CHARACTERISATION OF THE ELECTROCHEMICAL PROPERTIES OF MAIZE GRAIN CONTAMINATED WITH AFLATOXIN

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Submitted in fulfilment of the requirements

for the degree of MScEng

Bioresources Engineering

School of Engineering

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South Africa

June 2017

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PREFACE

The research contained in this dissertation was completed by the candidate while based in the Discipline of Bioresources Engineering, School of Engineering, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa. The research was financially supported by the Department of Science and Technology (DST), Republic of South Africa through the University of Venda. The work is part of an ongoing research project funded by the DST-ERAfrica. The project is titled SAPDRY – Development of grain drying facilities that use superabsorbent polymers (SAPs) and adjusting the properties of SAPs to optimise drying of grain and control of aflatoxin contamination (Project No. 011)

ABSTRACT

In this study, the influence of temperature and relative humidity on aflatoxin contamination of maize kernels during storage was investigated at different grain moisture contents. The effect of *Aspergillus flavus* (*A. flavus*) on the chemical composition of maize kernels was investigated. In addition, the study sought to establish the electrical properties of maize kernels at different levels of aflatoxin contamination. The first part of the study investigated the influence of temperature, relative humidity, and moisture content on aflatoxin contamination of maize kernels during storage. The experiment was designed as a full factorial experiment consisting of two temperature levels (20 ^{0}C and 30 ^{0}C), two relative humidity levels (60 % and 90 %), and five moisture content levels (14 %, 15 %, 16 %, 18 %, and 20 % wet basis). The moisture content of maize kernels was adjusted, and the samples inoculated with *A. flavus* and thereafter stored at the specified temperature and relative humidity for ten days in a climatic test chamber. The samples were evaluated for aflatoxin contamination at the beginning and the end of the storage period. The results indicated that temperature and relative humidity significantly ($p \leq 0.05$) affected aflatoxin contamination whereas moisture content had no significant ($p > 0.05$) effect. Aflatoxin contamination was observed at both 20 °C and 30 °C. The production of aflatoxin was pronounced at 30 °C, ranging between 0.3 $\mu g.kg^{-1}$ – 11179.7 $\mu g.kg^{-1}$, compared to 20 °C that ranged between 0.8 $\mu g.kg^{-1}$ – 733.7 $\mu g.kg^{-1}$. Relative humidity of 90 % had higher levels of aflatoxin contamination of between 3.9 $\mu g.kg^{-1}$ – 11179.7 $\mu g.kg^{-1}$, while a relative humidity of 60 % had levels of aflatoxin contamination of between 0.3 $\mu g.kg^{-1}$ – 2.4 $\mu g.kg^{-1}$. The interaction between temperature and relative humidity had a significant ($p \leq 0.05$) influence on the level of aflatoxin contamination. However, the two-way interaction of temperature and moisture content, relative humidity, and moisture content, as well as the three-way interaction of temperature, relative humidity and moisture content had no significant ($p > 0.05$) effect on the level of aflatoxin contamination. The second part of the study investigated the effect of *A. flavus* on the chemical composition of maize kernels. The experiment was designed as a 3 × 5 full factorial experiment. The moisture content of maize kernels was adjusted to 17 % (wet basis) and inoculated with three different inocula, viz. distilled water, *A. flavus*, and *Fusarium verticillioides* (*F. verticillioides*). The inoculated samples of maize kernels were incubated at 28 ^{0}C for 7, 14, 21, and 28 days. Sampling was done prior to incubation (day 0) as well as on days 7, 14, 21, and 28. The samples were thereafter analysed for aflatoxin contamination, moisture content, ash,

crude fibre, crude fat, crude protein, and carbohydrates. The results indicated that there was no change in the chemical composition of maize kernels inoculated with distilled water except for increased levels of aflatoxin contamination and moisture content. Maize kernels inoculated with *A. flavus* and *F. verticillioides* exhibited a significant decrease in fat and carbohydrate content and a marginal decrease in protein content. There was an increase in aflatoxin B1 (AFB1) and fumonisin B1 (FB1) contamination in maize kernels inoculated with *A. flavus* and *F. verticillioides*, respectively. Both ash and fibre content showed no changes across all treatments. The length of time of incubation and inoculum had significant ($p \leq 0.05$) effect on AFB1 and FB1 contamination, moisture content, fat, protein and carbohydrate. Aflatoxin contamination was highly correlated to fat ($R^2 = 0.82$) and carbohydrate ($R^2 = 0.92$) degradation whereas protein content showed a weak correlation ($R^2 = 0.50$). The third part of the study hypothesised that the changes in chemical composition due to *A. flavus* and subsequent aflatoxin contamination affects the dielectric properties of maize. A factorial experiment consisting of three levels of moisture content (13.3 %, 15.3 %, and 16.4 %), three frequencies (25 kHz, 50 kHz, and 100 kHz), and nine levels of aflatoxin (0 $\mu g.kg^{-1}$, 1.5 $\mu g.kg^{-1}$, 2.6 $\mu g.kg^{-1}$, 10 $\mu g.kg^{-1}$, 50 $\mu g.kg^{-1}$, 100 $\mu g.kg^{-1}$, 150 $\mu g.kg^{-1}$, 172 $\mu g.kg^{-1}$, and 230 $\mu g.kg^{-1}$), was employed. The maize kernels were poured into a custom-built sample holder comprising a shielded parallel plate capacitor. The capacitance measurements were done at a constant room temperature of 24 ^{0}C, using an ISO-TECH LCR-821 meter. The capacitance values obtained was used to compute the dielectric constant of the maize kernels. The results indicated that moisture content and frequency of the applied electric field significantly ($p \leq 0.05$) affected the dielectric constant. The dielectric constant increased with moisture content and decreased with increasing frequency. Aflatoxin contamination level had no significant ($p > 0.05$) effect on the dielectric constant of maize kernels. The coefficient of determination (R^2) of dielectric constant and aflatoxin contamination levels was low ($R^2 = 0.2687$). The low R^2 indicate that there is no correlation between the aflatoxin level and the dielectric constant of the maize kernels within the frequency range of 25 to 100 kHz. This study indicates that aflatoxin contamination can be controlled by storing maize at relative humidity below 60 %. While *A. flavus* infection leads to aflatoxin contamination and changes in chemical composition of maize, such changes have no impact on the dielectric constant of maize kernels. Dielectric properties of maize kernels are, therefore, less important for use in detecting aflatoxin contamination in maize kernels within the frequency range of 25 to 100 kHz.

DECLARATION ON PLAGIARISM

I, Francis Collins Muga, declare that:

(i) The research reported in this dissertation, except where otherwise indicated, is my original work.

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Dr MO Marenya

DECLARATION ON PUBLICATIONS

This section outlines the sections in this dissertation that have been presented/submitted to a conference, and submitted to peer-reviewed international journals for publication. The research reported is based on the data I collected from the various experiments. I designed the experiments, collected, analysed the data, and wrote the presentation and the manuscripts. This work was done under the supervision, guidance and review of my supervisors; Prof TS Workneh and Dr MO Marenya. The * indicates the corresponding author.

Chapter 3

F.C. Muga*, T.S. Workneh, M.O. Marenya (2017). Effect of temperature, relative humidity and moisture on aflatoxin contamination of stored maize. Oral presentation to 1st All Africa Postharvest Congress and Exhibition (28th to 31st March 2017, Nairobi, Kenya)

F.C. Muga*, T.S. Workneh, M.O. Marenya. Effect of temperature, relative humidity and moisture on aflatoxin contamination of stored maize.
Submitted to International Food Research Journal, Print ISSN 19854668

Chapter 5

F.C. Muga*, T.S. Workneh, M.O. Marenya. Electrical properties of maize kernels contaminated with aflatoxin.
Submitted to Pan African Society for Agricultural Engineers (PASAE) Conference (19th to 21st November 2017, Nairobi, Kenya)

F.C. Muga*, T.S. Workneh, M.O. Marenya. Electrical properties of maize kernels contaminated with aflatoxin.
Submitted to Journal of Agricultural Science and Technology, Print ISSN 16807073

ACKNOWLEDGMENTS

I thank the Almighty God for giving me good health and strength, and for His guidance throughout the course of my study. My heartfelt gratitude goes to my family and fiancé for their endless prayers, emotional support, and encouragement during my studies

I sincerely appreciate my supervisors, Prof Tilahun S Workneh and Dr Moses O Marenya for their keen interest and constant discussion that helped shape this research project. Their insightful suggestions and critique were instrumental in the preparation of this dissertation.

This work would not have materialised without the financial support of the Department of Science and Technology (DST), Republic of South Africa and the University of Venda. Their support was critical for the smooth running and completion of this research project.

I am entirely grateful to Dr Duncan O Mbuge for according me the opportunity to be part of the ERAfrica SAPDRY project. His constant encouragement and advice were invaluable throughout the course of my study.

I would also like to thank Dr Milindi Sibomana, Dr Medhin Hadish and Dr Benice Sivparsad for their advice as well as assistance in the acquisition of experiment materials, lab space and instruments. I am equally grateful to Messrs Alan Hill, Thabo Hlatshwayo and Mbuso Cele for their invaluable technical support and advice on the measurement of the electrical properties.

Finally, I thank my friends and fellow postgraduate students, Kipchumba Cherono, Luwam Ziena, Siphiwe Mdlalose, Tinashe Dirwai, Sabelo Shezi, Mubanga Chisenga, and Daniel Otim for their stimulating discussion, peer review and all the times they lent a hand in my research work.

SUPERVISORS' APPROVAL

Subject to the regulations of the School of Engineering, we the supervisors of the candidate, consent to the submission of this dissertation for examination.

Supervisor: _____ Date ……/ ……/ 2017

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Dr MO Marenya

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AFB1	Aflatoxin B1	18
AFB2	Aflatoxin B2	18
AFG1	Aflatoxin G1	18
AFG2	Aflatoxin G2	18
ANOVA	Analysis of variance	53
BEH	Ethyle bridge hybrid	55
C	Capacitance of maize filled sample holder	89
C_0	Capacitance of empty sample holder	89
CV	Coefficient of variance	93
C_2H_6O	Ethanol	74
DAD	Diode array detector	26
ECD	Electron capture detector	26
ELISA	Enzyme-linked immunosorbent assay	2
EMC	Equilibrium moisture content	61
ε_r	Relative permittivity	87
ε'	Dielectric constant	87
ε''	Dielectric loss factor	87
FB1	Fumonisin B1	67
FB2	Fumonisin B2	67
FIA	Fluorescence immunoassay	26
FID	Flame ionisation detector	26
FLD	Fluorescent detector	26
FTIR	Fourier transform infrared spectroscopy	27
GC	Gas chromatography	2
HgO	Mercuric oxide	73
H_2SO_4	Sulphuric acid	73

Abbreviation/Symbol	Meaning	Page
HPLC	High-performance liquid chromatography	2
HPTLC	High-performance thin layer chromatography	25
IAC	Immunoaffinity column	25
ICA	Immunoaffinity column assay	26
K_2SO_4	Potassium sulphate	73
LC	Liquid chromatography	55
LC-MS/MS	Liquid chromatography tandem mass spectroscopy	26
LSD	Least significant difference	76
MC	Moisture content	54
MMT	Million metric tonnes	9
MRM	Multiple reaction monitoring	55
MS	Mass spectroscopy	26
NaClO	Sodium hypochlorite	52
NaOH	Sodium hydroxide	74
NIR	Near infrared spectroscopy	86
OWLS	Optical waveguide light spectroscopy	27
QCMs	Quartz crystal microbalance sensor	27
RH	Relative humidity	54
RIA	Radio immunoassay	26
SAPs	Superabsorbent polymers	64
SPE	Solid phase extraction	24
SPR	Surface plasmon resonance	27
SSA	Sub-Saharan Africa	51
T	Temperature	54
TLC	Thin layer chromatography	2
UPLC	Ultra- performance liquid chromatography	55
UV	Ultra-violet	26
wb	Wet basis	13

1 INTRODUCTION

Maize is an important cereal for food, feed and industrial raw material (Awika, 2011; Ranum *et al.*, 2014). It is a staple food in many Sub-Saharan African countries, particularly Eastern and Southern Africa. The land under maize production in Sub-Saharan African countries accounts for about 50 % of the total land area under cereals (Erenstein *et al.*, 2011). Largely, maize production in Africa is for human consumption (Pingali, 2001). 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 countries wherein it provides income to resource-poor small-scale farmers (Reiter *et al.*, 2010).

Small-scale farmers are resource and land constrained farmers whose produce is majorly for subsistence consumption. The small-scale farmers are responsible for more than two-thirds of the total maize produced in Sub-Saharan Africa (Wu and Guclu, 2012). These farmers incur heavy post-harvest losses, particularly during storage (Tefera, 2012). Several factors including temperature, relative humidity, insect damage and growth of micro-organisms 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 insect pests and 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 Muthomi (2008), between 25 % and 50 % of crops worldwide are contaminated with mycotoxins.

Aflatoxin is one of the major mycotoxins in agriculture. Maize is significantly colonised by aflatoxin-producing *Aspergillus* species (Bandyopadhyay *et al.*, 2007). *Aspergillus flavus* (*A. flavus*) is the major producer of aflatoxin (Klich, 2007). The growth of *A. 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 *A. 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 *A. flavus.* Aflatoxin contamination in maize grain is prevalent in Sub-Saharan Africa (Wagacha and Muthomi, 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 and Muthomi, 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 fourier 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). Small-scale farmers have no access to these aflatoxin detection methods 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 small-scale farmers whose produce is usually for own consumption, hence never enters the formal grain market where testing 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 as well as bulk density (Al-Mahasneh *et al.*, 2001; Trabelsi *et al.*, 1998; Sacilik and Colak, 2010; Nelson, 2015).

The electrical properties of cereal grains are represented by their dielectric properties (Nelson and Trabelsi, 2012). The dielectric properties of cereal grains are significantly affected by grain moisture content, bulk density, temperature, and frequency of applied electric field (Jha *et al.*, 2011; Skierucha *et al.*, 2012; El Khaled *et al.*, 2016). There are no published articles on how the proximate composition of cereal grains affects their dielectric properties. However, Bhargava *et al.* (2013) reported that variations in proximate composition can influence the dielectric properties of cereals. This study, therefore, sought to investigate the influence of *A. flavus* and aflatoxin contamination on the chemical composition of maize kernels and the impact of these changes, on the dielectric properties of maize kernels at different levels of aflatoxin contamination.

The research questions for this study were:

(i) How do grain moisture content, temperature and relative humidity influence aflatoxin contamination of maize?

(ii) What is the effect of *Aspergillus flavus* and 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?

The specific objectives of this study were to:

(i) investigate the effect of temperature, relative humidity and grain moisture content on aflatoxin contamination of maize,

(ii) determine the effect of *Aspergillus flavus* and aflatoxin contamination on the chemical composition of maize grain, and

(iii) establish the electrical properties of maize grain under different levels of aflatoxin contamination.

1.1 Outline of dissertation structure

This dissertation is organised into six chapters.

Chapter 1 Provides a general overview of the study detailing its justification and the objectives.

Chapter 2 Details an overview of maize production and consumption in Africa. It reviews the literature on maize storage and the associated quality losses particularly aflatoxin contamination of maize. It discusses the factors affecting aflatoxin contamination of maize and the various methods for detecting aflatoxin contamination. This chapter finally presents literature of the electrical properties of grains, factors that influence electrical properties and the different applications of electrical properties on grain quality analysis.

Chapter 3 Focuses on the effect of temperature, relative humidity, and moisture on aflatoxin contamination of stored maize kernels.

Chapter 4 Investigated the deteriorative changes in maize kernel due to contamination with *A. flavus*.

Chapter 5 Presents the electrical properties of maize kernels contaminated with aflatoxin.

Chapter 6 This is the conclusion and recommendation chapter of this study. It highlights the major findings of this work and makes recommendations arising from the study.

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2 A REVIEW ON AFLATOXIN CONTAMINATION IN MAIZE AND THE DETECTION METHODS

This chapter presents an overview of maize production and consumption in Sub-Saharan Africa. Additionally, it presents reviews and critique on maize storage and associated quality losses, particularly aflatoxin contamination. Major aspects addressed herein include factors affecting aflatoxin contamination of maize during storage and aflatoxin detection methods. The literature on important electrical properties of maize is also reviewed with emphasis on the chemical composition of maize kernels.

2.1 An Overview of the Global Cereal Production

Cereals are a primary source of calories globally. Rice, wheat, and maize are the three most important food crops in the world (Awika, 2011). They account for 94 % of the global calorific intake (Ranum *et al.*, 2014). Maize also referred to as corn (*Zea mays Linnaeus*), is the largest cereal crop regarding production volumes (Table 2.1) and is the most domesticated of all field crops in the world (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 maize production (Taylor and Koo, 2013). Mexico, Argentina, India, Ukraine, Indonesia and South Africa are also large-scale producers of maize (Ranum *et al.*, 2014).

Table 2.1 Production statistics of major cereal

Cereal	Production (MMT)	International trade (MMT)	Grain entering international trade (%)	Reference
Maize	960	130	13	Wolf *et al.*, 2018
Wheat	735	170	23	Wolf *et al.*, 2018
Barley	140	30	20	Wolf *et al.*, 2018
Rice	715	35	7	Muthayya *et al.*, 2014
Sorghum	60	6.3	10.5	Awika, 2011

*MMT – Million metric tonnes

Globally, maize production and consumption continues to rise steadily (Pingali, 2001). Much of the increase in production is not only attributed to the use of genetically improved cultivars,

effective field practices, and fertiliser use but also due to increase in land under cultivation (Wariboko and Ogidi, 2014).

Maize 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). Human consumption accounts for about 70 % of maize utilisation in Sub-Saharan Africa whereas, in the developed countries, maize is primarily used as livestock feed and raw material for industrial products (Pingali, 2001).

2.2 Production of Maize 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 small-scale farmers except for South Africa that has a well-established commercial maize farming system (Reiter *et al.*, 2010; Suleiman *et al.*, 2013).

Maize covers approximately 29 million hectares which account for 30 % of the total cereal area in Sub-Saharan Africa (Smale *et al.*, 2013). It covers 29 % of the total cereal area in Eastern Africa; 65 % in Southern Africa; 19 % in West Africa and 61 % in Central Africa (Erenstein *et al.*, 2011). South Africa is the leading producer of maize in Africa with 14.9 million tonnes produced in 2014 (FAOSTAT, 2016a). Nigeria, Ethiopia, Tanzania and Egypt follow with 10.8, 7.2, 6.7 and 5.8 million tonnes respectively. 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). Ethiopia and Tanzania are the leading producers in Eastern Africa. Kenya, Zambia and Malawi also make a sizeable contribution with each producing well over 3 million tonnes annually (FAOSTAT, 2016a).

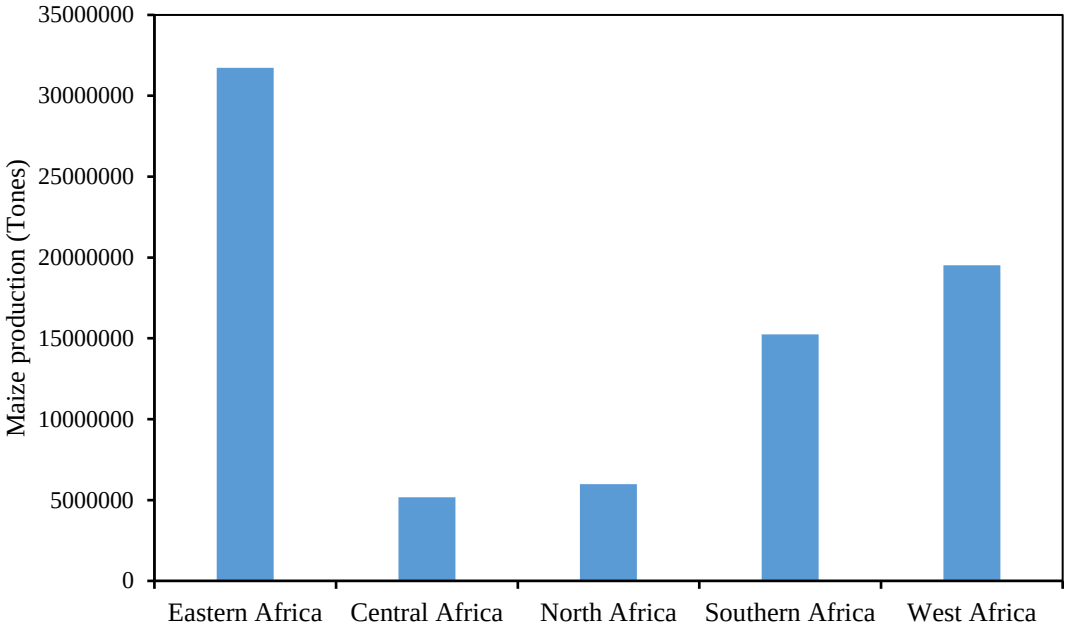

Figure 2.1 Maize production in Africa (drawn using data from FAOSTAT, 2016a)

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, 2016a). Maize production is not robust in West Africa. However, Nigeria is the second largest producer of maize in Africa with 10.8 million tonnes produced in 2014. Benin, Burkina Faso, Ghana, Mali and Senegal are the only other countries with annual production of over a million tonnes (FAOSTAT, 2016a).

2.3 Consumption of Maize in Africa

Maize dominates the food economy of Eastern and Southern Africa (Reiter *et al.*, 2010), providing income to millions of resource-poor small-scale 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).

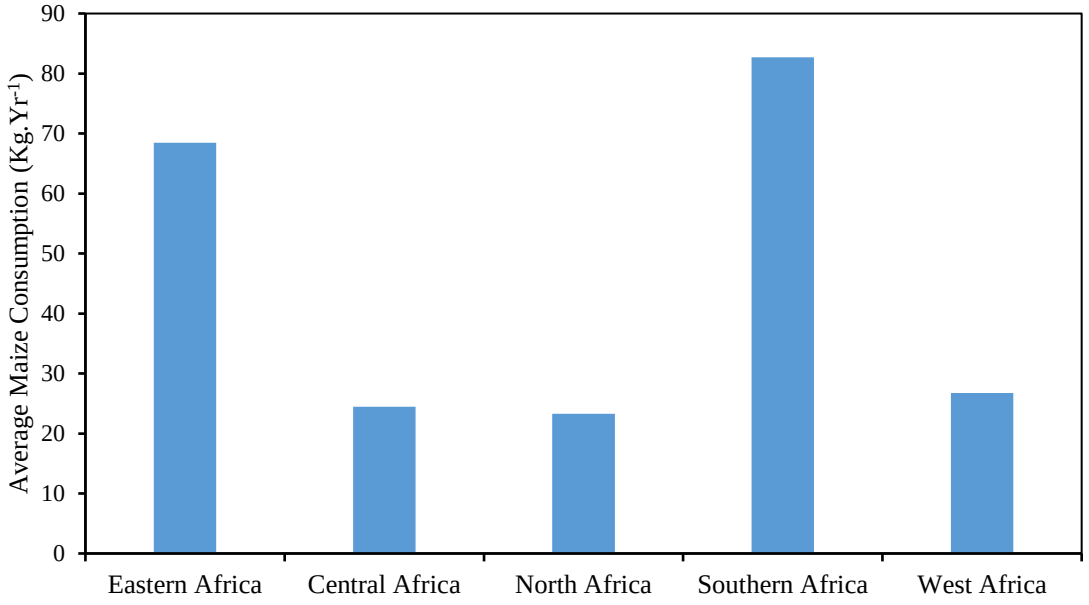

Figure 2.2 Maize consumption in Africa (drawn using data from FAOSTAT, 2016b)

2.4 Storage of Maize

Maize farming in Sub-Saharan Africa is predominantly done by small-scale farmers under rainfed conditions with limited inputs (Cairns *et al.*, 2013). These farmers have limited access to efficient storage technology (Kadjo *et al.*, 2013). Some of the storage methods used by farmers in West Africa include; raised platforms, jute or propylene bags, conical structures, clay structures and baskets (Motte *et al.*, 1998; Ofosu *et al.*, 1998; Hell *et al.*, 2000a; Addo *et al.*, 2002). The storage structures used by farmers in East and Southern Africa consist of roofed iron drums enclosed with mud, metal bins, pits, wooden open-air or roofed cribs, wood and wire cribs and raised platforms (Kankolongo *et al.*, 2009; Wambugu *et al.*, 2009). Most of the traditional storage methods expose the maize to open air conditions allowing rewetting, moulds, 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 moulds alone can cause a total loss in stored maize. The moulds 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).

2.5 Causes of Maize Quality Deterioration During Storage

Grain moisture content is critical in determining the storability of maize. Together with temperature and relative humidity, they determine the maximum amount of time maize can be stored without quality deterioration (Gonzales *et al.*, 2009). Insects and fungi also contribute immensely to quality and quantity degradation during storage.

2.5.1 Moisture content

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 supports 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). The grain moisture content can be expressed in wet basis as shown in Equation 2.1.

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

Where

$M.C_{wb}$ = moisture content wet basis (%).

The grain moisture content can also be expressed in dry basis as shown in Equation 2.2.

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

Where

$M.C_{db}$ = Moisture content dry basis (%).

2.5.2 Environmental factors

Temperature and relative humidity are the critical environmental factors that influence grain spoilage during storage. 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.

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 store maize safely. 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 change its moisture content (Devereau *et al.*, 2002). The interaction between the moisture content of the grain and the relative humidity within the storage unit results in the stored grains reaching the equilibrium moisture content (Volenik *et al.*, 2007; Samuel *et al.*, 2011).

2.5.3 Insects and fungi

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 within the storage unit (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, moulds, 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 can lead 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. They consume grain nutrients leading to dry matter losses and 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). Some of the common insects that attack maize are contained in Table 2.2.

Table 2.2 Common insect species and their optimal growth conditions (Jian and Jayas, 2012)

Insect	Relative humidity (%)	Temperature (°C)
Sitophilus zeamais (Maize weevil)	70	27-31
Prostephanus truncates (Large grain borer)	80	25-32
Rhyzopertha dominica	50-60	32-34
Sitotroga cerealella (Angoimois grain moth)	75	26-30
Plodia interpunctella (Indian meal moth)	70	26-29
Tribolium castaneum (Red flour beetle)	70-75	32-35
Cryptolesters ferrugineus (Rusty flour beetle)	70-80	33
Sitophilus oryzae (Rice weevil)	70	26-31
Oryzaephilus surinamenis (Sawtoothed grain beetle)	90	31-34

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 °C) (Williams and McDonald, 1983). Some field fungi can survive under storage conditions 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).

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 limiting 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 Muthomi, 2008).

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 contained in Table 2.3.

Table 2.3 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	Marín *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)

2.6 Aflatoxin

Aflatoxin is a toxic fungal metabolite produced by moulds 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). In culture, *A. flavus* is characterised by fast-growing yellow-green colonies, normally 65 – 70 mm in diameter (Figure 2.3).

Figure 2.3 Seven-day-old culture of A. flavus grown on potato dextrose agar at 25 ^{0}C in the dark

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 B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2) (Strosnider *et al.*, 2006; Lizárraga-Paulín *et al.*, 2011). The structural formula of AFB1, AFB2, AFG1, and AFG2 are shown in Figure 2.4.

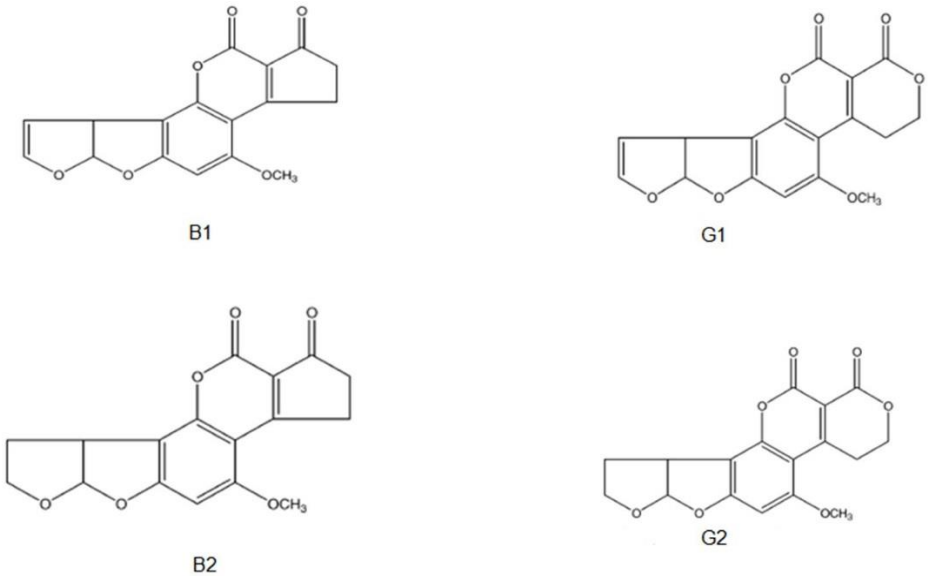

Figure 2.4 Structural formula of AFB1, AFB2, AFG1, and AFG2 (Feddern et al., 2013)

The International Agency for Research on Cancer (IARC) categorises AFB1 as the most potent naturally occurring carcinogen (Klich, 2007). AFB1 is the most prevalent in food resulting in most cases of aflatoxicosis (Lizárraga-Paulín *et al.*, 2011). Aflatoxins M1 (AFM1) and M2 (AFM2) are metabolic derivatives of AFB1 and AFB2 respectively and are found in dairy products, meat and urine (Strosnider *et al.*, 2006; Wild and Gong, 2010; Lizárraga-Paulín *et al.*, 2011).

2.6.1 Aflatoxin contamination in maize

Maize kernels are vulnerable to infection by toxigenic fungi (Abbas *et al.*, 2006) and are significantly colonised by aflatoxin-producing *Aspergillus* species (Bandyopadhyay *et al.*, 2007). High levels of aflatoxin contamination in maize are common in Sub-Saharan Africa (Wagacha and Muthomi, 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). Studies done by various authors in some African countries have shown significant aflatoxin contamination of maize way above the maximum tolerable limits set by the Codex Alimentarius Commission (Table 2.4).

Table 2.4 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;
		35	100	Wagacha and
		7	1000	Muthomi, 2008
Benin	Maize (before storage)	9-32	5	Hell *et al.* (2000b)
	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	Chilaka *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)

2.6.2 Factors affecting aflatoxin contamination of maize in storage

Aflatoxin 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.

A. 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 with the optimal growth and aflatoxin production observed between 25 ºC to 35 ºC (Atanda *et al.*, 2011). A study by Gbodi *et al.* (1986) analysed 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 at 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 vapour pressure over the substrate (P) to the vapour pressure over pure water at similar temperature and pressure (P_0) as shown in Equation 2.3.

$$a_w = \frac{P}{P_0} \tag{2.3}$$

Where

a_w = water activity,

P = vapour pressure over substrate (Pa), and

P_0 = vapour pressure over pure water (Pa)

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 whole 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.*, 2004) which raises the moisture content and temperature of grains to levels conducive for 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.

2.6.3 Effects of aflatoxin contamination on human health

AFB1 is the most toxic and prevalent aflatoxin in maize resulting in several cases of aflatoxicosis (Lizarraga-Paulin *et al.*, 2011). AFB1 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 AFM1 in the milk and urine samples of lactating mothers (Wagacha and Muthoni, 2008). AFB1 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).

Several studies in Sub-Saharan Africa have reported evidence of a widespread human exposure to aflatoxins (Ngindu *et al.*, 1982; Hendrickse, 1999; Henry *et al.*, 1999; Gong *et al.*, 2004; Strosnider *et al.*, 2006; Khlangwiset *et al.*, 2011). Table 2.5 highlights some of the published studies on aflatoxin exposure in Sub-Saharan Africa

Table 2.5 Evidence of human exposure to aflatoxin

Country	Population	Marker	Amount	Reference
Benin, Togo	Children (9 months – 5 yrs)	Aflatoxin albumin levels (Serum)	32.8 – 86.8 $pg.mg^{-1}$	Gong *et al.*, (2003); Gong *et al.*, (2004)
Cameroon	Children (1.5 – 4.5 yrs)	AFB1 (urine)	1.43 – 2.82 $ng.ml^{-1}$	Ediage *et al.*, (2013)
Egypt	Children (1-2.5 yrs)	AFB1 (urine)	13.2 $pg.ml^{-1}$	Polychronaki *et al.*, (2008)
Egypt	Pregnant women (Breast milk)			Wael *et al.*, (2011)
Gambia	Child (6 – 9 yrs)	Aflatoxin albumin levels (Serum)	22.3 $pg.mg^{-1}$	Turner *et al.*, (2003)
	Infant (> 1 yr)	Aflatoxin albumin levels (Serum)	8.7 $pg.mg^{-1}$	Turner *et al.*, (2007)
	Adults (18 – 70 yrs)	Aflatoxin albumin levels (Serum)	19.3 $pg.mg^{-1}$	Miele *et al.*, (1996)
Ghana	Adults	Aflatoxin albumin levels (Serum)	0.89 $pg.mg^{-1}$	Jolly *et al.*, (2006)
	Pregnant women	Aflatoxin albumin levels (Serum)	5 $pg.mg^{-1}$	Shuaib *et al.*, (2012)
Kenya	Adults	Aflatoxin albumin levels (Serum)	7.87 $pg.mg^{-1}$	Yard *et al.*, (2013)

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 AFB1 and total aflatoxin respectively (Kimanya *et al.*, 2008; Rheeder *et al.*, 2009; Kilonzo *et al.*, 2014).

2.6.4 Detection and analysis of aflatoxin

Accurate and sensitive determination of aflatoxins is essential to meet food safety requirements (Shephard, 2009). Aflatoxin analyses 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 (Köppen *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, immuno-affinity column, pressurised fluid extraction, solid phase micro-extraction and the quick easy cheap effective rugged safe approach (QuEChERs) (Bacaloni *et al.*, 2008; Nonaka *et al.*, 2009; Desmarchelier *et al.*, 2010).

The extraction solvent must ensure that aflatoxin is abstracted from the matrix without alteration (Bakırdere *et al.*, 2012). 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). Clean-up 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).

The cleaned sample extracts are usually analysed using chromatographic techniques coupled to an appropriate detector (Shephard, 2009). Thin-layer chromatography (TLC), gas chromatography (GC), and high-performance liquid chromatography (HPLC) are some of the frequently used chromatographic 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 ionisation detector (FID) or an electron capture detector (ECD) and mass spectrometer (MS) to identify volatile products (Pascale, 2009). It requires a preliminary clean-up step before analysis to eliminate matrix effects (Krska *et al.*, 2005). Matrix effect refers to the change in ionisation 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 of 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). AFB1 and AFG1 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 pre-treatment 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 (Cigić and Prosen).

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), immuno-affinity 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 AFB1 concentration in the range of 0.5–10 $\mu g.kg^{-1}$. Optical immuno-sensor such as surface plasmon resonance (SPR) and optical waveguide platform have been developed for aflatoxin detection. Daly *et al.* (2000) used SPR immuno-sensor with polyclonal antibodies to detect AFB1. Adányi *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 immuno-sensor is another form of biosensor whose bio-recognition element produces electroactive signals (Wacoo *et al.*, 2014). Most of the electrochemical methods developed for aflatoxin detection involve the use of antibodies immobilised 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 (Välimaa *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 coloured binding zones onto which aflatoxins bind causing a colour 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 analyses except for 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 (Fernández-Ibañez *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 analyse 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. Piermarini *et al.* (2009) used transmittance, and reflectance spectroscopy to detect aflatoxin in single maize kernels with more than 95 % of the kernels analysed being correctly categorised as having either high (>100 $\mu g.kg^{-1}$) or low (<10 $\mu g.kg^{-1}$) concentrations of aflatoxins.

All the aflatoxin analyses discussed require skilled personnel. Apart from lateral flow devices, these analyses are expensive 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 2.6.

Table 2.6 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
Immuno-dipstick	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 not accessible to resource-poor small-scale 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.*, 2014). 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 small-scale 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).

2.7 Electrical Properties of Cereal Grains

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 focused on the radio and microwave frequency dielectric properties of cereal grains (Nelson, 2010; Sacilik and Colak, 2010; Nelson and Trabelsi, 2012).

2.7.1 Dielectric properties of maize kernels

Maize kernels, like other cereal grains, are lossy insulators and are thus considered as dielectric materials (Mészáros, 2007). Dielectric properties are the electrical characteristics of poorly conducting materials that determine their interaction with electric fields (Nelson and Trabelsi, 2012). Dielectric properties 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.

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

Where

ε_r = relative permittivity

ε'_r = dielectric constant, and

ε''_r = dielectric loss factor.

The dielectric constant relates to the capacitance of the material when exposed to an electric field while the dielectric loss factor influences energy absorption and attenuation from an electric field (Sacilik and Colak, 2010). The loss tangent (dissipation factor) and power factor are also important dielectric properties (Nelson and Trabelsi, 2011). The loss tangent expresses the relative lossiness and is a measure of the power dissipation in a dielectric. It is a ratio of the dielectric loss factor to the dielectric constant (Equation 2.5).

$$\tan\delta = \frac{\varepsilon''_r}{\varepsilon'_r} \quad (2.5)$$

Where

ε'_r = dielectric constant, and

ε''_r = dielectric loss factor.

The power factor can be calculated as shown in Equation 2.6.

$$\text{Power factor} = \frac{\tan\delta}{\sqrt{1+\tan^2\delta}} \quad (2.6)$$

Where

$\tan\delta$ = loss tangent

Various factors influence the dielectric properties. These include; frequency of the applied alternating electric field, moisture content, bulk density, temperature, ionic nature, concentration (density), structure, and constituents of food materials (Zhang *et al.*, 2007; Jha *et al.*, 2011; Skierucha *et al.*, 2012; El Khaled *et al.*, 2016). The principal factors that influence the dielectric properties of cereal grains at a given electric field frequency are temperature, moisture content and bulk density (Nelson and Trabelsi, 2011; Nelson and Trabelsi, 2012).

The dielectric constant and dielectric loss factor varies linearly with moisture content, frequency of the applied electric field, temperature and bulk density (Skierucha *et al.*, 2012; Nelson, 2015). Dielectric constant increases with moisture content at any given frequency and decreases with increasing frequency (Jha *et al.*, 2011; El Khaled *et al.*, 2016). 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 5 GHz and 15 GHz. The dielectric constant of shelled yellow-dent field maize increased linearly with bulk density (Nelson, 1979). Similar trends were also reported by Trabelsi *et al.* (1998) on hard red winter wheat. Even though the dielectric constant becomes irregular over a wide range of bulk density, the square and cube root of dielectric constant remain linear with bulk density (Nelson, 1984).

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).

Apart from moisture content; carbohydrates, proteins, fats and fibre have low dielectric properties (Bhargava *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. The 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 (Ryynänen, 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. Although dielectric constant and loss factor increase with an increase in moisture content, 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.

2.7.2 Measurement techniques for determining dielectric properties

Measurement of the dielectric properties of agricultural materials is gaining importance due to their non-destructive nature of monitoring the quality attributes of these materials (El Khaled *et al.*, 2016). The suitability of a measurement technique depends on the electric field frequency and the degree of accuracy required (Venkatesh and Raghavan, 2004). Measurements at radio frequencies can be done through appropriated series or parallel circuits. The dielectric properties can be determined through equations that relate the radio frequency circuit parameters, impedance or admittance, to the relative permittivity of the material (Nelson, 1999).

Several bridges and resonant circuits have been used in the past for low, medium and high-frequency dielectric permittivity measurements (Field, 1954). Corcoran *et al.* (1970) measured dielectric properties of grain samples with a precision bridge from 250 Hz to 20 kHz. Nelson (1991) used Q-meter based on the resonant circuit in the 1 to 50 MHz range. Coaxial sample holders modelled as transmission line sections enabled higher frequency measurements of dielectric properties using RX – meter and admittance meter (Stetson and Nelson, 1968; Jorgensen *et al.*, 1970).

The Measurement techniques used for measuring the dielectric properties in the microwave frequency range can be grouped either as reflection or transmission types. These techniques use resonant or non-resonant systems, with open or closed structures (El Khaled *et al.*, 2017). Transmission measurement with closed structures includes the waveguide and coaxial line techniques, whereas the free-space transmission measurement and open-ended coaxial line system are open-structure techniques (El Khaled *et al.*, 2016). Commonly used microwave frequency, dielectric measurement techniques include; parallel plate capacitor, waveguide measurements, resistivity cell, resonant cavity, free space, cavity resonator, lumped circuit coaxial probe transmission line, and time domain spectroscopy (Nelson, 1991; Içier and Baysal, 2004; Venkatesh and Raghavan, 2004; Jha *et al.*, 2011; El Khaled *et al.*, 2016). The electric field frequency range appropriate for some of the measurement techniques is shown in Figure 2.8.

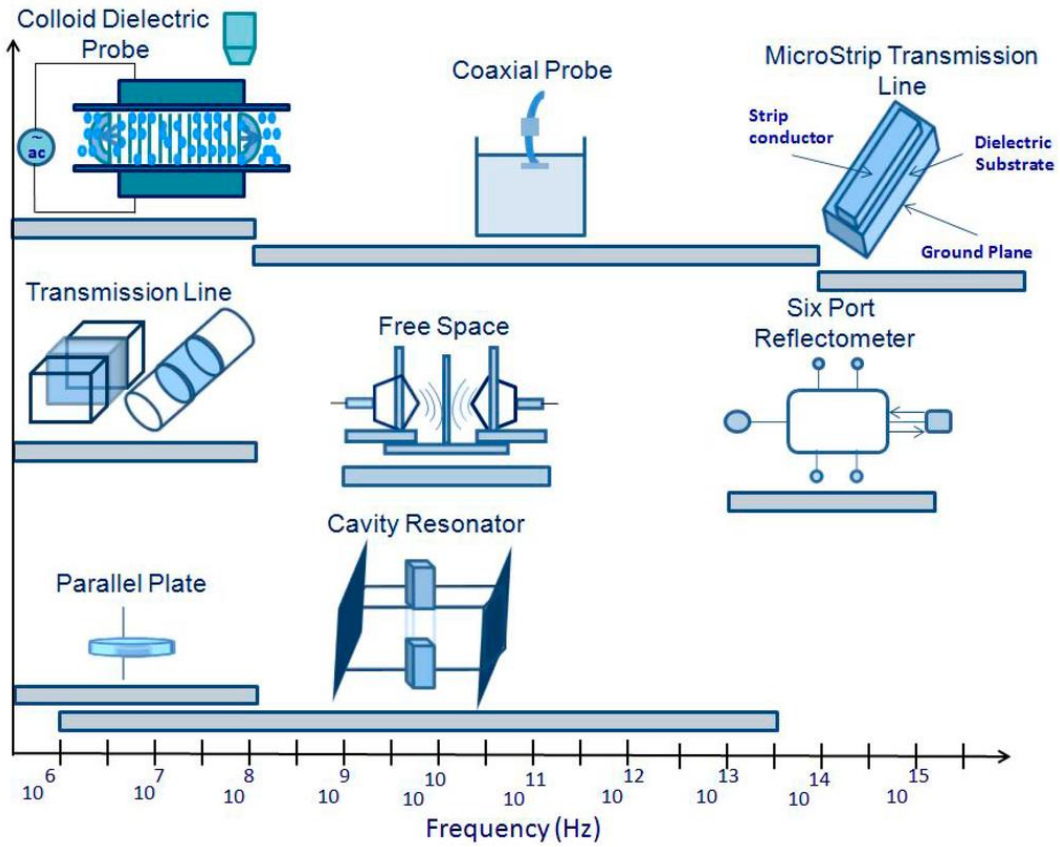

Figure 2.5 Dielectric properties measurement techniques versus the frequency range (El Khaled et al., 2016).

2.7.3 Application of dielectric properties

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.* (2001) 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).

2.8 Discussion

Maize is consumed in large quantities in Sub-Saharan Africa particularly in Eastern and Southern Africa. It is highly 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 are 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 conditions that promote the growth of fungi and mycotoxin production in maize during storage (Giorni *et al.*, 2007; Ngamo *et al.*, 2007).

It is important to understand the interaction between maize kernels and the storage environment to minimise aflatoxin contamination during storage (Ngamo *et al.*, 2007; Tefera, 2012). Currently, there is no literature on the effect of temperature, relative humidity and moisture content affect aflatoxin contamination of maize kernels during storage. There is need to establish how the storage environment and the grain moisture content influence the aflatoxin contamination of maize. This information will be crucial in developing storage structures that will safely preserve maize under conditions that limit/reduce the risk of aflatoxin contamination.

Besides proper storage structures, the safety of maize can only be guaranteed through adequate and constant monitoring of aflatoxin contamination levels during storage. The current aflatoxin detection and analysis methods are laboratory-based chemical analyses that require skilled personnel and expensive equipment. Consequently, these methods are inaccessible to small-scale farmers who are responsible for more than two-thirds of the total maize production in Sub-Saharan Africa (Wu and Guclu, 2012).

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 dielectric moisture meters and pH meters (Skierucha *et al.*, 2012).

Several published articles have reported the dielectric properties of cereal grains as a function of grain moisture content and bulk density which has enabled the detection of these properties through dielectric measurements (Jha *et al.*, 2011; Skierucha *et al.*, 2012; Nelson, 2015; El Khaled *et al.*, 2016). In as much as Zhang *et al.* (2007) reported that carbohydrates, ash and proteins can affect the dielectric properties of agricultural products, there is no documentation on the influence of the proximate composition of cereal grains on their dielectric properties. Nonetheless, Bhargava *et al.* (2013) reported variations in the dielectric constant and dielectric loss factor of different cereals which they attributed to their difference in proximate composition.

There is a need to study the effect of *A. flavus* and subsequent aflatoxin contamination on the chemical properties of grain. This information will be helpful in evaluating the effect of different levels of aflatoxin contamination on the dielectric properties of maize kernels contaminated with aflatoxin and consequently the suitability of dielectric properties for detecting aflatoxin contamination in maize.

2.9 Conclusion

Maize is an important staple food in most parts of Sub-Saharan Africa. Aflatoxin contamination of maize is, therefore, a risk to human health, food and financial security of many rural households involved in maize farming. Inadequate postharvest practices aggravate aflatoxin contamination of maize. It is evident from the literature review that the traditional storage methods used by small scale-farmers promote aflatoxin contamination of maize during storage. Consequently, it is important to establish how the storage environment and the grain moisture content affects aflatoxin contamination of maize. This would help in the development of new and cheap storage solutions to control/limit aflatoxin contamination during storage. Equally important, is the need to improve accessibility to aflatoxin detection methods for continuous

monitoring of aflatoxin contamination in maize. Increased accessibility to aflatoxin detection methods would help in early detection of aflatoxin contamination limiting severe losses and associated adverse health impacts to humans and animals. The literature review show that the current aflatoxin detection methods are out of reach of poor small-scale farmer hence the need to explore cheaper alternatives such as the use of electrical properties for detecting aflatoxin. Changes in the chemical composition of maize kernels due to aflatoxin contamination could influence their dielectric properties thus enabling aflatoxin detection.

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3 EFFECT OF TEMPERATURE, RELATIVE HUMIDITY AND MOISTURE ON AFLATOXIN CONTAMINATION OF STORED MAIZE KERNELS

Abstract

Maize kernels are vulnerable to attack by *Aspergillus flavus* (*A. flavus*) both in the field and during storage. *A. flavus* produces aflatoxin which is harmful to human and animal health. A combination of high temperature, relative humidity and grain moisture content promote *A. flavus* growth leading to aflatoxin contamination of maize during storage. In this study, aflatoxin contamination of maize kernels was investigated for selected temperature, relative humidity and moisture content levels. Samples of maize kernels at moisture content levels of 14, 15, 16, 18, and 20 % (wb) was inoculated with *A. flavus* spores. The inoculated samples were incubated in a climatic test chamber for ten days at 20 °C and 30 °C, and relative humidity of 60 % and 90 %. The results indicated that aflatoxin contamination was significantly ($p \leq 0.05$) affected by temperature and relative humidity whereas moisture content had no significant ($p > 0.05$) effect. Aflatoxin contamination occurred at both 20 °C and 30 °C. The production of aflatoxin was pronounced at 30 °C, ranging between 0.3 $\mu g.kg^{-1}$ – 11179.7 $\mu g.kg^{-1}$, compared to 20 °C that ranged between 0.8 $\mu g.kg^{-1}$ – 733.7 $\mu g.kg^{-1}$. Relative humidity of 90 % had higher levels of aflatoxin contamination of between 3.9 $\mu g.kg^{-1}$ – 11179.7 $\mu g.kg^{-1}$, while a relative humidity of 60 % had levels of aflatoxin contamination of between 0.3 $\mu g.kg^{-1}$ – 2.4 $\mu g.kg^{-1}$. The interaction between temperature and relative humidity significantly ($p \leq 0.05$) influenced aflatoxin contamination of maize. However, the interaction between temperature and moisture content, moisture content and relative humidity, as well as the combined interaction of temperature, moisture content, and relative humidity had no effect on the level of aflatoxin contamination. The results indicate that the level of aflatoxin contamination at a relative humidity of 60 % was lower than 5 $\mu g.kg^{-1}$. Consequently, maintaining storage conditions at a relative humidity level of less than 60 % results in minimal aflatoxin contamination of maize kernels, thus assuring its safety for consumption.

Keywords: *A. flavus*, aflatoxin, maize, storage.

3.1 Introduction

Sub-Saharan Africa (SSA) experiences severe yield losses in maize. Insufficient post-harvest practices exacerbate these losses. Losses of up to 50 % have been reported across many countries in Africa, most of which are experienced during storage (Hodges *et al.,* 2011). Insects and fungi collectively account for more than 50 % of grain lost during storage (Udoh *et al.,* 2000). Fungal spoilage of maize is a grave concern due to the mycotoxins associated with it.

Maize is very susceptible to fungal degradation, particularly *Aspergillus* and *Fusarium* which cause aflatoxins and fumonisins respectively (Tefera, 2012). These mycotoxins impact human and animal health and lower the economic value of produce (Gnonlonfin *et al.,* 2013). Aflatoxin contamination of maize causes significant grain losses in SSA (Dwivedi, 2011; Wagacha *et al.,* 2013). *A. flavus*, the primary cause of aflatoxin, attacks maize in the field and its effects are compounded by inappropriate post-harvest practices (Marín *et al.*, 2004).

Inadequate storage techniques and environmental conditions fuel fungal growth and aflatoxin contamination of maize. The complex interaction of the biotic and abiotic factors within the grain storage ecosystem determine the severity of the aflatoxin contamination of stored maize (Magan *et al.*, 2010). The primary factors that promote contamination of stored maize by *A. flavus* are high temperature, grain moisture content and relative humidity of the surrounding air (Alborch *et al.*, 2011; Mrema *et al.*, 2011; Tefera, 2012). Freshly harvested maize usually has a moisture content of between 18 % - 25 % which necessitates for rapid drying to reduce the moisture content to below 14 % to prevent fungal growth (Magan and Aldred, 2007). Majority of small-scale farmers in SSA depend on sun-drying. Sun-drying is largely based on the local weather conditions and often does not dry maize adequately or quickly to limit fungal attack (Wagacha *et al.*, 2013; Womack *et al.*, 2014). The prevailing conditions in large parts of SSA predispose the stored maize to attack by *A. flavus*, contaminating it with aflatoxins.

A. flavus is a mesophilic fungus that thrives in the temperature range of 10 ^{0}C – 43 ^{0}C and relative humidity levels greater than 85 % (Al-Shikli *et al.,* 2010; Giorni *et al.,* 2012). The traditional storage methods used by maize farmers in SSA do not offer environmental control of the storage environment. This lack of control exposes the stored maize to conditions that promote the growth

of fungi and mycotoxin production (Ngamo *et al.*, 2007; Giorni *et al.*, 2012). Inadequate ventilation that characterises most of the storage structures used by the resource-poor small-scale farmers leads to moist hot spots that exacerbate *A. flavus* and aflatoxin contamination.

Studies have established how moisture content and temperature (Giorni *et al.*, 2012), as well as relative humidity and temperature (Al-Shikli *et al.*, 2010; Pratiwi *et al.*, 2015), affect *A. flavus* growth and aflatoxin production. This study investigated the combined effect of grain moisture content and the ambient storage conditions, viz., temperature and relative humidity, on the aflatoxin contamination.

3.2 Materials and Methods

3.2.1 Inoculum preparation

A. flavus fungal strain was obtained from the Department of Plant Pathology, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, South Africa. The fungus was plated on potato dextrose agar (Merk, Darmstadt, Germany) at 25 °C for five days after which the conidia was harvested by flooding a single culture with distilled water and scraping the surface mycelia with a sterile scraper. The resulting suspension was filtered through a cheesecloth to obtain pure spore suspension. The spore suspension was then adjusted to 4×10^6 cells.ml^{-1} using a Neubauer hemocytometer to make the inoculum (Hruska *et al.*, 2015).

3.2.2 Preparation of maize samples

White maize variety SC411 was obtained from the Seed Co Pty Ltd (South Africa). The initial moisture content of the maize was 12.19 % ± 0.10 (w.b). The maize kernels were surface sterilised by immersing in 5 % (v/v) sodium hypochlorite (NaClO) and stirred for one minute then rinsed twice with distilled water (Reese *et al.*, 2011). The maize kernels were soaked in distilled water for a predetermined period of time to adjust the moisture content to five different levels, viz., 14 %, 15 %, 16 %, 18 % and 20 % (w.b). The rehydrated samples were sealed in Ziploc bags and refrigerated for 3 days at 4 °C. The samples were periodically shaken manually to ensure a uniform moisture distribution within the bags.

3.2.3 Experimental Design and Data Analysis

A completely randomised design with three replicates was employed in the experiment. A three-factor full-factorial design was used with the first two factors at two levels and the third factor at five levels. The factors studied were temperature (20 ^{0}C and 30 ^{0}C); relative humidity (60 % and 90 %) and moisture content (14 %, 15 %, 16 %, 18 %, and 20 %). Figure 3.1 details the treatment structure of the experiment design.

The data obtained from the study was subjected to analysis of variance (ANOVA) at 5 % significance level to determine the effect of the studied storage environmental parameters on maize kernel attack by *A. flavus* and the subsequent aflatoxin contamination. Where a significant ANOVA result was obtained, the mean comparison was done using Duncan's Multiple Range Test. GenStat® 17th Edition (VSN International Ltd, Hemel Hempstead, United Kingdom) was used to effect the statistical data analysis.

3.2.4 Inoculation and incubation of maize

The rehydrated maize kernels were retrieved from the cold storage and allowed to equilibrate to room temperature. 500 g of maize kernels at a moisture content of 14 %, 15 %, 16 %, 18 % and 20 %, were weighed into autoclaved plastic containers. 2 ml of the inoculum was sprinkled on each sample and mixed by hand. The samples were then randomly placed into the climatic test chamber (*CTS GmbH, Hechingen, Germany*). The climatic test chamber was used to regulate both temperature and relative humidity with an error margin of ± 1 °C and ± 5 % respectively. The samples were incubated for 10 days and fungal growth terminated by transferring the samples to a 70 °C forced-air drying oven for 72 hours.

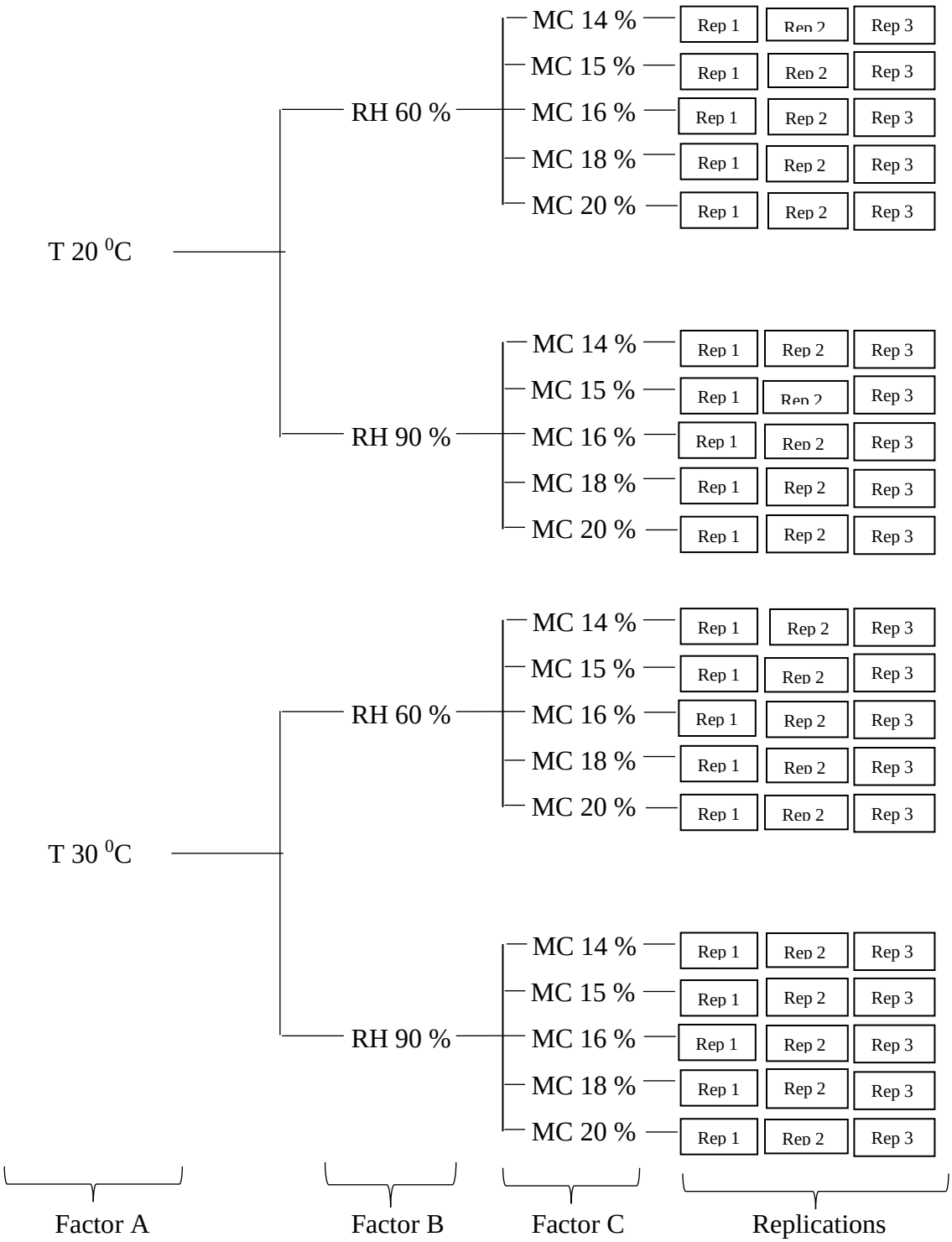

Figure 3.1 Schematic presentation of the experimental treatment structure with three factors (factor A= Temperature (T), factor B= Relative humidity (RH) C= Moisture content (MC) and three replications

3.2.5 Data collection

Sampling was done immediately prior to incubation (day zero) and at the end of incubation period (day ten). The samples were analysed for the presence and level of aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2) and MC.

Aflatoxin analysis was done using a liquid chromatography-tandem mass spectroscopy (LC-MS/MS) as outlined in de Kok *et al*., (2007). The liquid chromatography (LC) had an acquity, ultra-performance liquid chromatography, ethyle bridge hybrid column (UPLC BEH C_{18} 1.7 µm; 2.1×100 mm column). The mobile phase A and mobile phase B was 0.1 % formic acid in water and 0.1 % formic acid in acetonitrile respectively. The mobile phase gradients were as shown in Table 3.1.

Table 3.1 Mobile phase gradients

Time (minutes)	% A	% B
0 (initial)	90	10
3	90	10
10	30	70
10.1	10	90
12	10	90
12.1	90	10
15	90	10

The samples were ground using a retsch rotor mill (*SK 1, Germany*). 25 g of each sample of the ground maize was mixed with 80 ml of acetonitrile and 20 ml of distilled water for 2 hours. The extract was filtered and diluted four-times with distilled water. 20 µL of the diluted extract was fed into the LC-MS/MS. The LC flow rate was 0.4 $ml.min^{-1}$. The eluent from the LC column was directed to the mass spectrometer. The electrospray source was operated in a positive ionisation multiple reaction monitoring (MRM) mode (Table 4.2). The data acquired was analysed using Waters Masslynx^{TM} software. The limit of detection for the LC/MS/MS was 0.5 $\mu g.kg^{-1}$, whereas the quantification limit was 2 $\mu g.kg^{-1}$.

Table 3.2 MRM transition monitored for each type of aflatoxin

	Parent Ion (m/z)	Product Ion (m/z)	Con Voltage (v)	Collision voltage (v)
Aflatoxin B1	313	241	50	37
	313	285	50	23
Aflatoxin B2	315	259	50	30
	315	287	50	26
Aflatoxin G1	329	243	40	25
	329	283	40	25
Aflatoxin G2	331	245	50	30
	331	257	50	30

Authentic standards for AFB1, AFB2, AFG1, and AFG2 obtained from Sigma-Aldrich (Carlsbad, California, USA), were used to produce reference chromatogram for the four types of aflatoxins (Appendix A), as well as the standard calibration curves from which the aflatoxin content of the test samples was determined by interpolation.

3.3 Results

3.3.1 Rehydration of maize

The maize rehydration curve (Figure 3.2) was developed from the soaking experiment. The curve was used to determine the length of soaking time needed to obtain the desired MC. There was rapid moisture absorption by the grains from 0 minutes to 30 minutes after which there was very slow moisture absorption till 90 minutes. The near linear plot between 90 minutes and 360 minutes indicate a constant rate of moisture absorption at these time intervals.

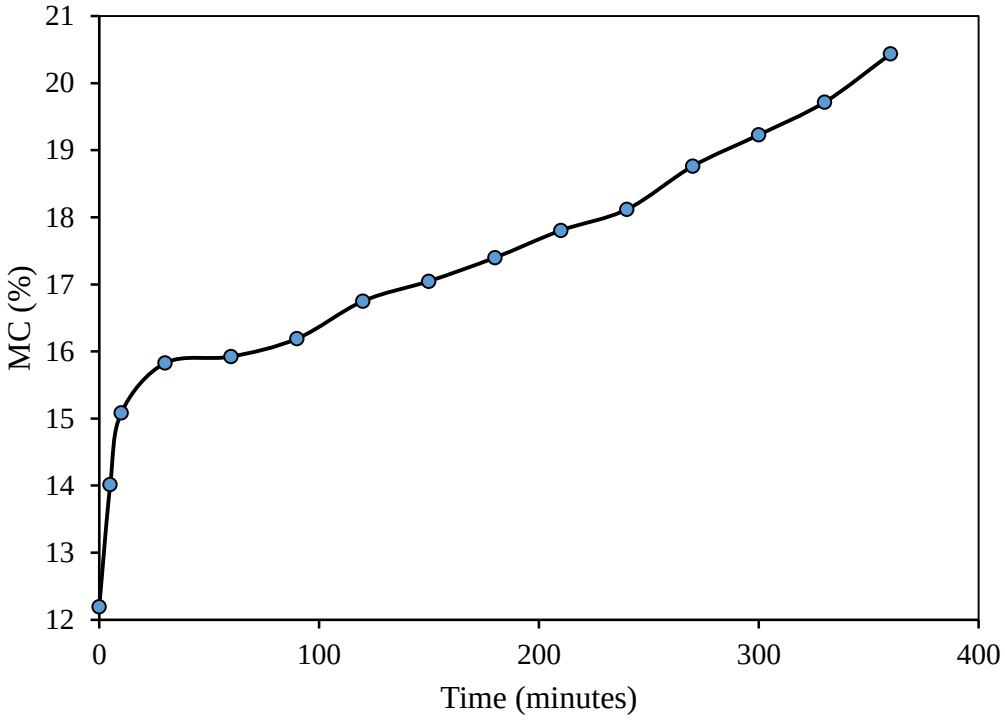

Figure 3.2 Maize rehydration curve

3.3.2 Effect of temperature, relative humidity, and moisture content on aflatoxin contamination of maize

Fungal growth was evident from visual examination at all the MC levels (Figure 3.3) for samples stored at 30 ^{0}C and 90 % RH. Aflatoxin levels were, therefore, highest at these conditions ranging from 4998.97 μg.kg^{-1} – 11179.67 μg.kg^{-1} for AFB1, 451.39 μg.kg^{-1} – 1404.27 μg.kg^{-1} for AFB2, 32030.47 μg.kg^{-1} – 53630 μg.kg^{-1} for AFG1, and 2043.13 μg.kg^{-1} – 5826.46 μg.kg^{-1} for AFG2. Samples for all the other T and RH combination showed no fungal growth from visual examination despite being contaminated with aflatoxin (Figure 3.4).

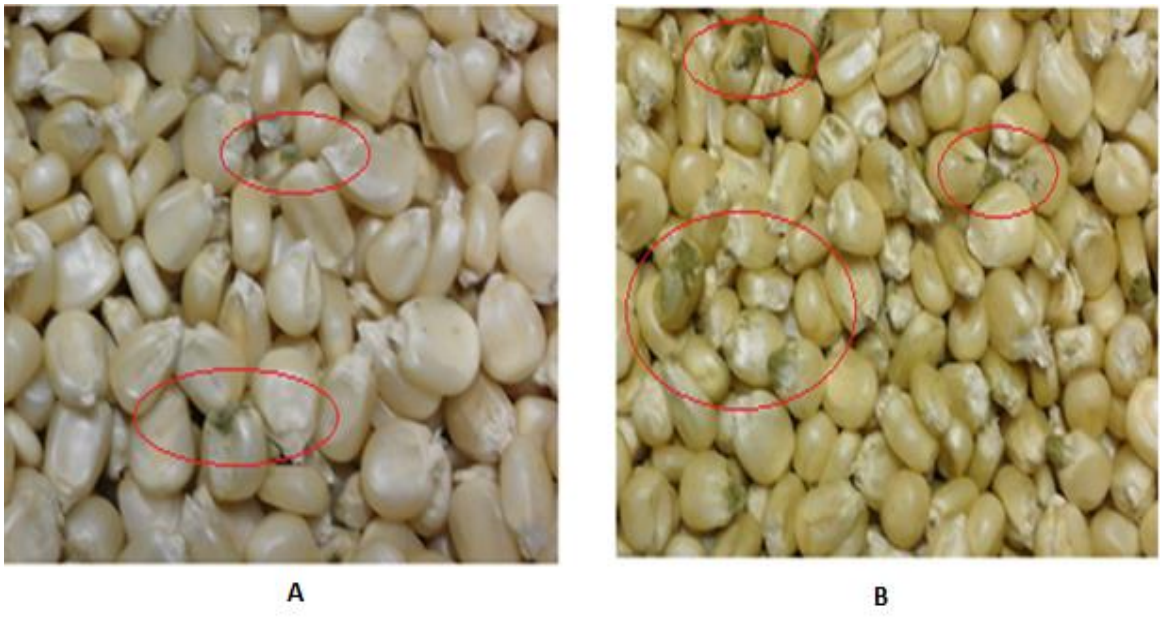

Figure 3.3 *A. flavus* growing on the surface of maize samples circled in red (A: MC = 14 %, T = 30 ^{0}C, RH = 90 %; B: MC = 20 %, T = 30 ^{0}C, RH = 90 %)

Figure 3.4 Maize samples with no sign of *A. flavus* growth (A: MC = 20 %, T= 20 ^{0}C, RH = 90 %; B: MC = 20 %, T= 30 ^{0}C, RH = 60 %; C: MC = 20 %, T = 20 ^{0}C, RH = 60 %)

AFB1 was detected in all treatments. AFG1 levels were the highest in all treatments except at MC levels of 15 %, 16 % and 18 % at 30 ^{0}C and 60 % RH where it was not detected. AFB2 levels were the lowest across all treatments. AFB1, AFB2 and AFG2 had low/undetectable (nd) levels at 60 % RH across all T and MC levels (undetectable – 2.81 μg.kg^{-1}). AFG1 showed relatively higher levels of between 11.16 μg.kg^{-1} - 17.08 μg.kg^{-1} at 20 ^{0}C and 60 % RH. Both AFB2 and AFG2 were not detected at 30 ^{0}C and 60 % RH at all MC levels. Higher aflatoxin levels were observed at 90 % RH although AFB2 was not detected at 14 % and 15 % MC whereas AFG1 was not detected at 14 % MC, all at 20 ^{0}C. The mean aflatoxin contamination levels for all the treatments is shown in Table 3.3.

Table 3.3 Mean aflatoxin contamination levels for all treatments (μg.kg^{-1})

T (^{0}C)	RH (%)	Type of Aflatoxin	MC (%)				
			14.0	15.0	16.0	18.0	20.0
20	60	AFB1	0.8	1.23	1.17	1.53	1.9
30	60		0.3	1.07	2.13	2.1	2.43
20	90		3.93	37.3	80.1	400.53	733.7
30	90		4998.97	7540.0	8338.23	11013.9	11179.67
20	60	AFB2	nd	0.12	0.66	2.81	0.14
30	60		nd	nd	nd	nd	nd
20	90		nd	nd	2.21	14.08	30.01
30	90		451.39	798.02	852.89	1031.51	1404.27
20	60	AFG1	12.88	11.16	14.17	17.08	16.23
30	60		1.49	nd	nd	nd	20.74
20	90		36.91	384.69	1180.8	7520.91	7739.77
30	90		32030.47	47459.79	50247.94	46588.12	53690.78
20	60	AFG2	nd	nd	1.59	nd	0.6
30	60		nd	nd	nd	nd	nd
20	90		nd	1.75	13.06	26.35	45.22
30	90		2043.13	3444.17	4075.16	5172.18	5826.46

*nd indicates not detected

The storage temperature significantly ($p \leq 0.05$) affected aflatoxin production in maize at 60 % and 90 % RH. Aflatoxin levels were greater at 30 ^{0}C than at 20 ^{0}C particularly at 90 % RH (Figure 3.5).

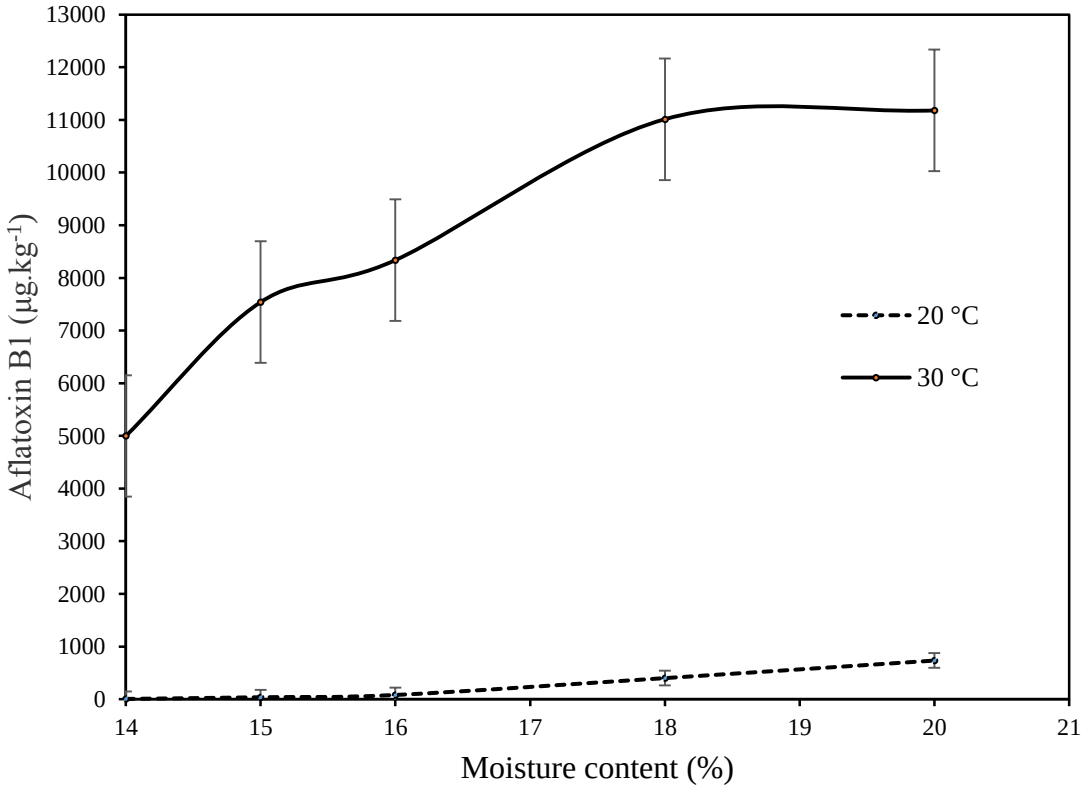

Figure 3.5 Aflatoxin levels at 90 % RH at both 20 °C and 30 °C (LSDp<0.05 = 2634.891, CV = 23.6)

Aflatoxin production was also significantly ($p \leq 0.05$) affected by relative humidity. Aflatoxin levels were higher at 90 % RH (3.9 µg.kg^{-1} – 11179.7 µg.kg^{-1}) than at 60 % RH (0.3 µg.kg^{-1} – 2.4 µg.kg^{-1}). At 60 % RH, 30 ^{0}C still resulted in higher levels of aflatoxin than 20 ^{0}C except at a MC of 14 % and 15 % as shown in Figure 3.6.

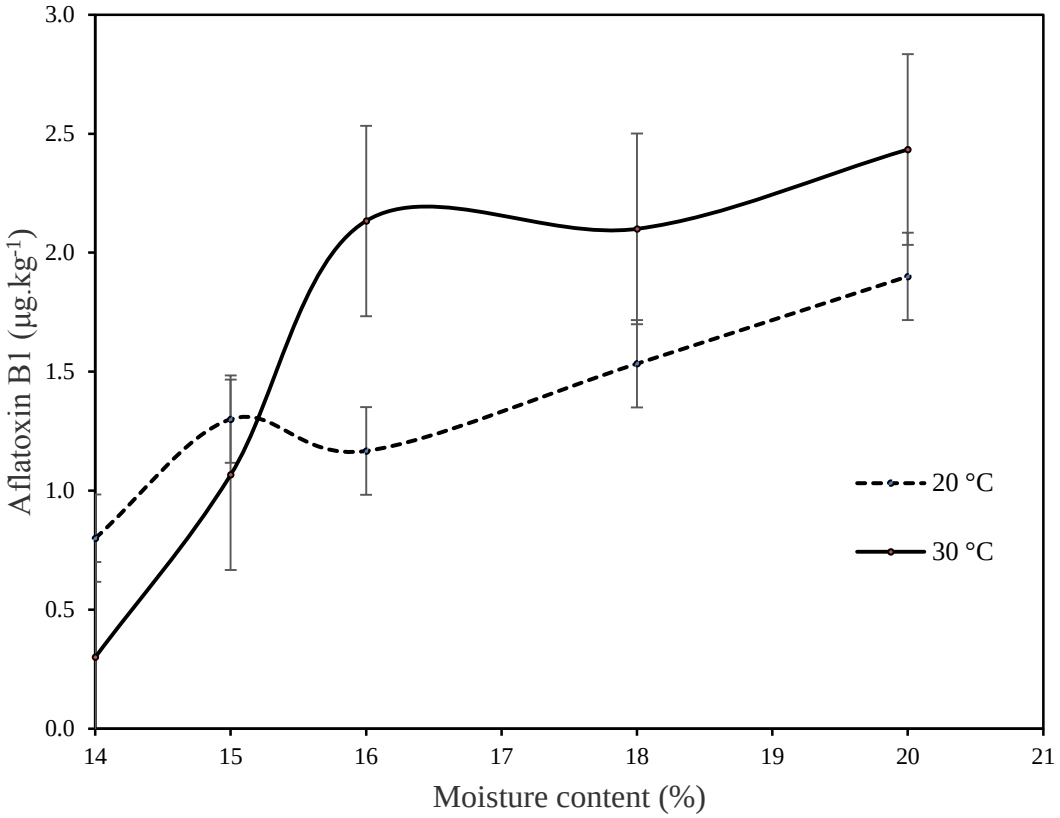

Figure 3.6 Aflatoxin levels at 60 % RH at both 20 °C and 30 °C (LSDp<0.05 = 2634.891, CV = 23.6)

MC was the only experimental factor that did not significantly ($p > 0.05$) affect the levels of aflatoxin contamination in the maize kernels. T and RH regulated the MC of the maize kernels setting up an equilibrium moisture content (EMC). The EMC at the end of day ten was; 10.23 %, 11.42 %, 14.12 % and 15.98 % for the samples stored at 20 °C and 60 %; 30 °C and 60 %; 20 °C and 90 % and 30 °C and 90 % respectively. High EMC corresponded to high levels of aflatoxin and vice versa. The interaction between T and RH significantly ($p \leq 0.05$) influenced the level of aflatoxin contamination in the maize kernels. However, the interaction between other factors such as; T × MC, RH × MC and T × RH × MC, had no significant ($p > 0.05$) effect on the level of aflatoxin contamination. The level of significance of various factors, as well as their interaction, is shown in Table 3.4.

There was no significant difference in the mean aflatoxin levels for all treatments, apart from those treatments at 30 °C and 90 % RH. However, at 30 °C and 90 % RH, the difference between

the means at MC of 18 % and 20 % were also not significant (Table 3.4). The same phenomenon is exemplified by the overlapping error bars at these treatments as shown in Figure 3.5.

Table 3.4 Mean AFB1 levels and the significance level for each experimental factor

T °C	RH %	MC %				
		14	15	16	18	20
20	60	0.8^{d}	1.23^{d}	1.17^{d}	1.53^{d}	1.9^{d}
30	60	0.3^{d}	1.07^{d}	2.13^{d}	2.1^{d}	2.43^{d}
20	90	3.93^{d}	37.3^{d}	80.1^{d}	400.53^{d}	733.7^{d}
30	90	4998.97^{c}	7540.0^{bc}	8338.23^{b}	11013.9^{a}	11179.67^{a}
Significance levels						
T		≤0.05				
RH		≤0.05				
MC		≥0.05				
T × RH		≤0.05				
T × MC		≥0.05				
RH × MC		≥0.05				
T × RH × MC		≥0.05				

Means within a row followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$).

3.4 Discussion

Environmental factors such as temperature and relative humidity influence the growth of *A. flavus* and aflatoxin production (Alborch *et al.*, 2011; Giorni *et al.*, 2012). Aflatoxin production in maize was detectable at 20 °C and 30 °C at 90 % RH, with maximum aflatoxin levels observed at 30 °C and 90 % RH. The results are in agreement with the findings of Das *et al.* (2012) and Al-Shikli et al. (2010). These previous studies reported that *A. flavus* grew well at 30 °C and relative humidity values greater than 85 %.

Aflatoxin contamination of maize was pronounced at 90 % RH compared to 60 % RH implying that RH significantly ($p \leq 0.05$) affects aflatoxin contamination in maize. These results are consistent with the findings by Cotty and Jaime-Garcia (2007) and Kusumaningrum *et al.* (2010). A 90 % RH provides sufficient water activity for the growth of *A. flavus* which then attacks the

maize kernels. The result of this attack is the production of aflatoxins. At 60 % RH, the water activity is below 0.65, which is the minimum water activity level necessary for microbial or fungal growth (Giorni *et al.*, 2012). The growth of *A. flavus* is, therefore, impeded at 60 % RH and the low aflatoxin content observed could have been caused by the high initial moisture content before the EMC is reached.

MC was found to have no significant effect on aflatoxin contamination of maize. T and RH influence EMC of the grain (Al-Shikli *et al*. 2010; Cotty and Jaime-Garcia 2007). Consequently, the interaction between T and RH significantly ($p \leq 0.05$) affected the level of aflatoxin in maize. The EMC for the maize kernels at 90 % RH was 15.98 % and 14.12 % for 30 °C and 20 °C respectively. High RH within the grain storage encourages moisture absorption by the grains, resulting in elevated levels of aflatoxin as reported by Kaaya and Kyamuhangire (2006) and Atehnkeng *et al.* (2008). At 60 % RH, the EMC was 10.23 % at 20 °C and 11.42 % at 30 °C. At such low EMC level, the fungal growth was limited resulting in low levels of aflatoxin recorded in this study.

The low levels of aflatoxin in maize kernels samples stored at 60 % RH at both 20 °C and 30 °C suggest that these conditions can be used to store maize without severe aflatoxin contamination occurring. AFB1 contamination at 60 % RH was below 5 $\mu g.kg^{-1}$ (0.3 $\mu g.kg^{-1}$ – 2.4 $\mu g.kg^{-1}$), which is the acceptable limit for AFB1 residue in food in South Africa, Kenya, Tanzania and Malawi (Kimanya *et al.*, 2008; Rheeder *et al.*, 2009; Kilonzo *et al.*, 2014). AFB1 contamination at 60 % RH was way below the international standard of 15 $\mu g.kg^{-1}$, set by the Codex Alimentarius Commision, and 20 $\mu g.kg^{-1}$ set by the United States Department of Agriculture (USDA) (Wu 2006).

Some techniques for reducing and controlling the moisture content of grain in storage have been reported. Mixing food grade super absorbent polymers (SAPs) with grain can help lower the MC of the grain (Mbuge *et al.,* 2016). Desiccants such as silica gel, quick lime, calcium chloride and zeolite seed drying beads have also been used in drying seeds for storage (Kiburi *et al.*, 2014). Both SAPs and desiccants absorb moisture from the air thus lowering the RH, and consequently, the EMC of the grains. Incorporating such techniques in storage structures can help lower the RH and MC of the grains within the storage structure. Low relative humidity below 70 % creates

a dry environment that stifles the growth of *A. flavus* and consequently inhibits aflatoxin production (Pratiwi *et al.*, 2015).

3.5 Conclusion

Aflatoxin contamination of maize is associated with inadequate post-harvest and storage practices. Typical traditional storage techniques used by small-scale farmers offer little or no control of the storage environment, thus no protection against factors that promote aflatoxin contamination. Aflatoxin contamination of maize can be aggravated at high relative humidity (90 %) at typical ambient conditions. Maize kernels stored at these conditions have extremely high levels of aflatoxin and are, therefore, not suitable for human or animal consumption. This study has shown that maize kernels stored at a relative humidity of 60 % had AFB1 levels below 5 $\mu g.kg^{-1}$, hence are safe for human consumption. Controlling aflatoxin contamination of maize during storage necessitates the development of simple storage facilities that can maintain the level of relative humidity below 60 %. This study, therefore, recommends research into appropriate technologies that can be used to regulate the relative humidity in storage structures such as the inclusion of super absorbent polymers and desiccants in the design such structures.

3.6 Reference

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4 DETERIORATIVE CHANGES IN MAIZE KERNELS DUE TO ASPERGILLUS FLAVUS

Abstract

A. flavus is a common pathogenic fungus that attacks maize leading to aflatoxin contamination and associated quality deterioration. The objective of this study was to establish the changes in chemical composition of maize kernels due to *A. flavus* infection and their relationship with aflatoxin production. The experiment was arranged as a 3 × 5 factorial design. The moisture content of maize kernels was adjusted to 17 % (wb) and inoculated with three different inocula, *viz.* distilled water, *A. flavus*, and *Fusarium verticillioides* (*F verticillioides*). The inoculated samples of maize kernels were incubated at 28 ^{0}C for 7, 14, 21, and 28 days. Sampling was done prior to incubation (day 0) and at the end of every incubation period. The samples were thereafter analysed for aflatoxin contamination, moisture content, crude fat, crude protein, carbohydrates, ash, and crude fibre. There was no change in the chemical composition of maize kernels inoculated with distilled water. Maize kernels inoculated with *A. flavus* and *F verticillioides* exhibited a significant decrease in fat and carbohydrate content and a marginal decrease in protein content. Aflatoxin B1 (AFB1) and fumonisin B1 (FB1) contamination levels increased in maize kernels inoculated with *A. flavus* and *F. verticillioides* respectively. Both ash and fibre content showed no changes across all treatments. The length of time of incubation and the inoculum had significant ($p \leq 0.05$) effect on AFB1 and FB1 contamination levels, moisture content, fat, protein and carbohydrate. Aflatoxin contamination was highly correlated to fat and carbohydrate degradation. Similar changes in fat and carbohydrate were also observed in maize kernels inoculated with *F. verticillioides* causing FB1 contamination. In conclusion, changes in chemical composition of maize kernels is a good indicator of the kernels' fungal degradation. However, such variations in the chemical composition cannot explicitly indicate *A. flavus* or aflatoxin contamination in maize kernels.

Keywords: *A. flavus*, aflatoxin, *F verticillioides*, fumonisin, maize kernels

4.1 Introduction

Maize is highly susceptible to *A. flavus* attack resulting in quality deterioration (Begum et al., 2013). Fungal development can cause a considerable modification in the chemical composition of stored grains (Kakde and Chavan, 2011). Fungal infection in grains is associated with losses in carbohydrates, proteins and total oil content while increasing moisture content and free fatty acid. The fungi produce hydrolytic enzymes including peroxidase, amylase, pectinases, proteases and lipases. These enzymes degrade biochemical components such as fat, protein, and carbohydrates leading to the loss of the dry matter (Begum *et al.*, 2013). Bhattacharya and Raha (2002), reported a decrease in carbohydrates and fat content in maize and soya beans due to storage fungi. Jain (2008), reported a rapid increase in free fatty acids in damaged grains due to fungal infestation. Embaby and Abdel-Galil (2006) observed a reduction in carbohydrates, reducing sugars and crude fat due to *Fusarium* in legumes. Kakde and Chavan (2011) concluded that *A. flavus* was responsible for the maximum depletion of fat content and reducing sugars in safflower, soya bean and sesame.

A. flavus is a common saprophytic fungus that attacks maize kernels resulting in the production of aflatoxins (Probst *et al.*, 2014). Nutrient composition is a key factor affecting aflatoxin production in maize by *A. flavus* (Ma *et al.*, 2015). The maize kernel provides a good natural substrate for the fungi, leading to aflatoxin contamination (Perrone *et al.*, 2014). Inherent materials in maize kernels such as starch, proteins and lipids represent significant carbon and nitrogen resources potentially available during seed infection by *A. flavus* (Mellon *et al.*, 2002). Saccharides provide the primary carbon source for mycelial growth and AFB1 production (Mellon *et al.*, 2005). Several researchers (Fanelli and Fabbri, 1989; Wilson *et al.*, 2004; Mellon *et al.*, 2005) have reported a relationship between lipid degradation and AFB1 production. Glucose, ribose, xylose, and glycerol are also good substrates for growth and aflatoxin production by *A. flavus* (Liu *et al.*, 2016).

The aim of this study was to evaluate the effect of *A. flavus* infection on the proximate composition of maize kernels. The relationship between the level of aflatoxin contamination and the proximate components of maize kernels was also studied.

4.2 Materials and Methods

4.2.1 Inoculum preparation

A. flavus and *F. verticillioides* fungal strains were obtained from the Department of Plant Pathology, School of Agriculture, Earth and Environmental Sciences, University of KwaZulu-Natal, South Africa. Both *A. flavus* and *F. verticillioides* inocula were prepared as outlined in section 3.3.1. Distilled water was used as the inoculum for the control samples.

4.2.2 Preparation of maize samples

The maize kernels were surface sterilised by immersing the kernels in a 5 % (v/v) sodium hypochlorite (NaClO) solution and stirring for one minute. The maize kernels were thereafter rinsed twice with distilled water. The moisture content of the maize kernels was then adjusted to 17 % (w.b) by soaking samples in distilled water for a duration of 2 hours. The samples were thereafter put in sealed plastic bags and refrigerated at a temperature of 4 ^{0}C for 72 hours to ensure uniform moisture distribution.

4.2.3 Experimental design and Data Analysis

A two-factor full-factorial design was used in this experiment, with the first factor at three levels and the second factor at five levels. The factors studied were inoculum (*A. flavus*, *F. verticillioides* and distilled water) and incubation period (day 0, day 7, 14, 21 and 28). Figure 4.1 details the treatment structure of the experimental design.

The data obtained from the study was subjected to analysis of variance (ANOVA) at 5 % significance level to determine the effect of *A. flavus* and *F. verticillioides* on mycotoxin contamination (aflatoxin and fumonisins), carbohydrates, crude fat, crude fibre, crude protein and moisture content of grains. Where a significant ANOVA result was obtained, the mean comparison was done using Duncan's Multiple Range Test. The analysis was done using GenStat® 17th Edition (VSN International Ltd, Hemel Hempstead, United Kingdom).

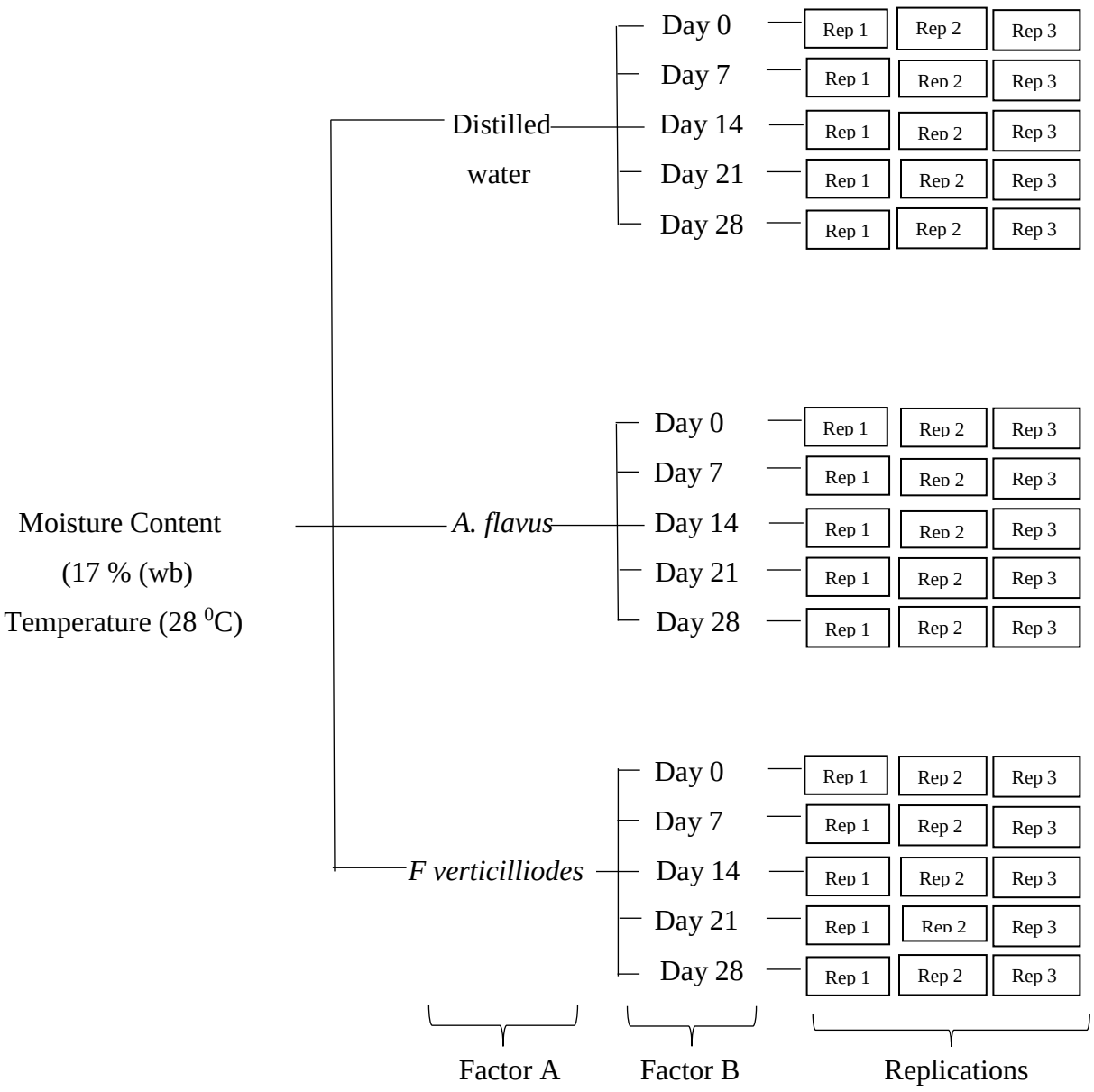

Figure 4.1 Experimental design (factor A= Inoculum, factor B= Incubation period)

4.2.4 Inoculation and incubation of maize

Maize was retrieved from cold storage and allowed to equilibrate to room temperature. A total of 45 samples of maize kernels each of mass 3 kg was weighed into sterilised plastic bag. 5 ml of each inoculum, *A. flavus, F. verticillioides* and distilled water, was sprinkled on the samples and mixed manually before being transferred to the incubator. The samples were incubated at a

temperature of 28 ^{0}C and sampling done on day 0, 7, 14, 21, and day 28. The samples were then analysed for aflatoxin and proximate composition.

4.2.5 Analysis of the chemical composition of maize kernels

AFB1 and FB1 analysis was done using a liquid chromatography-tandem mass spectroscopy (LC-MS/MS) as outlined by de Kok *et al.,* (2007). The liquid chromatography (LC) had an acquity, ultra-performance liquid chromatography, ethyle bridge hybrid column (UPLC BEH C_{18} 1.7 µm; 2.1×100 mm column). The mobile phase A and mobile phase B was 0.1 % formic acid in water and 0.1 % formic acid in acetonitrile respectively. The mobile phase gradients were as shown in Table 4.1.

Table 4.1 Mobile phase gradients

Time (Mins)	% A	% B
Initial	90	10
3	90	10
10	30	70
10.1	10	90
12	10	90
12.1	90	10
15	90	10

The samples were ground using a retsch rotor mill (*SK 1, Germany*). 25 g of each sample of the ground maize was mixed with 80 ml of acetonitrile and 20 ml of water for 2 hours. The extract was filtered and diluted four-times with distilled water. 20 µL of the diluted extract was fed into the LC-MS/MS. The LC flow rate was 0.4 ml.min^{-1}. The eluent from the LC column was directed to the mass spectrometer. The electrospray source was operated in a positive ionisation multiple reaction monitoring (MRM) mode (Table 4.2). The data acquired was analysed using Waters Masslynx^{TM} software. The limit of detection for the LC/MS/MS was 0.5 µg.kg^{-1}, whereas the quantification limit was 2 µg.kg^{-1}.

Table 4.2 MRM transition monitored for each type of aflatoxin

	Parent Ion (m/z)	Product Ion (m/z)	Con Voltage (v)	Collision voltage (v)
Aflatoxin B1	313	241	50	37
	313	285	50	23
Fumonisin B1	722	334	50	40
	722	352	50	40

To determine the moisture content, 10 g of ground maize kernels was placed on an aluminium dish and heated in a forced air oven for 24 hours at 105 ^{0}C. The heated sample was put in a desiccator and allowed to cool then reweighed. The wet basis moisture content was calculated using Equation 4.1.

$$\% \text{ Moisture content} = \frac{\text{Weight loss of sample}}{\text{Weight of the original sample}} \text{ x } 100 \quad (4.1)$$

Ash content was determined by placing 2 g dry ground maize kernels into a dry porcelain dish which was then heated at 600 ^{0}C for 6 hours in a muffle furnace. The sample was thereafter cooled in a desiccator and the final weight recorded. The ash content was calculated as shown in Equation 4.2 (AOAC, 2012a).

$$\% \text{ Ash} = \frac{\text{Weight ash}}{\text{Weight of the original sample}} \text{ x } 100 \quad (4.2)$$

Soxhlet extraction method was used to determine the crude fat content (AOAC, 2012b). 2 g of ground maize kernels was weighed and placed into the Soxhlet extraction thimble. The extraction thimble was plugged with cotton wool and placed in the Soxhlet extractor. 150 ml of petroleum ether was added and extraction done for 16 hours in the Soxhlet apparatus. The flask was thereafter transferred to a steam bath in a hood for 3 hours to evaporate the petroleum ether. This was followed by 1 hour of further drying in a hot air oven at 100 ^{0}C, then cooled in a desiccator and the final weight recorded. The fat content was calculated using Equation 4.3.

$$\% \text{ Fat } = \frac{\text{Weight of fat}}{\text{Weight of the original sample}} \text{ x } 100 \quad (4.3)$$

Khedjel method was used for protein content analysis (Enyisi *et al.*, 2014). 1 g of ground maize kernels was weighed into a digestion flask. 10 g of potassium sulphate (K_2SO_4), 20 cm^3 concentrated sulphuric acid (H_2SO_4), and 0.7 g mercuric oxide (HgO) was then added to the digestion flask and heated gently until foaming subsided. It was then boiled until the solution became clear. The solution was thereafter cooled and 90 ml of distilled water added and mixed. 80 ml of 2 M sodium hydroxide (NaOH) solution was then added. The flask was tilted to form two layer after which the condenser unit was connected and heated. Distilled ammonia was collected in a flask containing 50 ml boric acid/methyl red indicator. 50 ml of the distillate was placed in a buret and titrated against 0.1 M hydrochloric acid solution. Equation 4.4 and Equation 4.5 were used to calculate the crude protein content

$$\% \text{ Nitrogen} = \frac{(\text{Volume of acid x Molarity of standard acid}) \text{x } 0.014}{\text{Weight of the original sample}} \text{ x } 100 \quad (4.4)$$

$$\% \text{ Crude protein content } = \text{ nitrogen content x } 6.25 \quad (4.5)$$

Crude fibre was analysed according to AOAC (2012c). 2 g of the ground maize kernel and 0.5 g asbestos added was put in a 750 ml conical flask after which 200 ml of boiling 1.25 % sulphuric acid (H_2SO_4) was added. The flask was connected to a cold finger condenser and boiled on a hot plate for half an hour. The contents of the flask were thereafter filtered through a funnel laced with a linen cloth and washed with boiling water. The charge and asbestos were put back in the flask. 200 ml of boiling 1.25 % sodium hydroxide (NaOH) solution was added into the flask and connected to a condenser which was then heated for half an hour. The contents were filtered using a linen cloth and washed with boiling. The residue was placed in a Gooch crucible, 15 ml of 95 % ethanol (C_2H_6O) was added and thereafter dried at 100 °C in the oven for 1 hour. The flask was then cooled in a desiccator and its weight recorded, after which it was ignited in a preheated muffle furnace at 600 °C, then cooled again and reweighed. The percent crude fibre content was calculated as shown in Equation 4.6.

$$\% \text{ crude fibre} = \frac{\text{mass of fibre}}{\text{dry mass of sample}} \times 100 \quad (4.6)$$

4.3 Results

The initial proximate composition of the maize kernels was 0 µg.kg^{-1} for AFB1 and FB1, 1.18 % ± 0.01 ash, 3.97 % ± 0.05 crude fat, 4.51 % ± 0.07 crude fibre, 7.94 % ± 0.06 crude protein, and 72.05 % ± 0.27 carbohydrates. In this study, fat, carbohydrates and protein content were observed to decrease with time. AFB1, FB1 and MC increased over the course of the experiment. The ash and fibre content remained fairly stable. A summary of the chemical composition, on a dry matter basis, is presented in Table 4.3.

The MC was significantly ($p \leq 0.05$) affected by incubation period as well as the inoculum (Table 4.3). The MC increased with the length of time of incubation. The highest increase in MC was observed in samples inoculated with *A. flavus*, ranging from 17 % - 22.43 % (wb). The MC of samples inoculated with *F. verticillioides* ranged between 17 % - 20.68 % (wb). The lowest increase in MC was observed in samples inoculated with distilled water, ranging from 17 % - 18.54 % (wb).

There were no mycotoxins detected in the samples before incubation. Mycotoxin contamination was significantly ($p \leq 0.05$) affected by both inoculum and the length of time of incubation. Both AFB1 and FB1 were observed to increase as the length of time under incubation increased. The samples inoculated with distilled water showed no aflatoxin contamination at the end of day seven. 1.4 µg.kg^{-1} of AFB1 was observed on day 14, rising to 21.2 µg.kg^{-1} and 141.1 µg.kg^{-1} on day 21 and day 28 respectively. The maize kernels inoculated with *A. flavus* resulted in AFB1 contamination ranging from 409.1 µg.kg^{-1} on day 7 to 10508.2 µg.kg^{-1} on day 28, while those inoculated with *F. verticillioides* resulted in FB1 contamination ranging from 212.7 µg.kg^{-1} on day 7 to 2447.3 µg.kg^{-1} on day 28 (Table 4.3).

Table 4.3 Variation of chemical composition of maize with inoculum and time

Innoculum	Time (day)	MC (%)	Mycotoxin (µg.kg^{-1})	Ash (%)	Fat (%)	Fibre (%)	Protein (%)	Carb (%)
Distilled water	0	17.00^{g}	nd	1.18^{ab}	3.97^{g}	4.51^{a}	7.94^{f}	72.05^{fg}
Distilled water	7	17.29^{fg}	nd	1.18^{a}	3.98^{g}	4.49^{a}	7.95^{f}	72.18^{g}
Distilled water	14	17.53^{fg}	1.40^{a}	1.19^{ab}	3.92^{g}	4.49^{a}	7.93^{f}	71.92^{fg}
Distilled water	21	17.9^{e}	21.20^{a}	1.18^{a}	3.89^{fg}	4.52^{a}	7.84^{f}	71.91^{fg}
Distilled water	28	18.54^{e}	141.10^{a}	1.19^{a}	3.77^{ef}	4.51^{a}	7.63^{df}	71.04^{f}
A. flavus	0	17.02^{g}	nd	1.19^{ab}	3.97^{g}	4.52^{a}	7.9^{6f}	72.18^{g}
A. flavus	7	18.49^{e}	409.10^{a}	1.21^{b}	3.67^{e}	4.51^{a}	7.53^{cd}	71.4^{fg}
A. flavus	14	19.56^{d}	1259.80^{a}	1.20^{ab}	3.38^{cd}	4.51^{a}	7.43^{bc}	69.26^{e}
A. flavus	21	21.53^{b}	3032.20^{a}	1.24^{c}	3.11^{b}	4.48^{a}	7.33^{b}	66.73^{c}
A. flavus	28	22.43^{a}	10508.20^{b}	1.19^{ab}	1.91^{a}	4.52^{a}	7.17^{a}	60.94^{a}
F verticillioides	0	17.00^{g}	nd	1.19^{ab}	3.94^{g}	4.50^{a}	7.94^{f}	71.95^{fg}
F verticillioides	7	17.86^{f}	212.70^{a}	1.19^{ab}	3.68^{e}	4.50^{a}	7.71^{e}	71.39^{fg}
F verticillioides	14	18.36^{ef}	604.70^{a}	1.20^{ab}	3.52^{d}	4.52^{a}	7.50^{c}	69.41^{e}
F verticillioides	21	20.19^{cd}	1240.00^{a}	1.19^{ab}	3.37^{c}	4.51^{a}	7.42^{bc}	67.86^{d}
F verticillioides	28	20.68^{c}	2447.30^{a}	1.19^{ab}	3.18^{b}	4.50^{a}	7.39^{b}	64.77^{b}
Significance Level								
Inoculum			<.001	0.082	<.001	0.677	<.001	<.001
Time			<.001	0.349	<.001	0.991	<.001	<.001
Inoculum × Time			<.001	0.061	<.001	0.241	<.001	<.001
CV			0.433	0.001	0.017	0.002	0.004	0.002
SE			994.27	0.009	0.048	0.014	0.036	0.33
LSD $_{(p \leq 0.05)}$			2880.3	0.026	0.139	0.04	0.104	0.956

Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test (P<0.05).
nd = not detected

The fat content of maize kernels before incubation was 3.97 % ± 0.03. The fat content decreased with increase in the length of time of incubation across all treatment, except for the maize kernels inoculated with distilled water (Figure 4.2). Both the type of inoculum and the length of time of incubation significantly ($p \leq 0.05$) affected the fat content. The greatest reduction in fat content was observed in the maize kernel samples inoculated with *A. flavus.* The fat content ranged from 3.97 % on day zero to 1.91 % on day 28. The fat content for samples inoculated with *F. verticillioides* ranged from 3.94 % - 3.18 %, whereas that of samples inoculated with distilled water ranged from 3.97 % - 3.77 % (Table 4.3). The mean fat content of maize kernels inoculated with *A. flavus* and *F. verticillioides* were significantly different across all treatments. However,

there was no significant difference in the mean fat content of those treatments that resulted in approximately equal AFB1 and FB1 contamination.

There was a decrease in protein content as the length of incubation time increased (Figure 4.1). Both the inoculum type and the length of time of incubation significantly affected ($p \leq 0.05$) the protein content of all maize kernel samples across treatments. The protein content of the maize seeds inoculated with *A. flavus* decreased from 7.96 % - 7.17 % over the 28 days of incubation. In the samples inoculated with *F. verticillioides*, the protein content reduced from 7.94 % - 7.39 %. Duncan's Multiple Range Test showed significant differences between the mean protein content from all treatments in maize kernels inoculated with *A. flavus* and *F. verticillioides* (Table 4.3). The protein content of the maize samples inoculated with distilled water ranged between 7.94 % - 7.63 %. However, there was no significant difference in the mean protein content across treatments.

Carbohydrates showed the highest change amongst all the proximate components of maize kernel samples. The inoculum and the length of time of incubation significantly ($p \leq 0.05$) influenced the carbohydrate content of the maize kernels. The carbohydrate content decreased with increase in the length of time of incubation across all treatments (Figure 4.2 (d)). The greatest reduction in carbohydrate content was observed in samples inoculated with *A. flavus* ranging from 72.18 % - 60.94 %. The carbohydrate content for samples inoculated with *F. verticillioides* was in the 71.95 % - 64.77 % range (Table 4.3).

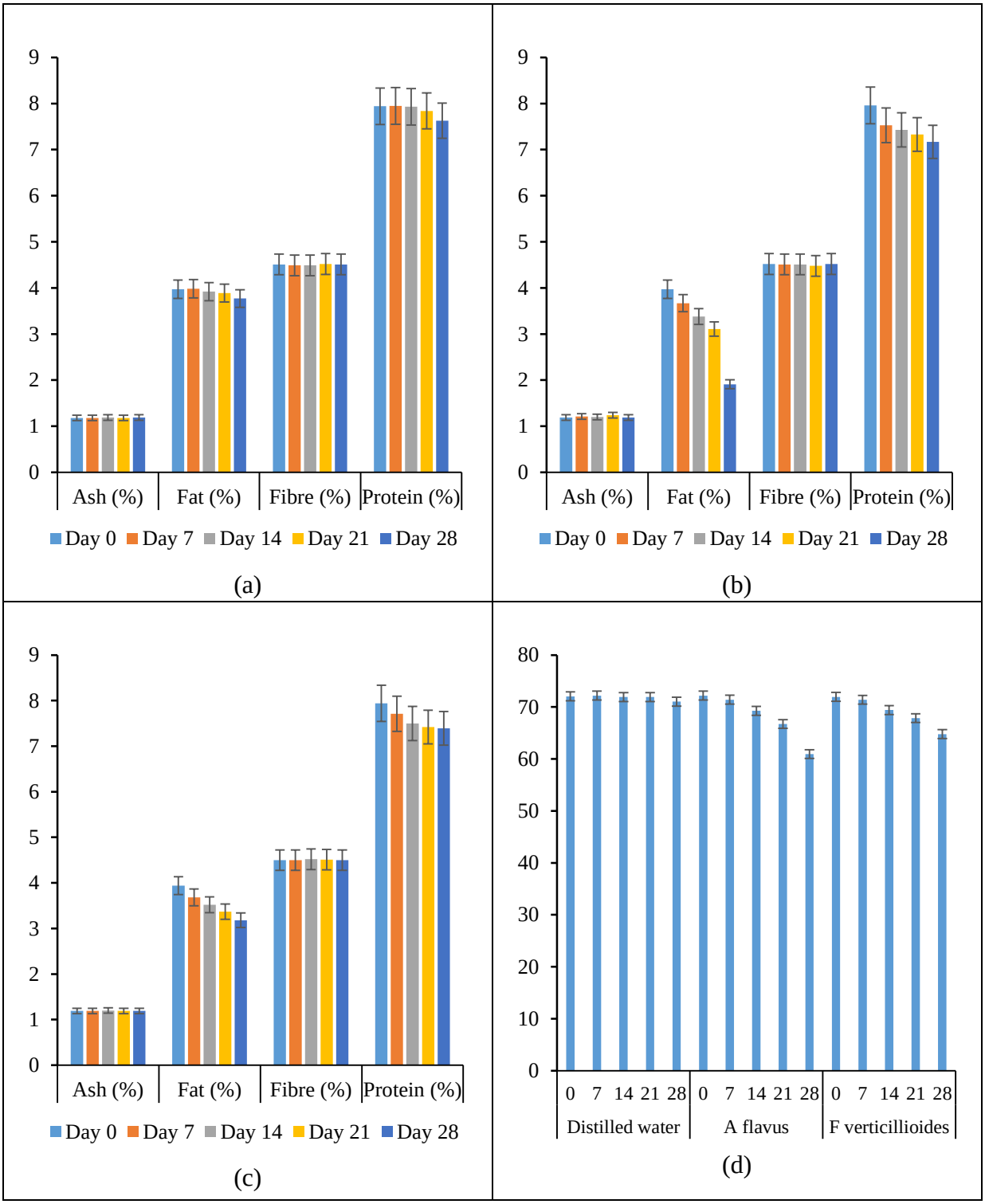

Figure 4.2 Proximate composition of maize kernels inoculated with (a) distilled water, (b) *A. flavus* and (c) *F. verticilliodes*. (d) Carbohydrate content of maize kernels inoculated with distilled water, *A. flavus*, and *F. verticillioides*

The mean carbohydrate content across treatments in maize kernel samples inoculated with *A. flavus* and *F. verticillioides* were significantly different. Maize kernel samples inoculated with distilled water showed the least reduction in carbohydrate content that was in the range of 72.05 % - 71.04 %. There was, however, no significant difference in the mean carbohydrate content across treatments in the maize kernels inoculated with distilled water.

The crude fibre content of the maize kernel samples was relatively stable across all treatments (Figure 4.2). The type of inoculum and the incubation time had no significant effect ($p > 0.05$) on the crude fibre content of the maize kernels. The fibre content of the samples ranged between 4.48 % – 4.52 % (Table 4.3).

There was negligible difference in the ash content of the maize kernel samples across all treatments (Figure 4.2). Both the inoculum and the length of time of incubation had no significant effect ($p > 0.05$) on the ash content of maize. The ash content of the maize ranged between 1.18 – 1.24 % (Table 4.1).

A strong correlation was observed between aflatoxin contamination and fat ($R^2 = 0.92$) and carbohydrate ($R^2 = 0.82$). However, there was a weak correlation between aflatoxin contamination and protein content ($R^2 = 0.50$). No correlation was observed between aflatoxin contamination and either ash or fibre content ($R^2 = 0.03$ and $R^2 = 0.02$, respectively).

4.4 Discussion

The MC of the maize kernels increased during incubation. These findings are similar to those reported by Islam (2016) on stored black gram. The increase in moisture content is attributed to respiration by both the maize kernels and the fungi (Magan *et al.*, 2004). The population of fungi increased with time as evidenced by the progressive increase in AFB1 and FB1. The increased fungal population escalated the respiration of fungi, hence high MC on day 28 compared to the minimal change in MC at the start of the experiment. The increase in MC was higher for maize kernels inoculated with *A. flavus* compared to *F. verticillioides*. The incubation temperature of

28 ^{0}C was optimal for the growth of *A. flavus* (Pratiwi *et al.*, 2015) but unfavourable for *F. verticillioides* which thrive at a temperature of 25 ^{0}C (Garcia *et al.*, 2012).

The AFB1 and FB1 contamination increased over time because of the increase in fungal population. There was a high AFB1 (409.1 µg.kg^{-1} - 10508.2 µg.kg^{-1}) contamination as compared to FB1 (212.7 µg.kg^{-1} - 2447.3 µg.kg^{-1}). *A. flavus* grows faster than *F. verticillioides* at the incubation temperature of 28 ^{0}C (Garcia *et al.*, 2012; Pratiwi *et al.*, 2015). Hence the higher levels of AFB1 compared to FB1. AFB1 contamination observed in the maize kernels inoculated with distilled water could have been caused by internal infection (Mellon *et al.*, 2007). It is important to note that the aflatoxin content of maize kernels inoculated with distilled water exceeded the acceptable limit for AFB1 (5 µg.kg^{-1}) allowed in food in South Africa, Kenya, Tanzania and Malawi (Kimanya *et al.*, 2008; Rheeder *et al.*, 2009; Kilonzo *et al.*, 2014).

The greatest depletion of fats occurred in maize kernels inoculated with *A. flavus*. This observation is consistent with the findings by Kakde and Chavan (2011) who reported that *A. flavus* was responsible for the maximum depletion of fat content in cereals and oilseeds. Embaby and Abdel-Galil (2006), also observed a reduction in crude fat content in legume seeds due to *Fusarium*. The decrease in crude fat content was due to the production of lipases, by both *A. flavus* and *F. verticillioides*. Lipases hydrolyse fats into fatty acids. Magan *et al.,* (2004) stated that an increase in fatty acids is an indicator of fungal degradation of maize. The increase in free fatty acids observed in this study is thus an indicator of fungal deterioration of the maize kernels.

The decrease in protein content is in agreement with the findings of Reed *et al.* (2007) that associated changes in the protein content of maize to fungal degradation. The depletion of protein is attributed to its utilisation during the growth and metabolism of fungi (Bhattacharya and Raha, 2002). Liu *et al.*, (2016) reported that amino acids such as glutamic acid, aspartic acid and arginine significantly promote AFB1 production by *A. flavus* indicating protein utilisation. Even though, Mellon *et al.* (2007), reported that *A. flavus* does not favour the use of proteins as carbon substrates, results from this study are in tandem with previous research findings (Bhattacharya and Raha, 2002; Reed *et al.*, 2007; Liu *et al.*, 2016) that associated protein depletion with fungal deterioration.

The decrease in carbohydrate content observed in this study is similar to previous research findings (Bhattacharya and Raha, 2002; Verma *et al.*, 2003; Embaby and Abdel-Galil, 2006; Rehman *et al.*, 2011; Chattha *et al.*, 2015). Carbohydrates are typically hydrolysed during respiration by both the maize kernel itself and fungi leading to their depletion (Magan *et al.*, 2004). The production of amylases by both *A. flavus* and *F. verticillioides* breaks down carbohydrates into simpler sugars for use during respiration. Soluble sugars such as glucose, sucrose and maltose have been positively correlated with aflatoxin production (Liu *et al.*, 2016).

Hydrolytic enzymes produced by *A. flavus* break down fats and carbohydrates for use in fungal growth and development, which in turn promotes the production of aflatoxins (Liu et al., 2016). This explains the strong correlation observed between carbohydrates and fats with aflatoxin contamination. Proteins are only utilised as carbon substrates in the absence of fats and carbohydrates hence their weak correlation with aflatoxin contamination (Mellon *et al.*, 2007). Carbohydrates and fats are the primary source of nutrients for fungal growth. The depletion of carbohydrates and fats correspond with increased fungal population, thus higher levels of aflatoxin contamination.

4.5 Conclusion

The results of this study affirm that changes in the chemical composition of maize are a good sign of fungal contamination. Both *A. flavus* and *F. verticillioides* caused significant ($p \leq 0.05$) variation in the carbohydrate, fat and protein content of maize kernels. Maize kernel samples that recorded approximately equal AFB1 or FB1 levels showed no significant ($p \geq 0.05$) difference in their chemical components (carbohydrates, fats and proteins). Therefore, it is not possible to identify the fungal strain responsible for maize degradation through chemical composition analysis. Although aflatoxin contamination was highly correlated to the depletion of carbohydrates and fats, such changes can also be caused by *F. verticillioides* which cause FB1. In addition, these in proximate composition manifested at high levels of mycotoxin contamination observed from day 7 to day 28. These high levels of AFB1 or FB1 are toxic. Consequently, changes in proximate composition especially fats and carbohydrates can only be used as an indicator of fungal contamination of maize kernels, but not as a way of determining aflatoxin contamination.

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5 ELECTRICAL PROPERTIES OF MAIZE KERNELS CONTAMINATED WITH AFLATOXIN

Abstract

Aflatoxin contamination of maize kernels is a serious health concern. Unfortunately, most of the existing aflatoxin detection methods are expensive and laboratory-based. Thus, alternative and inexpensive aflatoxin detection approaches are required. Dielectric spectroscopy is a technique that has been used to detect grain moisture content and bulk density rapidly. Therefore, it was hypothesised that dielectric spectroscopy is a possible technique for rapid detection of the presence of aflatoxin contamination in maize kernels. The objective of this study was to investigate the effect of aflatoxin contamination on the dielectric constant of maize kernels. A factorial experiment consisting of three levels of moisture content (13.3 %, 15.3 %, and 16.4 %), three frequencies (25 kHz, 50 kHz, and 100 kHz), and nine levels of aflatoxin (0 $\mu g.kg^{-1}$, 1.5 $\mu g.kg^{-1}$, 2.6 $\mu g.kg^{-1}$, 10 $\mu g.kg^{-1}$, 50 $\mu g.kg^{-1}$, 100 $\mu g.kg^{-1}$, 150 $\mu g.kg^{-1}$, 172 $\mu g.kg^{-1}$, and 230 $\mu g.kg^{-1}$), was employed. The maize kernels were poured into a custom-built sample holder comprising a shielded parallel plate capacitor. The capacitance measurements were done at a constant room temperature of 24 ^{0}C, using an ISO-TECH LCR-821 meter. The capacitance values obtained was used to compute the dielectric constant of the maize kernels. The results indicated that moisture content and frequency of the applied electric field significantly ($p \leq 0.05$) affected the dielectric constant. The dielectric constant increased with moisture content and decreased with increasing frequency. Aflatoxin contamination level had no significant ($p > 0.05$) effect on the dielectric constant of maize kernels. The coefficient of determination (R^2) of dielectric constant and aflatoxin contamination levels was low ($R^2 = 0.2687$), which indicate a lack of correlation between the aflatoxin levels and dielectric constant of the maize kernels. Based on the findings, the dielectric constant is unsuitable for predicting the level of aflatoxin contamination in maize kernels.

Keywords: Aflatoxin, capacitance, dielectric constant, maize kernels

5.1 Introduction

Aflatoxin is a highly potent carcinogen and has severe health impacts in humans and animals (Wu & Guclu, 2012). Maize, being an important staple for a vast majority of people in Sub-Saharan Africa (Wariboko et al., 2014), is a principal source of human exposure to aflatoxin (Strosnider *et al.*, 2006; Liu & Wu, 2010). Many nations have, therefore, set regulatory limits on the level of aflatoxin allowed in food, hence the need for aflatoxin detection and quantification methods (Wu & Guclu, 2012; Gnonlonfin *et al.*, 2013).

Screening and analytical methods have been developed to ascertain the levels of aflatoxin in maize. Chromatographic techniques are the most common analytical methods used for aflatoxin analysis (Hussain, 2011). Thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), liquid chromatography-tandem mass spectroscopy (LC-MS/MS), and high-performance thin-layer chromatography (HPTLC) are some of the commonly used techniques in aflatoxin detection and quantification (Rahmani *et al.*, 2009; Espinosa-Calderón *et al.*, 2011; Wacoo *et al.*, 2014). These methods are sensitive and provide precise and accurate analysis for aflatoxins. However, they are time-consuming and involve cumbersome sample preparation (Wacoo *et al.*, 2014).

Rapid screening methods offer quick and cheaper ways for detecting aflatoxins. Several rapid screening techniques are immunoassay based methods with enzyme-linked immunosorbent assay (ELISA) being the most notable approach (Shephard, 2009; Hussain, 2011). These methods give a qualitative result, and precise amounts of aflatoxin must always be confirmed by an analytical method. Both chromatographic and immunoassay methods involve laboratory-based chemical analyses that require huge capital investments (Wacoo *et al.*, 2014).

Spectroscopic techniques such as fluorometry, near-infrared reflectance (NIR), hyperspectral imaging, and Fourier transform infrared spectroscopy (FTIR), can provide qualitative and quantitative information on mycotoxin contamination with minimal sample preparation and pretreatment (Del Fiore *et al.*, 2010; Lee *et al.*, 2014). However, difficulties with the interpretation of the spectral data and spectral overlapping have limited the application of spectroscopic technology in mycotoxin detection and quantification (Lee *et al.*, 2014).

The currently used methods for aflatoxin analysis are inaccessible to small-scale farmers who are the majority producers of maize in Sub-Saharan Africa (Wacoo *et al.*, 2014). Most of the maize produce from small-scale farmers are for subsistence, thus not subjected to quality analysis (Wu & Guclu, 2012). Consequently, it is vital to develop portable devices that can be used in far-flung rural areas to provide quick and accurate aflatoxin analysis (Del Fiore *et al.*, 2010).

Electrical properties of grains have traditionally been used to develop portable devices that give quick quality estimates of cereal grains (Nelson, 2010). The electrical properties of grains are best represented by their dielectric properties (Skierucha *et al.*, 2012). Dielectric properties describe the interaction of a material with an electric field. The complex relative permittivity equation (ε_r), represents the dielectric properties (Equation 2.4). In this equation, the real part of ε_r represents the dielectric constant (ε'). The ε' is a measure of the amount of energy stored in the dielectric material due to the applied electric field. The imaginary part of ε_r is the dielectric loss factor (ε''). The ε'' is a measure of the energy loss of the external electric field (Nelson, 2010).

The molecular structure of a material defines its dielectric properties. Hence, they can be used to successfully diversify the physical and chemical properties (quality parameters) of a material under test (Skierucha *et al.*, 2012). Several researchers (Sosa-Morales *et al.*, 2010; Trabelsi & Nelson, 2012; Torrealba-Meléndez *et al.*, 2015; Noreña & Lescano-Anadón, 2017) have reported quality parameters of agricultural products based on dielectric properties. In cereals, dielectric properties have mostly been used in moisture content determination (Nelson & Trabelsi, 2012a). Other applications of dielectric properties in cereals include dielectric drying (Zhu *et al.*, 2012), bulk density measurement (Trabelsi *et al.*, 1998), prediction of grain damage levels (Al-Mahasneh *et al.*, 2001), and the control of pest in stored grains (Jiao *et al.*, 2011).

The dielectric properties of grains at a given electric field frequency are affected by moisture content, bulk density and temperature (Jha *et al.*, 2011; Nelson & Trabelsi, 2012a). Chemical composition can also affect the dielectric properties (Zhang *et al.*, 2007). Attempts to relate the weighted averages of the dielectric properties of the individual chemical components to the overall dielectric properties of food have so far not been successful (Bhargava *et al.*, 2013). Nonetheless, Sahin and Sumnu (2006), reported that physical changes that affect the proximate

composition such as moisture loss and protein denaturation, have an impact on the dielectric properties.

Maize kernels are susceptible to fungal attack, particularly *A. flavus*, which causes aflatoxin and grain deterioration. Fungal growth in stored grains can lead to losses in carbohydrates, proteins and total oil content while increasing moisture content and free fatty acids (Begum *et al.*, 2013). It was hypothesised that changes in chemical composition affect the dielectric properties of maize. Consequently, the dielectric properties can be used to detect the presence and predict the level of aflatoxin contamination in maize kernels. Therefore, the aim of the study presented in this chapter was to investigate the effect of the level of aflatoxin contamination on the dielectric properties of maize kernels.

5.2 Materials and Methods

Maize kernel samples inoculated with *A. flavus* was incubated for 7, 14, 21, and 28 days to obtain varying levels of aflatoxin contamination (Section 4.2.4). Nine samples each weighing 3.5 kg was selected for the measurement of electrical properties. The levels of aflatoxin contamination for the nine samples were; 0 $\mu g.kg^{-1}$, 1.5 $\mu g.kg^{-1}$, 2.6 $\mu g.kg^{-1}$, 10 $\mu g.kg^{-1}$, 50 $\mu g.kg^{-1}$, 100 $\mu g.kg^{-1}$, 150 $\mu g.kg^{-1}$, 172 $\mu g.kg^{-1}$, and 230 $\mu g.kg^{-1}$. The initial MC of the samples was determined by oven drying at 105 °C for 24 hours**.** The samples were dried in the oven at 55 °C to obtain three distinct levels of MC (16.4 %, 15.4 %, and 13.3 % (w.b). A calculated amount of water (Equation 5.1) was added to the samples where necessary. The samples put in Ziploc bags and refrigerated at 4 °C for 72 hours before electrical properties measurement. The bags were shaken manually at least three times a day to ensure uniform MC.

$$M_w = M_m \left(\frac{1 - MC_i}{1 - MC_f}\right) - M_m \quad (5.1)$$

Where;

M_w = mass of water needed (kg),

M_m = mass of maize kernels in (kg)

MC_i = initial moisture content (%)

MC_f = target moisture content (%)

5.2.1 Determination of dielectric constant

Dielectric constant is the ratio of the capacitance of a capacitor with a material as the dielectric to the capacitance of the capacitor with air as the dielectric as shown in Equation 5.2.

$$\varepsilon' = \frac{C}{C_0} \tag{5.2}$$

Where;

ε' = dielectric constant,

C = capacitance of sample holder filled with maize (pF), and

C_0 = capacitance of empty sample holder (pF)

The capacitance measurements were done using an ISO-TECH LCR-821 meter at three frequencies, viz., 25 kHz, 50 kHz and 100 kHz. The maize kernels were put in a custom-built, shielded parallel plate electrode assembly (Figure 5.1) as outlined by Lawrence *et al.* (1998). The sample holder had three aluminium plate electrodes each measuring 17.5 × 15 cm and 0.5 cm thick. The electrode spacing was 2.5 cm. The plate electrodes were attached to two 7.6 × 30.5 × 1.9 cm vertical support made from Perspex. The electrodes were fitted into 15 cm long, 1.27 cm deep grooves on the support members. The supports were screwed to an aluminium base plate measuring 15.3 × 25.4 × 0.6 cm. Perspex plate of dimensions 15.88 × 10.16 × 0.6 cm was used to seal the bottom of the electrode chamber by sliding it into grooves made on the Perspex supports.

The total volume of the electrode chamber was 1143 cm^3. A type N to APC-7 adaptor was attached to the middle electrode and used to connect the sample holder to the LCR meter through a 50 Ω coaxial cable.

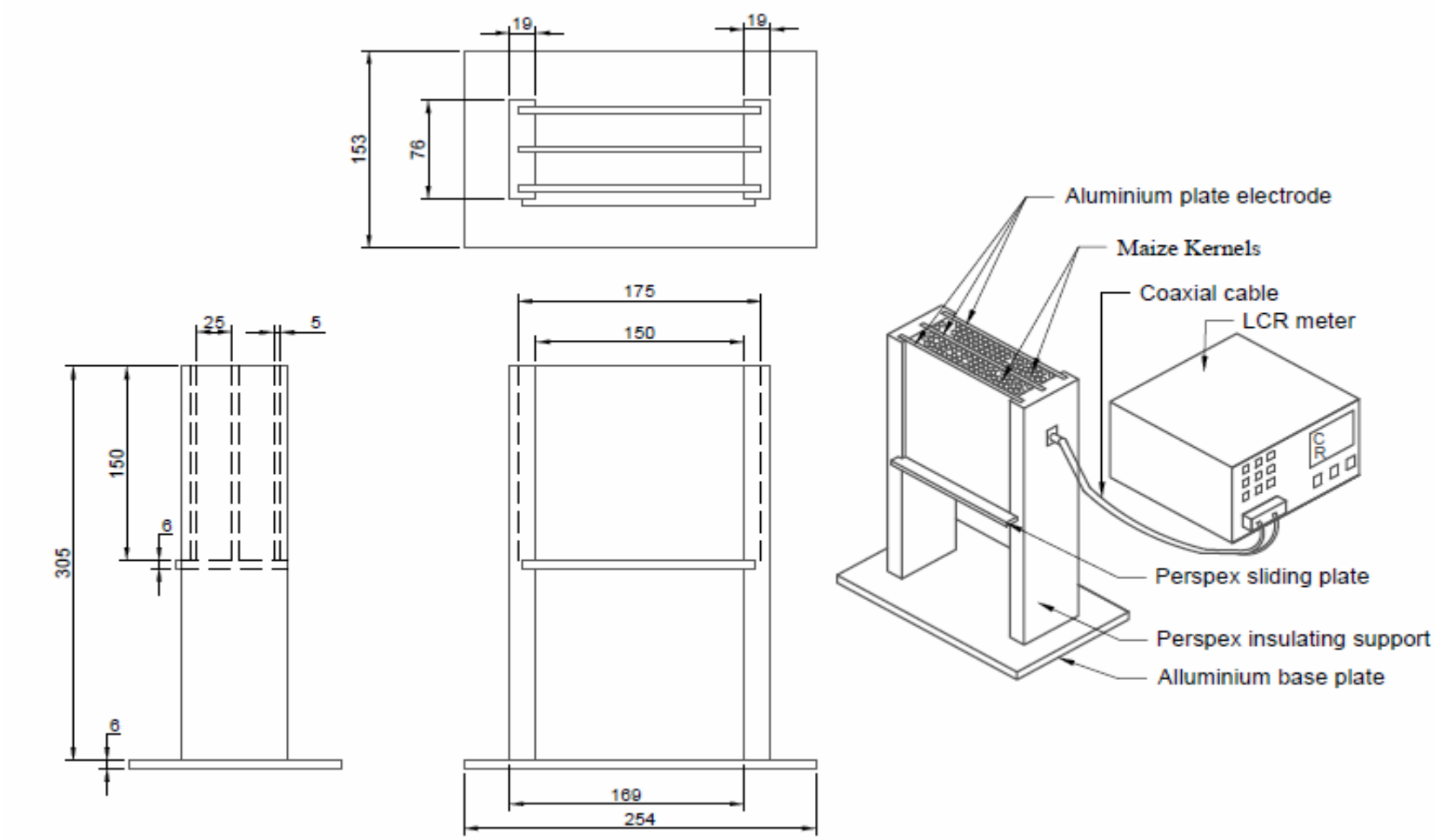

Figure 5.1 A modified custom-built shielded parallel plate electrode sample holder connected to an LCR meter

5.2.2 Experimental procedure

The maize kernel samples were retrieved from the refrigerator and allowed to equilibrate to room temperature for 6 hours. The LCR was calibrated through open and short calibrations before any tests were done. Capacitance measurements were first obtained with an empty sample holder. The obtained capacitance value was used as C_0 in Equation 5.1. Maize kernels were poured into the sample holder and the new capacitance, C, recorded. The dielectric constant, ε', for the maize kernels was then calculated using Equation 5.2.

5.2.3 Experimental design and data analysis

The design was a 3 × 3 × 9 factorial experiment. The three factors were MC (13.3 %, 15.4 %, and 16.4 %), frequency (25 kHz, 50 kHz, and 100 kHz), and aflatoxin level (0 $\mu g.kg^{-1}$, 1.5 $\mu g.kg^{-1}$, 2.6 $\mu g.kg^{-1}$, 10 $\mu g.kg^{-1}$, 50 $\mu g.kg^{-1}$, 100 $\mu g.kg^{-1}$, 150 $\mu g.kg^{-1}$, 172 $\mu g.kg^{-1}$, and 230 $\mu g.kg^{-1}$). All the treatments were replicated three times (Fig 5.1). Statistical analysis was done using GenStat® 17th Edition (VSN international Ltd, Hemel Hempstead, United Kingdom). Analysis of variance (ANOVA) was done at 5 % level of significance. Regression analysis was done to determine the correlation between dielectric properties and aflatoxin contamination.

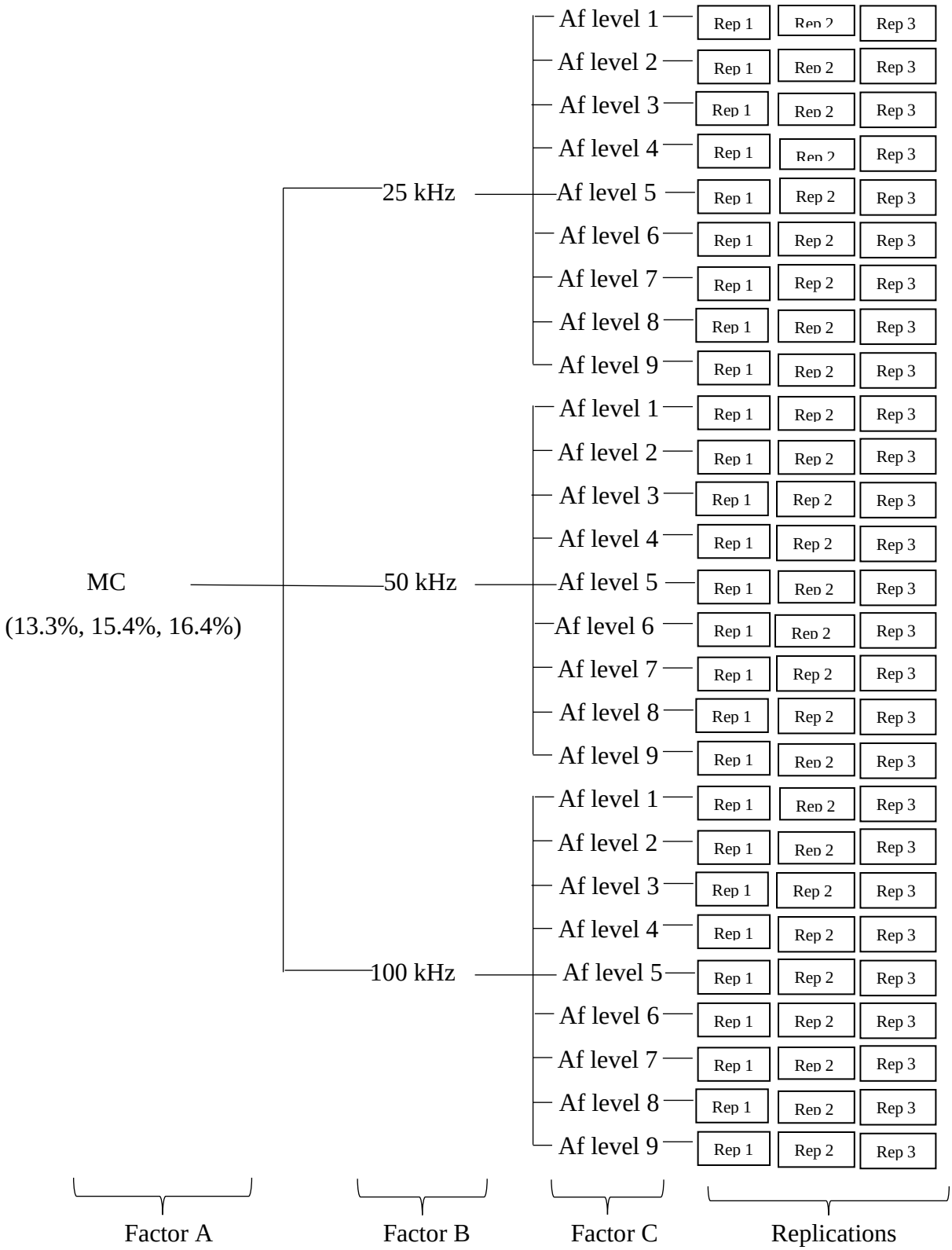

Figure 5.2 Experimental design for the dielectric constant determination (factor A= Moisture content (MC), factor B= Frequency, factor C= Aflatoxin level and three replications

5.3 Results

5.3.1 Capacitance of the empty sample holder

Capacitance values of the empty sample holder for the electric field frequency ranging from 1 – 200 kHz, varied between 0.03181 - 0.033715 nF (Figure 5.3). However, this change in capacitance was minimal with a standard deviation of 0.006 and coefficient of variation (CV) of 0.179. The capacitance decreased with increasing frequency with the highest and lowest capacitance values observed at 1 kHz and 200 kHz respectively. There was no change in capacitance between 100 kHz and 200 kHz.

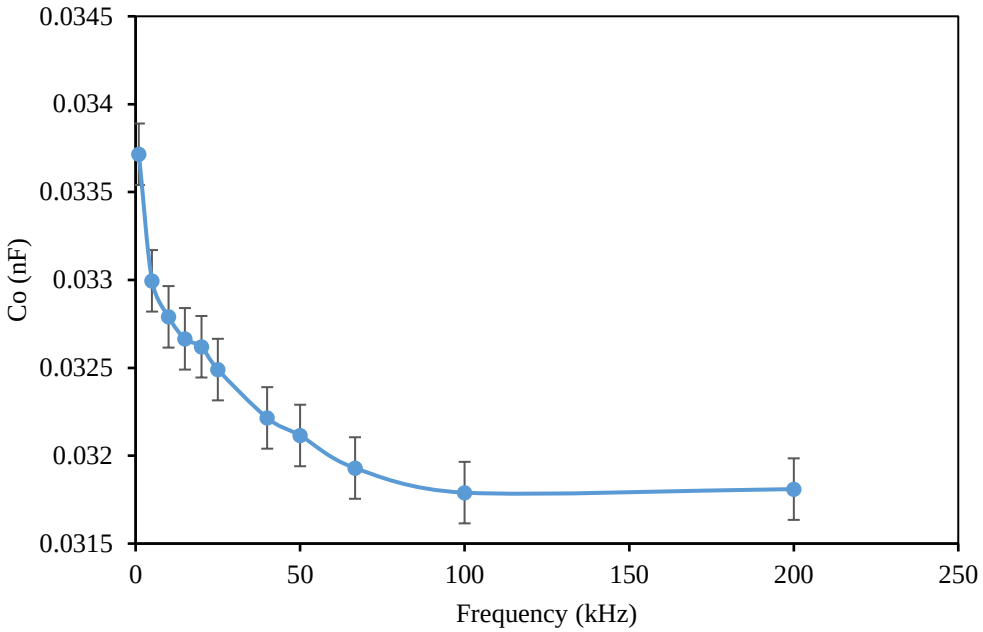

Figure 5.3 Capacitance values of the empty sample holder

5.3.2 Effect of moisture content on the dielectric constant

The dielectric constant was calculated using Equation 5.2. The dielectric constant ranged between 2.06 - 4.48 at 13.3 % MC, 3.28 - 9.10 at 15.4 % MC, and 4.47 - 14.47 at 16.4 % MC (Table 5.1). At a given frequency and aflatoxin level, the dielectric constant increased with increase in MC. Consequently, the greatest increase was observed at a MC of 16.4 %. MC, therefore, significantly ($p \leq 0.05$) influenced the dielectric constant. At every MC level, the rate

of increase in dielectric constant was greater at lower frequencies than at higher frequencies. There were significant differences between the mean dielectric constant at different MC levels as indicated by the results from Duncan's multiple range test (Table 5.1).

Table 5.1 Dielectric constant of maize kernels at specified level of aflatoxin, frequency and MC (wb)

Freq (kHz)	MC (%)	Dielectric constant (ε')								
		0 μg.kg^{-1}	1.5 μg.kg^{-1}	2.6 μg.kg^{-1}	10 μg.kg^{-1}	50 μg.kg^{-1}	100 μg.kg^{-1}	150 μg.kg^{-1}	172 μg.kg^{-1}	230 μg.kg^{-1}
25	13.3	4.26^{vwx}	4.48^{stuv}	4.35^{tuv}	4.03^{wxy}	4.01^{xy}	4.39^{tuv}	4.38^{tuv}	4.28^{vwx}	3.80^{yz}
	15.4	6.30^{jk}	8.16^{g}	7.25^{h}	5.76^{mn}	6.66^{i}	5.96^{lm}	7.21^{h}	6.05^{kl}	6.23^{kl}
	16.4	10.92^{c}	12.66^{a}	12.55^{a}	9.57^{e}	9.32^{ef}	10.16^{d}	11.90^{b}	9.13^{f}	9.52^{e}
50	13.3	3.27^{CDE}	3.58^{zAB}	3.25^{CDE}	3.11^{DEF}	3.07^{EF}	3.32^{BCDE}	3.53^{ABC}	3.49^{BC}	2.85^{F}
	15.4	4.82^{qrs}	6.11^{kl}	5.18^{op}	4.37^{uvw}	5.03^{pq}	4.53^{tuv}	5.60^{n}	4.51^{tuv}	4.71^{rst}
	16.4	7.42^{h}	8.29^{g}	8.30^{g}	6.63^{ij}	6.24^{kl}	7.17^{h}	8.47^{g}	6.83^{i}	6.39^{jk}
100	13.3	2.53^{G}	2.45^{G}	2.29^{GH}	2.33^{GH}	2.26^{GH}	2.44^{G}	2.42^{G}	2.43^{G}	2.06^{H}
	15.4	3.43^{BCD}	4.32^{vwx}	3.58^{ABC}	3.36^{BCDE}	3.67^{zAB}	3.29^{CDE}	3.86^{yzA}	3.43^{BCD}	3.28^{CDE}
	16.4	5.38^{o}	5.82^{mn}	5.81^{mn}	5.11^{opq}	4.71^{rstu}	5.36^{op}	5.78^{n}	4.94^{qr}	4.47^{tuv}

Significance levels (p)	
MC (%)	< 0.05
Frequency (kHz)	< 0.05
Aflatoxin (μg.kg^{-1})	> 0.05
MC × Frequency	< 0.05
Frequency × Aflatoxin	> 0.05
MC × Frequency × Frequency	> 0.05

$LSD_{p<0.05}$ = 0.5462 S.E = 0.1133 CV = 0.041

Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test (P<0.05).

5.3.3 Effect of frequency of the electric field on the dielectric constant

The dependence of the dielectric constant of maize kernels on the applied electric field as a function of aflatoxin level and MC is shown in Figure 5.4 (a), (b), and (c). The electric field frequency significantly ($p \leq 0.05$) affected the dielectric constant of the maize kernels at the three different MC and aflatoxin contamination levels. The dielectric constant decreased with increase in frequency. The rate of decrease of dielectric constant was greater at lower frequencies than at higher frequencies. The effect of frequency on the dielectric constant was pronounced at higher MC than at lower MC. The greatest change in dielectric constant was observed at 25 kHz, ranging between 3.80 and 12.66; whereas the least change in dielectric constant was observed at 100 kHz, in the range of 2.06 – 5.82. The dielectric constant at 50 kHz was in the range of 2.85 – 8.30 (Table 5.1).

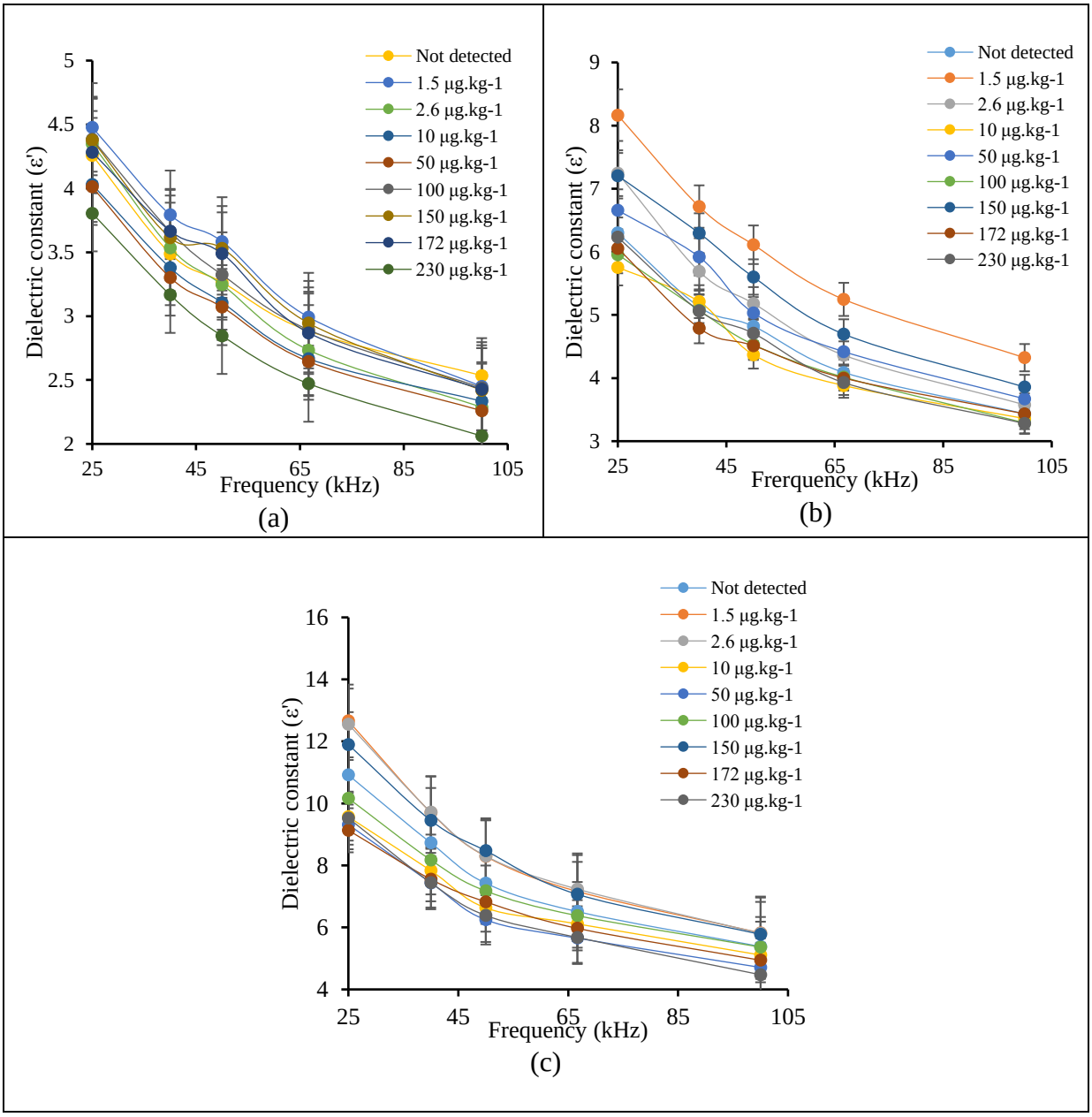

Figure 5.4 Variation of dielectric constant with frequency at specified aflatoxin levels for maize kernels at MC (w.b): (a) 13.3 %, (b) 15.4 %, and (c) 16.4 % ($LSD_{p<0.05}$ = 0.3154, CV = 0.014)

5.3.4 Effect of aflatoxin on the dielectric constant

The dielectric constant decreased with increase in frequency of the electric field and increased with MC, but there was no noticeable trend due to aflatoxin contamination level. The level of aflatoxin contamination had no significant ($p > 0.05$) effect on the dielectric constant of the maize kernels across all treatments. The dielectric constant for the maize kernels with a MC of 13.3 % and 16.4 % was fairly stable at a given frequency across all the levels of aflatoxin contamination. The dielectric constant for the maize kernels with a MC of 15.4 % kept fluctuating as the level of aflatoxin contamination increased (Figure 5.5). Generally, maize kernels with aflatoxin contamination level of 0 $\mu g.kg^{-1}$, 1.5 $\mu g.kg^{-1}$, and 2.6 $\mu g.kg^{-1}$ had relatively higher dielectric constant values, while maize kernels with aflatoxin contamination level of 172 $\mu g.kg^{-1}$ and 230 $\mu g.kg^{-1}$ had the lowest dielectric constant values.

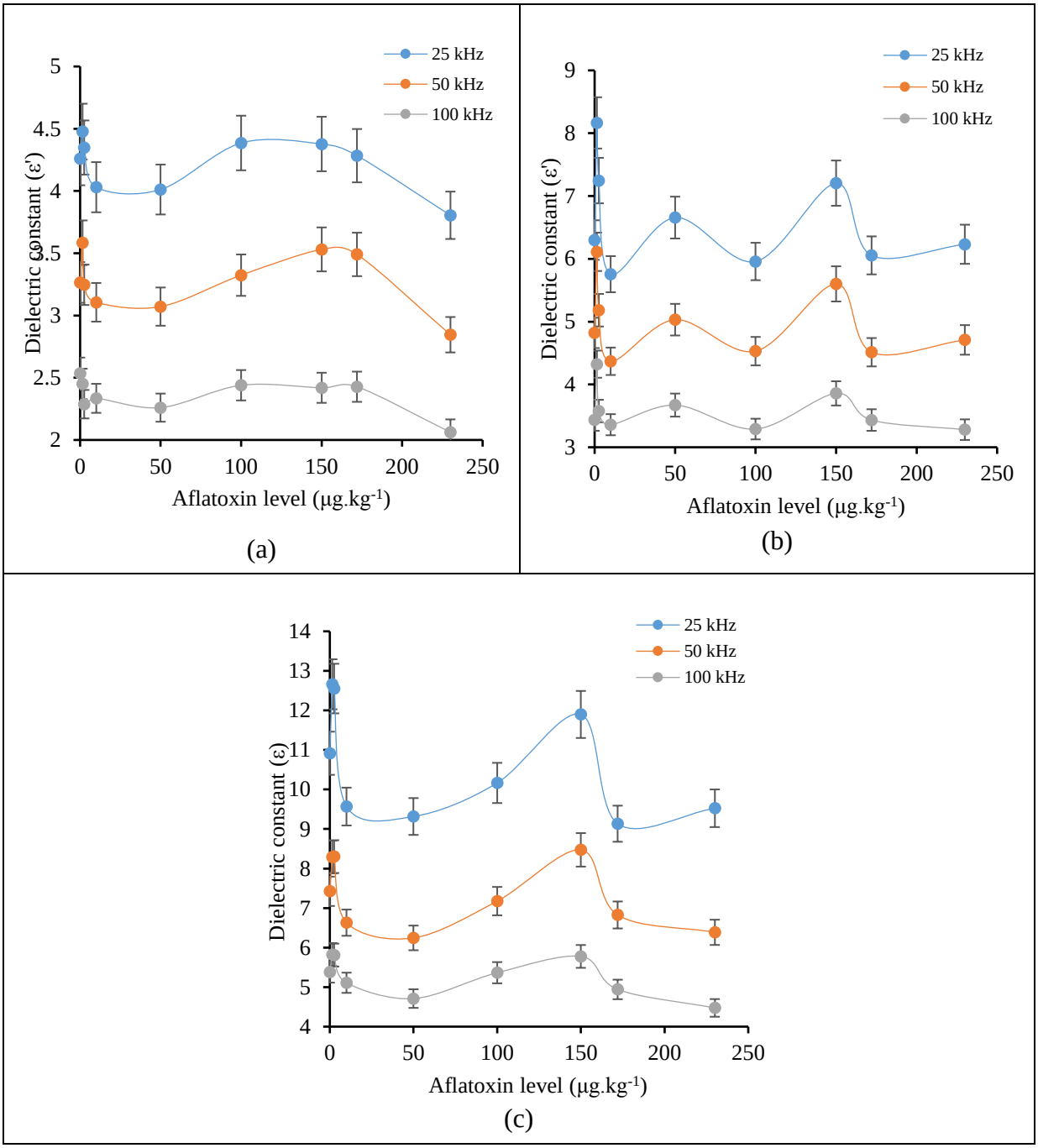

Figure 5.5 Variation of dielectric constant with aflatoxin contamination at specified frequencies for maize kernels at MC (w.b): (a) 13.3 %, (b) 15.4 %, and (c) 16.4 % ($LSD_{p<0.05}$ = 0.3154, CV = 0.014)

Results from a linear regression analysis of dielectric constant against aflatoxin contamination level are presented in Table 5.2. The highest value for the coefficient of determination (R^2 = 0.2687) was at 100 kHz at a MC of 16.4 %, whereas the lowest coefficient of determination (R^2 = 0.0300) was at 50 kHz and 13.3 % MC. The R^2 values are very low indicating a very weak correlation between dielectric constant and aflatoxin contamination level. At all electric field frequency and MC levels, aflatoxin had no significant effect ($p > 0.05$) on dielectric constant.

Table 5.2 Results of regression analysis for prediction of aflatoxin level in maize kernels

MC (%)	Frequency	R^2	Level of significance (p)
13.3	25	0.131394	$p > 0.05$
13.3	50	0.030006	$p > 0.05$
13.3	100	0.196642	$p > 0.05$
15.4	25	0.094377	$p > 0.05$
15.4	50	0.062869	$p > 0.05$
15.4	100	0.112516	$p > 0.05$
16.4	25	0.200319	$p > 0.05$
16.4	50	0.096801	$p > 0.05$
16.4	100	0.268654	$p > 0.05$

5.4 Discussion

The capacitance of the empty sample holder is in the same range to that obtained by Tomaraei (2010) using a shielded parallel plate sample holder with a volume of 936 cm^3. The empty sample holder has air acting as the dielectric material. Given that the dielectric constant of air is unity (Guo *et al.*, 2013), the capacitance of the empty sample holder is relatively stable.

Capacitance values increased when maize kernels were poured into the sample holder. The dielectric constant of maize kernels is higher than that of air, hence the higher values of capacitance for a sample holder filled with maize kernels compared to an empty sample holder. The MC of the maize kernels significantly ($p \leq 0.05$) affected the dielectric constant across all treatments. This is in agreement with Soltani and Alimardani (2011) who reported an increase in the dielectric constant of maize grains with increasing MC. Nelson and Trabelsi (2012b) and

Noreña and Lescano-Anadón (2017) also reported similar results on maize and wheat, and sorghum respectively. MC is the most important factor that influences the dielectric constant of maize (Shrivastava *et al.*, 2014). According to Singh *et al.* (2010), MC increases the dipoles in a material promoting total polarisation of the material.

Wang *et al.* (2013) attribute the progressive increase in dielectric constant with increase in MC to the transition of bound water state from monolayer or multilayer to free water state. At a MC of 13.3 %, most of the water is bound to the protein or starch, reducing the free water within the maize kernel, hence low values of dielectric constant at this level of MC. The high MC of 16.4 % increases the proportion of free water within the maize kernels leading to more water dipoles which contribute to polarisation hence higher values of dielectric constant.

Besides MC, the frequency of the electrical field significantly ($p \leq 0.05$) influenced the dielectric constant of the maize kernels. The findings from this study agree with those reported by Sacilik and Colak (2010) on maize kernels, Tomaraei (2010) on wheat, and Karjilova *et al.* (2013) on spelled grains. The frequency dependence of dielectric constant is also attributed to the polarisation of molecules with permanent dipole moments within the maize kernel. Polarisation follows the alteration of the electric field without any lag at low frequencies, hence larger dielectric constant values. The orientation of the dipoles cannot keep up with the rapid field reversals at higher frequencies resulting in low dielectric constant (Nelson & Trabelsi, 2012b).

The level of aflatoxin contamination had no significant ($p > 0.05$) effect on the dielectric constant of the maize kernels. The dielectric constant fluctuated with an increase in the level of aflatoxin contamination. These fluctuations in dielectric constant can be attributed to the small variation in MC among the samples. As reported in Section 5.3.2, MC significantly affects the dielectric constant, and its effects are pronounced at higher MC levels. It is evident from Figure 5.4 that fluctuations in dielectric constant are greater at 16.4 % and 15.4 % than at 13.3 % MC.

The relatively higher values of dielectric constant for maize kernels with aflatoxin levels of 1.5 $\mu g.kg^{-1}$, and 2.6 $\mu g.kg^{-1}$ and the low values of dielectric constant for maize kernels with aflatoxin level of 172 $\mu g.kg^{-1}$ and 230 $\mu g.kg^{-1}$, could have been caused by the difference in bulk density. The dielectric constant of maize increases with bulk density (Trabelsi *et al.*, 1998). The high

aflatoxin contamination of 172 μg.kg^{-1} and 230 μg.kg^{-1} indicates high *A. flavus* contamination which causes dry matter loss resulting in low bulk density. The maize kernels with aflatoxin contamination of 1.5 μg.kg^{-1}, and 2.6 μg.kg^{-1} undergo minimal dry matter loss due to low contamination with *A. flavus,* thus a relatively higher bulk density.

Aflatoxin content in the grain kernel constitutes an insignificant proportion of the total maize composition. However, *A. flavus* significantly degrades the fat, protein and carbohydrate, besides producing aflatoxin (chapter 4). Aflatoxin contamination positively correlates to changes in the proximate composition of maize. Some researchers (Ryynänen, 1995; Ndife *et al.*, 1998; Sahin & Sumnu, 2006; Zhang *et al.*, 2007) have reported that chemical composition influences the dielectric properties. Nonetheless, the changes in chemical composition of the maize kernels, associated with aflatoxin contamination had no impact on the dielectric constant of the maize kernels. This may be so because fats, proteins and carbohydrates have a low dielectric constant and their effect on the overall dielectric constant of the maize kernels was overshadowed by the effect of MC on the same as suggested by Nelson (1982).

5.5 Conclusion

The dielectric constant of maize kernels is significantly ($p \leq 0.05$) affected by the moisture content and the frequency of the applied electric field. The dielectric constant increased with increase in moisture content but decreased with increasing frequency. Aflatoxin contamination, however, does not have any significant influence on the dielectric constant of maize kernels. The coefficient of determination (R^2) of dielectric constant and aflatoxin contamination levels was low (0.03 - 0.2687), which indicate a lack of correlation between the aflatoxin levels and dielectric constant of the maize kernels. These findings imply that the dielectric constant of maize kernels is unsuitable for predicting the degree of aflatoxin contamination in maize kernels within the 20 – 200 kHz electric field frequency range. This study, therefore, recommends further research using higher frequencies of the applied electric field.

5.6 References

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6 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

This study sought to establish the electrochemical properties of maize kernels contaminated with aflatoxin. The study investigated the effect of the storage environment and moisture on aflatoxin contamination of maize kernels, after which the effect of *A. flavus* and aflatoxin contamination on the chemical composition of maize was evaluated. Finally, the electrical properties of maize kernels contaminated with different levels of aflatoxin were investigated.

The following conclusions were made:

a) Temperature and relative humidity have significant ($p \leq 0.05$) effects on the aflatoxin contamination of maize during storage. Aflatoxin contamination was higher at 30 ^{0}C than at 20 ^{0}C. Maize kernels stored at a relative humidity of 90 % showed higher levels of aflatoxin contamination than 60 %. The study established that storing maize at a relative humidity of 60 % resulted in non-toxic levels of aflatoxin contamination. Accordingly, there is need to design structures for storing of maize grains that are capable of maintaining the internal relative humidity of less than 60 %.

b) The chemical composition of maize varied significantly ($p \leq 0.05$) with both inoculum and the period of incubation. Both *A. flavus* and *F. verticilloides* caused significant depletion of carbohydrates, fats and proteins. Although AFB1 contamination was highly correlated to the reduction of fats and carbohydrate, such changes can also be caused by *F. verticilloides* which produces FB1. This study affirmed that the changes in proximate composition are a good indicator of fungal contamination of maize kernels. Nonetheless, such changes in chemical composition cannot explicitly infer the presence of aflatoxin.

c) The dielectric constant of maize is dependent on moisture content and frequency. However, the presence and level of aflatoxin contamination in maize kernels cannot be detected and predicted using the dielectric constant in the frequency range of 25 kHz to 100 kHz.

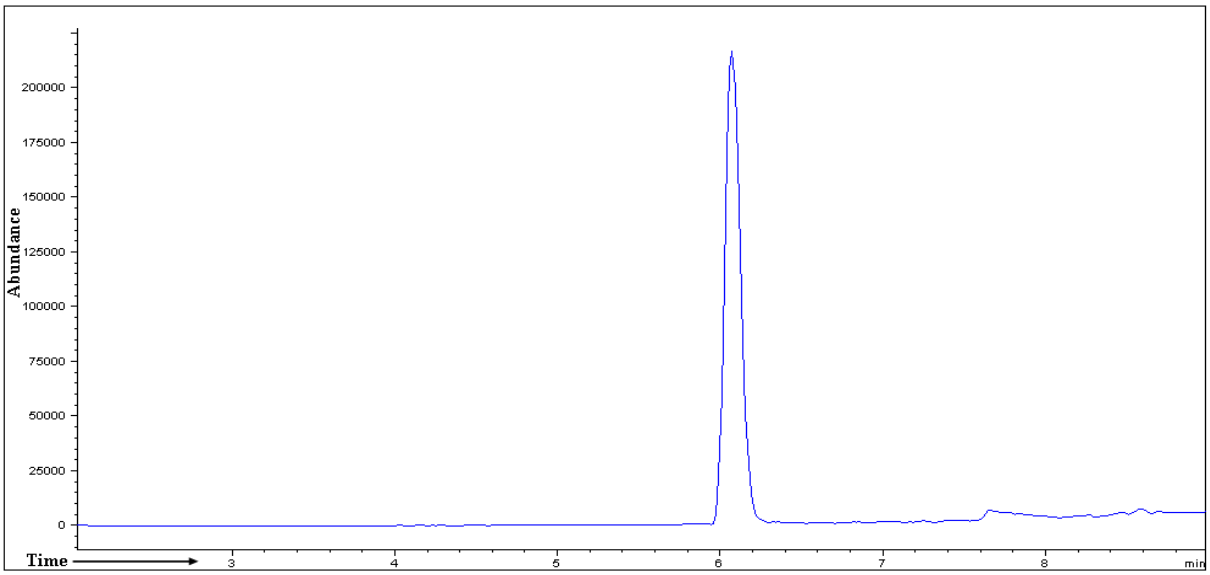
6.2 Recommendations for Further Research

a) Reducing aflatoxin contamination of maize during storage requires the development of storage facilities that can maintain the relative humidity within the storage structure below 60 %. Superabsorbent polymers (SAPs) and desiccants have shown the potential for use in seed drying and could be extended to grain storage. Incorporating such techniques in grain storage structures can help regulate the relative humidity, thus limit aflatoxin contamination.

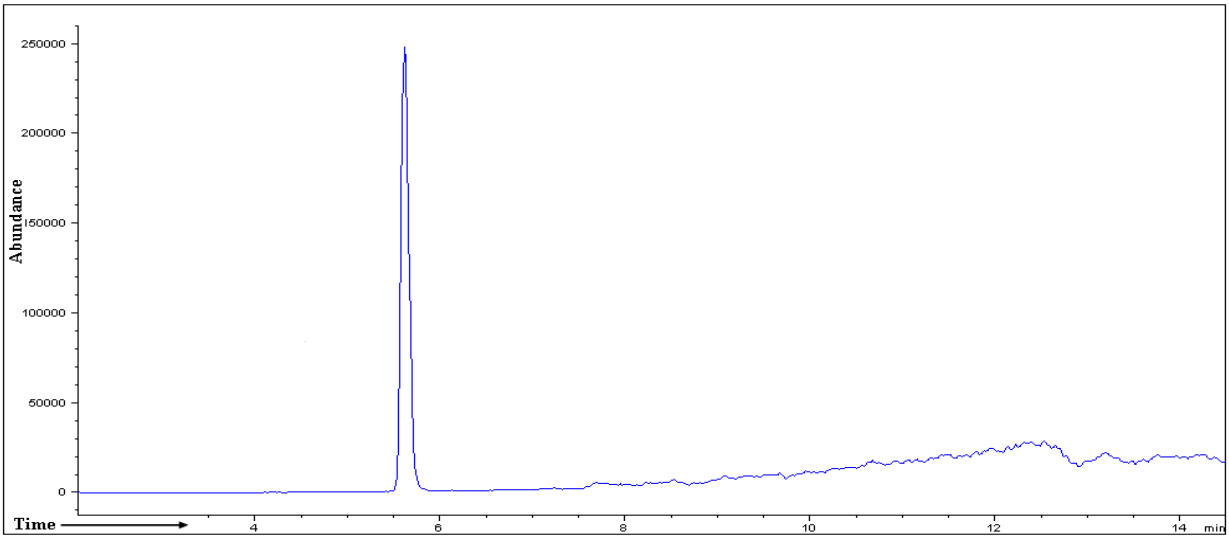
b) The frequency range (25 kHz – 100 kHz) used to determine the dielectric properties of maize kernels was limited by the equipment used. The use of higher frequencies to establish both the dielectric constant and dielectric loss factor of maize kernels would provide more information on the influence of grain composition on the dielectric properties of grains.

7 APPENDIX

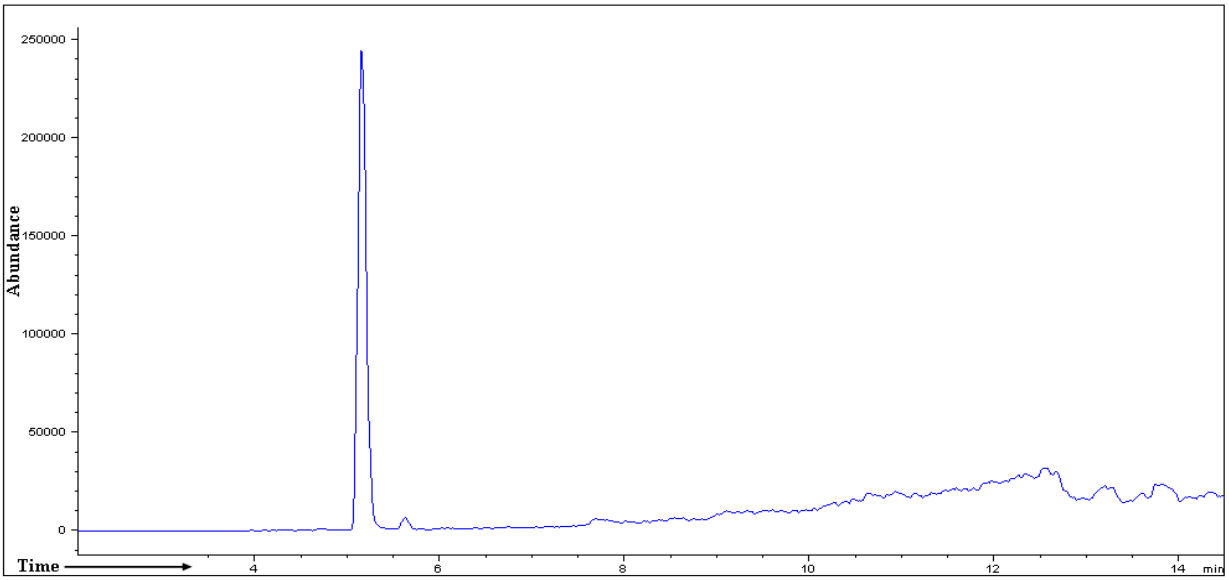
7.1 Appendix A



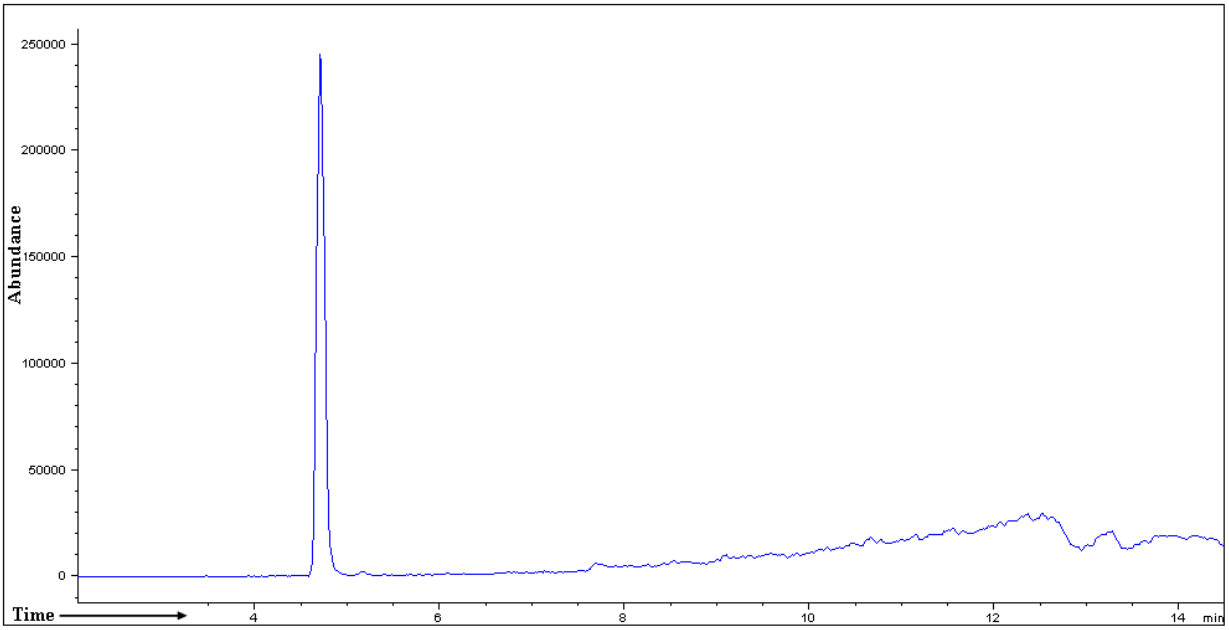
Appendix A 1 Total ion chromatogram and mass spectrum for aflatoxin B1 standard (m/z =313 and retention time 6.157 mins)



Appendix A 2 Total ion chromatogram and mass spectrum for aflatoxin B2 standard (m/z =315 and retention time 5.824 mins)



Appendix A 3 Total ion chromatogram and mass spectrum for aflatoxin G1 standard (m/z =329 and retention time 5.229 mins)



Appendix A 4 Total ion chromatogram and mass spectrum for aflatoxin G2 standard (m/z =331 and retention time 4.87 mins)