CHARACTERIZATION OF THE ELECTROCHEMICAL PROPERTIES OF MAIZE GRAIN CONTAMINATED WITH AFLATOXIN

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ABSTRACT

Aflatoxin contamination of maize is a serious problem in Sub-Saharan Africa since it causes several negative health implications and in some cases death. A number of methods have been developed to detect the presence of aflatoxin in maize and thus limit dietary exposure to the toxin. However, these methods require skill and expensive equipment which makes them inaccessible to smallholder farmers who make up the majority of maize producers in Sub-Saharan Africa. This review presents literature on maize production, consumption and storage in Africa. It also examines the aflatoxin contamination of maize, its health effects and methods of detection. Finally the review presents literature on the electrical properties of maize with the aim of identifying the properties that are sensitive to aflatoxin contamination.

The review indicates that maize consumption is predominant in Eastern and Southern Africa where it is the most important source of calories. The hot and humid conditions across Sub-Saharan Africa coupled with poor grain storage promote the growth of *Aspergillus flavus* and aflatoxin production in maize. Aflatoxin detection is usually done using chromatographic techniques or immunological methods for rapid screening. A few spectroscopic techniques have also been developed for aflatoxin screening. These methods are however expensive and require skilled personnel. It is necessary to develop easier and cheaper methods for aflatoxin detection.

A. flavus consumes nutrients from the maize grain besides producing aflatoxin hence changing the chemical composition of the grain. The dielectric properties of grains can be influenced by these changes in chemical composition. Moisture content has the greatest influence on the dielectric properties of maize while starch, proteins and fats generally have low dielectric properties. The research proposal presented in this document seeks to characterize the chemical and electrical properties of aflatoxin contaminated maize by assessing the effect of different maize sample treatments on its chemical compositing and dielectric properties under different conditions.

DECLARATION

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Francis Collins Muga

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1. INTRODUCTION

Maize is an important cereal for food, feed and industrial raw material (Farnham *et al.*, 2003). It is a staple food in many Sub-Saharan African countries, particularly Eastern and Southern Africa, where it accounts for about 55% of the total land area under cereals (Erenstein *et al.*, 2011). According to Pingali (2001), a large proportion of maize production in Africa is mainly for human consumption. Smale and Jayne (2003) estimated the average per capita consumption of maize at 94 $kg.year^{-1}$ in East Africa and over 100 $kg.year^{-1}$ in Southern Africa. Maize dominates the food economy of Eastern and Southern Africa (Reiter *et al*., 2010), providing income to millions of resource-poor smallholder farmers in these regions.

Several factors including temperature, relative humidity, insect damage and growth of microorganisms affect the quality of maize in storage (Oyekale *et al.*, 2012). These factors singly or in combination make maize kernels vulnerable to infection by toxigenic fungi such as *Aspergillus* and *Fusarium* (Abbas *et al*., 2006). Close to 50% of maize grain lost in tropical countries is attributed to storage fungi (Fandohan *et al*., 2004). Mycotoxins produced by fungi pose significant food safety risks and health hazards which limit the marketability of grain supply worldwide (Woloshuk and Shim, 2013). According to Lewis et al. (2005) and Wagacha and Muthoni (2008), between 25% and 50% of crops worldwide are contaminated with mycotoxins.

Aflatoxin is one of the major mycotoxins in agriculture. Maize is significantly colonized by aflatoxin-producing *Aspergillus* species (Bandyopadhyay *et al*., 2007). *Aspergillus flavus* is the major producer of aflatoxin (Klich, 2007). The growth of *Aspergillus flavus* and aflatoxin production in the field is influenced by high temperature, high humidity and drought stress (Cotty and Jaime-Garcia, 2007). The moisture content of grain is critical in controlling the growth of *Aspergillus flavus* during storage. Temperature and relative humidity are also important storage factors since they influence the equilibrium moisture content of the grain

(Giorni *et al*., 2012). Improper storage of maize will increase aflatoxin contamination by promoting proliferation of *Aspergillus flavus.*

Aflatoxin contamination in maize grain is common in Sub-Saharan Africa (Wagacha and Muthoni, 2008; Mutiga *et al*., 2015). The consumption of such contaminated maize is harmful to both human and animal health (Fellinger, 2006). Cases of fatal aflatoxicosis have been reported in India, Nigeria and Kenya (Krishnamachari *et al*., 1975; Wagacha & Muthoni, 2008). The 2004 aflatoxin poisoning in Kenya has been directly linked to consumption of homegrown maize stored in damp conditions (Lewis *et al*., 2005). Dietary intake of aflatoxins has also been associated with the high incidences of liver cancer in Africa (Strosnider *et al*., 2006). Studies by Gong *et al*. (2004) have linked malnutrition, impaired growth and immune suppression to aflatoxin intake.

The serious health consequences of consuming food contaminated with aflatoxin have necessitated the establishment of regulatory levels to limit exposure to aflatoxins. The Codex Alimentarius Commission proposed 15 $\mu g.kg^{-1}$ as the maximum tolerable level for total aflatoxin in food (Van Egmond *et al*., 2007). A few African countries such as South Africa, Kenya and Tanzania have set the maximum acceptable limits for aflatoxin at 5 $\mu g.kg^{-1}$ and 10 $\mu g.kg^{-1}$ for aflatoxin B1 and total aflatoxin respectively (Kimanya *et al*., 2008; Rheeder *et al*., 2009; Kilonzo *et al*., 2014).

Analytical and screening methods have been developed for detecting aflatoxin levels in food to conform to the strict regulations on the acceptable limits. Chromatographic techniques such high-performance liquid chromatography (HPLC), thin layer chromatography (TLC) and gas chromatography (GC) are the conventional analytical methods (Shephard, 2009). Several immunological methods such as enzyme-linked immunosorbent assay (ELISA) have been developed to provide faster and cheaper analysis (Ostadrahimi *et al*., 2014). Spectroscopic methods such as fluorescence spectroscopy and frontier infrared spectroscopy have also been used to detect aflatoxins since they require little sample manipulation (Wacoo *et al*., 2014).

Apart from lateral flow devices, all other aflatoxin detection methods are laboratory-based techniques. These analyses are expensive and require skilled personnel to perform (Shephard

2009). The smallholder farmers who produce more than two-thirds of maize in Sub-Saharan Africa have no access to aflatoxin screening because of their meagre resource (Wu *et al*., 2013). It is, therefore, necessary to develop simple, cheap and portable instruments that can be used for aflatoxin screening of maize produced by smallholder farmers since their maize is usually for own consumption and never enters the formal grain market where screening methods are established (Del Fiore *et al*., 2010).

Electrical properties have been used to devise simple techniques for estimating quality attributes of agricultural products (Skierucha *et al*., 2012). Electrical properties of grains have predominantly been used to provide quick estimates of their moisture content (Nelson, 2010; Sacilik and Colak, 2010). The electrical properties of cereal grains are represented by their dielectric properties (Nelson and Trabelsi 2012).

The dielectric properties of grains are greatly affected by grain moisture content, bulk density, temperature and frequency of applied electric current (Trabelsi and Nelson 1998; Nelson, 2008; Skierucha *et al*., 2012). However, proximate composition can also have an impact on the dielectric properties of cereal grains (Zhang *et al*., 2007; Bhargava *et al*., 2013). Apart from moisture content, carbohydrates, proteins, fats, ash and fibre are the other chemical compounds that make up maize (Iqbal *et al*., 2006). These compounds have very low dielectric properties. Nonetheless, variations in carbohydrate, protein, fat, ash and fibre content are capable of influencing the dielectric properties (Bhargava *et al*., 2013).

This document provides a review of the literature (Chapter two) on maize production and consumption across Africa and the factors that lead to quality and quantity deterioration of maize during storage. It also presents literature on aflatoxin contamination of maize and the various methods used for aflatoxin analysis. Finally, the review examines the electrical properties of grains with emphasis on the factors that affect dielectric properties of maize that could be sensitive to aflatoxin contamination of maize. Chapter three describes a project proposal which focuses on characterizing the electrochemical properties of maize contaminated with aflatoxin.

2. LITERATURE REVIEW

2.1 The Global Perspective

Maize is the second largest cereal crop regarding production volumes and is the most domesticated of all field crops in the world (Wariboko and Ogidi, 2014). It is an important staple for more than 1.2 billion people in Sub-Saharan Africa, Latin America and Asia (Aoudou *et al*., 2012; Wariboko and Ogidi, 2014). The United States of America produces about 40% with China, Brazil, and the European Union accounting for another 20% of the global production (Taylor and Koo, 2013).

Globally, maize consumption has risen steadily. In the developed countries, maize is primarily used as livestock feed and raw material for industrial products whereas in the Sub-Saharan Africa human consumption accounts for about 70% (Pingali, 2001).

2.2 Maize Production in Africa

Maize production in Africa has expanded significantly because of its importance as a source of food and feed (Ranum *et al*., 2014). It has become the preferred cereal in many parts of Sub-Saharan Africa, displacing traditional grains such as sorghum and millet (Hell *et al*., 2010). More than two-thirds of the maize produced in Sub-Saharan Africa comes from smallholder farmers except for South Africa that has a well-established commercial maize farming system (Reiter *et al*., 2010; Suleiman *et al*., 2013).

South Africa is the leading producer of maize in Africa with 14.9 million tonnes produced in 2014 (FAOSTAT, 2015a). Nigeria, Ethiopia, Tanzania and Egypt follow with 10.8, 7.2, 6.7 and 5.8 million tonnes respectively (FAOSTAT, 2015a). Regionally, Eastern Africa accounts for the largest amount of maize produced in Africa with the least production observed in Central and North Africa (Figure 2.1). Maize accounts for 30% of the total cereal area in Sub-Saharan Africa with 29% produced in Eastern; 65% Southern Africa; 19% in West Africa and 61% in Central Africa (Erenstein *et al*., 2011). Ethiopia and Tanzania are the leading producers in Eastern

Africa. Kenya, Zambia and Malawi also make a sizeable contribution with each producing well over three million tonnes.

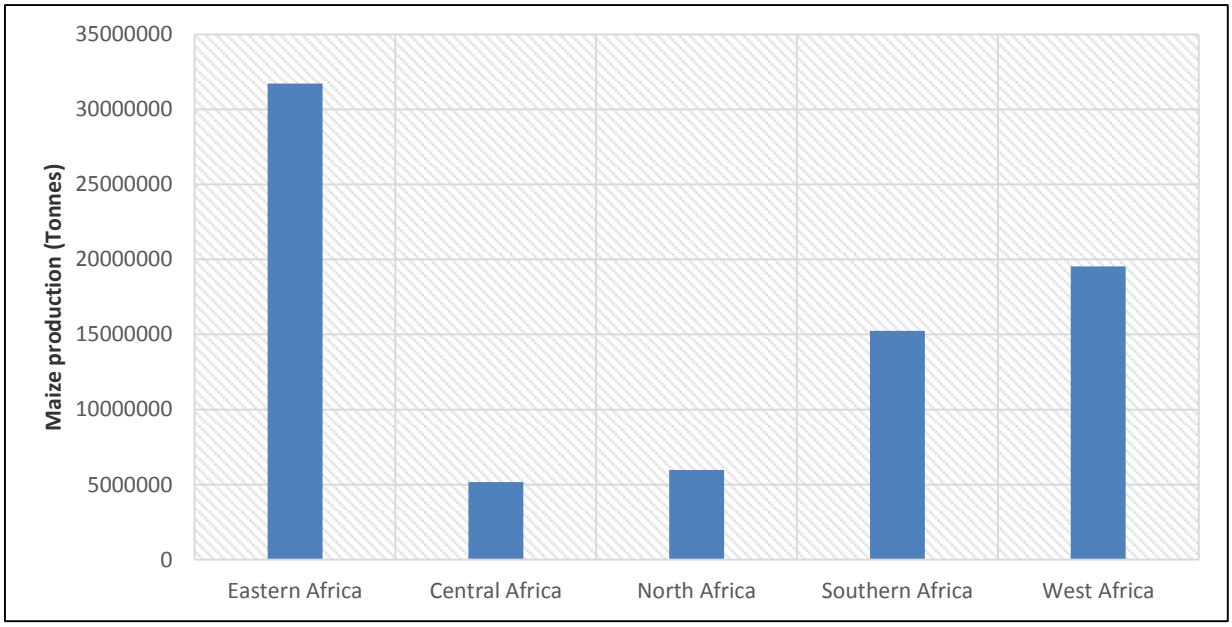

Figure 2.1: Maize production in Africa by region (FAOSTAT, 2015a)

Some recent statistics indicate that South Africa produces almost all the maize in Southern Africa. It accounted for 14.9 million tonnes of the total 15.3 million tonnes produced in Southern Africa in 2014. Similarly, Egypt produces virtually all the maize in North Africa accounting for 5.8 million tonnes of the total 5.97 million tonnes produced in North Africa (FAOSTAT, 2015a). Maize production is not robust in West Africa. Benin, Burkina Faso, Ghana, Mali and Senegal are the only countries with annual production of over a million tonnes except Nigeria, which is the second largest producer of maize in Africa with 10.8 million tonnes produced in 2014 (FAOSTAT, 2015a).

2.3 Maize Consumption in Africa

Maize dominates the food economy of Eastern and Southern Africa (Reiter *et al*., 2010), providing income to millions of resource-poor smallholder farmers in these two regions (Tefera, 2012). It is by far the dominant staple crop and accounts for 50% and 30% of the total calories

consumed in Eastern and Southern Africa respectively (Langyintuo *et al*., 2010; Reiter et al., 2010). North, Central and West Africa have very low per capita consumption of maize of less than 30 kg.year^{-1}, compared to 68 kg.year^{-1} in Eastern Africa and 82 kg.year^{-1} in Southern Africa (Figure 2.2).

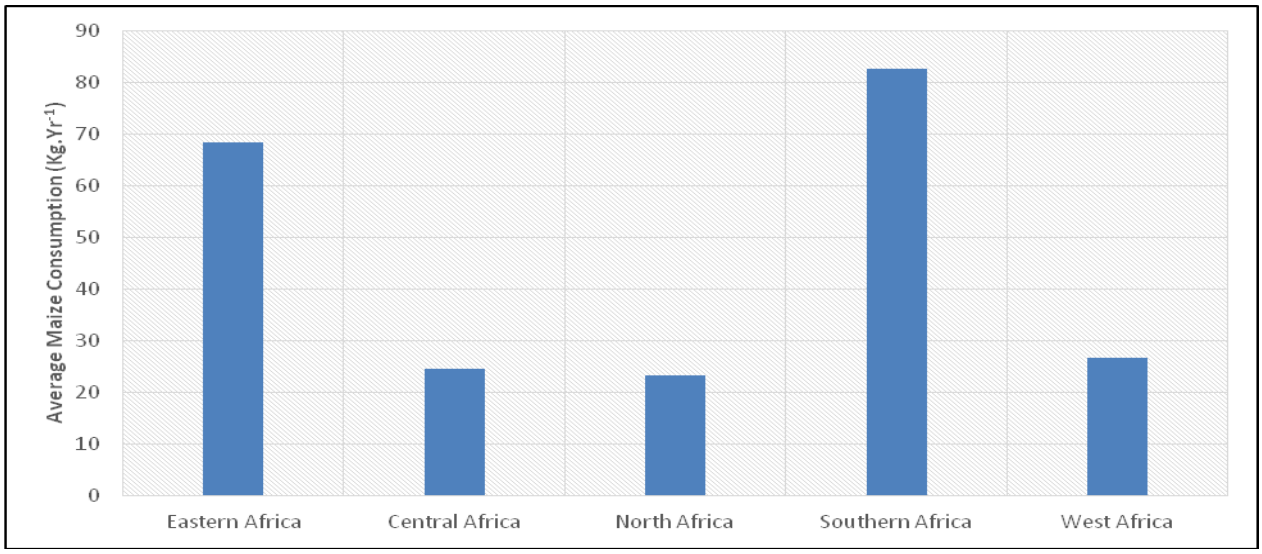

Figure 2.2: Average per capita consumption of maize by region (FAOSTAT, 2015b).

About 80% of maize produced by smallholder farmers is for own consumption with less than 20% marketed (Jayne et al., 2010). Maize accounts for 63% and 55% of the total cereal consumption in Eastern and Southern Africa respectively compared to 26%, 18% and 12% in Central, West and North Africa respectively (FAOSTAT, 2015b).

2.3 Maize Storage

Maize farming in Sub-Saharan Africa is predominantly done by smallholder farmers under rainfed conditions with limited inputs (Cairns *et al*., 2013). These farmers have limited access to efficient storage technology (Kadjo *et al*., 2013). They make use of traditional storage technologies which expose the maize to open air conditions allowing rewetting, molds, rodents and insect attack that lead to quality and quantity deterioration during storage (Golob, 2002; Oyekale *et al*., 2012; Yakubu, 2012).

Ineffective storage remains one of the most critical problems throughout the maize postharvest chain (Kadjo *et al*., 2013). Studies by Demissie et al. (2008) and Weinberg et al. (2008) have shown that maize weevil and molds alone can cause a total loss in stored maize. The molds promote mycotoxin contamination which endangers human health. These losses also threaten food security as well as dent the economic potential of the local communities leading to hunger and poverty (Thamaga-Chitja *et al*., 2004).

2.3.1 Factors affecting maize storage

Temperature and moisture content are the principal factors that influence the quality of stored grain (Gonzales *et al*., 2009). Relative humidity within the storage environment also plays a significant role in preserving the quality of stored grain.

Moisture content is the most important physiological factor in grain storage (Volenik *et al*., 2007). High grain moisture content promotes grain respiration, insect and fungal problems. Heat produced during respiration enhances water vapour presence in the stored grain promoting further grain deterioration (Freer *et al*., 1990). Grain moisture content of 14% or higher support the proliferation of insect and fungal attack (Govender *et al*., 2008). The moisture content of maize grains must, therefore, be reduced to ensure its safe storage (Jayas and White, 2003). Grain moisture content can be expressed in wet basis or dry basis as shown in Equation 2.1 and Equation 2.2, respectively.

$$M.C_{wb} = \frac{weight\ of\ water\ in\ sample}{weight\ of\ wet\ sample} \times 100\% \quad (2.1)$$

$$M.C_{db} = \frac{weight\ of\ water\ in\ sample}{weight\ of\ dry\ sample} \times 100\% \quad (2.2)$$

Another major factor that influences grain spoilage during storage is temperature. The increase in temperature increases the biological and chemical reactions that promote grain deterioration. It is important to lower temperatures in storage structures to reduce the metabolic rates of insects and

fungi as well as grain respiration thus extending the safe storage period of maize (Suleiman and Rosentrater, 2016). Respiration from grains, insects and fungi produce heat and moisture creating damp hot spots that accelerate the degradation of maize in storage.

In as much as grain moisture content is the most important physiological factor in grain storage, storage unit temperature and relative humidity are critical in maintaining grain quality (Manickavasagan *et al*., 2006). It is important to understand the interplay between these three factors to safely store maize. Temperature significantly affects the relative humidity which in turn affects the grain moisture content. Maize is a hygroscopic material, and it undergoes sorption and desorption processes that can increase its moisture content (Devereau *et al*., 2002). The interaction between the moisture content of the grain and the relative humidity of the storage unit results in the stored grains reaching the equilibrium moisture content (Volenik *et al*., 2007; Samuel *et al*., 2011).

2.3.2 Causes of storage losses in maize

Insects and fungi are the predominant cause of maize losses during storage (Tefera, 2012). The proliferation of insects and fungi during storage is influenced by the environmental conditions of the storage (Nukenine, 2010). Fungi, in particular, produce mycotoxins that are harmful to human and animal health. Respiration within the grain storage environment also leads to dry matter loss in stored grains.

Viable maize kernels, molds, insects and mites consume oxygen and nutrients during respiration producing carbon dioxide, water and heat. The water increases the moisture content of grains, and the heat leads to caking of grains (Ngamo *et al*., 2007). Higher moisture content increases the respiration rates compromising the safe storage of maize grain (Hayma, 2003).

Insect pests cause the greatest loss in maize during storage. Insects consume grain nutrients leading to dry matter losses and also contaminate the grains with filth (Paliwal *et al*., 2000). Between 20-50% of stored grain is lost to insect pests in developing countries (Ileleji *et al*.,

2007; Nukenine, 2010). About half of the 500 insect species associated with grains are linked to both field and storage attack on maize grain (Jian and Jayas, 2012).

Fungal contamination of maize grain is a serious food safety concern in tropical countries and the world over (Kaaya and Kyamuhangire, 2006). Maize is attacked by both field and storage fungi. Field fungi attack and produce toxins before maize is harvested. They thrive under high relative humidity (R.H > 80%) and high grain moisture content (22% - 33%) over a wide temperature range (10 ± 35 ºC) (Williams and McDonald, 1983). Some field fungi can survive under storage conditions (Sanchis *et al*., 1982) causing yield reduction and quality loss especially in hot and humid environments (Moturi, 2008). Storage fungi invade stored grains and require a relative humidity between 70% to 90% and corresponding equilibrium moisture content (Suleiman *et al*., 2013).

Fungal infestation during storage severely reduces maize grain quality through dry matter losses, grain discoloration as well as chemical and nutritional changes (Chuck-Hernández *et al*, 2012). Approximately 50% of maize grain lost in tropical countries is attributed to in-storage fungal attack (Fandohan *et al*., 2004). Storage fungi rank second after insects as the leading cause of deterioration and loss in maize (Suleiman *et al*., 2013). Grain damage due to insects, predisposes maize kernels to fungal infection (Sone, 2001; Fandohan *et al*., 2006).

Mold and fungal growth leads to mycotoxins contamination of maize both in the field and during storage (Ngamo *et al*., 2007). Mycotoxins are secondary metabolites produced by fungi on food and feedstuff (Kilonzo *et al*., 2014). They are toxic in very small concentrations hence pose significant food safety risks and health hazards and ultimately limit the marketability of grain supply worldwide (Woloshuk and Shim, 2013). Approximately 25% to 50% of crops worldwide are contaminated with mycotoxins (Lewis *et al*., 2005; Wagacha and Muthoni 2008). Maize grown in warm and humid environments is highly prone to mycotoxin contamination (Kaaya and Kyamuhangire, 2006).

Aflatoxins, fumonisins, ochratoxins, zearalenone and trichothecenes are the most important mycotoxins that occur in cereal grains (Pittet, 1998). Aflatoxins and fumonisins are the most

common and toxic mycotoxins found in maize in tropical and sub-tropical regions (Krska *et al*., 2008; Tefera, 2012). Some common mycotoxins in agriculture and the fungi that produce them are listed in Table 2.1.

Table 2.1: Common mycotoxins and the fungi that produce them

Fungus	Mycotoxin	Reference
Aspergillus flavus, aspergillus parasiticus	Aflatoxin	Campbell and White, 1995
Furasium moniliforme, F. proliferatum	Fumonisins	Marin *et al*., 2004
Furasium graminearum	Deoxynivalenol	Krska *et al*., 2003
Furasium graminearum, F. culmorum, F. poae	Trichothecenes	Adejumo *et al*., 2007
Penicillium verrucosum, aspergillus ochraceus	Ochratoxins	Lattanzio *et al*., 2007
Penicillium sp. Aspergillus sp.	Citrinin	Prasad, 1997

2.4 Aflatoxin

Aflatoxin is a toxic metabolite produced by some molds of the genus *Aspergillus* (Fountain *et al*., 2015). It is one of the major mycotoxins in agriculture that contaminates a large number of world foods (Masoero *et al*., 2007). Aflatoxin is primarily produced by *Aspergillus flavus* and partly by *Aspergillus parasiticus* (Pittet, 1998). However, it can also be produced by other strains of *Aspergillus* such as *A. fumigatus, A. bombycis, A. nomius, A. pseudotamari*, and *A. parvisclerotigenus* (Frisvad *et al*., 2005).

A. flavus is an opportunistic pathogen of plants, animals, and insects (Fountain *et al*., 2015). It causes storage rots in numerous crops and produces aflatoxin as a secondary metabolite (Klich, 2007). *A. flavus* has a broad economic impact among the aspergilli causing mycoses in humans (Stevens *et al*., 2000). The losses due to the infection of maize by *A. flavus* are primarily due to the subsequent contamination of the grain with aflatoxin.

There are several types of aflatoxin. The naturally occurring types are aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2) (Strosnider *et al*., 2006; Lizarraga-Paulin *et al*., 2011). The International Agency for Research on Cancer (IARC)

categorizes AFB_1 as the most potent naturally occurring carcinogen (Klich, 2007). AFB_1 is the most prevalent in food resulting in most cases of aflatoxicosis (Lizarraga-Paulin *et al*., 2011). Aflatoxins M_1 and M_2 are metabolic derivatives of AFB_1 and AFB_2 respectively and are found in dairy products, meat and urine (Strosnider *et al*., 2006; Wild and Gong, 2010; Lizarraga-Paulin *et al*., 2011).

2.4.1 Aflatoxin contamination in maize grain

Maize kernels are vulnerable to infection by toxigenic fungi (Abbas *et al*., 2006) and are significantly colonized by aflatoxin-producing *Aspergillus* species (Bandyopadhyay *et al*., 2007). High levels of aflatoxin contamination in maize are common in Sub-Saharan Africa (Wagacha and Muthoni, 2008; Mutiga *et al*., 2015). Eastern Africa, where maize is a staple food, has experienced severe aflatoxin contamination leading to fatal aflatoxicosis cases (Manjula *et al*., 2009; Kang'ethe, 2011). Aflatoxin contamination is also a problem in other developing regions of the world (Kensler *et al*., 2011). Aflatoxin contamination of maize across various African countries is way above the maximum tolerable limits set by the Codex Commission (Table 2.2).

Table 2.2: Aflatoxin contamination of maize in some African countries

Country	Commodity	% of positive samples	Contamination ($\mu g.kg^{-1}$)	Reference
Kenya	Maize	55	20	Lewis et al., 2005 Wagacha and Muthomi 2008
		35	100	
		7	1000	
Benin	Maize (before storage)	9-32	5	Hell et al., 2000
	Maize (6-month storage)	15-32	5	
	Maize	38	105	Shephard, 2003
Ghana	Stored maize	100	20-355	Kpodo et al., 1996
	Fermented maize	95	6-196	Shephard 2003
South Africa	Commercial maize	80	0-762	Adaku et al., 2012
Nigeria	Maize	45	200	Shephard 2003
Tanzania	Maize	_	1-158	Kimanya et al., 2008
Mozambique	Maize	46	16-363	Warth et al., 2012
Burkina Faso	Maize	50	3-636	Warth et al., 2012

2.4.2 Factors affecting growth of *A. flavus* and aflatoxin contamination in stored maize

Contamination of stored maize is affected by the storage environment and grain moisture content (Alborch *et al*., 2011). Temperature and relative humidity are the primary environmental conditions that influence the growth of *A. flavus* and aflatoxin production in stored maize (Giorni *et al*., 2012). Grain damage also has a profound impact on the contamination of maize.

Aspergillus flavus is a mesophilic fungus that grows well at temperatures above 30 ºC (Das *et al*., 2012). It grows within a temperature range of between 10 ºC to 43 ºC. The optimal growth of *A. flavus* and aflatoxin production is observed between 30 ºC to 35 ºC (Atanda *et al*., 2011). A study by Gbodi *et al*. (1986) analyzed maize samples from farmers stores at three different periods of the year in Langtang, Nigeria and reported the highest aflatoxin contamination in samples collected during the hot, humid and wet period between June and September.

Relative humidity significantly affects the growth of *A. flavus* in maize (Pratiwi *et al*., 2015). Although minimal growth of *A. flavus* and production of aflatoxins has been observed at a relative humidity of 78%, 82% and 83%, relative humidity above 85% support optimal growth of *A. Flavus* and toxin production (Al-Shikli *et al*., 2010).

Pratiwi *et al*. (2015) reported maximum *A. flavus* growth and toxin production at 90% relative humidity and 30 ºC. High temperatures with low relative humidity limit the growth *A. flavus* and consequently the toxin production. The growth of *A. flavus* is inhibited by 70% relative humidity and 40 ºC (Atanda *et al*.2011).

Apart from temperature and relative humidity, grain moisture content is critical in controlling *A. flavus* and aflatoxin contamination in maize (Giorni *et al*., 2012). *Aspergillus* species can grow well when the moisture content of maize is above 15% producing a significant increase in temperature and spontaneous heating (Giorni *et al*., 2007). The available moisture for microbial growth can be measured using the water activity (a_w) (Abdel-Hadi *et al*., 2011). Water activity is the ratio of vapor pressure over the substrate (P) to the vapor pressure over pure water at similar temperature and pressure (P_0) as shown in Equation 2.3.

$$a_w = \frac{P}{P_0} \quad (2.3)$$

Fungal growth requires a water activity above 0.65 which is equivalent to an equilibrium relative humidity of 65% (Giorni *et al*., 2012). Lacey and Magan (1991) reported that 0.78 and 0.95 are the minimum and maximum water activity required for the growth of *A. flavus*. Trucksess *et al*. (1983) observed the growth of *A. flavus* in maize at a water activity of 0.80 at 16 ºC. Ferna and Vaamonde (1991) reported minimal aflatoxin production at a water activity of 0.895 at 20 ºC and a maximum toxin production at a water activity of 0.95 at a temperature of 37 ºC. Cuero *et al*. (1987) observed maximum aflatoxin production at a water activity of 0.98 and 0.95 at a temperature of 25 ºC. Faraj *et al*. (1991) confirmed 0.95 and 0.98 as the optimum water activity at a temperature of 30 ºC.

Fungal growth is more rapid in damaged kernels than in intact kernels (Tuite *et al*., 1985). Insects damage grains providing entry points for fungal spores and they also act as vectors, transmitting fungal spores within the stored grains (Giorni *et al*., 2012). Respiration by insects generates moisture and heat (Magan *et al*., 2003) which raises the moisture content and temperature of grains to levels conducive to fungal growth and toxin production. Sinha and Sinha (1991) found strong correlations between stored maize infested with the maize weevil, *Sitophilus Zea mays*, and *A. flavus* contamination. Sinha and Sinha (1992) also reported a high incidence of *A. flavus* fungi and aflatoxin in insect-damaged maize samples from different localities in India than in insect free samples.

2.4.3 Health effects of aflatoxins

Aflatoxin B_1 is the most toxic and prevalent aflatoxin in maize resulting in several cases of aflatoxicosis (Lizarraga-Paulin *et al*., 2011). AFB_1 causes acute and chronic toxicity, teratogenicity, carcinogenicity, genotoxicity and immunotoxicity (Klich, 2007). Kenya has experienced several cases of fatal human aflatoxicosis with the worst case reported in 2004 (Ngindu *et al*., 1982; Lewis *et al*., 2005; Wagacha and Muthoni 2008). Other cases of fatal

aflatoxicosis have been reported in India (Krishnamachari *et al*., 1975), Brazil and Netherlands (Dvorackova, 1989).

The chronic exposure to aflatoxin in diets is evident from the presence of AFM_1 in the milk and urine samples of lactating mothers (Wagacha and Muthoni, 2008). AFB_1 has long been linked to hepatocellular carcinoma (Strosnider *et al*., 2006). A study by Khlangwiset et al. (2011) reported stunted growth and immune suppression in children exposed to aflatoxin. Aflatoxin exposure in pregnant women results in neonatal jaundice and reduced birth weight (Hendrickse, 1999).

The serious health effects of human exposure to aflatoxin have prompted various national and international bodies to regulate the amount of aflatoxin allowed in food to limit exposure to this category of mycotoxins (Van-Egmond *et al*., 2007). According to Henry *et al*., (1999), the permissible limit of aflatoxin in human food ranges between 4 $\mu g.kg^{-1}$ and 30 $\mu g.kg^{-1}$ across various countries. The Codex Alimentarius Commission proposed 15 $\mu g.kg^{-1}$ as the maximum tolerable level for total aflatoxin in food (Van-Egmond *et al*., 2007). A total of seventy-seven countries all over the world have set the maximum tolerable limits for aflatoxin in food (Makun *et al*., 2011). South Africa, Kenya, Malawi, Zimbabwe, and Tanzania are among the few countries in Africa that regulate aflatoxin in food. They have all set their maximum tolerable limits at 5 $\mu g.kg^{-1}$ and 10 $\mu g.kg^{-1}$ for aflatoxin B_1 and total aflatoxin respectively (Kimanya *et al*., 2008; Rheeder *et al*., 2009; Kilonzo *et al*., 2014).

2.4.3 Aflatoxin analysis

Accurate and sensitive determination of aflatoxins is essential to meet food safety requirements (Shephard, 2009). Aflatoxin analyzes are laboratory based physicochemical methods (Krska *et al*., 2005). These methods range from analytical chromatographic techniques to rapid immunological methods (Wacoo *et al*., 2014). The majority of aflatoxin determination methods consist of three steps namely extraction, separation, and detection (Bakırdere *et al*., 2012). However, before any analysis can be done, proper sampling is necessary because aflatoxins are heterogeneously distributed in grains (Koppen *et al*., 2010). Sample plans have therefore been designed for aflatoxins determination in grain (Krska *et al*., 2005).

An efficient extraction step is critical in the detection and quantification of aflatoxin (Wacoo *et al*., 2014). Organic solvents such as acetone, methanol, chloroform, and acetonitrile can dissolve aflatoxins hence are usually mixed in different proportions with water and used as extraction solvents (Bertuzzi *et al*., 2012). Liquid-liquid extraction and solid phase extraction (SPE) have been used extensively to extract aflatoxins from different food matrix (Alcaide-Molina *et al*., 2009; Bertuzzi *et al*., 2012). Other aflatoxins extraction methods include accelerated solvent extraction, ultrasound assisted extraction, immunoaffinity column, pressurized fluid extraction, solid phase micro-extraction and QuEChERs (Bacaloni *et al*., 2008; Desmarchelier *et al*., 2010; Nonaka *et al*., 2009).

The extraction solvent must ensure that aflatoxin is abstracted from the matrix without alteration (Bakırdere *et al*., 2012). However, most sample extracts contain several co-extracts that make them unsuitable for direct analysis (Shephard, 2009). Co-extracts such as fats, proteins, and pigments affect the sensitivity of aflatoxin analyses (Krska *et al*., 2005). Sample clean-up is, therefore, necessary to remove these co-eluting impurities that interfere with spectrophotometric detection (Spanjer *et al*., 2008). Cleanup techniques employed include liquid–liquid partitioning, Solid Phase Extraction (SPE), Ion-exchange columns, immunoaffinity columns (IAC) and multifunctional cleanup columns (Bacaloni *et al*., 2008; Huang *et al*., 2009; Piermarini *et al*., 2009).

Analytical Methods

The cleaned sample extracts are usually analyzed using chromatographic techniques coupled with an appropriate detector (Shephard, 2009). Thin-layer chromatography (TLC), gas chromatography (GC), and high-performance liquid chromatography (HPLC) are the frequently used chromatography techniques.

Several types of mycotoxins can be detected using TLC in a single test sample (Trucksess *et al*., 1983). Although TLC has excellent sensitivities, accumulated errors during sample application, plate development, and plate interpretation compromise its precision. It is therefore commonly used as a screening method (Shephard, 2009). The advent of high-performance thin-layer

chromatography (HPTLC) has overcome some of these challenges and is currently one of the most efficient and precise methods for detecting aflatoxins (Ramesh *et al*., 2013).

Gas chromatography (GC) uses a flame ionization detector (FID) or an electron capture detector (ECD) and mass spectrometer (MS) to identify volatile products (Pascale, 2009). It requires a preliminary cleanup step before analysis to eliminate matrix effects (Krska *et al*., 2005). Matrix effect refers to the change in ionization efficiency of the analyte of interest due to contaminating compounds (Kruve *et al*., 2008). Other challenges associated with GC include; nonlinearity of calibration curves, memory effects from previous samples, drifting responses and high variation in reproducibility and repeatability (Liang *et al*., 2005). The existence of other cheaper chromatographic methods limits the use gas chromatography for the analysis of aflatoxins.

HPLC is the preferred chromatographic method for aflatoxin separation and detection (Wacoo *et al*., 2014). Reversed phase high-pressure liquid chromatography with C_{18} columns is popular in aflatoxin analysis (Rahmani *et al*., 2009). The detection of aflatoxins is done using a fluorescent detector (FLD), ultraviolet (UV) detector or diode array detector (DAD). AFB_1 and AFG_1 may at times need chemical derivatization to enhance their fluorescence and hence improve their detection (Papadopoulou-Bouraoui et al., 2002). Using a mass spectroscopy together with HPLC eliminates the need for sample clean-up and derivatization processes (Krska *et al*., 2005). Liquid chromatography-tandem mass spectroscopy (LC-MS/MS) uses small amounts of sample to provide multiple toxin analysis at very low detection limits (Wacoo *et al*., 2014).

Although chromatographic techniques are very sensitive and reliable, they require a skilled technician, cumbersome pretreatment of the sample, and expensive equipment (Sapsford *et al.*, 2006). This has led to the development of screening methods which provide quick analysis, are cost-effective, easy to use, and some can be used in the field (Cigik and Prosen., 2009).

Screening Methods

Several rapid screening methods have been developed based on immunoassay techniques (Shephard, 2009). These immunological methods make use of the high affinity and specificity of antibodies and receptors to antigens and ligands respectively (Sargent and Sadik, 1999).

Radioimmunoassay (RIA), immunoaffinity column assay (ICA), enzyme-linked immunosorbent assay (ELISA), and fluorescence immunoassay (FIA) are some of the immunoassay based methods (Wacoo *et al*., 2014).

ELISA is the most established and commercially available screening method (Shephard, 2009). It provides a fast and efficient method for routine aflatoxin analysis (Lequin, 2005). It has low detection limits comparable to chromatographic methods; however, it is significantly affected by impurities in the sample extracts hence, its results must always be confirmed with a more selective chromatographic method (Bakırdere *et al*., 2012). ELISA kits based on a competitive immunoassay format have been developed and are widely used for the detection of aflatoxins in foods (Ostadrahimi *et al*., 2014).

Biosensors are another form of immunological methods that use an antigen or antibody species as a biological recognition element (Wacoo *et al*., 2014). The antigen or antibody is coupled to a signal transducer that helps to detect the binding of the complementary species (Ricci *et al*., 2007). Spinella *et al*. (2014) developed a piezoelectric quartz crystal microbalance sensor (QCMs) capable of detecting AFB_1 concentration in the range of 0.5–10 $\mu g.kg^{-1}$. Optical immunosensor such as surface plasmon resonance (SPR) and optical waveguide platform have been developed for aflatoxin detection. Daly et al. (2000) used SPR immunosensor with polyclonal antibodies to detect AFB_1. Adanyi *et al*. (2007) detected aflatoxin and ochratoxin in the range of 0.5 and 10 $\mu g.kg^{-1}$ in barley and wheat flour samples using optical waveguide light spectroscopy (OWLS).

Electrochemical immunosensor is another form of biosensor whose biorecognition element produces electroactive signals (Wacoo *et al*., 2014). Most of the electrochemical methods developed for aflatoxin detection involve the use of antibodies immobilized on the surface of an electrode (Liu *et al*., 2006; Owino *et al*., 2007; Linting *et al*., 2012). The signals are measured in the form of electrochemical impedance spectroscopy, differential pulse voltammetry, linear sweep voltammetry or cyclic voltammetry (Valimaa *et al*., 2010).

The simplest and fastest immunological methods are the lateral flow devices (Shim *et al*., 2007). They use labels coated with antibodies to provide colored binding zones onto which aflatoxins bind causing a color change (Ostadrahimi *et al*., 2014). They are simple, portable devices in the form of a strip or dip stick hence can be used in the field (Shim *et al*., 2007).

The chromatographic and rapid screening methods are destructive laboratory-based chemical analyzes except lateral flow devices which can be used out in the field (Shephard, 2009). These methods require a huge sample size, are time-consuming and are not suitable for online detection of aflatoxin in whole grain sample during processing operations (Fernandez-Ibanez *et al*., 2009). The use of spectroscopic methods such as fluorometry, infrared spectroscopy, and hyperspectral imaging, have provided qualitative aflatoxin analysis with limited sample manipulation (Del Fiore *et al*., 2010).

The natural or induced fluorescence of aflatoxins makes them detectable by spectroscopic methods (Shephard, 2009). Babu (2010) used fluorescence to analyze aflatoxins in grains and raw peanuts and reported detection limits between 5 to 5000 ppb which is a very wide range and higher than 4 $\mu g.Kg^{-1}$ set by the European Union. Pearson *et al*. (2001) used transmittance and reflectance spectroscopy to detect aflatoxin in single maize kernels with more than 95% of the kernels analyzed being correctly categorized as having either high (>100 ppb) or low (<10 ppb) concentrations of aflatoxins.

All the aflatoxin analyses discussed require skilled personnel. These analyses are costly laboratory-based methods with cumbersome sample preparation techniques (Sapsford *et al*., 2006). A summary of these methods showing the sample preparation methods required in each case, the limit of detection and the need for a skilled operator is outlined in Table 2.3.

Table 2.3: Summary of aflatoxin detection methods (after Wacoo *et al*. 2014)

Method	Sample preparation	Limit of Detection ($\mu g.Kg^{-1}$)	Multiple analyses	Skilled operator	Field use
TLC	SPE	1-20	Yes	Yes	No
HPTLC	Extraction only		Yes	Yes	No
HPLC	IAC/SPE	2	Yes	Yes	No
LC-MS/MS	Extraction only	0.8	Yes	Yes	No
Fluorometer	IAC	5-500	Yes	Yes	No
FTIR		< 10	Yes	Yes	No
RIA	Extraction only	1	Yes	Yes	No
ELISA	Extraction only		Yes	Yes	No
Immunodipstick	Extraction only	5	Yes	Yes	Yes
QCMs	Extraction only	0.01-10	Yes	Yes	No
SPR	Extraction only	3-98	Yes	Yes	No
OLWS	Extraction only	0.5-10	Yes	Yes	No
Electrochemical	Extraction only	2	Yes	Yes	No

These methods are therefore not accessible to resource-poor smallholder farmers who are responsible for more than two-thirds of the total maize produced in Sub-Saharan Africa (Strosnider *et al*., 2006; Wacoo *et al*., 2013). It is necessary to develop cheap and easy to use techniques that can be applicable in the field for aflatoxin surveillance in maize produced by smallholder farmers (Del Fiore *et al*., 2010; Wu *et al*., 2013).

Simple devices for quality evaluation of agricultural materials have been developed by exploiting the electrical properties of these materials (Skierucha *et al*., 2012). Electrical properties can detect the changes in the moisture content as well as changes in the chemical composition of foods and agricultural products (Zhang *et al*., 2007).

2.5 Electrical Properties of Maize

The electrical properties of cereal grains have been of interest for many years because of their usefulness in providing quick estimates for grain moisture content (Nelson, 2006). Electric conductivity, resistance, capacitance and dielectric properties have been used extensively in rapid grain moisture content determination (Nelson, 2010). Recent studies have focussed on the radio and microwave frequency dielectric properties of cereal grains (Nelson, 2010; Sacilic and Colak, 2010; Nelson and Trabelsi, 2012).

2.5.1 Dielectric properties of maize

Maize and all other cereals are lossy insulators hence considered as dielectric materials (Meszaros, 2007). Dielectric properties are the electrical characteristics of poorly conducting materials that determine their interaction with electric fields (Nelson and Trabelsi, 2012), and influence the distribution of electromagnetic field and current in the region occupied by the material (Nelson, 2010). The dielectric properties are derived from the relative complex electrical permittivity as shown in Equation 2.4.

$$\varepsilon_r = \varepsilon_r' - j\varepsilon_r'' \quad (2.4)$$

The real part, ε_r', is the dielectric constant and relates to the capacitance of the material when exposed to an electric field. The imaginary part ε_r'', is the dielectric loss factor, and it influences energy absorption and attenuation from an electric field (Sacilik and Colak, 2010).

The relationship between grain moisture content and both dielectric constant and dielectric loss factor has been exploited extensively in the development of portable moisture meters for rapid grain moisture measurements (Nelson, 2008). Trabelsi *et al*., (1998) developed a density independent method for online monitoring of grain moisture content and bulk density in moving grain using the microwave dielectric properties of the grains. A study by Al-Mahasneh *et al*. (1998) reported the use of dielectric properties of artificially damaged maize to develop a damage level prediction sensor. The dielectric variables used also provided a reliable prediction of the moisture content and bulk density. Knowledge of dielectric properties is critical in the design of equipment and processes for dielectric heating applications and potential agricultural applications such as grain drying, seed treatment to improve germination and insect control in stored grain using radio-frequency and microwave electric fields (Nelson, 2010).

The principal factors that influence the dielectric properties of grains at a given frequency are temperature, moisture content and bulk density (Jha *et al*., 2011; Nelson and Trabelsi, 2012)

However, Zhang *et al*., (2007) reported that carbohydrates, ash and proteins can affect the dielectric properties. Several attempts have been made to relate the dielectric properties of food to the weighted averages of the dielectric properties of the individual chemical components (Bhargava *et al*., 2010).

2.5.1 Effect of proximate composition on dielectric properties

Physical changes that affect the proximate composition such as moisture loss and protein denaturation also have an impact on the dielectric properties (Sahin and Sumnu, 2006). Carbohydrates, fats, proteins, fibre and moisture content are the major components of maize grain (Iqbal *et al*., 2006). Proteins and starches have low dielectric activities while free water and monosaccharides have higher dielectric properties (Shukla and Anantheswaran, 2001).

Moisture content is the most dominant factor affecting dielectric properties of cereal grains. Dielectric constant has been shown to increase with moisture content at any given frequency (Nelson and Trabelsi, 2012). Nelson and Trabelsi (2012) reported that both dielectric constant and loss factor for shelled yellow-dent field maize increased with increase in moisture content at frequencies of 5GHz and 15GHz.

Apart from moisture content, carbohydrates, proteins, fats and fibre have low dielectric properties (Barghava *et al*., 2013). Starch is the major carbohydrate found in maize, and it makes up approximately 70% of the grain (Nuss and Tanumihardjo, 2010). The dielectric properties of starch have been studied by several researchers (Moteleb, 1994; Ndife *et al*., 1998). A study by Ndife *et al*. (1998) reported that both dielectric constant and loss factor of different starches increased with temperature. The variation in the values of dielectric properties for the various starches was attributed to the difference in bulk density as is typical for most granular materials (Ndife *et al*., 1998).

Free amino acids are dielectrically reactive. Amino acids influence the protein dipole and hence their dielectric properties (Sahin and Sumnu, 2006). According to Shukla and Anantheswaran (2001), proteins adsorb and retain water which significantly affects their dielectric properties.

Increase in temperature also has an influence on the dielectric properties of proteins since heat causes denaturation of proteins (McWilliams, 1989).

Lipids are hydrophobic hence dielectrically inactive (Mudgett and Westphal, 1989). Fats and oils have very low dielectric properties. Fat content reduces the free water in food and consequently its dielectric properties (Ryynanen, 1995).

Bhargava *et al*. (2013) conducted a study to establish a link between the dielectric properties and the proximate composition of sorghum, pearl millet and barley. The cereals were ground to ensure constant bulk density and particle size distribution. The proximate composition and the dielectric properties of the three different kinds of cereal are indicated in Table 2.4. Although dielectric constant and loss factor increase with an increase in moisture content (Nelson, 2010), Bhargava *et al*. (2013) reported that barley had the lowest dielectric constant despite having the highest moisture content. They attributed this to the high fibre content of barley. Pearl millet had the highest value of dielectric loss despite having the lowest moisture content, and this was attributed to its high fat content (Bhargava *et al*., 2013).

Several studies have reported that dielectric properties increase with moisture content (Narayan *et al*., 2011; Nelson and Trabelsi, 2012). However, according to the study by Bhargava *et al*. (2013), both dielectric constant and loss factor do not follow the same trend as the moisture content when there is variation in the proximate composition of the sample being analyzed (Table 2.4). Iqbal *et al*. (2006) reported that the growth of *A. flavus* on maize affects its proximate composition and this could potentially influence its dielectric properties. *A. flavus* significantly decreases the fat content. The ash content, starch and protein were reduced as well (Table 2.5).

Table 2.4: Proximate composition of sorghum, pearl millet and barley and their dielectric properties (after Bhargava *et al*., 2013)

Sample	Moisture (%)	Protein (%)	Fat (%)	Crude fibre (%)	Carbohydrate (%)	ε_r'	ε_r''
Sorghum	11.46	11.20	2.34	2.0	70.34	3.16	0.24
Pearl millet	10.66	11.64	5.23	2.54	65.50	3.03	0.41
Barley	11.69	9.32	2.04	6.04	66.99	2.28	0.28

Table 2.5: Percentage change in the proximate composition of *A. flavus* inoculated samples (after Iqbal *et al*., 2006)

Proximate composition	% change		
	Popcorn	Fresh maize	Kashmir maize
Moisture content (increase)	39.9	10.8	6.5
Crude protein (decrease)	5.1	8.2	15.3
Crude fat (decrease)	170	70.5	66.6
Ash (decrease)	11.1	15.4	22
Starch (decrease)	16.9	8.3	3.1

2.6 Summary and Conclusion

Maize is consumed in large quantities in Sub-Saharan Africa and especially in Eastern and Southern Africa. It is very susceptible to infection by aflatoxin producing *A. flavus*. This makes maize a significant source of aflatoxin exposure in human beings through dietary consumption (Kilonzo *et al*., 2014).

Several factors contribute to *A. flavus* growth and aflatoxin contamination in maize, key among them being grain moisture content, temperature and relative humidity (Klich, 2007). These factors promote the proliferation of *A. flavus* and aflatoxin production if not controlled. The traditional storage methods used by maize farmers in Sub-Saharan Africa do not offer control of the storage environment hence exposes the maize to insects, high temperature and high relative humidity which promote the growth of fungi and mycotoxin production in maize during storage (Ngamo *et al*., 2007; Giorni *et al*., 2012).

Most of the smallholder farmers do not have access to grain drying facilities. They make use of sun drying to reduce the moisture content of their maize produce. Sun drying relies on the prevailing atmospheric conditions which in most cases achieve a grain moisture content of about 14% (Folaranmi, 2008). Grain moisture content above 13% makes maize vulnerable to fungal infestation and consequently mycotoxin contamination (Giorni *et al*., 2014).

Several instances of aflatoxin contamination of maize have been reported across Sub-Saharan Africa leading to adverse health effects. Some cases of fatal aflatoxicosis have been directly linked to excessive consumption of aflatoxin contaminated maize (Wagacha and Muthoni, 2008).

The situation is made worse by the fact that smallholder farmers have no access to aflatoxin screening to help determine the suitability of their produce for human consumption (Wu *et al*., 2013).

Aflatoxin analysis methods are lab based chemical analyses that require skilled personnel and expensive equipment. Although these methods are well established in formal grain market systems, maize produced by smallholder farmers never enters the formal market since the produce is predominantly for own consumption hence such regulatory systems have no effect on the level of aflatoxin exposure in the population (Wu *et al*., 2013).

It is critical to develop cheap and simple instruments that can be used to detect the presence of aflatoxin in maize in far-flung rural households where the majority of maize farmers are based (Jayne *et al*., 2010). Such instruments have previously been made through the correlation of the quality attributes of interest to the electrical properties of agricultural products, for instance, the electrical moisture meters and P.H meters (Skierucha *et al*., 2012).

The dielectric properties represent the electrical properties of cereal grains. The dielectric properties of maize are influenced by several factors, among them being the chemical properties (Zhang *et al*., 2007; Bhargava *et al*., 2013). *A. flavus* causes a change in the proximate composition of the grain besides producing aflatoxin (Iqbal *et al*., 2006). These variations in the chemical composition of maize could influence the dielectric properties of grain and possibly have a correlation with the aflatoxin contamination in maize.

3. PROJECT PROPOSAL

The project proposal focuses on the characterization of the electrochemical properties of maize grain contaminated with aflatoxin but will also establish the effect of temperature and relative humidity on aflatoxin contamination of maize.

3.1 Rationale

The main causes of post-harvest aflatoxin contamination are high grain moisture content, high temperature and high relative humidity of the surrounding air (Giorni *et al*., 2012). Poor grain storage facilities with no control of the storage environment encourage the proliferation of *A. flavus* and aflatoxin production in stored maize.

The growth of *A. flavus* and aflatoxin production affects the chemical composition of maize and consequently its electrical properties (Iqbal *et al*., 2006). According to Zhang *et al*. (2007), dielectric properties can be related to the changes in chemical properties of agricultural products.

3.2 Research Questions

The main issues to be explored in this study are:

i. How do grain moisture content, temperature and relative humidity influence aflatoxin contamination of maize?
ii. What is the effect of aflatoxin contamination on the chemical composition of maize grain?
iii. How does the change in chemical composition due to aflatoxin contamination influence the electrical properties of maize grain?

3.4 Aims and Objectives

The overall goal of this study is to characterize the electrochemical properties of maize grain contaminated with aflatoxin. The specific objectives are:

i. To investigate the effect of grain moisture content, temperature and relative humidity on aflatoxin contamination of maize.
ii. To determine the effect of aflatoxin contamination on the chemical properties of maize grain.
iii. To establish the electrical resistivity, capacitance and the dielectric properties of maize grain under different levels of aflatoxin contamination.

3.5 Materials and Methods

This study will be conducted in two phases. Experiment I will focus on the influence of grain moisture content, temperature and relative humidity on aflatoxin contamination of maize as well as the effect of aflatoxin contamination on the proximate composition of maize grain. Experiment II will focus on establishing the electrical properties of maize grain samples obtained from the first experiment.

3.5.1 Inoculation and incubation of maize grains

Toxigenic and non-toxigenic *Aspergillus flavus* strains will be obtained from the Department of Plant Pathology, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal (South Africa). The fungi will be plated on potato dextrose agar at 25 °C for five days after which the conidia will be harvested by flooding a single culture with ultrapure water and scraping the surface mycelia with a sterile scraper. Conidial suspensions will then be adjusted to 4×10^6 cells.ml-1 using sterile distilled water to prepare inoculum for toxigenic *A. flavus* and non-toxigenic *A. flavus* separately (Hruska *et al*., 2015).

The moisture content of maize obtained from Link Seed (Pty) Ltd will be adjusted by spraying with a calculated mass of distilled water to obtain three moisture contents of 14%, 17% and 20% (wet basis). The maize kernels will then be surface sterilized by immersing in 5% (v/v) sodium hypochlorite and stirred for one minute. The kernels will be rinsed with distilled water and left to air dry (Reese *et al*., 2011). The kernels will then be subjected to three different treatments by:

1. immersing in inoculum with toxigenic *A. flavus* spores
2. immersing in inoculum with non-toxigenic *A. flavus* spores
3. immersing in distilled water.

The inoculated seeds will then be spread out on paper towels to air dry before being transferred to the climatic test chamber where they will be incubated at two storage temperature (20 °C and 30 °C) and two relative humidity (60% and 90%) with three replicates. All the experimental parameters are summarized in Figure 3.1.

The samples will be incubated for seven days and fungal growth terminated at the end of day seven by transferring samples to a 60 °C forced-air drying oven for 24 hours. The samples will then be cooled to room temperature and maize samples intended for electrical properties determination will be stored at 4°C to ensure no microbial activity (Hruska *et al*., 2015). The remaining samples will be taken for chemical composition analysis.

3.5.2 Determination of the chemical composition of maize grain

Aflatoxin analysis

25 g of ground maize will be mixed with 100 ml (80:20) acetonitrile/water for 2 hours and the extract filtered and diluted four folds with water. 20 µL of the extract will be injected into the LC-MS/MS for analysis. The LC has an acquity UPLC BEH C_{18} 1.7 µm; 2.1×100 mm column. The mobile phase A will be 0.1% formic acid in water, and mobile phase B will be 0.1% formic acid in acetonitrile. The flowrate of the LC will be set at 0.4 $ml.min^{-1}$. The eluent from the column will then be directed to the electrospray source of a Quattro Premier XE tandem quadrupole mass spectrometer operated in a positive ionization multiple reaction monitoring (MRM) mode (de Kok *et al*., 2007). The data will then be acquired and analyzed using Waters Masslynx^{TM} software. The limit of detection for the LC/MS/MS is 0.5 $\mu g.kg^{-1}$, and the limit of quantification is 2 $\mu g.kg^{-1}$ (de Kok *et al*., 2007).

Moisture content (AOAC 925.10)

The moisture content will be estimated by drying triplicate 10g mass of the sample at 105^0C for 24hr in a forced air oven after which the samples will be cooled in a desiccator and reweighed. The moisture content will be calculated as a percentage of the dry weight using Equation 3.1 (AOAC, 1992).

$$\% \text{ Moisture content} = \frac{\text{Weight loss of sample}}{\text{Weight of the original sample}} \text{ x } 100 \quad (3.1)$$

Ash content/mineral content (AOAC 923.03)

Two grams of the dried sample will be weighed into a dry porcelain dish and heated in a muffle furnace at 600^0C for six hours. The sample will then be cooled in a desiccator and reweighed (AOAC, 1992). The percentage ash content will be calculated as in Equation 3.2.

$$\% \text{ ash} = \frac{\text{Weight ash}}{\text{Weight of the original sample}} \text{ x } 100 \quad (3.2)$$

Fat content (AOAC 920.39)

Two grams of the sample will be weighed and placed into the Soxhlet extraction thimble. The extraction thimble will be plugged with cotton wool to avoid loss of sample and then transferred to the Soxhlet extractor, and sufficient petroleum ether added until the latter is siphoned into a weighed receiving flask. More ether will be poured to cover the thimble completely and flask placed with the extractor on the electric heating mantle. The reflux condenser will be heated gently for 3 hours, switched off and allowed to cool for 10 minutes.

Recovered solvent will be transferred into an air oven (100 ^{0}C) for 1 hour and then cooled in a desiccator and weighed (AOAC, 1992). The amount of oil produced will be calculated and expressed as a percentage of the original sample as shown in Equation 3.3.

$$\%\text{Fat} = \frac{\text{Weight loss of sample}}{\text{Weight of the original sample}} \times 100 \quad (3.3)$$

Crude protein

One gram of the sample will be weighed into a digestion flask. Ten grams of potassium sulphate, 0.7 g mercuric oxide and 20 cm^3 concentrated sulphuric acid will then be added to the sample in the digestion flask. The flask will be heated gently at an inclined angle until frothing subsides and boiled until the solution becomes clear for half an hour. A small amount of paraffin wax will be added if the frothing is excessive. On cooling, 90 ml of distilled water will be added and mixed and a little piece of pumice added to prevent bumping. 80 ml of 2 M sodium hydroxide solution will be added while tilting the flask so that two layers are formed. The condenser unit will then be connected, heated and the distilled ammonia collected in 50 ml boric acid/methyl red indicator. Fifty milliliters of the distillate will be collected and titrated against 0.1 M hydrochloric acid solution (Enyisi *et al.*, 2014). The percentage nitrogen content will be calculated as shown in Equation 3.4 and Equation 3.5.

$$\%\ \text{N} = \frac{(\text{Volume of acid x Molarity of standard acid}) \text{x } 0.014}{\text{Weight of the original sample}} \times 100 \quad (3.4)$$

$$\%\ \text{Crude protein content} = \text{nitrogen content x } 6.25 \quad (3.5)$$

Crude fibre (AOAC 962.09)

Two grams of the ground sample will be transferred to a 750 ml Erlenmeyer flask and 0.5 g asbestos added. 200 ml of boiling 1.25 % H2SO4 will be added to the flask and the flask connected to cold finger condenser. The flask will immediately be brought to boil on a hot plate for 30 minutes. The content of the flask will then be filtered through a funnel laced with a linen cloth and washed with boiling water until no longer acidic. The charge and asbestos will be

washed back into the flask with 200 ml of boiling 1.25 % NaOH solution and the flask connected to a condenser again and boiled for 30 min. The content will be filtered through linen cloth and thoroughly washed with boiling water. The residue will be transferred into a gooch crucible, washed with 15 ml of 95% ethanol and dried in the oven for one hour at 100 °C. The flask will be cooled in a desiccator weighed and ignited in a preheated muffle furnace at 600 °C and then cooled again and reweighed. The percent crude fibre content will be calculated as shown in Equation 3.6 (AOAC, 1992).

$$\%\ crude\ fibre = \frac{mass\ of\ fibre}{dry\ mass\ of\ sample} \times 100 \quad (3.6)$$

Determination of mineral elements in maize

One gram of ground sample will be weighed and added to a 250 ml conical flask followed by addition of 15 ml of HNO_3 and 5 ml of concentrated H_2SO_4. The mixture will be shaken and heated on a hot plate preset at 160^0C until brown fumes disappear and white fumes begin to show. 10 ml of H_2O_2 will be added carefully and heated to dryness. The digest will then be allowed to cool and 20 ml of deionized water added to dissolve the residue. This will be filtered quantitatively into a 100ml volumetric flask and the residue in the conical flask washed with 10 ml deionized water into the filtrate. The filtrate will be made up to 100 ml mark for trace metal analysis using atomic absorption spectrophotometer (Barefoot and Van Loon, 1996).

3.5.3 Determination of electrical properties

Sample holder fabrication

The sample holder will be designed as outlined by Sacilik and Colak (2010). It will consist of a coaxial cylinder, with the outer electrode internal diameter of 89 mm and inner electrode diameter of 16 mm both made of stainless steel. Two Teflon rods of dimensions 89 × 17 × 8.5 mm will hold the electrodes in place from top and bottom. The total volume of the capacitor will be 1512 cm^3. The sample holder will be connected to the 50-ohm output terminal of the signal

generator using BNC clip connectors. Oscilloscope probes will then be used to connect the sample holder to channel one, of the oscilloscope as shown in the circuit diagram in Figure 3.1.

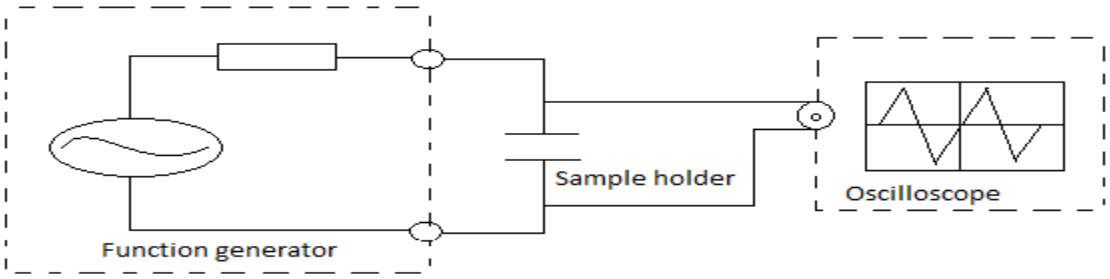

Figure 3.1: Circuit diagram for electrical property measurements.

Sample preparation and measurement procedure

The refrigerated maize samples (from Experiment 1) will be allowed to equilibrate to room temperature for at least six hours. Each sample will be conditioned to obtain three moisture contents of 11%, 14% and 17% wet basis by spraying with a calculated mass of distilled water. The samples will then be stored in sealed polythene bags at 4 °C for 72 hours and mixed periodically to obtain uniform moisture content (Trabelsi *et al.*, 1998).

The sample to be analyzed will again be allowed to equilibrate to room temperature. Before tests, calibrations will be performed to ensure accuracy and precision of the measurement system. An initial measurement sequence will be completed with the empty sample holder after which the prepared sample will be slowly poured into the sample holder from a height of 150 mm from the top of the sample holder at a constant rate and excess amount removed with a strike-off stick in a "Z" motion. The measurement of resistance (Equivalent series resistance: ESR) and capacitance of the sample holder will be taken at 40 frequencies ranging from 1 to 40 MHz at 1 MHz intervals from which three frequencies will be chosen to perform the rest of the experiment. All measurements will be performed at room temperature (Sacilik and Colak, 2010). Dielectric

properties of maize kernels will then be computed from the measurements of resistance and capacitance using Equations 3.1 and 3.2.

$$\varepsilon' = \frac{C}{C_0} \tag{3.1}$$

Where;

ε' is the dielectric constant,

C is the capacitance of sample holder filled with maize in pF

C_o is the capacitance of empty sample holder in pF

$$\varepsilon'' = \frac{G - G_o}{2\pi f C_o} \tag{3.2}$$

$$G = \frac{R}{R^2 + X^2} \tag{3.3}$$

$$X = X_C = \frac{1}{2\pi f C} \tag{3.4}$$

Where;

ε'' is the dielectric loss factor,

G is the conductance of the sample holder filled with maize in S,

G_o is the conductance of the empty sample holder in S,

f is the frequency of the applied electric current in Hz,

R is the resistance

X the capacitive reactance.

3.6 Experimental Design and Statistical Analysis

A factorial design with three treatments, three moisture contents, two temperatures and two relative humidity with three replicates will be used for Experiment I (Figure 3.2). A total of 36, 4 kg maize samples will be analyzed for each treatment resulting in a total of 108 samples for Experiment I. An analysis of variance (ANOVA) will be performed to test for the effect of sample treatment, moisture content, temperature and relative humidity on the aflatoxin contamination of maize. ANOVA will also be used to verify the effect of the sample treatment on the chemical composition of maize. Mean comparisons and separations will be done using the least significant difference (LSD) test at a 95% probability level.

Experiment II will also be a factorial design using samples obtained from the three treatments in Experiment I, five sample points (corresponding to 5 different levels of aflatoxin obtained from maize inoculated with toxigenic *A. flavus*) and three moisture contents analyzed at three frequencies with three replicates (Figure 3.3). 135 samples will be analyzed for all the three treatments. Analysis of variance will once more be used to test for the effect of chemical composition on the electrical properties of maize at each of the three chosen frequencies for the three moisture contents.

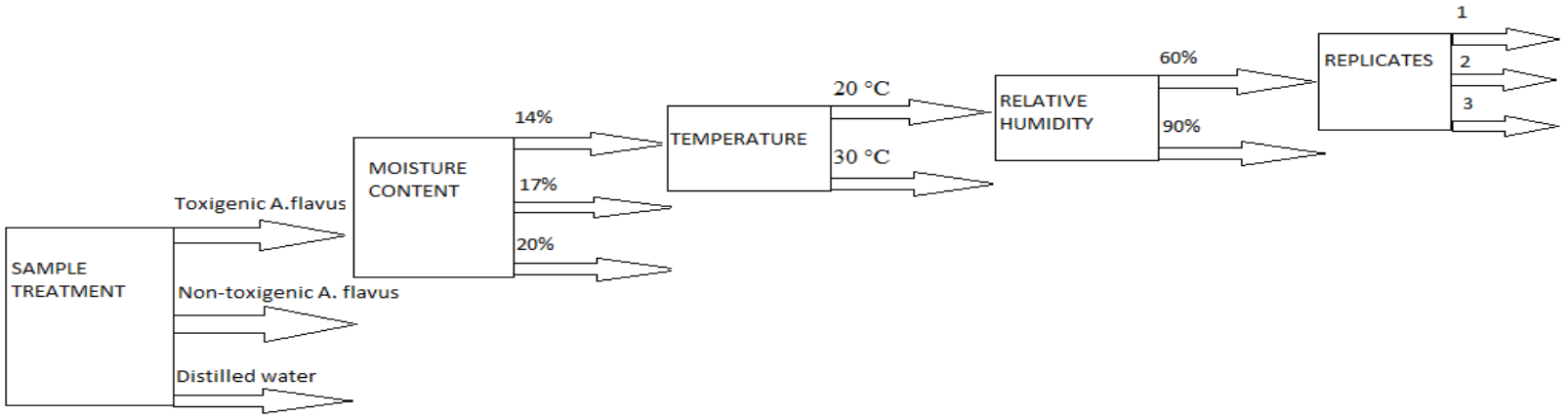

Figure 3.2: Maize treatments, moisture content, temperature and relative humidity to be investigated in Experiment 1.

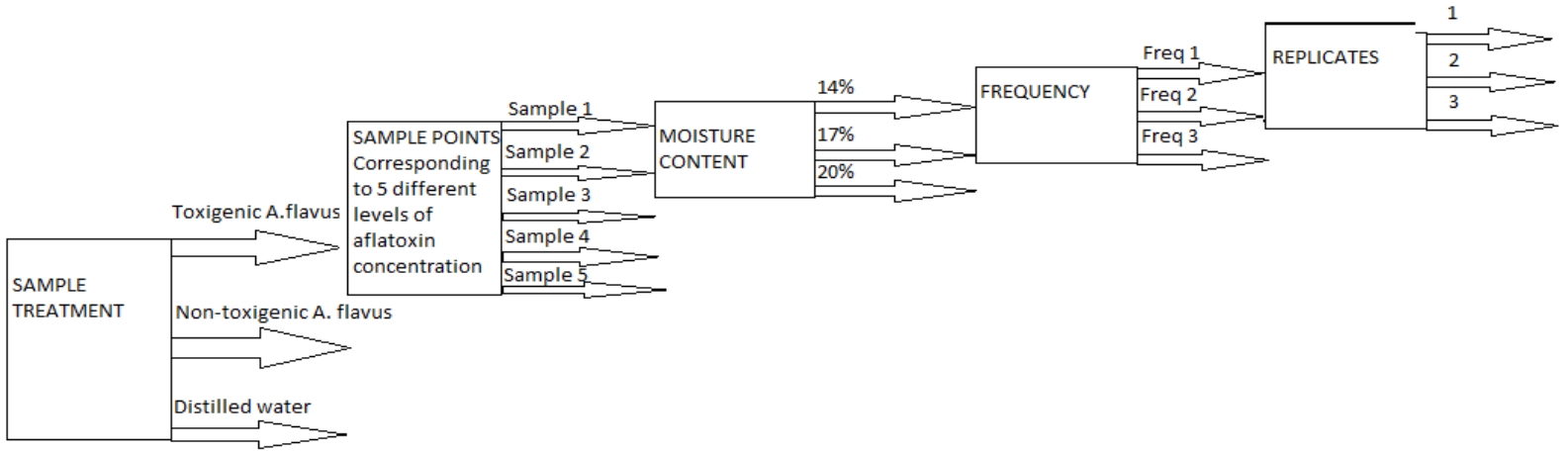

Figure 3.3: Experimental design for investigating the effect of proximate composition and aflatoxin contamination on the dielectric properties of maize (Experiment II).

3.7 Resources

The equipment and instruments needed for various experiments are listed in Table 3.1 while the project budget is presented in Table 3.2. The study is wholly funded by the DST-ERAfrica project, an international four-country collaborative research project that is housed at the University of Venda's Department of Agricultural and Rural Engineering for the South Africa.

Table 3.1: Equipment required for assessments and the means of acquisition

Equipment	Quantity	Means of acquisition
Laboratory mill	1	Available at UKZN
CTS Climatic test chamber	1	Available at UKZN
Weighing balance	1	Available at UKZN
Storage freezer	1	Available at UKZN
Forced air oven	1	Available at UKZN
Moisture balance	1	Available at UKZN
Autoclave	1	Available at UKZN
Oscilloscope	1	Available at UKZN
Function generator	1	Available at UKZN
BNC clip connectors	1	Available at UKZN
Oscilloscope probes	1	Available at UKZN

Table 3.2: Project budget

Item	**Quantity**	**Unit**	**Unit price (R)**	**Total price (R)**
PDA	4	Bottle	600	2 400
Barley seeds	2	Kg	60	120
NaClO	5	Lt	30	150
ELISA kit	1	Kit	7000	7 000
Maize	300	Kg	100	30 000
Chemical composition analysis	108	Samples	270	29 160
Aflatoxin analysis	80	Samples	370	29 600
Electrical analysis				7 000
Sealable polythene bags	4	Packet	200	800
Plastic trays	20	Piece	20	400
Labels	1	Roll	100	100
Gloves	2	Packet	100	200
Respirator masks	2	Packet	100	200
Total				107 130

3.8 Project Plan

A proposed project plan with activities and milestones to be achieved within scheduled timeframes is presented in Figure 3.4

Year	2015				2016												2017		
Task	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M
Literature review																			
Project proposal																			
Proposal write-up																			
Presentation of Literature review and project proposal																			
Inoculation and incubation of maize																			
Inoculum preparation																			
Inoculation and incubation of maize																			
Analysis of chemical composition of maize																			
Aflatoxin detection and quantification																			
Determination of proximate composition																			
Data analysis																			
Electrical properties measurement																			
Fabrication of sample holder																			
Development of electrical measurement system																			
Measurement of electrical properties																			
Data analysis																			
Thesis development																			
Thesis write-up																			
Submission of first draft																			
Submission of final draft																			

Figure 3.4: Proposed project plan.

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