Gu, Y., and Qian, S.Y. (2012). Analysis of deoxynivalenol and deoxynivalenol-3-glucoside in wheat. *Food control,* 26 (2): 287–292.

Singh, P. and Cotty, P.J. 2017. Aflatoxin contamination of dried red chilies: Contrasts between the United States and Nigeria, two markets differing in regulation enforcement. *Food Control*. 80: 374-379.

Škrbić, B., Živančev, J., Đurišić-Mladenović, N., and Godula, M. (2012). Principal mycotoxins in wheat flour from the Serbian market: Levels and assessment of the exposure by wheat-based products. *Food Control,* 25 (1): 389–396.

Stackebrandt, E., and Goebel, B. M. (1994). Taxonomic note: a place for DNA–DNA association and 16S rRNA sequence analysis in the present species definition in bacteriology. *International Journal of Systematic Bacteriology*, 44: 846-849.

Statistics South Africa (2011). Census 2011 Statistical Release: Gauteng Province. Available from: http://www.statssa.gov.za/?page_id=1021andid=city-of-johannesburg-municipality [Accessed 27 September 2016].

Susca, A., Moretti, A., Stea, G., Villani, A., Haidukowski, M., Logrieco, A., Munkvold, G. (2014). Comparison of species composition and fumonisin production in *Aspergillus* section Nigri populations in maize kernels from USA and Italy. *International Journal of Food Microbiology,* 188: 75–82.

Temba, B. A., Sultanbawa, Y., Kriticos, D. J., Fox, G. P., Harvey, J. J. W., and Fletcher, M. T.

(2016). Tools for defusing a major global food and feed safety risk: Non-biological postharvest procedures to decontaminate mycotoxins in foods and feeds. *Journal of Agricultural and Food Chemistry*, 64(47): 8959-8972. https://doi.org/10.1021/acs.jafc.6b03777.

Temba, M. C., Njobeh, B. P., and Kayitesi, E. (2017). Storage stability of maize groundnut composite flours and an assessment of aflatoxin B_1 and ochratoxin: A contamination in flours and porridges. *Food Control*, 71: 178-186. https://doi.org/10.1016/J.FOODCONT.2016.06.033.

Tsitsigiannis, D., Dimakopoulou, M., Antoniou, P., and Tjamos, E. (2012). Biological control strategies of mycotoxigenic fungi and associated mycotoxins in Mediterranean basin crops. Special section on Mycotoxicological risk in Mediterranean countries, *Phytopathologia Mediterranea,* 51(1): 158-174

Turner, P. C., Collinson, A. C., Cheung, Y. B., Gong, Y., Hall, A. J., Prentice, A. M., and Wild, C. P. (2007). Aflatoxin exposure in utero causes growth faltering in Gambian infants. *International Journal of Epidemiology*, 36: 1119–1125.

Udomkun, P., Wiredu, A. N., Nagle, M., Müller, J., Vanlauwe, B., and Bandyopadhyay, R. (2017). Innovative technologies to manage aflatoxins in foods and feeds and the profitability of application: A review. *Food Control*, 76: 127–138.

Uyttendaele, M., De Boecka, E., and Jacxsensa, L. (2016). Challenges in food safety as part of food security: Lessons learnt on food safety in a globalized world. *Procedia Food Science,* 6: 16 – 22.

Van Asselt, E. D., Azambuja, W., Moretti, A., Kastelein, P., De Rijk, T. C., Stratakou, I., and Van Der Fels-Klerx, H. J. (2012). A Dutch field survey on fungal infection and mycotoxin concentrations in maize. *Food Additives and Contaminants: Part A,* 29 (10): 1556-1565.

Varga, J., Frisvad, J. C., Kocsubé, S., Brankovics, B., Tóth, B., Szigeti, G., and Samson, R. A. (2011). New and revisited species in Aspergillus section Nigri. *Studies in Mycology,* 69: 1–17.

Wagacha, J.M., and Muthomi, J.W. (2008). Mycotoxin problem in Africa: Current status, implications to food safety and health and possible management strategies. *International Journal of Food Microbiology*, 124 (1): 1–12.

Waliyar, F., Umeh, V.C., Traore , A., Osiru, M., Ntare, B.R., Diarra, B., Kodio, O., Kumar , K.V.K., and Sudini, H. (2015). Prevalence and distribution of aflatoxin contamination in groundnut (Arachis hypogaea L.) in Mali, West Africa. *Crop Protection*, 70: 1-7.

Wang, X., Lien, K. and Ling. M. (2018). Probabilistic health risk assessment for dietary exposure to aflatoxin in peanut and peanut products in Taiwan. *Food Control*, 91: 372-380.

Waśkiewicz, A., Beszterda, M., Bocianowski, J., and Goliński, P. (2013). Natural occurrence of fumonisins and ochratoxin A in some herbs and spices commercialized in Poland analyzed by UPLC–MS/MS method. *Food Microbiology*, 36 (2): 426-431.

World Health Organization (WHO). (2006). Mycotoxins in African foods: Implications to food safety and health. *AFRO Food Safety Newsletter World Health Organization Food safety (FOS),* Issue No. 2. Avaliable from: http://www.afro.who.int/index.php?option =com_docman&task=doc_download&gid=1724. [Acessed 21 May 2014].

Wild C., and Gong Y. (2010). Mycotoxins and human disease: A largely ignored global health issue. *Carcinogenesis*, 31: 71-82.

Wu, F. and Khlangwiset, P. (2010). Health economic impacts and cost-effectiveness of aflatoxin reduction strategies in Africa: Case studies in biocontrol and postharvest interventions. *Food Additive Contaminant Part A Chemistry Analysis Control Exposure and Risk Assessment,* 27 (4): 496–50.

Wu, F., and Guclu, H. (2012). Aflatoxin Regulations in a Network of Global Maize Trade. *PLoS ONE*. 7(9): e45151. [Accessed 29 July 2014]. Available from:http://www.plosone.org/article/ fetchObject.action?uri=info%3Adoi%2F10.1371%2Fjournal.pone.0045151&representation=pdf.

Wu, F., Narrod, C., Tiongco, M., and Liu, Y. (2011). The health economics of aflatoxin: Global burden of disease. *International Food Policy Research Institute*. [Accessed 21 May 2014]. Avaliable from: http://www.ifpri.org/sites/default/files/publications/aflacontrol_wp04.pdf.

Yu-jiao, W., Ji-yun N., Zhen, Y., Zhi-xia, L., Yang, C., Farooq, S. (2018). Multi-mycotoxin exposure and risk assessments for Chinese consumption of nuts and dried fruits. *Journal of Integrative Agriculture*, 17 (7):1676–1690.

Zain, M.E. (2011). Impact of mycotoxins on humans and animals. *Journal of Saudi Chemical Society,* 15 (2): 129–144.

Zaki, M.M., El-Midany, S.A., Shaheen, H.M. and Rizzi, L. (2012). Mycotoxins in animals: Occurrence, effects, prevention and management. *Journal of Toxicology and Environmental HealthSciences*,4(1):13-28.

APPENDIX 1: RESEARCH PROPOSAL APPROVAL

UNISA university of south africa

Department of Life and Consumer Sciences
School of Agriculture and Life Sciences
College of agriculture and Environmental Sciences
Private Bag X6
Florida
1710

To: Chinenye Kate Okaekwu (Student no: 50019074)

Subject: Outcome of your research proposal

It gives me great pleasure to inform you that your MSc research proposal titled: "Microbial and aflatoxin quality of small-scale processed foods of African origin". has been approved by the research committee of the Department of Life and Consumer Sciences.

Although you were awarded an average score of 67% for your research proposal, you are advised to pay special attention to the comments raised by the review committee. These comments will be communicated to you by your supervisor.

Good luck with the rest of your studies.

Best regards

.....Date: ...03/02/2015.....
Prof PT Matjila.
Post-graduate coordinator

.....Date: 2015 -02- 11
Dr SL Lebelo
COD: Department of Life and Consumer Sciences

APPENDIX 2: ETHICS APPROVAL

UNISA university of south africa

CAES RESEARCH ETHICS REVIEW COMMITTEE

Date: 01/04/2015

Ref #: **2015/CAES/022**
Name of applicant: **Ms CK Okaekwu**
Student #: **50019074**

Dear Ms Okaekwu,

Decision: Ethics Approval

Proposal: Microbial and aflatoxin quality of small-scale processed foods of African origin

Supervisor: Dr FT Tabit

Qualification: Postgraduate degree

Thank you for the application for research ethics clearance by the CAES Research Ethics Review Committee for the above mentioned research. Final approval is granted for the duration of the project.

The researcher is required to adhere to the stipulation in point 4 during the research project.

The application was reviewed in compliance with the Unisa Policy on Research Ethics by the CAES Research Ethics Review Committee on 01 April 2015.

The proposed research may now commence with the proviso that:

1) *The researcher/s will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.*
2) *Any adverse circumstance arising in the undertaking of the research project that is relevant to the ethicality of the study, as well as changes in the methodology, should be communicated in writing to the CAES Research Ethics Review Committee. An amended application could be requested if there are substantial changes from the existing proposal, especially if those changes affect any of the study-related risks for the research participants.*
3) *The researcher will ensure that the research project adheres to any applicable*

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national legislation, professional codes of conduct, institutional guidelines and scientific standards relevant to the specific field of study.

4) *Should serious cases of contamination of food be found, the researcher must inform the relevant people accordingly so that they can take action. In the case of food purchased from retail outlets, the Head Office of the relevant retailer must be informed. In the case of street vendors, the umbrella association that oversees the vendors must be informed. If there is no such association or the vendor does not belong to the existing association, the researcher should speak to the vendor directly. This should be done with discretion so that it does not lead to loss of income for the vendor. The researcher must therefore have a coding system in place for the retail outlets and vendors so that samples can be traced to a specific source. However, the researcher must take care that this information is held secure, and that the confidentiality and anonymity of the participants are not compromised.*

Note:

The reference number [top right corner of this communiqué] should be clearly indicated on all forms of communication [e.g. Webmail, E-mail messages, letters] with the intended research participants, as well as with the CAES RERC.

Kind regards,

Signature
CAES RERC Chair: Prof EL Kempen

Signature
CAES Executive Dean: Prof MJ Linington

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