EFFECT OF MAIZE STORAGE AND MILLING PRACTICES ON AFLATOXIN LEVELS IN MAIZE FLOUR

HALIFA HAMIS SUME

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN FOOD QUALITY AND SAFETY ASSUARANCE OF THE SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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ABSTRACT

The presence of aflatoxins in foods and feeds is respectively a health hazard to human and animals. Cereals, especially maize have been reported to be susceptible to aflatoxin contamination. This study was carried out to investigate aflatoxin levels in maize flour as influenced by maize storage and milling practices in Gairo district, Morogoro region. Quantification was preceded by a purposive cross-sectional survey focusing on storage and milling practices. Based on the survey, the predominant storage types were Indoor Storage Practice (ISP), Outdoor Storage Practice (OSP) and Hermetic Storage Practice (HSP). Prominent milling practices were “dehull-mill” milling (DMM), whole maize milling (WMM) and “dehull-soak-mill” milling (DSM). Millers (42.9%) reported that DMM was the most preferred milling process. Samples for aflatoxin analysis were also collected during the survey while embracing the storage and milling practices. Aflatoxin detection and quantification was done using High Performance Liquid Chromatography (HPLC). In general it was found that about 98% of the samples were contaminated with total aflatoxin above permitted levels in accordance with the East Africa Community standards for which the acceptable limit is 10 ppb. HSP was shown to have good effect in avoiding aflatoxin contamination in maize during storage. On the other hand DMM milling showed interesting trend in minimizing aflatoxin levels in maize flour. Maize stored according to ISP practice had the highest level of total aflatoxin (452 ppb). Whereas maize stored according to HSP practice had the lower level (47 ppb). Whole milled maize (WMM) had 216.5 ppb and 91.1 ppb for DM maize (57% decrease). Interactive effect showed significant decrease in levels for instance maize located in Chakwale and stored by HSP practice had just 9.3 ppb total aflatoxin. Similarly maize milled according to process DMM and stored by HSP practice had 17 ppb level which was lower compared to its individual treatments. Therefore it can be concluded that interactive strategies for the storage practices using HSP and milling practices using DMM is effective in minimizing the aflatoxin contamination.

DECLARATION

I, **Halifa Hamis Sume** do declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor concurrently being submitted in any other institution.

______________________ ________________________

Halifa Hamis Sume Date

(MSc. FQSA Candidate)

The above declaration is confirmed by:

______________________ ________________________

Prof. E.E. Maeda Date

(Supervisor)

______________________ ________________________

Prof. B.K. Ndabikunze Date

(Supervisor)

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DEDICATION

To my daughters, my mother Sikudhani Mkondo, my father Hamis Sume, uncle Kasim Sume and my late father Mohamed M. Lunyalile, fellow Msc. students Food Quality & Safety Assurance (FQSA) and future research work in combating aflatoxin contamination of food.

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LIST OF ABBREVIATIONS AND SYMBOLS

AFB_1	Aflatoxin B_1
ANOVA	Analysis Of Variance
CRD	Completely Randomized Design
DF	Degree of Freedom
DMM	De-hull-Mill Milling
DSM	De-hull, Soak, Mill
FAO	Food and Agriculture Organisation
EAS	East African Standards
GDC	Gairo District Council
HPLC	High Performance Liquid Chromatography
HSP	Hermetic Storage Practice
IARC	International Agency for Research on Cancer
ISP	Indoor Storage Practice
ISO	International Standard Organization
LSD	Least Significant Difference
NBS	National Bureau of Statistics
PBS	Phosphate Buffer Saline
PICS	Purdue Improved Crop Storage
ppb	parts per billion
SUA	Sokoine University of Agriculture
TFDA	Tanzania Food and Drugs Authority
µg	Microgram
WMM	Whole Maize Milling

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Mycotoxins are low-molecular-weight compounds synthesized during secondary metabolism by filamentous fungi (Mollea and Bosco, 2015). These moulds belong to various genera including *Aspergillus, Penicillium, Fusarium and Byssochlamys* (Abdulaziz, 2011). Abdulaziz (2011) has also reported that the metabolites are produced during mould growth in response to stress factors. Mycotoxins intake at low concentration have adverse health effects on vertebrates, including human. Some mycotoxins have been reported to cause autoimmune illnesses, interference with hormonal activity, allergic reactions; teratogenic reactions, carcinogenic and mutagenic reactions (Bezerra *et al.*, 2014). Out of the currently 400 known mycotoxins, about 20 are serious food and feed crop contaminants (Mollea and Bosco, 2015; Eshetu *et al.*, 2016). Six of the 20 mycotoxins are of great importance as they may co-exist with aflatoxins (Kimanya *et al*., 2014). The mycotoxins that have been reported to co-exist with aflatoxins include ochratoxins, fumonisins, zearalenone, deoxynivalenol, trichothecenes, and Patulin (Shephard, 2008; Mollea and Bosco, 2015).

The aflatoxins are secondary metabolites from mainly fungi of genus *Aspergillus* (Wild and Gong, 2010) and are produced by two major species; *Aspergillus flavus* which produce aflatoxins B_1 and B_2, and *Aspergillus parasiticus* which produce aflatoxins B_1, B_2, G_1 and G_2 (Omar, 2013). Aflatoxin B_1 in particular has been reported as a carcinogen to human beings (International Agency for Research on Cancer, 2002). Aflatoxin B_1 occurs in diverse groups of crops, including the major cereal staples (e.g. maize), edible nuts and legumes, and their products. In general, its concentration and toxicity are both highly prominent. Contamination with aflatoxin takes place in both preharvested and postahrvested maize grains (Kabak *et al.*, 2006; Diao *et al.*, 2014).

In general, mycotoxin production is enhanced by poor food handling and storage methods and especially if there is lack of stipulated regulatory standards that focus on consumer protection. Nevertheless, even in developed countries, specific subgroups may be susceptible to mycotoxin exposure attributed to either high consumption of some contaminated products or favourable growth conditions for mycotoxin producing moulds in storage facilities (Mollea and Bosco, 2015). It is interesting to know that maize storage practices (such as traditional or modern storage, storage time and storage percentage moisture content) and milling practices (such as whole grains milling soaked and dehulled maize milling) if they can exert some influence on aflatoxin levels.

1.2 Problem Statement and Study Justification

In the tropic and sub-tropic regions, maize grain contamination by mycotoxins is a major health problem. This is because maize is prone to contamination by mycotoxin variants including aflatoxins, deoxynivalenol and fumonisins (Kimanya *et al.,* 2014). It is exacerbated by the fact that maize based diets are staples consumed in Tanzania irrespective of quality due to food scarcity problems. Excessive consumption of a single cereal diet is also reported in many other African diets (Shephard, 2008). The health risks arising from consumption of contaminated cereals are compounded by lack of regulatory standards that provide legislation and permissible aflatoxin levels in cereals and related foods.

The maize storage practices in Tanzania are conducive to fungal growth, toxin production and therefore compromise its flour safety on account that products based on the flour are consumed by relatively high percentage of the population (Wagacha and Muthoni, 2008). Furthermore, milling practices in community settings raise curiosity to study their influence on total aflatoxin. Considering that maize is a staple food for the majority of Tanzanians, it is necessary to estimate the aflatoxin contamination levels in its flour in relation to storage and milling practices, with a view of initiating intervention measures and recommendations.

Identification and rating of existing storage and milling practices and how they either singly or in combination influence aflatoxin levels would probably serve and complement existing consumers' protection measures.

1.3 Objective of the study

1.3.1 General objective

The overall objective of this study was to assess the influence of storage and milling practices on aflatoxin levels in maize flour.

1.3.2 Specific objectives

(i) Identification of storage and milling practices by farmers and millers respectively

(ii) Assessment of aflatoxin levels in maize flour produced by identified milling practices

(iii) Assessing the degree by which storage and milling practices interact to influence aflatoxin levels in maize flour

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Aflatoxin

Studies conducted on mycotoxin have been conclusive that mycotoxin pose a public health threat such as vomiting, diarrhea, mycotoxicoses, immunosuppression, cancer, mutagenicity, teratogenicity, death and impaired growth and development, (Shephard, 2008; Eshetu *et al.*, 2016). Aflatoxins are highly toxic with aflatoxin B_1 being reported by the International Agency for Research on Cancer (IARC) as a causal agent for human being liver cancer. It is argued that Aflatoxin B_1 acts synergistically with Hepatitis B infection (International Agency for Research on Cancer, 2002). Aflatoxin contamination occurs at any stage during farming, harvesting, storage, transportation and during processing (Hell *et al.*, 2010).

According to Cole and Cox (1981), the four major aflatoxins: - B_1, B_2, G_1 and G_2 differ in chemical structure (Figure 1). The colour of emitted fluorescence upon being irradiated by ultraviolet light (λ = 365 nm) can either be “B” for Blue and “G” for Green. Their distinction as aflatoxins B_1, B_2, G_1 and G_2 are based on their relative retention factors (Rf) upon being separated by thin layer chromatography. This distinction is also manifested in their inherent structural differences as illustrated in Figure1.

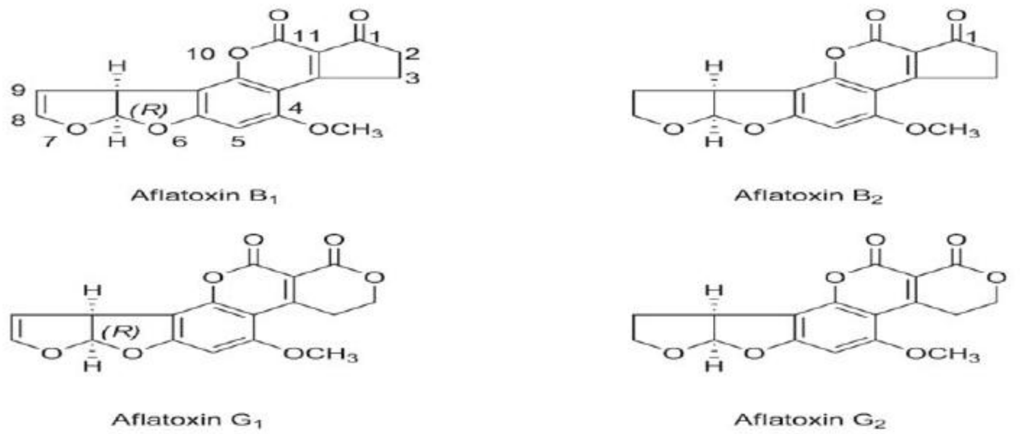

Figure 1: The chemical structures of some aflatoxins (Cole and Cox, 1981)

2.2 Maize

Maize is one of the primary staple cereal crops consumed in Tanzania and many African countries (Wilson and Lewis, 2015). In Africa more than 70 million metric tons are produced annually (Macauley, 2015). Maize is prone to contamination by multiple mycotoxins notably aflatoxins, deoxynivalenol and fumonisins. The risk of exposure to mycotoxins in Africa is higher than in other parts of the world because of the relatively high maize/cereal based food consumption in the African continent as well as environmental conditions (Kimanya *et al*., 2014). In the developed world, human diets are extremely diverse and commercial food suppliers in their market economies exploit quality index as means to compete. Setting of standards and legislation spelling mycotoxins tolerable limits are additional consumer protection advantages in those countries. By contrast, diets consumed by the population in developing countries tend to be less diverse and less emphasis on legislating maximum tolerable levels and even when such legislation exist; the capacity for enforcement is frequently lacking (Shephard, 2008).

In many counties in sub-Saharan Africa maize is the preferred cereal crop for food, feed and industrial use, displacing traditional cereals such as sorghum and millets (Macauley, 2015). Maize accounts for 31 % of the total food production and more than 75

% of the cereal consumption in Tanzania (Suleiman and Rosentrater, 2015). Suleiman and Rosentrater further reported that estimated, annual per capita maize consumption in Tanzania is around 128 kg. Other studies by Nyoro *et al.* (2004) and Peter *et al.* (2014) have reported that in Tanzania the per capital maize consumption is nearly 400 grams per day. The observed potential in maize has been overshadowed by reports that maize is more susceptible to mycotoxin (Aflatoxins) contamination than other cereals such as sorghum or millet (Bandyopadhyay *et al.,* 2007).

2.2.1 Maize storage

Aflatoxins and their control strategies during maize grains storage to reduce impact of aflatoxins contamination in the final maize flour is reported by studies conducted by Eshetu *et al.* (2016) and Kabak *et al.* (2006); that mycotoxins reduction in food could be carried out during storage in conjunction with control storage moisture below 15%, low temperature and controlled atmosphere storage with oxygen levels (not exceeding 51%).

2.3 Maize Grain Hermetic and Traditional Storage

Suleiman and Rosentrater (2015) reported that hermetic storage technology is the best approach to combat post-harvest cereal losses due to insects, birds and physical damages as well as damages by fungi/moulds. It is a cost-effective storage that principally works by exclusion of oxygen and create physical barrier. Murdock *et al*. (2014) and Villers *et al.* (2010) have reported that hermetic storage bags can be of different types such as Purdue Improved Crop Storage (PICS) and Grain Pro Super Bags. PICS bags are triple-layered by having three plastic linings. The Super Grain bag is a portable hermetic sack consisting of a single reusable plastic film made from 2 plain polyethylene films between which is laminated with a plastic layer that acts as a gas and moisture barrier (Baoua *et al.,* 2014).

Notwithstanding existence of these storage structures, they are seldom found in Tanzania farming communities (Shabani *et al.,* 2015) in which there are two classical maize storage approaches namely, roof and sack storage. In the roof method, after harvesting, farmers store the maize in the ceiling for several months. Whereas in the sack storage, farmers tend to shell the maize, and store the grains in polypropylene bags. The major materials used for constructing the stores are wood and clay (Shabani. *et al*., 2015). According to Ajani and Onwubuya (2012), maize is stored in different forms; such as with husk, as cobs without husks and as shelled grains.

2.4 Milling Practices

Visconti *et al*. (2004) concluded that washing food or grain can reduce mycotoxin levels. For example, the first step in spaghetti preparation using wheat flour, by washing the grains the researchers reported 23% deoxynivalenol removal (Visconti *et al*., 2004). Soaking and dehulling the grain has been reported to remove 40-80% of aflatoxins (Fandohan *et al.*, 2005). Furthermore Karlovsky *et al.* (2016) cited Mutungi (2008) who reported that de-hulling led to aflatoxin decrease by 46.6%. According to Siwela *et al.* (2005) dehulling maize grain can reduce aflatoxin contamination by 92%.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was conducted in Gairo district which is one of the major maize growing areas in Tanzania. Gairo is one of the seven districts in Morogoro region, with an area of 1 974.46 km² and a population of 193 011 according to 2012 Census (NBS, 2013). The district has two divisions and nine wards namely; Gairo, Kibedya, Chakwale, Chagongwe, Rubeho, Iyogwe, Idibo, Chanjale and Mandege. The district has two rain seasons with an average rainfall of about 600 to 1400 mm per annum. The dry season starts from the middle of May to October (GDC strategic plan, 2015). The survey and maize sampling for this study was done in October, 2016 during which an average temperatures were between 23 - 30 degrees centigrade and a relative humidity of 68.8% (Weatherbase, 2016).

3.2 Research Design

3.2.1 Storage and milling practices

3.2.1.1 Cross-sectional survey

Maize farmers and millers were interviewed using two independent structured questionnaires (with closed ended questions) to gather information on the prevalent storage and milling practices respectively (Appendices 2 and 3). Responses were elicited on farmers' storage practices and milling practices, storage structures, storage treatment, length of storage and milling preferences. Basic demographic details of farmers and millers were also collected.

3.2.1.2 Selection of farmers and millers

In the course of wards, farmers and millers selection, stratified sampling was adopted on account of information from the District Agricultural officer. Based on the information from the District officer, sampling focused on the wards whose agricultural engagement was maize farming. The selected wards were Gairo, Kibedya and Chakwale (Appendix 1). Cross-sectional surveys were conducted in the three wards to gather information on storage practices, milling practices and in purchasing of samples from farmers. Only maize stored for two months after harvest was considered for sampling.

Since the actual number of the farmers was unknown parameters, the sample size was estimated using the formula for infinite population proposed by Kothari, (2014).

$$n = \frac{z^2 \text{ x } p.q}{e^2} = \frac{z^2 \text{x } p(1\text{-}p)}{e^2}$$

Where by n = size of sample, P = Sample proportion, assuming 5% (0.05) for this study e = acceptable error (the precision), set at 5% (0.05) for this study and z = standard variate at a given confidence level, for this study 95% confidence level= 1.96 (Kothari and Gaurav, 2014).

$$\text{Thus, } \frac{1.96^2 \text{ X } 0.05(1\text{- } 0.05)}{0.05^2} = 72.99 \approx 73$$

But due to unavoidable circumstances for storage practices only 69 farmers participated in the study (95%). The selection for questioning criteria was formulated such that after every three households or maize storage place (house or farm) possibly sharing similar storage and farming characteristics were interviewed. District health inspectors reported that approximately 24 maize millers were operating in Gairo. Although survey for assessing milling practices aimed at covering all 24 premises. However only 21 millers were available and participated in the survey.

3.2.2 Maize sampling

3.2.2.1 Completely randomized design with factorial arrangement

Completely randomized design (CRD) in a *3* x *3* factorial arrangement was adopted. Location served as a primary factor (A), storage practices as secondary factor (B) and milling practices as third factor (C). The maize grains for each storage practice (B) was milled in accordance with maize milling practices (C). Two trials represented the 2 replicates for the CRD model. The design was deployed for samples earmarked for aflatoxin analysis with a view of establishing the extent by which location, storage, milling practices, the second order interactions influence aflatoxin levels in maize flour. According to CRD model (3 x 3 x 3 x 2), 54 samples were collected and whose detailed analytical description is shown in section 3.2.2.2.

3.2.2.2 Sample collection and management

Sampling was done according to ISO 24333 (2009) procedure and followed the CRD model in which 18 maize samples (10kg each) were collected from maize farmers' storage areas embracing three locations, three identified storage practices and their two respective replications. The samples were then divided according to the three identified milling practices. From that it came up with 3 locations x 3 prominent storage practices x 2 replicates = 18. When this was divided to 3 milling practices it gave 54 samples for laboratory analysis. In order to have a representative sample from each location, sampling from each bag was randomly done repeatably using triers culminating to a gross sample weighing about 10 kg. Each gross sample weighing 10 kg was packed and tightly closed in polypropylene bags, internally lined with polyethylene lining.

During milling process care was taken to avoid contamination between samples and between processes. Before undertaking any milling process involved, respective maize sample approximately 3 kg was passed through the processing line to clear the previous sample residue so as to have meaningful representative output.

3.2.3 Aflatoxin Analysis

Aflatoxin analysis was conducted at the TFDA Laboratory, located at Mabibo External, Dar es salaam. Each maize flour sample was sub-divided according to IUPAC sampling scheme (Horwitz, 1990) to obtain a representative analytical sample for analysis.

3.2.3.1 Sample extraction

The test portions sample flours were extracted with methanol/water. During extraction approximately 12.5g of sample was put in 100ml Erlenmeyer flask and mixed with 50 ml methanol/water 3/2 solvent, the mixtures were then shaken for 60 minutes using gyrating shaker (Talboys shaker, model 3500 by Henry Troemner, USA). The mixture was then filtered through a qualitative filter paper (prefolded).

3.2.3.2 Dilution of extract

The extract was diluted with phosphate buffer saline (PBS) in which 10 mL of extract (sample) was added 30 mL PBS. Before the diluted sample was applied to the column the pH was adjusted to 7.4 using 0.1 M NaOH and H_3PO_4 solutions.

3.2.3.3 Clean up/ sample application

Clean up was done using ready-made immunoaffinity column called AflaStar. The AflaStar immunoaffinity column was put on an adapter. The column and extract were kept at room temperature. The adapter with the syringe barrel was attached. The diluted extract was applied until all has passed over the column by gravity. Before the column ran dry the column was washed down with HPLC-grade distilled water (20mL) making sure all extract passed through the column (Plate 1).

Plate 1: Aflastar immunoaffinity and sample application

3.2.3.4 Elution

The syringe barrels were removed from the column and vials placed under the column for collection of the eluates. The HPLC grade methanol was used as eluent. During elution the total volume of 1.5 mL of methanol was applied to the column in several small portions (i.e. 250 µL x 2 and 500 µL x2), Methanol was left for short period of time before elution as shown in Plate 2.

Plate 2: Elution and collection of eluates in vials

3.2.3.5 Aflatoxin analysis by HPLC coupled to a fluorescence detector

The analysis was done using High Performance Liquid Chromatography, HPLC (Shimazdu Corp., USA) coupled to a fluorescence detector in accordance with ISO 16050:2003: quantification of aflatoxin in cereal and cereal products. The individual aflatoxins B_1, B_2, G_1 and G_2 were detected, quantified and later on summed to represent total aflatoxin.

The mobile phase for HPLC was made by preparing 1000mL of Methanol: Acetonitrile:Water in a 15:20:65 ratio respectively. The mixture was also added with 119mg Potassium Bromide (KBr) and 100 µL of 65% Nitric acid (HNO_3) for derivatisation. Fluorescence detector with wavelength of 363 nm excitation filter and a wavelength of 440 nm cut off emission filter were applied. Kobra cell which is electrochemicaly generated bromine was used for post-column derivatisation with flow rate of 0.9mL/min (mobile phase) and a current of 100µA. Calibration curves were prepared using the working calibration solutions which were supplied standard solution containing known concentrations of aflatoxins B_1, B_2, G_1 and G_2 in HPLC grade acetonitrile solutions. Detection and quantification limits were set at 0.2µg/kg and 0.5µg/kg respectively.

3.2.4 Determination of moisture content

Despite the fact that HPLC would detect aflatoxins regardless of moisture content of samples and the results was expressed in percentage dry basis. Just for curiosity, samples were tested for moisture content using the standard oven method in which 27 samples were kept in an oven (Genlab oven) set at 105 º C for 5 hours (AOAC, 1990).

3.3 Statistical Data Analysis

Data obtained from survey (data on storage and milling practices) were analysed using Statistical Package for the Social Sciences (SPSS) software version 20 (IBM SPSS Statistics, 2015).Whereas Laboratory data were analysed using Microcomputer Statistical Package (MSTAT-C) version 2.0 (Freed, 1985) for Analysis of Variance to determine the significant ($p<0.05$) variations in the location, storage, milling and interaction effects (Appendix 6). Means were separated by Duncan's Multiple Range Test (Freed, 1985).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Prevalent Maize Storage and Milling Practices

4.1.1 Storage practices

There were several storage practices identified from different wards. Survey revealed three prevalent storage practices which were considered as shown in Table 1. The prevalent storage types were Indoor storage practices, ISP, (61% of the surveyed farmers), Outdoor /field storage practices, OSP, (23% of the surveyed farmers) and Hermetic storage practices, HSP (9% of the surveyed farmers). Whereas ceiling, wooden and metallic silos storage contributed only 7% of all surveyed stores.

Table 1: Identified storage practices from different surveyed locations in Gairo

Storage method	Ward			Total
	Gairo	**Kibedya**	**Chakwale**	
Indoor storage Practice (ISP)	17	15	10	42
Outdoor storage Practice (OSP)	7	5	4	16
Hermetic Storage Practice (HSP)	2	1	3	6
Ceiling Storage	0	2	0	2
In wooden silos	0	1	1	2
In metallic silos	0	0	1	1
Total	26	24	19	69

Key: **$\chi 2$ =** 62.7, **df** = 25 and **P-value =** 0.00. Chi-square test shows that χ^2 was significant ($P \leq 0.05$) i.e. Storage practices are dependent of locations.

Traditional storage practices were independent of the wards upon being subjected to a chi-square test ($P \leq$ 0.05), (Table 2). This relationship could be due to the fact based on the intervention by One Acre Fund on storage practices; approximately 31% of the surveyed areas, Chakwale farmers had attended training on good storage practices using Purdue Improved Crops Storage (PICS) bags. Whereas in Gairo farmers practiced mixing storage (Indoor Storage practices, Outdoor storage practices and few Hermetic storage practices).

Approximately 47% of surveyed Kibedya farmers do postharvest drying of their produce. A climatic condition among the surveyed wards was similar and over 94 % of the farmers had their maize stored for two months during the survey, as earlier observed and reported by Shabani *et al.* (2015).

The three outstanding storage practices i.e. ISP, OSP and HSP revealed in this study were contrary to research done by Shabani *et al.* (2015), on maize storage and consumption practices by farmers in Handeni district, Tanzania who reported two basic storage methods namely; roof and sack storage. This shows that storage practices differ between different cultural practices and among different communities. With respect to Gairo the tendency is to store maize outdoor or indoor in polypropylene bags or using Purdue Improved Crop Storage (PICS) bags.

Table 2: Relationship of location and storage practices

Storage practices	**Location**		
	Gairo n (%)	Kibedya n (%)	Chakwale n (%)
Indoor storage Practice (ISP)	16 (64)	13(72.2)	6(42.9)
Outdoor storage Practice (ISP)	7(28)	4(22.2)	2(14.3)
Hermetic Storage Practice (HSP)	2(8)	0(0)	4(28.6)

Key: $\chi 2$ = 62.7, df = 25 and **P-value** = 0.00. Chi-square test shows that χ^2 was significant (*P<0.05*) i.e. Storage practices and locations were dependent.

Note: Figures in bracket show are percentage of farmers within wards who apply the respective storage type.

4.1.1.1 Outdoor Storage Practice (OSP)

The study showed that 23% of the surveyed farmers applied outdoor storage practice and the majority were from Gairo (54%) and Kibedya (31%) wards. The study revealed that, farmers stored their maize outside the house (Plate 3) in polypropylene bags, while others leave maize in the field due to either lack of in-house storage space or exploitation of sunlight exposure for increased produce dehydration. Even though extended sun drying after harvest was minimal as the majority (82%) appeared to be satisfied with short sunlight exposure of their produce at the field level. Nevertheless, these farmers did not do any moisture test to make sure maize was harvested at the recommended moisture content by East Africa Standards which is 13.5 percent (EAS, 2013).

Plate 3: Maize kept outside on ground at Gairo market place

4.1.1.2 Indoor Storage Practice (ISP)

The study showed that about 61% of the surveyed farmers were applying indoor storage practice for which the majority were from Gairo and Kibedya wards (Table 1). Indoor storage (ISP) was the most preferable storage practice in which maize was packed in polypropylene bags which were subsequently stored in either special stores or within residential room (Plate 4).

Plate 4: Indoor maize storage in residential room using polypropylene bags

4.1.1.3 Hermetic Storage Practice (HSP)

Six out of 69 surveyed farmers mainly from Chakwale and Gairo were applying HSP using Pardue Improved Crop storage (PICS) bags (Table 1). Apart from polypropylene bags which were used for ISP and OSP, storage practice using PICS bags was only applied by 9% of all farmers who participated in the survey (Table 1). The bags were sold at a cost ranging from 4000 to 6000 Tanzania shilling. This cost was unaffordable by majority (91%) of the surveyed farmers. Yet PICS bags (Plate 5) have been proven to be the most effective storage bags against vermin and mould and thereby upholding cereal quality (Suleiman and Rosentrater, 2015). One Acre Fund project implemented in Tanzania over several trial phases on the use of PICS bags (One Acre Fund, 2013), only few farmers mainly from Chakwale (6%) were sensitized on the importance and use of the bags.

Plate 5: Hermetic maize storage using PICS bags:

4.1.1.4 Other storage practices

Approximately 3% of the farmers store maize in wooden silos made from bark of the trees. Ceiling storage was also practiced by 2.9% of the surveyed farmers in which maize grains, some with cobs were stored on ceiling preferably above the kitchen place so as to enhance smoke drying. Few (1%) used airtight metallic silos.

4.1.2 Maize milling practices

Results show that there were different milling practices preferred by millers' customers. These include; whole maize milling (WMM), "dehull-mill" milling (DMM) and "dehull-soak-mill milling" (DSM) as shown in Table 3.

Table 3: Maize milling practices among the surveyed millers

Practice	Frequency (%)
Whole Maize milled (WMM)	5 (23.8)
Dehulled-Milled Millling (DMM)	9(42.9)
Dehulled, soaked, milled (DSM)	7(33.3)

The $\chi2$ (3.19), df = 4 and the P-value (0.53) show that $\chi2$ is not significant at p = 0.05

Whereas 42.9% of the millers preferred DMM practice, 23.8% were practicing whole maize milling (WMM). The three prominent milling practices (WMM, DMM and DSM) were adopted and samples from the three practices were collected for laboratory analysis. Also sorting, washing and sieving were practiced before milling into flour. The similar processes were reported by Karlovsky *et al.* (2016).

The majority of millers from three wards namely Gairo, Kibedya and Chakwale operate in the Gairo town and with the rest operating in remote villages within the Gairo district (Table 4). The distribution of the millers within wards did not influence the choices of milling practices as when the relationship of the location and milling practices was tested (at $P< 0.05$) using chi-square test there was no significant difference between milling preference and location of the milling machine (Table 4).

Table 4: Relationship of location and milling preferences

Milling method	Location Gairo n (%)	Kibedya n (%)	Chakwale n (%)	Total (n)
WMM	4 (26.7)	1(33.3)	0(0)	**5**
DMM	5(33.3)	2(66.7)	2(66.7)	**9**
DSM	6(40)	0(0)	1(33.3)	**7**
Total (n)	**15**	**3**	**3**	**21**

Key: WMM = Milled (wholly milled maize), DM = Dehulled- milled maize,

DSM = De-hulled-Soaked-Milled.

The $\chi 2$ (3.19), df = 4 and the P-value (0.53) show that $\chi 2$ is not significant at p = 0.05.

This indicates that the milling practices are independent of locations.

Among the millers surveyed, 42.9% of the millers' customers prefer DMM, the finding relates in some way with the study by Shabani *et al*. (2015) who report that most of the farmers (42%) surveyed in Handeni consumed dehulled maize while 35% and 12% consumed non-dehulled and mixed (dehulled and non-dehulled), respectively. In this study it was also found out that about 33.3% of the millers were dehulling maize, soak for two days, dry it and bring back for milling which was identified as DSM milling. However, studies done by Mutungi (2008) and Kirui (2016) on similar process reported that soaking resulted to mycotoxin decontamination. Whereas Fandohan *et al.* (2005) reported that soaking and dehulling the grain removes 40-80% of mycotoxins.

4.2 Moisture Content

There were variations of moisture contents of the samples taken from different storage and milling practices. The results show average moisture content of 10%. Most moist samples were collected from Chakwale. Samples milled according to DSM milling practices had the highest moisture content (11%) followed by whole milled (M) maize flour (10%). Whereas DM milled maize were the most dried samples with an average moisture content of 8.7% (Table 5). The recommended safe moisture storage level for maize must not exceed 13.5 percent (EAS, 2013).

Table 5: Average percentage moisture content of the samples based on location, storage and milling practices

Factors	Type/practice	Moisture content (%)	Average (%)
Location	Gairo	9.78	9.7
	Kibedya	8.75	
	Chakwale	10.57	
Milling practices	WMM	10.25	
	DMM	8.70	10.1
	DSM	11.38	
Storage practices	ISP	10.01	
	OSP	10.22	10.1
	HSP	10.11	

Key: ISP = Indoor Storage Practice, OSP = Outdoor Storage Practice and HSP = Hermetic Storage Practice. WMM = Wholly Milled Maize), DMM = Dehulled- Milled Maize, DSM = De-hulled-Soaked-Milled.

4.3 Aflatoxin Content and Levels in Maize Flour due to Identified Storage and Milling practices

The results revealed different mean levels of aflatoxin G_2, G_1, B_2 and B_1 in maize flour whose grains were stored and milled in accordance to the three identified practices i.e. location, storage and milling practices being primary, secondary and tertiary factors respectively.

4.3.1 Effect of location on aflatoxins levels

Maize samples from Gairo, Kibedya and Chakwale show various levels of aflatoxin in maize flour (Table 6). The results show that Aflatoxin B_1 occurs in highest levels in all locations (Table 6) for which maize flour in Gairo had the highest level. For this aflatoxin, and the rest of the aflatoxin types i.e. B_2, G_1 and G_2, there is significant ($P<0.05$) locational differences. Similar findings have been reported by Kabak *et al*. (2006) and Diao *et al*. (2014). Indeed the prevalence of aflatoxin B_1 in flour in the study area is critical as this is the most deadly aflatoxin when consumed.

Table 6: Effect of location on aflatoxin levels

Location	Aflatoxin levels (µg/kg)				
	B_1	B_2	G_1	G_2	Total Aflatoxin
Gairo	198.5[a]	9.7[a]	64.0[a]	3.6[a]	275.8[a]
Kibedya	100.1[c]	5.7[c]	38.0[b]	2.8[c]	146.6[c]
Chakwale	106.2[b]	6.2[b]	36.9[c]	3.1[b]	152.4[b]
LSD value	0.8657	0.1103	0.3893	0.2195	1.107 (at alpha = 0.05)

Means within columns not superscripted by the same lower case letter are significantly different following separation by Duncan Multiple Range Test (DMR) at P≤0.05.

As shown in the Table 6 there were decrease in levels of aflatoxins G_1, B_2 and G_2 in that order. Most of Kibedya farmers were practicing post-harvest drying process while others did not dry again their maize. According to Pratiwi *et al.* (2015) well dried maize has lesser chance for mould growth than moist one.

4.3.2 Effect of maize storage on mean aflatoxin levels in maize flour

Maize samples taken from three storage practices i.e. ISP, OSP and HSP gave different levels of aflatoxin in maize flour (Table 7). Maize stored in accordance with the three practices did not conform to the East Africa permissible level for total aflatoxin which is 10 ppb (Table 7). HSP storage system had flours with significantly ($P<0.05$) low Aflatoxin levels (47 ppb) compared to ISP and OSP storage systems. These observation concurs with studies on hermetic PICS storage that have had impact on reducing post-harvest loss arising from mycotoxin, pests and moisture (Murdock *et al.*, 2014; Villers *et al.*, 2010). Despite the finding that HSP show good trend in minimizing aflatoxin levels, presence of aflatoxin in maize stored by HSP would probably indicate maize being contaminated before storage.

Table 7: Effect of maize storage on various forms of aflatoxin levels

Storage practice	Aflatoxin levels (µg/Kg)				
	B_1	**B_2**	**G_1**	**G_2**	**Total Aflatoxin**
ISP	247.2[a]	13.4[a]	85.8[a]	5.6[a]	352.0[a]
OSP	122.5[b]	6.6[b]	43.2[b]	3.4[b]	176.1[b]
HSP	35.2[c]	1.6[c]	10.0[c]	0.5[c]	47.3[c]
LSD value	0.8657	0.1103	0.3893	0.2195	1.107 (at alpha = 0.05)

Key: ISP = Indoor Storage Practice, OSP = Outdoor Storage Practice and HSP = Hermetic Storage Practice.
Means within columns not superscripted by the same lower case letter are significantly different following separation by Duncan Multiple Range Test (DMR) at $P \leq 0.05$.

During data collection storage temperatures ranged from 23 to 30 º C which is favourable temperature condition for optimum growth of aflatoxigenic fungi such as *Aspergillus spp*. (Pratiwi *et al.,* 2015; Somjaipeng and Ta-uea, 2016). Whereas according to Roy and Chourasia (1989) and Hassan and Aziz (1998) the optimum temperature for aflatoxin production by *A. flavus* ranges between 25 and 35°C which was within the range found in the study areas.

4.3.3 Effect of milling process on mean aflatoxin levels

The three milling practices had variable and yet significant effect (*P<0.05*) on each aflatoxin type in maize flour (Table 8). Whereas the DSM practice had significantly high levels (*P<0.05*) with respect to Aflatoxin B_1 and G_1. Aflatoxin B_2 and G_2 had the significantly low levels (*P<0.05*) for the same milling practice. Indeed Aflatoxin G_2 manifested an opposite trend on comparing with the two earlier mentioned Aflatoxins (B_1 and G_1) for unexplained reasons (Table 8). According to Lahouar *et al.* (2015) the favourable conditions for *aspergillus spp.* mycelial growth and production of aflatoxins variants are almost the same i.e. the minimum a_w needed for such mycelial growth was 0.91 at 25 and 37 °C.

Table 8: Effect of milling on mean aflatoxin levels in maize flour

Milling practice	Aflatoxin levels (μg/kg) B_1	B_2	G_1	G_2	Total Aflatoxin
WMM	149.7[b]	10.1[a]	52.0[b]	4.8[a]	216.6[b]
DMM	56.1 [c]	5.7[c]	25.6[c]	3.7[b]	91.1[c]
DSM	199.1[a]	5.8[b]	61.4[a]	1.0[c]	267.3[a]
LSD value	0.8657	0.1103	0.3893	0.2195	1.107 (at alpha = 0.05)

M = Wholly Milled Maize, DMM = Dehulled- milled maize, and DSM =De-hulled-Soaked-Milled. Means within columns not superscripted by the same lower case letter are significantly different following separation by Duncan Multiple Range Test (DMR) at P≤0.05.

The observed significantly high levels in wholly milled maize flour compared to that in de-hulled maize flour was reported in the previous studies. According to Siwela (2005) dehulling maize reduced total aflatoxin by 92%. In the current study whole milled maize (M) had 216.6 ppb of total aflatoxin which was significantly higher (*P<0.05*) than 91.1 ppb for dehulled-milled (DM) maize corresponding to 57% decrease. The implication here is that the fungal mycelia do not just end in the bran but also penetrate the endosperm and thus explaining residual aflatoxin in dehulled maize flour. Similar arguments have been reported by Siwela *et al*. (2005). Strangely enough, dehulled-soaked-milled maize flour (DSM) in the study had the highest levels of aflatoxin contradictory to earlier reports by Muthoni (2008). This could be due to challenges in attempt to decontaminate cereals with aflatoxins; challenge on unhygienic handling and contamination with more mould in a myriad ways during soaking and sun-drying. Garcia *et al*. (1994) reported that aflatoxin is only slightly soluble in water but very soluble in organic solvents such as chloroform and that the process that involved soaking has just a little effect to decontaminate cereals contaminated with aflatoxin.

Soaking and drying has a lot of challenges, including utensils, quality of water, dust during sun exposure and light intensity during sun drying. Otherwise this could bring more levels than its reduction. The most important remark to note following unexpected finding for DSM process is that de-hulling process does not decontaminate aflatoxigenic producing residual moulds rather it is an attempt to decontaminate aflatoxins. Soaking especially with contaminated water would probably provide conducive environment (water activity,a_w) for more mould growth and therefore toxin production as reported by Lahouar *et al.* (2016) that increased water activity (aw) between 0.85 and 0.99 led to the increased colony diameters for *A. flavus* isolates.

It is not even practical for Gairo people to attempt to decontaminate aflatoxins with heat treatment as the study on impact of food processing and detoxification treatments on mycotoxin contamination by Karlovsky *et al. (*2016) reported that most mycotoxins are thermally stable for which conventional food preparation with temperatures up to 100 °C have little effect on most mycotoxins. Karlovsky *et al.* (2016) further revealed that pure aflatoxin B_1 (AFB1) was destroyed by temperatures above 160 °C. Practically this temperature cannot be attained by Gairo communities as an appropriate aflatoxin decontamination procedure. So far success in destroying moulds by heat treatment have not yielded convincing results. Even attempt to pasteurize mould spores at 62.8ºC for 30 minutes disappointingly culminated to survival of all *Aspergillus* species strains (Thom, 1996).

4.4 Interactive Effect of Location, Storage and Milling Practice on Mean Aflatoxin Levels in Maize Flour

When the two factors interaction was considered between location with storage practice, location with milling practice and storage with milling practice on aflatoxin levels results are shown on Tables 9-11.

4.4.1 Location and maize storage practice on mean aflatoxin levels

When location and storage practices were integrated maize from Gairo and stored according to practice ISP gave the highest total aflatoxin levels (452.4 ppb) whereas maize from the same location (Gairo) but stored according to HSP had the lower level (107 ppb). Maize samples from Chakwale and stored by HSP gave significant (P<0.05) lowest total aflatoxin level (9.3 ppb). This is the only mean level which falls within EAC allowable limit for total aflatoxin in cereals (10 ppb). Among all surveyed areas HSP usage in Chakwale is high compared to other wards.

Table 9: Effect of location and maize storage practices on mean Aflatoxin levels (µg/kg) in maize flour

Aflatoxin	**Storage practice**	Location: Gairo	Location: Kibedya	Location: Chakwale	**LSD**
B_1	ISP	324.4^{a}	245.0^{b}	172.1^{c}	
	OSP	191.8^{a}	36.7^{c}	139.1^{b}	1.50
	HSP	79.3^{a}	18.6^{b}	7.5^{c}	
B_2	ISP	15.3^{a}	14.3^{b}	10.5^{c}	
	OSP	10.5^{a}	1.4^{c}	8.0^{b}	0.19
	HSP	3.4^{a}	1.2^{b}	0.2^{c}	
G_1	ISP	106.9^{a}	95.9^{b}	54.5^{c}	
	OSP	61.6^{a}	13.2^{c}	54.9^{b}	0.67
	HSP	23.6^{a}	5.0^{b}	1.4^{c}	
G_2	ISP	5.8^{a}	7.0^{a}	4.0^{b}	
	OSP	4.3^{b}	0.9^{b}	5.1^{a}	0.38
	HSP	0.9^{c}	0.6^{c}	0.1^{c}	
Mean	ISP	452.4^{a}	362.2^{a}	241.1^{a}	
total	OSP	268.2^{b}	52.2^{b}	207.0^{b}	1.92
aflatoxin	HSP	107.2^{c}	25.4^{c}	9.3^{c}	

Key: ISP = Indoor Storage Practice, OSP = Outdoor Storage Practice and HSP = Hermetic Storage Practice. Means within rows for each listed aflatoxin type and storage practice not superscripted by the same lower case letter are significantly different following separation by Duncan Multiple Range Test (DMR) at P≤0.05.

4.4.2 Location and maize milling practice on mean aflatoxin levels in maize flour

Interactive effect of location and milling practice did not give any significant decrease in total aflatoxin for samples taken from Gairo (Table 10). However Kibedya's samples gave results with a little bit lower levels. Kibedya's lowest levels could be attributed by the fact that maize samples were well dried. When these samples were milled according to DM process there was decrease in aflatoxin levels (Table 10).

Table 10: Effect of location and maize milling practices on mean Aflatoxin levels (µg/kg) in maize flour

Aflatoxin	milling practice	Gairo	Location Kibedya	Chakwale	LSD
B_1	M	239.6^a	118.2^b	91.3^c	
	DM	73.2^a	33.3^c	61.7^b	
	DSM	282.6^a	148.8^c	165.8^b	1.50
B_2	M	12.9^a	9.3^b	8.0^c	
	DM	7.6^a	3.6^c	5.9^b	0.19
	DSM	8.6^a	4.1^c	4.8^b	
G_1	M	71.4^a	59.3^b	25.2^c	
	DM	31.7^b	10.0^c	35.1^a	0.67
	DSM	89.0^a	44.7^c	50.5^b	
G_2	M	4.9^b	6.1^a	3.3^c	
	DM	4.5^b	1.5^c	5.1^a	0.38
	DSM	1.5^a	0.7^c	0.8^b	
Mean total aflatoxin	M	328.8^a	192.9^b	127.8^c	
	DM	117.0^a	48.5^c	107.8^b	1.92
	DSM	381.7^a	198.3^c	221.8^b	

Key: WMM = Milled (wholly milled maize), DMM = Dehulled- milled maize, DSM = De-hulled-Soaked-Milled.
Means within rows representing aflatoxin type, milling practice and location not superscripted by the same lower case letter are significantly different following separation by Duncan Multiple Range Test (DMR) at P≤0.05.

4.4.3 Effect of maize storage and milling practice on mean aflatoxin levels in maize flour

Interaction of storage and milling practices showed interesting trend of aflatoxin levels in maize flour; For example maize stored in HSP had total aflatoxin mean level of 47.3 ppb while maize milled according to the process DMM had total aflatoxin mean level of 91.1 ppb, interaction effect of maize stored in HSP and milled by DM gave the total aflatoxin level 17.7 ppb (Table 11) which shows promising decrease compared to individual treatment. Interaction between the storage practices HSP and the milling practice DM show a promising strategic intervention measures in avoiding aflatoxin contamination in maize storage and decontamination during processing to permissible aflatoxin levels in maize flour. Combination of factors in attempt to reduce aflatoxin in cereals were also reported by Pratiwi *et al.* (2015) in which storage temperature and relative humidity were controlled factors. Whereas a study by Karlovsky *et al.* (2016) reported that interaction of the physical processing on cereals such as sorting, sieving, washing, dehulling flotation and density segregation reduced the chances of having mycotoxins in a final product.

Table 11: Effect of maize storage and milling practices on mean Aflatoxin levels (µg/Kg) in maize flour

Aflatoxin	milling practice	Storage practice			LSD
		ISP	**OSP**	**HSP**	
B_1	WMM	329.4^a	96.2^b	23.5^c	
	DMM	86.9^a	68.2^b	13.1^c	
	DSM	325.3^a	203.1^b	68.8^c	1.50
B_2	WMM	20.0^a	8.4^b	2.0^c	
	DMM	9.7^a	6.5^b	0.9^c	
	DSM	10.4^a	5.0^b	2.0^c	0.19
G_1	WMM	115.2^a	33.6^b	7.1^c	
	DMM	37.4^a	36.2^b	3.3^c	0.67
	DSM	104.8^a	59.8^b	19.6^c	
G_2	WMM	9.2^a	4.2^b	1.0^c	
	DMM	5.7^a	5.2^b	0.3^c	
	DSM	1.9^a	0.8^b	0.3^c	0.38
Mean	WMM	473.6^a	142.4^b	33.6^c	
total	DMM	139.7^a	116.1^b	17.7^c	
aflatoxin	DSM	442.4^a	268.8^b	90.7^c	1.92

Key: WMM = Milled (wholly milled maize), DMM = Dehulled- milled maize, DSM = De-hulled-Soaked-Milled.
Means within rows for each listed aflatoxin type, milling and storage practice not superscripted by the same lower case letter are significantly different following separation by Duncan Multiple Range Test (DMR) at $P \leq 0.05$.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The conclusion drawn from the research findings is that, storage practices, locations and milling practices of maize influence the levels of aflatoxin contamination and/or distribution. Several storage factors that may help to reduce aflatoxin levels in stored maize in Gairo were identified in this study. The predominant storage practices i.e. ISP, OSP and HSP whereas milling practices i.e. WMM, DMM and DSM were identified in this study and indeed showed impactful aflatoxin contamination and decontamination trend in maize flour. HSP storage showed significantly ($P<0.05$) good trend in combating mould growth and therefore reducing aflatoxin production. Whereas dehull- mill process for maize grains (DMM) has been known and here again revealed to have mycotoxin decontamination effect on contaminated grains. Interactive effect of the factors such as location, storage and milling procedures have shown to supplement safety measures on avoiding aflatoxin. Dehull and soaking of maize (DSM) before milling could not provide evidence that it is a safe practice in this study as it might require precautionary procedures during soaking and drying, may have encouraged more mould growth and therefore more aflatoxin contamination. Interventions through training to build capacity of maize farmers in Gairo and elsewhere in Tanzania on Post-harvest handling techniques that will reduce occurrence of aflatoxin in flour is very important.

5.2 Recommendations

i. Government should initiate a countrywide campaign on the importance of dehulled-milled maize flour to avoid deadly impact of aflatoxin. It should be clear that wholly maize flour despite its known importance in providing fibre and proteins which can be obtained from other sources has more deadly impact than benefit. The countrywide campaign should also address the issue of good maize storage practices as well as safe and hygienic handling milling practices.

ii. Government in collaboration with NGOs should put effort to educate communities about aflatoxin and their impact on health, safe maize farming, harvesting, storage and milling practices.

iii. More research should be undertaken in other areas on the issues that could not be covered in this study; studies that address and compare storage structures and levels of aflatoxin in different locations, climatic variation of the area and their impact on aflatoxin levels and studies that investigate attitude and behavioral practices on maize storage and processing

iv. In collaboration with One Acre Fund project the Government should intervene to subsidize the PICS bags which are expensive for a regular farmer. PICS bags do not only prevent mould growth and mycotoxin contamination, they also provide barrier to moisture migration, and cereal loss due to grain damage and infestation.

v. Gairo district council should regularly conduct inspection of the milling machines and storage areas just to ensure adherence to safety and hygiene requirements of the facilities.

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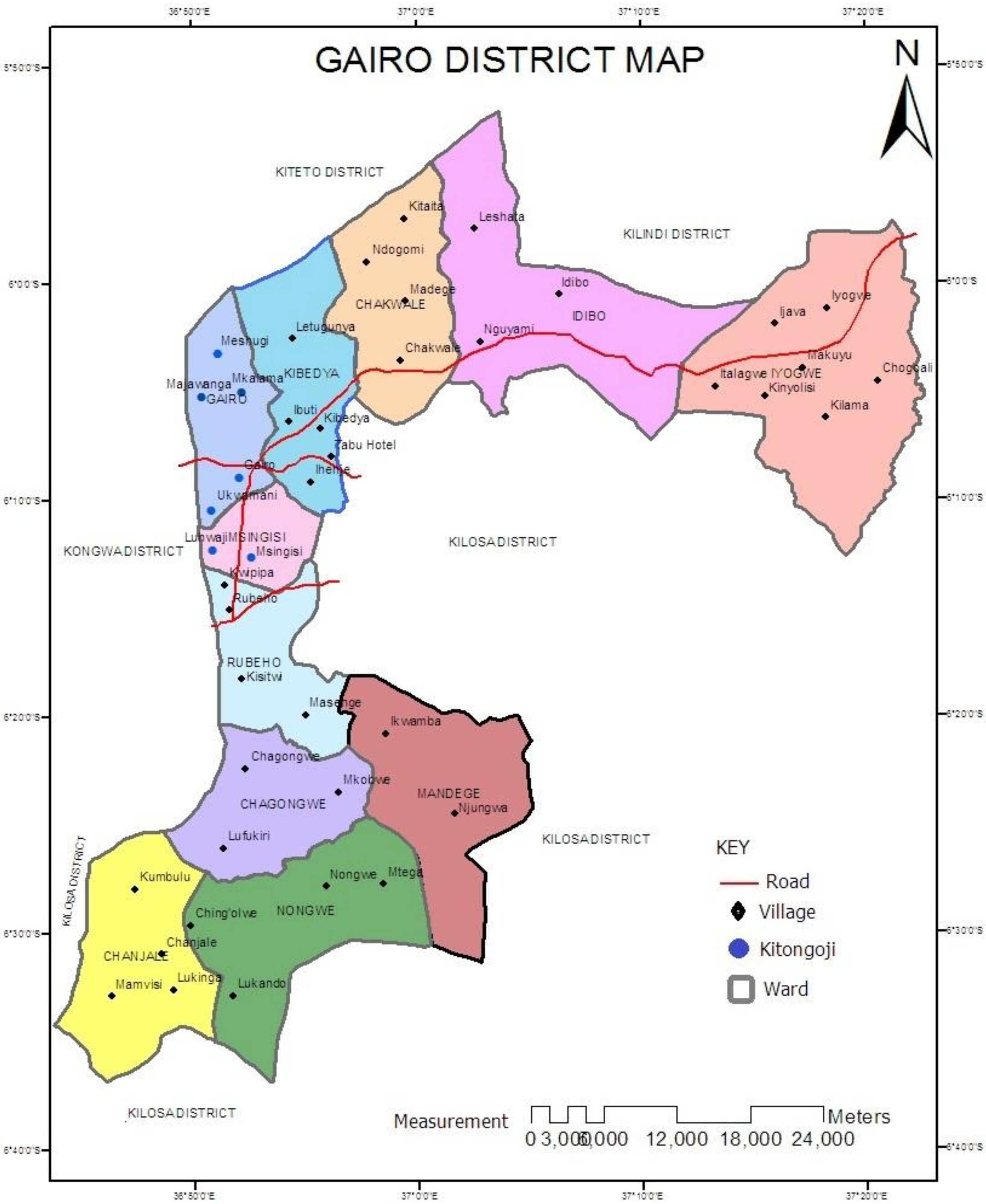
APPENDICES

Appendix 1: District map of Gairo showing surveyed wards



Appendix 2: Questionnaire on maize storage practices

Qnr……..

My name is Halifa H. Sume, a student from Sokoine University of Agriculture (SUA). I am currently doing my research on effect of maize storage and milling practices on total aflatoxin in maize flour as a requirement for the completion of Msc. Food Quality and Safety Assurance degree programme. The purpose of this interview is to collect information on the storage practices. This information will be useful in improving maize farming sector as far as the maize storage is concerned. You will be interviewed on your post-harvest maize storage practices. The interview will be recorded in a questionnaire. No one else but the interviewer will be present unless you would like someone else to be there. The information recorded will be confidential and no one else except the researcher (s) will be able access. Please feel free to participate and if you have any question regarding the research please ask to the interviewer and he /she will explain to you.

A. Demographic information

1. Interviewee (farm head) name……………2. Sex………..3.Ward…………4 Village…….

B. Maize storage (circle the appropriate answer)

5. What type of storage do you use among the following?
   (you can circle more than one if applicable)
   (a) Roof storage (b) Sack storage (c) Open field storage (d) Hermatic storage
6. If sack storage is used what type of storage bag? (a) Polypropylene
   (b) Sisal-woven bag
7. In what form do you preferably store maize? (a) Husked maize (b) De-husked maize cobs (c) Shelled grains

8. Do you store maize in the store every season

(a) No why………………………………….

(b) Yes Why………………………………..

9. Do other cereal products being stored together with maize?

(a) No (b) Yes

10.If Yes list them.………………………………………………………………….................

C. Condition of building/ store

11. Which storage building is more descriptive of your storage structure?

(a) Clay walls and thatch roofed (b) Clay walls and Iron sheet roofed (c) Wood and Iron sheet roofed (d) wood and thatch roofed (e) Block/concrete walls and iron sheet roofed

(f) Block/concrete walls and thatch roofed (g) Metal/ Iron sheet silo

12. For how many seasons have you used the store?

13. Does the store roof have leakages? (a) Yes (b) No

14. How often do you subject your storage structure to routine maintenance?

(a) Once a month (b) Once every 3 months (c) Once every 6 months (d) Once per year (e) Whenever necessary (f) never done

15. With the aid of visual observation does the storage area looks clean? (a) Yes (b) No

D. Storage time state of maize grains (circle more than one response if applicable)

16. For how long the grains were stored after harvest?...............month(s)

17. Rate the dry state of harvested grains (a) Moist (b) dry (c) Not sure

18. What drying process is adopted prior to storage (a) Mats (b) roof (c) floor (d) smoke

19. Describe state of stored grains (a) clean (b) spoiled (c)dried (d) moist (e) moulded

20. Any sorting before storage? (a) Yes (b) No

21. If yes, how do you sort……………………………………………

22. What criteria do you use when sorting? (a) Colour (b) Size (c) Shape (d) insect infested (e) Physical damaged (f) mould

23. Are the pesticides applied for grain treatment prior to storage? (a) Yes (b) No

24. If the answer above is Yes, name the type of pesticide used…………

25. In general opinion: how do you rate the storage condition (a) Good (b) Fair (c) Poor

26. How do you consider store aeration (a) Good (b) Fair (c) Poor?

Thank you for your response

Appendix 3: Questionnaire on maize milling practices Qnr……..

My name is Halifa H. Sume, a student from Sokoine University of Agriculture (SUA). I am currently doing my research on **effect of maize storage and milling practices on total aflatoxin in maize flour** as a requirement for the completion of Msc. Food Quality and Safety Assurance degree programme. The purpose of this interview is to collect information on the **milling practices.** This information will be useful in improving maize flour quality and safety. You will be interviewed on your maize milling practices. The interview will be recorded in a questionnaire. No one else but the interviewer will be present unless you would like someone else to be there. The information recorded will be confidential and no one else except the researcher (s) will be able access. Please feel free to participate and if you have any question regarding the research please ask to the interviewer and he /she will explain to you.

A. Demographic information

2. Interviewee (premises) name………………..2.Ward…………3. Village…………..

B. Condition of building and milling machines (You can circle more than one response if applicable)

4. What are the building materials for the premises?

(a) Clay walls and thatch roofed (b) Clay walls and Iron sheet roofed (c) Wood and Iron sheet roofed (d) wood and thatch roofed (e) Block/concrete walls and iron sheet roofed

(f) Block/concrete walls and thatch roofed (g) Metal/ Iron sheet silo

5. How often you clean the machines and equipment subject to the processing?

(a) Everyday (b) Every week (c) Once a month (e) never done (f) more than twice a day (g) Twice a week (h) prior to every milling

C. Maize milling

6. Any sorting before milling? (a) Yes (b) No

7. If sorting is conducted what criteria do you use when sorting?

(a) Colour (b) Size (c) Shape (d) insect infested (e) Physical damaged (f) mould

8. Is the maize being stored before milled (a) Yes (b) No

9. If Yes, how long is maize being stored prior to milling?...................Months

10. For how many years have you used the store?...

11. Do other cereal products being stored together with maize? (a) No (b) Yes

12. If Yes list them………………………………………………

13. Do you mill, label and pack the maize flours for selling? (a) No (b) Yes

14. If yes, to whom are you selling your products? (a) local residents

(b) retail traders (c) other merchants

(specify)…………………………………………………………………

15. How is the maize processing done among the following (a) dry dehulling then milling

(b) Wet (soaked) dehulling then milling (c) Whole grains milling (no dehulling)

16. Are the pesticides applied to stored grains prior to milling (a) Yes (b) No

17. If Yes what is the name/type of the pesticide used……………………

18. If pesticide is used for stored maize grains how long does it stay before milling?............

19. In approximation what is the higher milling preference of customers among the following

(a) dehulled milling and (b) whole maize milling

20. In general how do you rate the milling practices? (a) Good (b) Fair (c) Poor

21. How do you rate the state of machines, premises and equipment cleanliness?

(a) Good (b) Fair (c) Poor

Thank you for your cooperation

Appendix 4: HPLC Analysis report sample IND-03-M

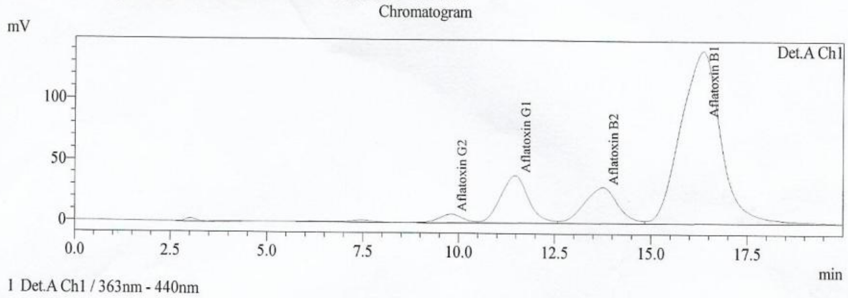
===Shimadzu LC Solution Analysis Report===

Sample Information C:\Mycotoxins\SUME-SUA\BATCH-\DATA 30-1-17\24.lcd

Acquired by	: Ezekiel Mubito
Sample Name	: Maize Flour
Sample ID	: IND-03-M
Tray#	: 1
Vail#	: 25
Injection Volume	: 10 uL
Data Filename	: 24.lcd
Method Filename	: Total Aflatoxin Method in Cereals.lcm
Batch Filename	: Postrun batch 16-3-2017.lcb
Report Filename	: Report RF 10AXL.lcr
Date Acquired	: 06/02/2017 21:40:49
Data Processed	: 16/03/2017 15:40:18

<Chromatogram>

Chromatogram
Maize Flour C:\Mycotoxins\SUME-SUA\BATCH-\DATA 30-1-17\24.lcd



1 Det.A Ch1 / 363nm - 440nm

<Results>

Quantitative Results

Detector A

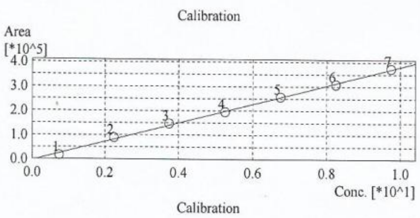
ID#	Name	Ret. Time	Area	Height	Conc.	Units	Mark
1	Aflatoxin G2	9.804	285011	6835	7.512	ng/g	V
2	Aflatoxin G1	11.471	1951465	38828	59.738	ng/g	V
3	Aflatoxin B2	13.752	1797594	29510	17.971	ng/g	V
4	Aflatoxin B1	16.343	10235559	140502	202.883	ng/g	V

Calibration Curve

ID#	: 1
Name	: Aflatoxin G2
Quantitative Method	: External Standard
Function	: f(x)=38539.7*x-4500.25

Rr1=0.9988065 Rr2=0.9976143
MeanRF:37385 RFSD:4098.1 RFRSD:10.9619

FitType	: Linear
ZeroThrough	: Not Through
Weighted Regression	: None
Detector Name	: Detector A



#	Conc. (Ratio)	MeanArea	Area
1	0.750	15921.4	15921
2	2.250	89742.8	94677
			88059
			86492
3	3.750	146547.3	147303
			144436
			147903
4	5.250	194829.8	195944
			197865
			190681
5	6.750	256757.0	258523
			258885
			252864

Appendix 5: HPLC Analysis report sample IND-03-DM

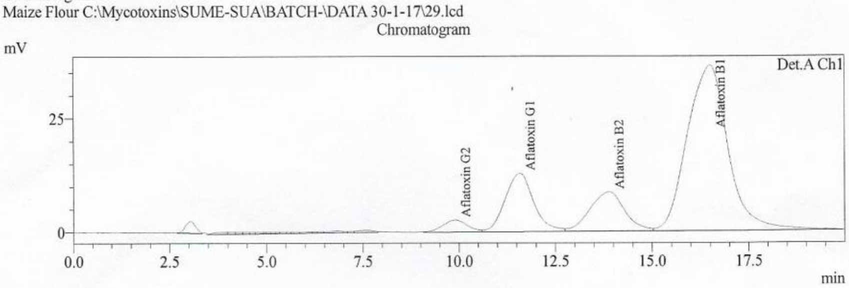
====Shimadzu LC Solution Analysis Report===

Sample Information C:\Mycotoxins\SUME-SUA\BATCH-\DATA 30-1-17\29.lcd

Acquired by	: Ezekiel Mubito
Sample Name	: Maize Flour
Sample ID	: IND-03-DM
Tray#	: 1
Vail#	: 28
Injection Volume	: 10 uL
Data Filename	: 29.lcd
Method Filename	: Total Aflatoxin Method in Cereals.lcm
Batch Filename	: Postrun batch 16-3-2017.lcb
Report Filename	: Report RF 10AXL.lcr
Date Acquired	: 06/02/2017 23:23:10
Data Processed	: 16/03/2017 15:40:20

<Chromatogram>

Chromatogram
Maize Flour C:\Mycotoxins\SUME-SUA\BATCH-\DATA 30-1-17\29.lcd



1 Det.A Ch1 / 363nm - 440nm

<Results>

Quantitative Results

Detector A

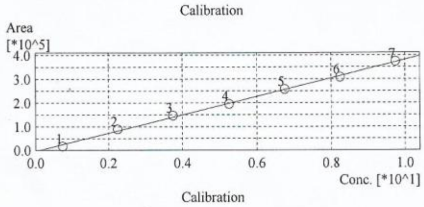
ID#	Name	Ret. Time	Area	Height	Conc.	Units	Mark
1	Aflatoxin G2	9.906	112439	2569	3.034	ng/g	V
2	Aflatoxin G1	11.588	656225	12817	20.142	ng/g	V
3	Aflatoxin B2	13.886	535645	8615	5.359	ng/g	V
4	Aflatoxin B1	16.506	2674748	36363	53.192	ng/g	SV

Calibration Curve

ID#	: 1
Name	: Aflatoxin G2
Quantitative Method	: External Standard
Function	: f(x)=38539.7*x-4500.25

Rr1=0.9988065 Rr2=0.9976143
MeanRF:37385 RFSD:4098.1 RFRSD:10.9619

FitType	: Linear
ZeroThrough	: Not Through
Weighted Regression	: None
Detector Name	: Detector A



#	Conc. (Ratio)	MeanArea	Area
1	0.750	15921.4	15921
2	2.250	89742.8	94677
			88059
			86492
3	3.750	146547.3	147303
			144436
			147903
4	5.250	194829.8	195944
			197865
			190681
5	6.750	256757.0	258523
			258885
			252864

Appendix 6: Analysis of Variance (ANOVA) Tables

Three Factor Completely Randomized Design

Data case no. 1 to 54.

Factorial ANOVA for the factors:
Replication (Var 3: REPLICATION) with values from 1 to 2
Factor A (Var 9: STORAGE) with values from 1 to 3
Factor B (Var 1: LOCATION) with values from 1 to 3
Factor C (Var 2: MILLING TYPE) with values from 1 to 3

A N A L Y S I S   O F   V A R I A N C E   T A B L E

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value
2	Factor A	2	229.447	114.724	1111.9486
4	Factor B	2	6.743	3.372	32.6801
6	AB	4	81.701	20.425	197.9707
8	Factor C	2	134.869	67.435	653.6029
10	AC	4	87.855	21.964	212.8825
12	BC	4	64.896	16.224	157.2502
14	ABC	8	249.012	31.127	301.6906
-15	Error	27	2.786	0.103	
	Total	53	857.311		

Coefficient of Variation: 10.12%

$s_{\bar{y}}$ for means group 2: 0.0757 Number of Observations: 18

$s_{\bar{y}}$ for means group 4: 0.0757 Number of Observations: 18

$s_{\bar{y}}$ for means group 6: 0.1311 Number of Observations: 6

$s_{\bar{y}}$ for means group 8: 0.0757 Number of Observations: 18

$s_{\bar{y}}$ for means group 10: 0.1311 Number of Observations: 6

$s_{\bar{y}}$ for means group 12: 0.1311 Number of Observations: 6

$s_{\bar{y}}$ for means group 14: 0.2271 Number of Observations: 2

Variable 5: AFLAT G1 MICR /KG

Grand Mean = 46.337   Grand Sum = 2502.187   Total Count = 54

A N A L Y S I S   O F   V A R I A N C E   T A B L E

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value
2	Factor A	2	51934.414	25967.207	80081.3028
4	Factor B	2	8471.917	4235.958	13063.4403
6	AB	4	10645.835	2661.459	8207.7785
8	Factor C	2	12375.292	6187.646	19082.3279
10	AC	4	12399.680	3099.920	9559.9667
12	BC	4	7602.335	1900.584	5861.2860
14	ABC	8	7293.609	911.701	2811.6315
-15	Error	27	8.755	0.324	
	Total	53	110731.837		

Coefficient of Variation: 1.23%

s_ for means group 2: 0.1342 Number of Observations: 18
y

s_ for means group 4: 0.1342 Number of Observations: 18
y

s_ for means group 6: 0.2325 Number of Observations: 6
y

s_ for means group 8: 0.1342 Number of Observations: 18
y

s_ for means group 10: 0.2325 Number of Observations: 6
y

s_ for means group 12: 0.2325 Number of Observations: 6
y

s_ for means group 14: 0.4027 Number of Observations: 2
y

Variable 6: AFLATOXIN B2 MICR/KG

Grand Mean = 7.203   Grand Sum = 388.949   Total Count = 54

ANALYSIS OF VARIANCE TABLE

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value
2	Factor A	2	1254.251	627.125	24179.0637
4	Factor B	2	174.530	87.265	3364.5345
6	AB	4	197.401	49.350	1902.7138
8	Factor C	2	225.515	112.757	4347.4082
10	AC	4	204.070	51.018	1967.0008
12	BC	4	24.151	6.038	232.7880
14	ABC	8	349.078	43.635	1682.3541
-15	Error	27	0.700	0.026	
	Total	53	2429.695		

Coefficient of Variation: 2.24%

$s_{\bar{y}}$ for means group 2: 0.0380 Number of Observations: 18

$s_{\bar{y}}$ for means group 4: 0.0380 Number of Observations: 18

$s_{\bar{y}}$ for means group 6: 0.0657 Number of Observations: 6

$s_{\bar{y}}$ for means group 8: 0.0380 Number of Observations: 18

$s_{\bar{y}}$ for means group 10: 0.0657 Number of Observations: 6

$s_{\bar{y}}$ for means group 12: 0.0657 Number of Observations: 6

$s_{\bar{y}}$ for means group 14: 0.1139 Number of Observations: 2

Grand Mean = 134.945 Grand Sum = 7287.047 Total Count = 54

A N A L Y S I S O F V A R I A N C E T A B L E

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value
2	Factor A	2	408792.740	204396.370	127561.2127
4	Factor B	2	109415.730	54707.865	34142.4928
6	AB	4	52779.142	13194.786	8234.7003
8	Factor C	2	189916.190	94958.095	59262.1569
10	AC	4	112763.693	28190.923	17593.6019
12	BC	4	34325.498	8581.374	5355.5282
14	ABC	8	38930.683	4866.335	3037.0189
-15	Error	27	43.263	1.602	
	Total	53	946966.937		

Coefficient of Variation: 0.94%

$s_{\bar{y}}$ for means group 2: 0.2984 Number of Observations: 18

$s_{\bar{y}}$ for means group 4: 0.2984 Number of Observations: 18

$s_{\bar{y}}$ for means group 6: 0.5168 Number of Observations: 6

$s_{\bar{y}}$ for means group 8: 0.2984 Number of Observations: 18

$s_{\bar{y}}$ for means group 10: 0.5168 Number of Observations: 6

$s_{\bar{y}}$ for means group 12: 0.5168 Number of Observations: 6

$s_{\bar{y}}$ for means group 14: 0.8951 Number of Observations: 2

Variable 8: TOTAL AFLT MICR/KG

Grand Mean = 191.658   Grand Sum = 10349.545   Total Count = 54

A N A L Y S I S   O F   V A R I A N C E   T A B L E

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value
2	Factor A	2	841845.076	420922.538	160798.9387
4	Factor B	2	191999.104	95999.552	36673.3179
6	AB	4	124648.166	31162.041	11904.3832
8	Factor C	2	295953.553	147976.776	56529.4239
10	AC	4	210076.854	52519.214	20063.1542
12	BC	4	70518.614	17629.653	6734.8011
14	ABC	8	73170.061	9146.258	3494.0123
-15	Error	27	70.678	2.618	
	Total	53	1808282.105		

Coefficient of Variation: 0.84%

$s_{\bar{y}}$ for means group 2:   0.3813   Number of Observations: 18

$s_{\bar{y}}$ for means group 4:   0.3813   Number of Observations: 18

$s_{\bar{y}}$ for means group 6:   0.6605   Number of Observations: 6

$s_{\bar{y}}$ for means group 8:   0.3813   Number of Observations: 18

$s_{\bar{y}}$ for means group 10:   0.6605   Number of Observations: 6

$s_{\bar{y}}$ for means group 12:   0.6605   Number of Observations: 6

$s_{\bar{y}}$ for means group 14:   1.1440   Number of Observations: 2