UNIVERSITY OF NAIROBI

PREVALENCE AND FACTORS ASSOCIATED WITH AFLATOXIN CONTAMINATION OF STAPLE FOODS IN RURAL BUSIA COUNTY, KENYA

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Reg. No.: H80/92361/2013

Thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in Public Health in the Department of Public and Global Health

August 2022

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University for examination.

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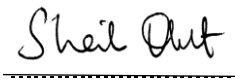
Signature:  **Date:** Sept 2nd 2022

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ACKNOWLEDGEMENTS

The assistance of the following people and organizations without whom this project would not have been possible is gratefully acknowledged:

1. Technically - My very patient supervisors Dr Faith Mugure Thuita and Professor Sheila Okoth for the tireless guidance, insights, positive criticism and encouragement that they provided over the years in the course of my work. My professional work supervisors Joel Montgomery, PhD and Marc-Alain Widdowson, PhD of Centers for Disease Control, Atlanta and Kenya respectively for shepherding me through the process.

2. Intellectually (assistance, advice) - My dear parents Engineer Akisoferi N. Okello and Mwalimu Bethseba Rose Okello.

3. Financially (CDC for partial support towards my tuition and field activities) and Helica Biosystems for support towards my field activities and laboratory testing

4. The data team comprising Leonard Cosmas Otieno of WHO, Fred Otiato and Maureen Ondire of KEMRI and Janet Adhiambo and Molly Atieno who ensured that all data was monitored, entered, cleaned and analyzed all in good time.

5. The lab assistants, Kevin and Hesbon for their tireless effort and input during samples testing.

6. Research team led by James Omondi – CDC Kenya, Ms Rosemary Okuku and Pius Irukan - MoH Busia, Mr. Alphonse Otiato, Constant Emukule, Martin Luciano – UoN, Alfred Papai, Douglus Odera, Zelda Nasirumbi.

7. The CDC Kenya drivers who maneuvered through challenging terrains and ensured we were in the field in time and safe.

DEDICATION

Dedicated to God from whom all wisdom flows and my lovely family: my dear husband Fred J.O Awuor, my daughter Sandra Hawi Awuor and son Sheldon Hera Awuor.

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ABBREVIATIONS AND ACRONYMS

AFB_1	Aflatoxin B1
ANOVA	Analysis of Variance
aW	Water activity
CDC	Centers for Disease Control & Prevention
cELISA	Competitive ELISA
CFR	Case Fatality Rate
CHU	Community Health Unit
CHV	Community Health Volunteer
CAC	The FAO/WHO Joint Codex Alimentrius Commission
DNA	Deoxyribonucleic acid
FAO	Food and Agricultural Organization
FBD	Food Borne Disease
FGDs	Focus Group Discussions
GAPs	Good Agricultural Practices
GAC	Granulated Activated Carbon
GI	Gastro-intestinal
GIS	Geographic Information Systems
GPS	Global Positioning System
HCC	Hepato-cellular Carcinoma
HSCAS	Hydrated Sodium Calcium Alumino Silicate

IARC	International Agency for Research on Cancer
ICBT	Informal Cross Border Trade
ISO	International Standards Organization
KEBS	Kenya Bureau of Standards
KIIs	Key Informant Interviews
KM	Kilometers
KS	Kenya Standards
LOD	Limit of Detection
LMIC	Low- and Middle-Income Countries
M_1	Aflatoxin M1, is a hydroxylated metabolite of Aflatoxin B_1
MLs	Maximum Limits
MTL	Maximum Tolerable Limits
NCEH	National Center for Environmental Health
ODK	Open Data Kit
PI	Principal Investigator
PPB	Parts Per Billion
PPT	Parts Per Trillion
RA	Research Assistants
SPS	Sanitary and Phytosanitary
USDA	United States Department of Agriculture

PAPERS PUBLISHED FROM THIS THESIS

1. **Prevalence of aflatoxins in dietary staples in the border county of Busia, western Kenya**

   Awuor, A. O., Thuita, F. M., & Okoth, S. D. (2021). Prevalence of aflatoxins in dietary staples in the border County of Busia, Western Kenya. African Journal of Food, Agriculture, Nutrition and Development, 20(7), 17045-17062.

   DOI:10.18697/ajfand.95.19815

2. **Community knowledge and awareness of aflatoxin in dietary staples in rural Busia County, Kenya: A mixed methods study**

   Awuor, A. O., Okoth S. D., & F. M. Thuita. (2021). Community knowledge and awareness of aflatoxin in dietary staples in rural Busia County, Kenya: A mixed methods study. African Journal of Food, Agriculture, Nutrition and Development 21(103),18414-18434

   DOI:10.18697/ajfand.103.21325

ABSTRACT

Food safety is a major challenge in many Low- and Middle-Income Countries including Kenya. The most common food safety challenge in Kenya is aflatoxin contamination. Long term dietary exposure to low doses of these toxigenic fungi have been associated with chronic disease like liver cancer while exposure to high doses has been linked to hepatic failure in humans. This study aimed to determine the prevalence of aflatoxin in staple foods including maize, sorghum, millet, groundnuts and cassava consumed in households within Budalang'i, Nambale and Teso- South sub-counties in Busia County; describe factors associated with aflatoxin contamination of the cereals, groundnuts and cassava; to evaluate the effect of selected preparation methods on aflatoxin contamination levels in the cereals and to determine the consumption patterns of maize, sorghum, millet, groundnuts and cassava in the study households.

This study utilized a convergent mixed methods design. Both quantitative and qualitative data were collected in parallel, analyzed separately and then merged at results and discussion levels. Household geo-coordinates, respondents' socio demographic profile, food sources, storage practices and food consumption patterns were collected and entered data into an Open Data Kit (ODK). A household survey was conducted. Dietary diversity data, food frequency and 24 hr food recall data were collected and analyzed descriptively. Food samples collected from sampled households were tested to determine the levels of total aflatoxins using competitive ELISA method. Some contaminated food samples were either boiled, fermented and boiled or boiled in locally made alkaline solutions and levels of aflatoxin assessed after each process. Quantitative data were analyzed using SAS 9.4 software. Six focus-group discussions each with 11-12 participants and sixteen key informant interviews were also conducted and recorded using

Olympus recorder and analyzed using Nvivo version 10. In-depth information on the food sources, community diets, food storage and food preparation practices and awareness of aflatoxin among a sub-set of community members was gathered.

Maize, sorghum, cassava and millet are the staples. However, frequency of maize consumption was highest of all the grains. All food samples collected and tested (n=493) had detectable levels of aflatoxin. The levels of aflatoxin ranged from 1-1584ppb in maize, 0.3 to 740ppb in sorghum, 0.5 to 15ppb in cassava, from 0.5 to 12 ppb in millet and 0.1 to 2.8 in groundnuts. Overall, maize recorded the highest level of contamination (mean 100ppb; SD 252.9; range 1-1584ppb) with 31% of samples above East African Community regulatory limits (10ppb). Aflatoxin contamination in maize was seemingly higher than in sorghum though not statistically significant *(p=0.0568).* Homegrown maize was less likely to have aflatoxin levels >10ppb when compared to market sourced grain (OR 1.185, CI 0.554, 2.534) but difference was not statistically significant *(p= 0.0760).* Sorghum stored in buckets had a 12.81 likelihood of having higher than allowable limits of aflatoxin (OR 12.82, CI 2.566, 63.992) *(p=0.0096)* relative to sorghum stored in nylon sacks. Though maize stored in a bucket had a 1.61 likelihood to have less aflatoxin than that stored in a nylon sack (OR 1.650, CI 0.840, 3.247) the association was not significant *(p=0.2398).* Residents of Teso South and Nambale were at highest risk of acute exposure as demonstrated by the hotspot analysis. Boiling of maize in alkaline solution (ash salt) recorded a 72-91% reduction of aflatoxin level while 24 hour fermentation then boiling of maize, sorghum and cassava composite flours recorded a 64% reduction of aflatoxin. Residents of Busia County exhibited very low levels of awareness of aflatoxins, aflatoxin mitigation practices and the possible negative health effects resulting from the exposure to these toxins mainly as a result of limited education and sensitization provided by the county's ministries of agriculture and health.

Aflatoxins are prevalent in maize, sorghum millet, groundnuts and cassava in Busia County and residents are at risk of possible chronic exposure therefore there is need for instigation of aflatoxin mitigation measures. Surveillance of populations exposed to aflatoxin levels beyond KEBS acceptable limits is warranted and an aflatoxin sero-survey and a health impact assessment of this population is recommended. Awareness creation among farmers and retailers on pre and post-harvest food handling practices and on causes of aflatoxin occurrence and health implications, with the objective of encouraging voluntary compliance to public health regulations and improved food handling practices is advisable. Regulation of formal cross-border trade of these grains is also needed.

Knowledge on current food safety situation and trends of occurrence in the food chain is vital. This knowledge should be continually updated through systematic food sample collection, analysis and interpretation of data and dissemination to guide public health decision making. Lastly, a longitudinal study that would collect samples at different times of the year from the same sites in addition to socioeconomic, temporal, and biophysical data to assess for other determinants of contamination is highly recommended. There is also need for further research and document prevalence of contamination of animal source products such as eggs, milk and pork in Busia.

SECTION ONE

This section comprises of three chapters, the introduction, the literature review and statement of the problem. Chapter one, the introduction, gives general description of the food safety situation in the country with an emphasis on the challenge of aflatoxin in staple foods. A synthesis of relevant background literature is presented in chapter two. Herein is a synopsis of the state of food security and safety in Kenya, a description of the current efforts to tackle food safety and the influence of culture on food patterns. The importance of staples such as maize, sorghum, millet, cassava and groundnuts in diets of Kenyans is outlined. Prevalence of aflatoxin in foods consumed in Kenya is reported. Means through which aflatoxins get to humans are captured and the impact of exposure is reported. The public health and economic implications of aflatoxin contamination and exposure are also enumerated. A review of the common post- harvest decontamination strategies and regulatory limits of aflatoxin in foods for human consumption in Kenya is included. Chapter three presents the problem statement. The theoretical and conceptual frameworks are illustrated, research questions listed, and the study rationale explained. The study objectives, literature reviewed and statement of the research problem and study hypothesis that are derived from gaps identified in the study are enumerated.

CHAPTER ONE: INTRODUCTION

Food security as defined by the Food and Agricultural Organization (FAO) assumes all year-round access by households to sufficient quantity, quality, safe and nutritious food (FAO, 2001). About 2 billion people in the world experience moderate to severe food insecurity with the majority living in Low- and Middle-Income Countries (LMICs) (FAO, 2019). An estimated 51% of Kenyans lack enough food and 3 million are severely food insecure (Welborn, 2018; National Council for Population and Development, 2017). This situation has been largely attributed to low productivity and rapid population growth among other reasons (Population Action International, 2017).

Staple foods which are the foods that dominate a community's diet and are their major source of energy and nutrition are core to food security (FAO, 2012). However, if the production of such foods is affected by either climatic variations, pest infestation or disease, the food security situation is at risk (Intergovernmental Panel on Climate Change (IPCC), 2014). Maize is one of Kenya's key staples foods that has been affected by poor weather conditions and disease in the past few years. As a result, there have been significant maize shortage which has prompted government to import maize to fill the deficit (FAO, Unpublished). Additionally, poor or inadequate drying of seeds and poor storage can lead to pest attack and contamination by fungi, thus rendering it unfit for human consumption (Waliyar, et al., 2005a). In 2012, several metric tonnes of maize in the National Cereal Board warehouses were contaminated by aflatoxin and declared unfit for human consumption. The consignment was later destroyed by incineration (Kilonzo R. , Head of Environment Health Division and secretary of Food Safety Coordination Committe, Kenya Ministry of Health, 2012). This had a negative effect on the country's economy.

Globally and nationally, foodborne diseases like aflatoxicosis are important causes of morbidity and mortality as has been reported in Kenya and lately in Tanzania (Azziz-Baumgartner, et al.,

2005; Kamala, et al., 2018). Of the 317 reported aflatoxicosis cases from Kenya in 2004, in Makueni and Kitui districts of Eastern Province, 39% died and exposure was attributed to ingestion of contaminated maize. Aflatoxin levels of food samples from case households were as high as 20000ppb (Centers for Disease Control and Prevention, 2004; Wagacha & Muthomi, 2008; Muthomi, Njenga, Gathumbi, & Chemining'wa, 2009; Lewis, et al., 2005). Tanzania reported a case fatality rate of 29% (Kamala, et al., 2018). Indeed, aflatoxins compromises the safety of food and exacerbates food insecurity. The 2004 Kenya aflatoxicosis outbreak was traced back to ingestion of contaminated maize (Lewis, et al., 2005). Food is considered safe if it does not cause harm to the consumer when it is prepared or eaten and is thus an integral part of food security (FAO, 2001). According to WHO estimates reported in 2019, about 600 million people in the world fall ill upon ingesting contaminated food but children aged less than 5 years are reported to carry 40% of the foodborne disease burden. However, the true burden of Foodborne Diseases (FBD) in Low- and Middle-Income Countries (LMICs) remains underestimated due to underreporting (World Health Organization, 2019).

Aflatoxin compromises quality and safety of cereals which are a major component of the diet of Kenyans. Aflatoxins are harmful fungal metabolites produced mostly by *Aspergillus flavus* and *A. parasiticus* (Baranyi, Kocsube, Vagvolgyi, & Varga, 2013). These molds are prevalent in latitudes between 16° and 35°, around the equator and are major contaminants of cereals, grains and roots at several stages of food production (Scheidegger & Payne, 2003). They are ubiquitous in the environment but high temperatures and humidity, unseasonal rains during harvest, and improper harvesting and storage practices combine to create conditions suitable for fungal growth resulting in aflatoxin contamination pre- and post-harvest (Brown, Brown-Jenco, & Payne, 1999).

Exposure to aflatoxin in humans is mainly through ingestion. Residents of Busia County rely heavily on foods prone to aflatoxin contamination, such as maize, groundnuts and sorghum (Kang'ethe E. , 2011). In addition, results from an aflatoxin sero-survey showed all participants from Busia had detectable levels of aflatoxin (Yard, et al., 2013). In this sero-survey, a sub-set of Kenya Aids Indicator Survey (KAIS) specimen of 2007 were analyzed for aflatoxin-B1-lysine with the objectives of characterizing exposure in the Kenyan population and identifying high risk population. The samples were stratified by province and by sex. Indeed, over 75% of the population had detectable levels of aflatoxin. Exposure also varied regionally. However, dietary data was not collected thus exposure could not be directly associated with levels of contamination in the foods consumed. There is limited data on the prevalence of aflatoxin of most dietary staples in this agricultural rich county of Busia.

While grains can be contaminated by aflatoxin pre-harvest, contamination mostly happens post-harvest. Grains are prone to contamination if they are stored with high moisture levels, in storage spaces that can allow for pest infestation thus grain damage and if grains are in poorly aerated storage containers. The aim of this study was to determine the prevalence of aflatoxin contamination in selected food staples in three sub-counties in Busia County and to describe risk factors associated with contamination.

CHAPTER TWO: BACKGROUND LITERATURE

2.1 State of Food Security and Safety in Kenya

While Kenya has enjoyed decades of an impressive food production index, (World Data Atlas, 2018), a sharp decline in food production has been observed in the past decade. Kenya has a land mass of 580,367 square kilometers but the population of 47,564,296 is growing at 2.2 per cent annually (Kenya National Bureau of Statistics, 2019). Overcrowding reduces total land available per person for agricultural use thereby reducing the amount of farm produce available per person. Additionally, overcrowding degrades the environment and reduces agricultural productivity, but also bolsters the spread of diseases, which influence labor productivity (Riely, Mock, Cogill, Bailey, & Kenefick, 1999). Indeed, the increase in population has driven up the demand for more food and water (Population Action International, 2017). This phenomenon concurs with the Malthusian theory where population growth is postulated to increase rapidly and thus creating a problem for food production and supply (Malthus, 1826). Increase in population densities in Kenya has resulted in land fragmentation, which has in turn contributed to inefficient and destructive farming practices, and increased usage of the available 20% marginal arable land thus has reduced food production (Ministry of Environment and Natural Resources, 2016).

Food insecurity is closely linked to poverty. According to the national estimates, Kenya's poverty index was at 36.1% in 2015/16. The budget estimates during the same time also indicated that poverty levels were higher in the rural areas (40%) compared to urban areas (29%) (KNBS, 2018). Poverty limits this population to access food due to having a poor resource base.

Agriculture is the mainstay of the rural population in Busia County yet only 20% of the land is arable (Campbell, Lusch, Smucker, & Wangui, 2003). Most farmers rely heavily on rain fed

agriculture and as a result of irregular and erratic rainfall patterns, a reduction in food production has been observed. This has been worsened by plant diseases, armyworm infestation and high global food prices and low purchasing power for large proportion of the population due to high levels of poverty (FAO, 2018). Unfortunately, during times when surplus produce is realized, post-harvest loses result from either inadequate storage facilities or poor storage practices. It has been estimated that between 30-40% of surplus maize is lost through weevil damage, discoloration leading to quality loss and poor shelling and on farm drying practices (Ministry of Agriculture, Livestock and Fisheries, 2017). Post- harvest contamination with mycotoxins is also rampant (Okoth., 2016). This is exemplified by the disposal of aflatoxin contaminated maize worth $US 5 million that Government of Kenya had to undertake in 2014 (Bandyopadhyay, et al., 2016).

While women are pivotal to agricultural production, they do not have access to new agricultural technologies and other resources for production like land when compared to the male counterparts. Low women's empowerment in agriculture has exacerbated food insecurity (Government of Kenya, 2011).

Food security remains inextricably linked to food safety. Bacteria like *Salmonella*, *Campylobacter*, and *Enterohemorrhagic Escherichia coli* (*EHE coli*), Hepatitis A virus, parasites and worms are the most common foodborne pathogens and toxins like aflatoxins that cause foodborne morbidity and mortality (Fung & Clark, 2004). Whereas there are challenges in estimating mortality attributed to FBDs, the WHO, approximately half a million lives are lost annually as a result of consumption of unsafe food (World Health Organization, 2015). In Kenya, while infections due to *Salmonella typhi*, *Shigella spp* and *Vibrio cholerea* are closely monitored by the ministry of health, there is limited monitoring of aflatoxin in agricultural produce sold in the informal markets. Lack of enforcement of food safety regulations in these domestic informal

markets amplifies the food safety challenge (Grace, Makita, Kang'ethe, & Bonfoh, 2010; Ombui, 2001). This is compounded by the heterogeneity and fragmented domestic market system. The weak systems expose the consumer to all foodborne threats.

2.2 Current efforts to tackle food insecurity and safety in Kenya

To help mitigate food insecurity among its people, the Kenya government formulated the Food and Nutrition Security Policy (FNSP 2011). The objective of this policy was to create synergy among different sectors and among government and other partner initiatives in order to achieve adequate nutrition for optimum health of all Kenyans; increase the quantity and quality of food available, accessible and affordable to all Kenyans at all times; and protect vulnerable populations using creative, feasible and affordable safety nets linked to long-term development. The FNSP Implementation Framework (FSNP-IF) identified areas of food availability, food accessibility, household resource productivity, food safety standards and quality control, nutrition improvement, food nutrition in crisis and emergency, food and nutrition security data and information management among others as core components that needed to be addressed in a synchronized approach. This would in turn enhance prioritization of food security agenda at national and county levels. Generally, many IF activities relate to supply, prices and income to help mitigate the accessibility and availability crisis (Ministry of Agriculture, Livestock and Fisheries, 2017). Some of the supply related policies include: i) encouraging diversification of crops planted; ii) encouraging citizens to diversify their eating habits to avoid over reliance on maize and iii) providing farmers with seeds especially for the drought tolerant crops to be grown in arid and semi-arid areas among others (Kenya Agricultural Research Institute, 2012). Indeed, realization of this policy requires significant financial investment. Various donors under the umbrella of Scaling Up Nutrition (SUN) have assisted in the the implementation of various components of the FNSP.

With the support from donor agencies like UKaid, USAID, DFID, World Bank and European Union, the County Government of Busia has one medium sized irrigation scheme in Budalangi and several other smaller ones operated by groups of farmers. Sisenye irrigation scheme, Mudembi irrigation scheme and Budalang'i National irrigation scheme all operated by the National Irrigation Board while Ludacho community irrigation in Sio Port, Samia Fruits irrigation, Nandikinya irrigation scheme are operated by farmer groups (Busia County, 2018).

The government of Kenya identified expansion of the agricultural sector as the key to improved productivity. Some interventions that the government initiated to moderate food insecurity include waiver of duty on imported maize in times of need, importation of maize by government to replenish Strategic Grain Reserves, increased maize producer prices, procuring fertilizers in bulk, and reduction of fertilizer prices. However, as food security remains tenuous, some targeted programmes addressing food security include: (i) National Accelerated Agriculture Inputs Access Programme (NAAIAP) for improved access and affordability of key inputs to small holder farmers living below the absolute poverty line; (ii) Orphaned Crops Programme for diversification of sources of food through promotion of drought tolerant indigenous crops; (iii) Revitalization of Agricultural Mechanization Services for better agricultural infrastructure and land development to Kenyan farmers; and (iv) utilizing irrigation for food production (Government of Kenya, 2011). Government has invested heavily in large irrigation schemes in both Hola and Bura with an aim of addressing the rampant short fall in maize availability (Bandyopadhyay, et al., 2016).

USAID Kenya in collaboration with the GOK has had projects like Kenya Agricultural Value Chains Enterprises Project (KAVES) in Busia a project that helped promote value chain growth and diversification in order to increase the productivity and incomes of smallholder farmers (Thuita, 2016).

The implementation of this policy framework has not been without some challenges. For instance, while 90% of rural women contribute to agricultural production by working on family farms and provide 80% of labor in smallholdings, they face exclusion and economic injustice (World Bank, 2003). Also, while direct funding to the agricultural sector has increased, recipients of these funds are the middle class and elite and not the rural women who contribute largely to food production (Kilonzo P. , 2019).

Currently, issues on food availability and access, including storage and processing and food safety standards and quality control have been addressed in the Kenya Food Security and Nutrition Policy (Government of Kenya, 2011). While the responsibility of ensuring food safety and quality in the county is scattered amongst various regulatory ministries and departments, the major responsibility resides with the Department of Public Health, Government Chemist, Kenya Bureau of Standards, Department of Veterinary Services and Kenya Plant Health Inspectorate Service. The food safety laws are enshrined in 3 main Acts of the Laws of Kenya namely: i) Public Health Act Cap 242; ii) Food, Drugs and Chemical Substances Act Cap 254, and iii) the Standards Act Cap 496. Clearly, several government agencies have a mandate to ensure food safety in Kenya under the stewardship of the National Food Safety Coordination Committee (NFSCC). The various agencies and laws that guide food safety in Kenya are shown in appendix 1. Unfortunately, the NFSCC has not been able to effectively execute its mandates. This is exemplified by the frequent media reports showcasing flaws in the food safety and uncoordinated efforts among government agencies to ensure food safety especially for local consumers is maintained (Nation Media, 2019; Andae, 2019). Additionally, there are recommendations for the establishment of the National Food Safety Authority, a body that will be mandated to coordinate all the existing food control infrastructure and services and redefine their roles in order to eliminate areas of overlap and conflict beside other

roles because the NFSCC in unable to effectively carry out this role (Kilonzo & Gathura, Kenya Food Control System, 2018).

Efforts to address food safety challenges in Kenya date back to 1981 with the development of the National Food Policy sessional paper No. 4, which was improved and merged into sessional paper No. 1 of 1986 to create the Economic Management for Renewed Growth. This policy aimed at sustaining self-sufficiency in major foodstuffs and ensuring equitable distribution of food of nutritional value to all citizens. This 1986 sessional paper has since metamorphosed to the Kenya Food Security and Nutrition Policy of 2011. Kenya's food safety agenda is hinged on the Sanitary and Phytosanitary (SPS) measures agreement of the World Trade Organization (WTO), which sets out the basic rules for food safety and animal and plant health standards. KEBS has developed standards on food technologies, food safety, fertilizers, agricultural produce, livestock and livestock products, poultry and poultry products (Kenya Bureau of Standards, 2019). The roles of KEBS include quality assurance and inspection of commodities, market surveillance to ensure products conform to the requirements, testing for conformity and training on quality related courses. However, these duties have not been exercised fully due to constrains such as human and financial resource limitations, lack of the latest testing equipment for products and lack of trained staff of international standards (Chitembwe, 2012). Consumption of contaminated food over time chronically exposes the public to aflatoxin thus predisposes them to effects of aflatoxin like hepatocellular carcinorma (Yan & Wu, 2010; Ross, et al., 1992), impaired immunity (Jiang 2005) and stunting among children (Gong Y. , et al., 2002; Khlangwiset, Shephard, & Wu, 2011). In 2018 alone, there were 11,550 reported new liver cancer cases and 11,251 liver cancer related deaths in East African alone (IARC, 2018). To this end, Government of Kenya through the Kenya Agriculture and Livestock Research Organization (KALRO), working in collaboration with

International Institute of Tropical Agriculture (IITA) has built a manufacturing plant for Aflasafe KE01 in Machakos to ensure for the production of aflasafe for sell to farmers use to control of aflatoxin on farm (Bandyopadhyay, et al., 2016).

2.3 Common foods in Kenyan diets

Common foods in Kenyan diets include maize, wheat, beans, sorghum, millet, cassava and groundnuts. The choice and consumption of specific staple foods varies across region and is largely influenced by culture. Culturally conveyed classifications of food determine what potential foods are included in the household's regular diet (Helman, 2007). This in turn influences the choice of food to produce and how it is produced. Certain traditional crops can be used as substitutes in times of environmental stresses (Milburn, 2004). Additionally, some traditional pest management systems are well adapted to local environments and risks (Jaenicke & Höschle-Zeledon, 2006). Food processing and storage is also determined by culturally transmitted knowledge and practices related to food processing and storage techniques (Chipungu, Ambali, Kalenga Saka, Mahungu, & Mkumbira, 2012).

Maize (*zea mays*) is a staple of most households in Kenya with a daily consumption rate of 258g/person (ACDI/VOCA, 2019). It is an important source of carbohydrates, protein, vitamin B and minerals. Maize in Busia County is a staple and is grown by all communities. Cultivation happens during both the long and the short rain seasons. Maize in this region many at times is intercropped with sorghum, another staple in the county and beans. Among all the communities in Busia, dried maize mill flour is consumed as either stiff porridge also known as *ugali*, or thin porridge (*uji*) (Ebere, Kimani, & Imungi, 2017). Maize is also used in the preparation of local brews commonly known as "*busaa*" and "*changaa"* after undergoing fermentation. Green maize, fresh on the cob is either roasted or boiled. Green maize off the cob is also mixed with beans to

make a mixture commonly referred to as *mayengere* (luhyia) or *nyoyo* (luo) while the cob and damaged grains are used for animal feed production.

Production and consumption of traditional food crops like millet and sorghum has declined over the past decades with greater consumption of wheat and rice (Kennedy, 1994). Kennedy (1994) also reported food patterns are homogenous within a given area in the rural areas in Kenya. Finger millet (*Eleusine coracona*) is an important staple in East Africa. The grains comprise protein (8%), fat (1.3%), calcium (0.3%), phosphorus (0.3%), minerals (2.7%), carbohydrates (73%), fibre (3%) and moisture (13%) (Food and Agricultural Organization, 2001). Finger millet is mainly grown in Western Kenya by small holder farmers to meet their subsistence food requirements and the surplus is sold. Millet is predominantly grown around Lake Victoria and parts of the Rift Valley. The production of finger millet has been declining in in Kenya and in Busia County though there is still a significant demand for the crop. Additionally, finger millet prices in Kenya have been far above maize prices or any other cereal prices over the past years (Oduori, 2005). Dry milled finger millet flour, fermented or unfermented is used to prepare thin porridge, commonly known as *uji* in Kenya. Sorghum (*sorgum bicolor (*L*)*) is the only cereal species indigenous to Kenya. Sorghum flour is blended with cassava flour to make thin porridge (fermented or unfermented) or stiff porridge, known as '*ugali*'. The by-products from sorghum processing are typically used for animal feed production (Food and Agricultural Organization, 2013). Millet flour is often blended with sorghum, cassava, groundnuts in order to make weaning gruel for children (Okoth & Ohingo, 2004). Finger millet and sorghum remain major food crops among the Iteso and also important crops among the luhyia and luo communities.

Cassava (*manihot esculenta Crantz*) is an energy rich tuber with carbohydrate (38.06g), protein (1.36) g, (which carries vitamin B complex group of vitamins and chief source of zinc (0.34mg),

magnesium (21mg), iron (0.27mg) and manganese (0.383mg). It also has potassium (271mg per 100g). Young cassava leaves, which contain up to 25% protein, are used as vegetables (Food and Agriculture Organization of the United Nations, 2013). Of all the national cassava production, 60% is grown and consumed in Western region (Obiero, et al., 2007). Cassava is usually intercropped with beans, maize and bananas. Cassava flour is typically blended with other cereals like sorghum and maize to make either thin or stiff porridges. It also used to make cassava chips. In the 1920s colonial officials introduced cassava as a supplement to millet and sorghum as a famine-relief food and maize was then later introduced as cash crops (GoVisitKenya, 2014). To date, these foods are still used in the luhyia, Iteso and luo communities. Cassava is currently cooked with finger millet and sorghum

Groundnut (*Archis hypogea*) is a legume with high nutritional value: fat (47 to 59 per cent), protein (26 to 39 percent) and carbohydrate (11 percent), sodium (42.0 mg/100g), potassium (705.11mg/100g), magnesium (3.98mg/100g), calcium (2.28mg/100g), iron (6.97mg/100g, zinc (3.2mg/100g), phosphorus (10.55mg/100g) (Atasie, Akinhanmi, & Ojiodu, 2009). Most groundnuts are grown in Teso South sub-county. The area of land in Busia County under groundnut production is 950 hectares in Teso South, 350 hectares in Budalang'i and 250 hectares in Nambale sub-counties (Masira, 2017). Groundnuts are mostly eaten roasted or boiled while in shells or as a stew or as a paste.

2.4 Culture as a determinant of food consumption practices

While defining culture remains a challenging task, there is consensus that transmission of information like values, beliefs and behavioral norms both within and across generations is one of its mains traits (Alesina & Giuliano, 2015). It is also important to note that for a concept to be considered as cultural, then it has to be shared by at least two or more people and is also subject to

gradual change (Becker & Ghimire, 2003). Food consumption practices and food choices are examples of such concepts. Household food choice is factor of household dietary diversity. Indeed, there are several factors that influence household food consumption practices and food choices. These include taste preferences (Drewnowski & Levine, 2003; Birch, 1999), social factors like other family members (Evans, et al., 2011), time and acculturation (Dhokarh, et al., 2011) among other.

While food consumption practices are a function of key known factors such as food access and availability, socio-economic status and environmental conditions in addition to culture (National Research Council, 2013), food habits are the most intensely entrenched characteristics of many cultures that cannot be easily changed (Reddy & Anitha, 2015). These aspects would include food production, processing, storage and cooking. It is also noteworthy that in practice, culture is heterogeneous thus cultural differences may occur within small groups and intra-group differences are generally larger than inter-group differences (Shweder, 2000).

Cultural information has been documented as a predicator of dietary behavior (Johns & Kuhnlein, 1990). Johns and Kuhnlein (1990) have reported ethnicity as one of the major predictors of dietary behavior. These scholars have identified culture as "the pattern of knowledge, concepts, values attitudes, beliefs and traditions that are inherited, often from generation to generation". Dietary habits attained in childhood tend to persist in adulthood. Beside culture, food choices and consumption have been investigated and reported to be also influenced by psychological and social determinants (Roudsari, et al., 2017). Roudsari et al (2017) in a study they conducted in Iran among adults aged 30-64 years reported that a people's belief about the benefits of indigenous and traditional food, the inspiration that certain foods offered traditional and alternative medicine thus had positive effects on health status on the consumers and religious beliefs and principals of some

religious principals like "Halal" in Muslim were major determinants of food choice and consumption practices. In the same way, food preservation and storage are influenced by similar cultural aspects. However, it has also been noted that much as dietary behavior may display a certain pattern, some determinants like belief may only be upheld by a small fraction of the community (Bernard, 1988). Additionally, beliefs and values may vary from sub-culture to the next (Johns & Kuhnlein, 1990).

Kenya is a multi-ethnic and multi-cultural state which is inhabited by Bantu, Nilotes and Cushitic speaking ethnic speakers with majority being Bantu (Kenya National Bureau of Statistics, 2011). Each of these ethnic groups have their unique cultural practices which influence food production and consumption habits. Busia County is endowed with rich cultural diversity. While majority of the inhabitants of Busia County are Bantus, Nilotes and Nilo-hamites have overtime settled in the county.

This study was conducted among Bantu, Nilotes and Nilo-hamites, ethnic groups living in Busia County's Nambale, Budalang'i and Teso-south sub-counties. The main ethnic groups residing in these sub-counties are the Luhyia, Luo and Iteso respectively. The study sites were located in rural settings where the populations rely more on traditional grains and less on meat, dairy, fruit and vegetables because they tend to have lower incomes and have poorer access to a wide variety of food compared to their urban counterparts. Of the different foods, maize is most commonly consumed by the three ethnic groups.

The luhya are a Bantu ethnic sub-group who primarily settled in Western province in the 1450s. The Abaluyia are mostly farmers who keep cattle, but in precolonial times men hunted and animal husbandry was even more important. The Banyala of Budalang'i who live along the shores of Lake

Victoria were known for fishing. Finger millet, sorghum, sesame, pumpkins, sweet potatoes, yams, beans, and bananas were the most important crops in precolonial times. In addition to the traditional crops, other important contemporary crops currently include green beans, red beans, bananas, groundnuts (peanuts), *sukuma wiki* (kale), cabbages, potatoes, and cassava (Forum, 2018).

The Iteso people are a nilo-hamitic ethnic group in Western Kenya. They inhabit Teso South and Teso North Sub-counties with some living in some parts of Bungoma and Trans Nzoia Counties (Wikimedia Foundation, 2019). They originated from what is now Egypt through Ethiopia. The Kenyan Iteso are part of the Nilo-hamitic southern iteso who leave in Busia County, south of Mount Elgon. Agriculture has played a significant role in their social and economic lives. The Iteso have a history of long-standing ethnic interactions with the Bantu as a result of living among the Bantu thus have been subject to a variety of cultural influences (GoVisitKenya, 2014).

The luos are a nilotic ethnic group in western Kenya who migrated from the Sudan in the 19th century and settled around the lake. Just like the luhyia, the luo comprise a number of communities made up of various clans and sub-clans. The luo practice both crop farming and cattle keeping.

Migration and settlement of these ethnic groups in Kenya has brought about both accidental and or intentional changes in their cultures. Some groups have borrowed cultural practices from the people they interact with. Some of these cultural practices include adoption of economic practices like cultivation of crops. It is noteworthy that the cultures and practices of these ethnic groups are dynamic and continually changing over time.

2.5 Prevalence of aflatoxin in food and animal feed consumed in Kenya

Aflatoxin contamination can occur in developing crops when environmental conditions favor both fungal and crop susceptibility. Factors that influence fungal growth and aflatoxin production include humidity, the genotype of the crop planted, soil type, minimum and maximum daily temperatures and daily net evaporation both before and after harvest (Strosnider, et al., 2006; Mannaa & Kim, 2017). *Aspergillus* has been reported to grow more rapidly under the combination of 0.995-0.85 a_w and temperature of 15-25℃ and maximum aflatoxin production was observed at 30℃ and was suppressed at 40℃ (Marin, Companys, Sanchis, Ramos, & Magan, 1998). Therefore, at the farm level, the mechanical disruption of crops by birds, insects and mammals or the stress of hot dry conditions, result in significant fungal crop infection (Cotty & Lee, 2007). The aflatoxin problem in Kenya is longstanding and seemingly inextricable. Of the major agricultural products in Kenya, maize is most susceptible to aflatoxin colonization as demonstrated by findings from some studies previously conducted in various regions (Azziz-Baumgartner, et al., 2005; Muture & Ogana, 2005; Wagacha & Muthomi, 2008; Bandyopadhyay, Kumar, & Leslie, 2007; Lewis, et al., 2005). Aflatoxin is also prevalent in sorghum, millet and groundnuts in some parts of the country (Mutegi C. , 2010; Kang'ethe E. , et al., 2017; Sirma A. , et al., 2016). Estimated daily groundnut consumption rate is 1.1g/person (Okoth., 2016).

Under-reporting of aflatoxin contamination in food commodities is possible as toxicity in these foods is not regularly monitored (Ombui, 2001). However, previous epidemiologic investigations in Kenya, have demonstrated that maize is a major source of human exposure (CDC, 2010). This is attributed to its greater susceptibility to fungal attack. Ranajit et al (2007) established that maize in Nigeria was significantly more colonized by aflatoxin-producing aspergillus species than either sorghum or millet. Additionally, they concluded that if the primary cereal was sorghum rather than

maize, the risk of aflatoxin-related health problems would be reduced 4-fold, while an 8-fold effect would be observed for pearl millet (Bandyopadhyay, Kumar, & Leslie, 2007).

Consumption of maize grains, possession of homegrown maize that is discolored or visibly contaminated with mold, storage of damp maize, inside storage of maize rather than outside granary storage have been reported as some of the risk factors of aflatoxin contamination and exposure (Azziz-Baumgartner, et al., 2005). Additionally, a study conducted in Kisii and Homa Bay, Upper Eastern Embu and Mbeere, Machakos and Makueni districts, established that the incidence of aflatoxin in farmer stores in 2009 was higher in Upper eastern especially in the 2nd month of postharvest (ACDI/VOCA, 2019). However, the market samples had higher levels of aflatoxin (>10 ppb) in Upper and Lower Eastern than South Western region (Kang'ethe, 28th to 30th September 2011.).

Table 1 below shows variability in prevalence of aflatoxin based on selected studies conducted in the East African region namely, Uganda and certain parts of the country such as Makueni, Homabay, Kisii, Nandi. The prevalence of aflatoxin contamination of most foods in Busia County however remains unknown. The only documented study so far conducted to establish aflatoxin prevalence in the county was that done by Mutegi (Mutegi C. , 2010) where 345 groundnut samples were tested. The levels of aflatoxin ranged from 0 to 2,687.6 µg /kg. Prevalence of 7.54% were contaminated based on Kenya Bureau of Standards (KEBS).

Table 1: Aflatoxin prevalence in parts of Kenya and Uganda

District	Crop/Produce	Number of samples tested	Aflatoxin Prevalence/mean AFB in ppb	Reference
Makueni	Maize	87	72%	(Kangeth'e 2012)
	Sorghum & millet (farm)	204	29.3%	(Kangeth'e 2012)
	Sorghum & millet (market samples)	11	31.4%	(Kangeth'e 2012)
	Maize	98	41.5	(Kang'ethe E. , et al., 2017)
	Sorghum	46	20.43	(Kang'ethe E. , et al., 2017)
Lower Eastern Kenya	Maize	519	49.1%	(Mahuku, Henry, Charity, Kanampiu, & Narrod, 2019)
Upper Eastern Kenya	Maize	356	92.1%	(Mahuku, Henry, Charity, Kanampiu, & Narrod, 2019)
South Western Kenya (Homabay/Rongo)	Maize	469	5.6%	(Mahuku, Henry, Charity, Kanampiu, & Narrod, 2019)
South Western Kenya (Kisii Central)	Maize	283	57%	(Mahuku, Henry, Charity, Kanampiu, & Narrod, 2019)
Nandi	Maize	78	66%	
	Sorghum & millet (farm samples)	105	26.6%	(Kangeth'e 2012)
Busia	Groundnuts	79	7.54%	(Mutegi C. , 2010)
Different parts of Uganda	Various food samples	480	29.6%	(Alpert 1968)
Uganda	Maize	49	44.9%	(Kaaya & Warren, 2005)
Uganda	Groundnuts	152	17.8	(Kaaya & Warren, 2005)
Uganda	Sorghum	69	37.7	'' ''
Uganda	Millet	55	16.4	'' ''

2.5.1 Aflatoxin contamination and exposure pathways to humans and animals

Maize and groundnuts are the main sources of human exposure because of their greater susceptibility to contamination (Bandyopadhyay, Kumar, & Leslie, 2007). These cereals contain AFB_1 which is the most prevalent and potent type of the aflatoxins (Okoth., 2016; IARC Working Group Report No. 9, 2015). Unmonitored food and feed production and sale poses a major challenge to both human and animal health. Upon maturity, crops may be contaminated when exposed to warm and moist conditions either in the field or during transportation and storage. The moisture content of the grain and temperature determine the extent of contamination (Bandyopadhyay, Kumar, & Leslie, 2007). These molds are also widespread especially during extended periods of drought (Keeler, 1983). The tropical climate of many developing countries favors the propagation of pests and naturally occurring toxins like mycotoxins.

Contamination of feeds could happen at different stages along the food chain. While many countries have set MLs for food for human consumption, the same cannot be said for animal feed. Table 2 shows guidelines of the maximum allowable aflatoxin levels of contents for feed by the United States Food Drug Authority (USFDA) and the European Union (EU). Aflatoxin B_1 and B_2 is converted in the liver to the M1 and M2, metabolites.

In Kenya, just like in other countries, maize (corn) is used as an energy source in animal feed. Maize and rice by-products are also used as feed preparation ingredients. Animal feed in Kenya is pervasively contaminated with aflatoxin. The study by Kang'ethe and Langa' (2009) reported prevalence of AFB_1 in animal feeds in the farmer, producer and retailer chain in Nyeri, Eldoret, Machakos and Nakuru (Kang'ethe & Lang'a, 2009). Contaminated grains may have been used to manufacture the animal feed. As a result, aflatoxins have been reported in both commercial and household milk in parts of the country (Okoth S. , 2016; Sirma A. , et al., 2014). Kagera et al found

64% of processed milk samples in Kasarani sub-county with AFM_1 levels above the EU limit of 50ppt while Kirino et al found 55% in Dagoretti (Kagera, et al., 2018; Kirino, K., Delia, & Johanna, 2016). Table 2 below shows the guidance by the United States Food and Drug Authority (US FDA) and the European Union on the maximum limits of aflatoxin allowable in raw materials meant for animal various feed.

Humans are exposed to aflatoxins when they ingest contaminated grains or contaminated animal products. AFB_1 is metabolized to Aflatoxin M_1 and is found in breast milk and when consumed by animals, AFM_1 is found in animal milk with an estimated conversion ratio between AFB_1 and AFM_1 of 1-3% (Barbieri, Bergamini, Ori, & Pesca, 1994). Infants and young children may hence be exposed to aflatoxin B1 or M1 by consuming contaminated breast milk, legumes and cereals and animal products, especially at weaning (Okoth & Ohingo, 2004; Gong, et al., 2002; Gong, et al., 2004; Shirima, et al., 2015) (Figure 1). Exposure to these fungal toxins can lead to acute or chronic aflatoxicosis, based on the duration and amount of exposure.

Table 2: Regulatory guidance for feed and feed ingredients*

US FDA		
Intended use	***Grain, grain by-product, feed or other products***	***AFB_1 maximum level (ppb)***
Immature animals	Corn, peanut products, and other animal feeds and ingredients, excluding cottonseed meal	20
Dairy animals, animals not listed above, or unknown use	Corn, peanut products, cottonseed, and other animal feeds and ingredients	20
Breeding cattle, breeding swine and mature poultry	Corn and peanut products	100
Finishing swine 100 pounds or greater in weight	Corn and peanut products	200
Finishing (i.e., feedlot) beef cattle	Corn and peanut products	300
Beef, cattle, swine or poultry, regardless of age or breeding status	Cottonseed meal	300

European Commonwealth	
Matrix	***AFB_1 maximum level (ppb)***
All feed materials	20
Complete feeding stuffs for cattle, sheep and goats (except dairy animals)	20
Complete feeding stuffs for dairy animals	5
Complete feeding stuffs for calves and lambs	10
Complete feeding stuffs for pigs and poultry (except young animals)	20
Other complete feeding stuffs	10
Complementary feeding stuffs for cattle, sheep, and goats (except complementary feeding stuffs dairy animals, calves, and lambs)	20
Complementary feeding stuffs for pigs and poultry (except young animals)	20
Other complementary feeding stuffs	5

* United States Food Drug Authority and European Union

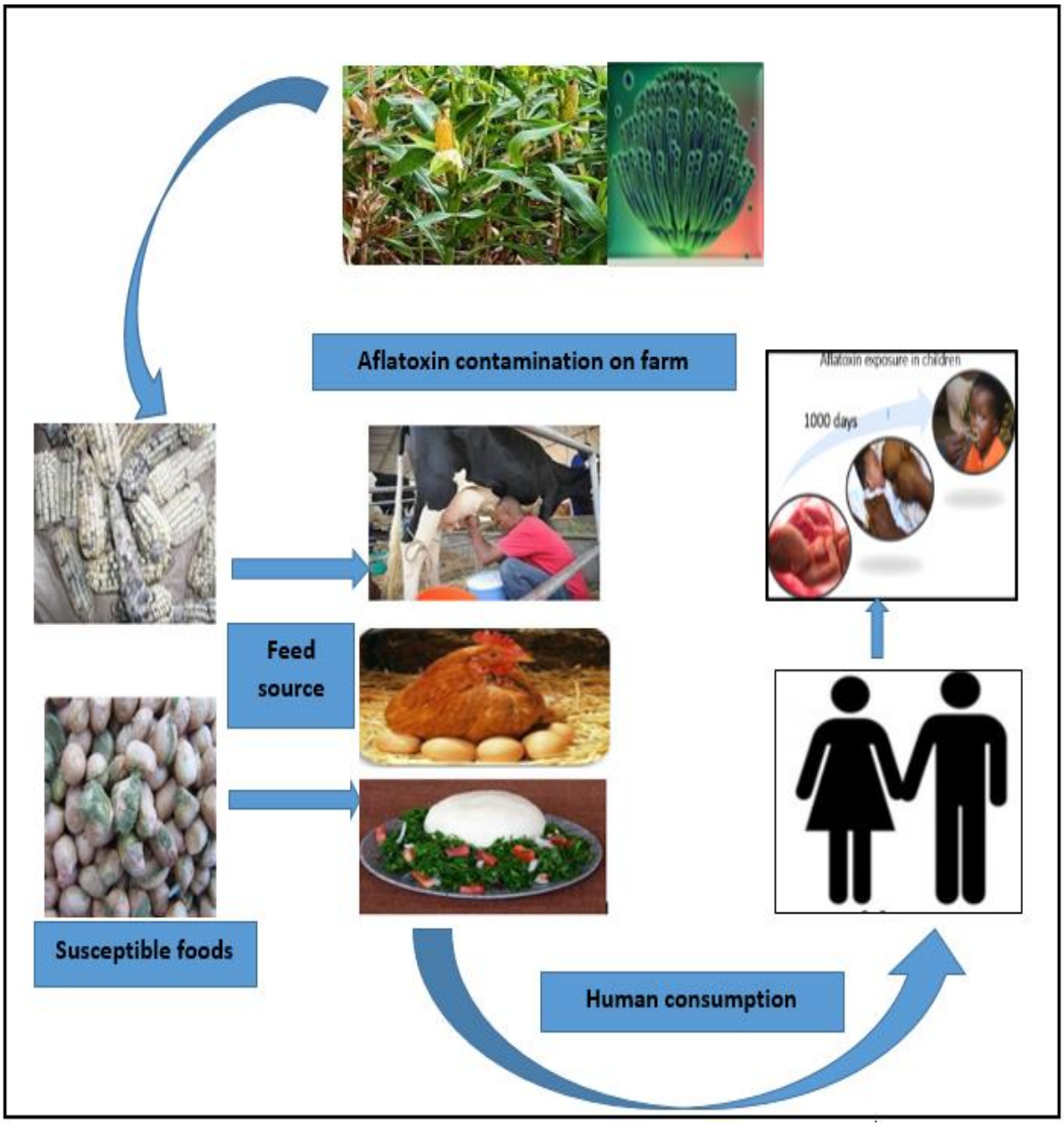


Figure 1: Aflatoxin exposure pathways to humans and animals

Source: Author

2.6 Public health implications of aflatoxin contamination

2.6.1 Aflatoxin and human health

Dietary patterns represent a broader picture of food and nutrient consumption and may be predictive of disease risk (Hu, 2002). Risk of negative health effects resulting from ingesting

aflatoxin-contaminated food is determined by the frequency of ingestion and severity of contamination of the grain. Reference is made to studies conducted in Togo and Benin which assessed dose-response relationship between aflatoxin exposure and child impairment. A relationship was found between the mean AF albumin levels and lower height-for-age (HAZ) and weight-for-age (WAZ) scores. Stunted children had 30-40% higher mean AF albumin levels than the non-stunted ones (Gong Y. , et al., 2002; Gong, et al., 2004). Aflatoxin B_1 has been classified as a Group 1 carcinogen in humans by International Agency for Research on Cancer (IARC) parameters. Furthermore, the FAO and WHO Joint Expert Committee on Food Additives concluded in 1997 that aflatoxins should be considered as human liver carcinogens. Carcinogenic properties of aflatoxins, in particular, AFB_1, have been characterized in many experimental systems and epidemiological surveys carried out over the past 25 years in Asia and Africa revealed a strong statistical association between aflatoxin ingestion and primary liver cancer (PLC) incidence (Liu & Wu, 2010). Findings from a meta-analysis of 17 case-control and cohort studies carried out in sub-Saharan Africa, China and Taiwan showed that the population attributable risk (PAR) of aflatoxin-related HCC was 17% and was even 20% higher in HBV positive populations (Liu, Chang, Marsh, & Wu, 2012). Prevalence of hepatomegaly, a firm form of liver enlargement, has been reported in another study conducted in Kenya among children with high aflatoxin exposure (YunYun & Wilson, 2012). A nested case control study in Shanghai China conducted among a cohort of 18,000 middle aged men, found 55 incident cases of HCC with highly significant association between the presence of urinary aflatoxins, serum hepatitis B surface antigen positivity and HCC risk (Qian, et al., 1994). In another study conducted in the Gambia that sort to investigate the environmental exposures and cirrhosis in 97 individuals, investigators

reported the possible synergistic interaction between aflatoxin and hepatitis B virus to substantially increase the risk of cirrhosis (Kuniholm, et al., 2008).

Aflatoxins remain a threat to the health of humans and livestock by their continued intermittent occurrence in both foods and animal feeds. An estimated 5 billion people globally are chronically exposed to aflatoxin by ingestion of low doses of these aflatoxins (Strosnider, et al., 2006; Council of Agricultural Science And Technology, 1989). Populations living in the tropical regions, specifically sub-Saharan Africa and East Asia are at higher risk of exposure since aflatoxins are most prevalent in these regions because of the hot and humid conditions that stimulate fungal growth (Wild & Gong, 2010).This is evident from high aflatoxin prevalence 78% from a sero-survey of adults from Kenya (Yard, et al., 2013), 93% from a cross-sectional study among children aged 6-9 years in The Gambia (Turner, Moore, Hall, Prentice, & Wild, 2003) and 99% from a cross-sectional study among children aged 9 months to 5 years in Benin and Togo (Gong Y. , et al., 2002).

Clinical symptoms of acute aflatoxicosis include jaundice, abdominal pain, distended abdomen, vomiting, and fever within 30 days of exposure (CDC, 2010). Aflatoxicosis can eventually lead to liver failure, with documented fatality rates as high as 40% as was observed in Makueni and Kitui aflatoxicosis outbreaks, the worst ever documented aflatoxicosis outbreak in the region (Lewis, et al., 2005; Wild & Gong, 2010; Centers for Disease Control and Prevention, 2004). In theafore mentioned investigations, all 317 cases identified had acute hepatic failure yet all seven samples which were analyzed were all negative for hepatic disease viruses (American Public Health Association, 2000). A survey administered in 2008 in Kitui and Makueni districts to adult heads in households that reported any case of aflatoxicosis from 2004-07 demonstrated how outbreaks are costly. At household-level direct medical and nonmedical costs, and productivity loss incurred

had estimated average costs for treatment at 267 US$ for inpatient treatment at a health facility and 14 US$ for self-treatment (Mumma & Awuor, 2007).

Chronic aflatoxin exposure in humans has been linked to hepatocellular carcinoma (Liu & Wu, 2010; Ross, et al., 1992) and impaired immunity (Jiang, 2005). Of 550,000 new hepatocellular carcinoma cases (HCC) globally, 4.6-28.2% of these cases may be attributable to aflatoxin exposure (Liu & Wu, 2010). Most agricultural produce in Kenya, including maize, are sold in unregulated informal markets (Grace, Makita, Kang'ethe, & Bonfoh, 2010). The local population, who are chronically infected with hepatitis B virus, are also chronically exposed to the most potent form of aflatoxin, aflatoxin B_1, (AFB_1). This is the result of the synergistic action between aflatoxin and HBV which increases risk of (HCC) (Groopman, Johnson, & Kensler, 2005).

2.6.2 Aflatoxin Exposure on Child Health and Nutrition Status

Aflatoxins have deleterious effects on fetal and child growth through several mechanisms. Aflatoxin exposure can occur through the placental pathway. The foetus can be exposed to aflatoxin in utero through maternal food intake because aflatoxin B_1 is lipophilic therefore it crosses the placental barrier and this can have significant effect on faltering in fetal growth (Turner, et al., 2007; Wild, et al., 1991). High aflatoxin levels in-utero have been associated with low birth weight. Low birth weight was observed in 20.3% of infants born to singleton mothers with high aflatoxin exposure levels in Ghana (Shuaib, et al., 2010). Aflatoxin exposure has also been found to be significantly correlated with stunting, (Gong Y. , et al., 2002). In the study that was conducted in Benin among 479 children aged 9 months to 5 years, the authors reported AF–albumin exposure levels ranging 5–1064 pg/mg (geometric mean of 32.8 pg/mg). Of these 479 children, 33% were stunted, 29% underweight and 6% wasted.

Young infants may be exposed to aflatoxin through the excretion of aflatoxin metabolite - aflatoxin M1 (AFM_1) in breast milk. The WHO recommends exclusive breast feeding of infants for the first 6 months, in order to achieve optimal growth, development and health. Aflatoxin M1 of 1.8 pg/ml has been reported in Ghana (Lamplugh, Hendrickse, Apeagyei, & Mwanmut, 1988). However, AFM_1 is less toxic than AFB_1 – which is ingested – and the impact of M1 exposure in neonates and young infants is unknown (Gong, et al., 2004).

Furthermore, young children are more vulnerable to aflatoxin because the daily intake of food per kg body weight in small children is 6.1 times higher than in adults (Armstrong, Zaleski, Konkel, & Parkerton, 2002). When children are chronically exposed, they may suffer disproportionately from the long-term effects of aflatoxin exposure like impaired growth (Christopher & Kleinjans, 2003; Gong, et al., 2004).

2.7 Socio-economic implications of aflatoxin contamination

Aflatoxin contamination inflicts a heavy economic burden on both human health and on the economy. The human health burden can be demonstrated by several studies that report estimates of aflatoxin induced liver cancer burden. Treatment of an aflatoxicosis case in Kenya in 2004 to 2005 was estimated at an average cost of 267 US$ for inpatient treatment from both private and public health facilities in the districts of Makueni, Makindu, Kitui and Mutomo and 14 US$ for self-treatment during an outbreak (Mumma & Awuor, 2007). Additionally, because aflatoxin is carcinogenic the risk of cancer is high (International Agency of Research on Cancer, 1976; Liu & Wu, 2010). This is further exacerbated by the endemic hepatitis B virus (HBV) infection in Kenya estimated at 2–5%, in 2007, which works synergistically with aflatoxin to increase risk of hepatocellular carcinoma. Indeed, the risk of liver cancer in individuals exposed to both aflatoxin

and HBV has been reported to be 30 times greater than the risk of individual exposed to aflatoxin only (Groopman, Kensler, & Wild, 2008).

Ingestion of aflatoxin contaminated feed has detrimental effects on livestock health. Decreased feed utilization leading to poor weight gain, reduced growth and poor production are some of the clinicopathological effects of aflatoxin in livestock (Yaroshenko, J, & P., 2003). Aflatoxins lower the value of farm produce thus farmers suffers great economic loses when their produce is contaminated. It has been estimated that 1.2 billion US$ worth of trade is lost globally due to aflatoxin contamination with African economies losing approximately 750 million US$ (Udomkun, Wiredu, M., Ranajit Bandyopadhyay, & Vanlauwe, 2017). . Indeed, thousands of tons of maize in the national cereals and produce board have in the past been condemned and declared unfit for human consumption (Kilonzo R. , 2012). In 2014, aflatoxin contaminated maize worth 5 million US$ was destroyed by the government (Bandyopadhyay, et al., 2016). Indeed, it is notable that there has been a significant drop in earnings from agriculture from 2013 to 2017 (Kenya Markets Trust, 2018).

2.8 Aflatoxin mitigation strategies

The level of food insecurity in Kenya cannot be overemphasized. This situation is further exacerbated by widespread recurrent problem of aflatoxin contamination of major staples like maize and aflatoxicosis outbreaks (Okoth., 2016; CDC, 2010; Daniel J. H., et al., 2011). Consequently, 47 million Kenyans are at risk of low dose, long term aflatoxin exposure. Aflatoxin related health effects pervade the Kenyan population despite the fact that these can be prevented or controlled (YunYun & Wilson, 2012; Okoth & Ohingo, 2004).

There are different strategies that can be used in mitigation of aflatoxin challenge. These range from those that target stopping the infection, to those that focus of controlling the environmental factors that influence fungal growth and toxin production to post harvest crop management. Aflatoxin control and mitigation interventions on farm differ from those used after harvest. These interventions also vary in their cost, applicability, labor intensiveness and effectiveness. These strategies can be categorized as either physical methods of mycotoxin removal or methods of decontamination.

2.8.1 Pre-harvest strategies

Aflatoxin contamination of plant material can happen on farm. Breeding for resistance and biocontrol are the two main pre-harvest strategies that have been explored to prevent aflatoxin infection and contamination. Menkir et al (2008) in their study at the USDA- ARS in New Orleans reported tropical maize bred germplasm lines that had significantly lower levels of aflatoxins when compared to the aflatoxin resistant U.S inbred check, M182 (Menkir, Brown, & Bandyopadhyay, 2008). Therefore, the choice of crops resistant to growth of fungi, drought, disease, pest infestation and are genetically more resistant to drought are recommended (Cotty & Bhatnagar, 1994). Unfortunately, these lines of aflatoxin resistant breeds are yet to be commercialized in Kenya since and environmental impact assessment is yet to be completed.

The use of native atoxigenic strains of aflatoxin fungi to competitively exclude toxigenic strains is another option that has been studied with promising degrees of success and is currently being employed in some countries like Nigeria, Senegal and Kenya (Cotty P. J., 1990). The use of Aflasafe KE01, the product produced and registered in Kenya for use in maize was reported to have reduced aflatoxin on farms in Bura and Hola to levels of no more than 4ng/g production in 99% of the treated farms (Bandyopadhyay, et al., 2016).

Other strategies that help with the control of aflatoxin are those that focus on regulating the factors that increase the risk of aflatoxin contamination. These include planting at the right time of crops, avoiding overcrowding of plants, avoiding adequate irrigation, proper plant nutrition, controlling other plant pathogens and weeds, insect pest control and proper harvesting (Bruns, 2003). Use of farmyard manure and application of lime has been reported to reduce *A. flavus* by 50-90% (Waliyar, et al., 2008).

Additionally, control of fungal infection by controlling vector insects that cause grain damage has been investigated and proposed as a strategy that can be readily used. Vector insect damage on grains on the farm allows fungi to gain access. Indeed, high incidence of the *Mussidia nigrivenella* insect borers were positively correlated with aflatoxin contamination in maize in a study conducted in Benin (Sétamou, Cardwell, Schulthess, & Hell, 1998).

2.8.2 Post harvest strategies

2.8.2.1 Physical methods of mycotoxin removal

Sorting and floatation

Sorting or segregation is a non-invasive mycotoxin procedure through which visually damaged grains are identified and is normally the first control option for decontamination (FAO, 2014). This can either be done manually or by use of electronic sorting. Separation of mold-damaged maize and/or screening can significantly reduce aflatoxin concentrations (Bennett, Rottinghaus, & Nelson, 1992). In an experiment that Matumba et al (2015) conducted with the objective of assessing effectiveness of hand sorting, flotation/washing, dehulling and combinations thereof on the decontamination of mycotoxin-contaminated white maize, they reported greatest effect on mycotoxin removal with hand sorting that only left less than 6 percent of aflatoxin B1 compared

to flotation (Matumba, Poucke, Ediage, Jacobs, & saeger, 2015). These investigators however recommend hand sorting as a method that should be used as a last option for aflatoxin exposure. Therefore, although some contamination may persist, physical removal represents an alternative for both industry and domestic use. The advantage with sorting is that it does not interfere with the nutritional properties of the grains and does not produce any toxin degradation products. The disadvantage is that it is time consuming, especially with large amounts of grains.

Dehulling

Dehulling is the removal of the outer layer from maize grains. The effect of this process as an aflatoxin decontamination strategy has been investigated. A significant reduction has been reported by some investigators. Siwela et al (2005) reported a 92% reduction while another study conducted in Kenya reported a 46.6% reduction during the preparation of muthokoi – "mixture of dehusked maize, peas and beans" (Siwela, Siwela, Matindi, Dube, & Nziramasanga, 2005; Mutungi C. , Lamuka, Arimi, Gathumbi, & Onyango, 2008). Wet and dry milling processes, which are widely used for maize and cereal grains, have been shown to result in reduced aflatoxin levels in several fractions such as milling solubles, gluten, fiber, starch and germ (Lopez-Garcia R. , 1998). Washing, dehulling, dry screening, milling and fermenting raw maize resulted in a 93% reduction of aflatoxins (Fandohan P. , Zoumenou, Hounhouigan, Marasas, & Wingfield, 2005).

2.8.2.2 Physical methods of decontamination

Thermal treatment

Of the physical methods investigated and found to be efficacious in aflatoxin decontamination is the thermal treatment. Solid AFB_1 has been reported to be stable to dry heat up to 260℃ (Ciegler & Vesonder, 1983). However, temperature of 300℃ has been observed to degrade AFB_1

(Fischbach & Campbell, 1965). It has also been noted that the presence of moisture at critical concentrations in foods can enhance degradation of AFB_1. Some experimental studies have shown that microwave roasting of groundnuts at 0.7kw for 8.5 minutes resulted in a 48-61% degradation (Pluyer, Ahmed, & Wei, 1987), boiling corn resulted in a 40% degradation (Price & Jorgensen, 2006). In some more recent studies, investigators subjected aflatoxins in their pure form to thermal treatment and found that Aflatoxin B_1 was almost completely degraded by heat treatment at temperatures of 160℃ over a period of 30 minutes (Matissek & Raters, 2008) while Njapau et al (1998) also found that some phases of industrial processes could reduce specific mycotoxins to a certain degree through thermal inactivation.

Roasting of some grains like groundnuts is a process that is extensively used in Busia County (Mutegi C. , 2010). Mutegi (2010) reported a reduction of 11.9 per cent of aflatoxin when groundnuts were roasted for 9 to 15 at 110 to 150℃ while a 35.9 per cent decline in levels of aflatoxin was a result of roasting and dehusking peanuts after roasting. Njapau et al. (1998) also showed reduction of aflatoxin levels by up to 80% in roasted peanuts (Njapau, Muzungaile, & Changa, 1998). Traditional aflatoxin removal and processing methods like sorting and roasting respectively are recommended to be the first choice for aflatoxin management and decontamination if they are effective since they are more acceptable and affordable (Lopez-Garcia R. , 1998). Besides these few studies, not much has been studied on use of traditional salt especially in Busia County.

2.8.2.3 Biological decontamination

Fermentation

Biological methods have been explored as options for mycotoxin decontamination. The use of organisms to reduce the incidence of toxigenic Aspergilli has been used for instance in the fermenting industry. It has been found that aflatoxins are not degraded during fermentation, although the toxins are absent from the alcohol fraction after distillation. Aflatoxins are usually concentrated in the spent grains (Lopez-Garcia R. , 1998). During the preparation of Akassa, a West African meal:- pre- cooking, steeping, milling fermenting raw maize a 92% reduction of aflatoxins was recorded while owo preparation:- milled cooked raw maize recorded a 40% reduction of aflatoxins (Fandohan P. , et al., 2005)

2.8.2.4 Chemical decontamination of aflatoxin in food and animal feed

Chlorination

Chlorine is one chemical that has been screened and found to have the ability to degrade pure AFB1 and is recommended for removing aflatoxins from contaminated surfaces (Stoloff & Trager, 1965).

Ammoniation

Ammoniation is another chemical method that has received the most research attention (Park, Lee, Price, & Pohland, 1984). Ammoniation is the injection of aqueous or gaseous ammonia with or without heat and pressure to contaminated grains. Lopez et al (1999) indicate that extensive evaluation of this procedure has demonstrated that it is an efficacious and safe way of decontaminating aflatoxin-contaminated feeds. The method has been reported to degrade 77-99% AFB_1 under laboratory conditions (Shantha, Murthy, Rati, & Prema, 1986). A reduction of 79 -

90% AFM1 content has been reported with ammoniation (Sipos, et al., 2021). Ammoniation has been used selectively with success in the United States, France, Senegal, the Sudan, Brazil, Mexico and South Africa, in some cases for almost 20 years (Lopez-Garcia, 1999). It is noteworthy that nutritional quality of ammoniated feed is affected. However, ammoniation has not been approved for use for human food commodities.

Use of alkaline base

Nixtamalization, the traditional alkaline treatment of maize used to manufacture tortillas in Latin America, has been found to partially degrade aflatoxins (Price & Jorgensen, 2006). The addition of oxidizing agents, such as hydrogen peroxide, has been shown to be an effective aid in nixtamalization (Lopez-Garcia R. , 1998). Nixtamalization is a process for preparation of maize in which the maize is soaked and cooked in an alkaline solution, which could be limewater or wood ash lye. The traditional nixtamalization process has been also reported to reduce levels of aflatoxin B_1 by 94% and aflatoxin M_1 by 90% (Elías-Orozco, 2002). In Africa, preparation methods of *mawe, makume, ogi, akassa,* and *owo,* maize-based foods common in Benin, West Africa have been evaluated. Aflatoxin levels were significantly reduced during the preparation of makume (Fandohan P. , et al., 2005). In another study, the use of *magadi*, an alkaline mineral salt, in cooking reduced levels of aflatoxin by 22-78% in muthokoi (dehulled maize) (Mutungi, Lamuka, Arimi, Gathumbi, & Onyango, 2008).

2.9 Use of adsorbents for reduction to aflatoxin exposure

Aflatoxins are ubiquitous thus avoiding consumption of the same is almost impossible therefore reduction of dietary exposure is important (Phillips, Afriyie-Gyawu, Wang, Williams, & Huebner, 2006). Another approach to minimize exposure to aflatoxins in both humans and animals is

detoxification. Several studies have documented the efficacy of the use of clay binders both in humans and animals (Afriyie-Gyawu, et al., 2005; Mitchell, et al., 2014; Phillips T. , 1999). In this approach, aflatoxins are bound in the gut by this adsorbent hence reducing their bioavailability. In Kenya, the Ministry of Health in collaboration with the Centers for Disease Control and Prevention, Kenya and the County government of Makueni conducted a Phase 11 clinical trial to investigate the efficacy and acceptability of Air Classified Calcium Silicate 100 (ACCS100), a refined montmorillonite clay as a potential sustainable human intervention for aflatoxins. Participants of this clinical trial were healthy volunteers among a population with recurring aflatoxicosis outbreaks. ACCS100 was found to be effective and acceptable (Awuor, et al., 2016). However, this intervention is yet to be conducted among children and expectant women in Kenya.

To date, disposal or decontamination of the contaminated maize in the cereals warehouses in the country still remain a challenge due to high financial implications on the appropriate decontamination or disposal technique and limited funds (Felicia & Yan, 2008). It has been observed growers spend much money on disposal of contaminated corn in the United States (Njapau, Muzungaile, & Changa, 1998; Kaaya & Kyamuhangire, 2006).

Since conventional decontamination processes are very expensive for poor developing countries, then alternative cheaper decontamination methods need to be sought to help communities manage exposure to aflatoxin thus reduce their health risks. This study aims at improving the knowledge and understanding of extent of contamination of aflatoxins in local staples and how food source, storage and preparation methods either exacerbate or contribute to the reduction of aflatoxins in maize (*Zea mays*), sorghum (*Sorghum bicolor* (L), millet, cassava and groundnuts (*Archis hypogea*) and eventual exposure to humans in Busia County.

2.10 Country's efforts to mitigate aflatoxin

Kenya is one of the -FAO/WHO member states that has adopted the maximum limits (MLs) of aflatoxins in foods formulated by Codex Alimentarius Commission (Codex). However, Codex has not been able to formulate an internationally acceptable ML for maize because of the huge differences in perceived risks, food consumption patterns, and in the levels of aflatoxin contamination in food produced from different agro-ecological regions around the globe. Kenya formulated national MLs for total aflatoxins of 10 ppb and ML of 5 ppb for AFB1 in selected foods, cereals, and pulses. The East African Community recently adopted these limits as harmonized MLs for the region (International Institute of Tropical Agriculture Technical Policy Paper 8, 2015).

Kenya has adopted pre- and post-harvest strategies to help mitigate aflatoxin contamination. As first line intervention, there are current breeding trials of aflatoxin resistant inbred maize lines that Kenya has identified through the collaboration of Kenya Agricultural Livestock and Research Organization (KALRO), The University of Nairobi (UoN) and Stellenbosch University. These lines are reported to have low susceptibility to aspergillus ear rot and aflatoxin accumulation (Okoth., 2016).

In addition, Kenya has also registered Aflasafe^{TM}, an atoxigenic biocontrol of *Aspergillus flavus* that can out compete closely related toxigenic strains on farm thus reducing levels of aflatoxins in the produce. Aflasafe KE01^{TM}, is reported to have upto 90% efficacy (Ranajit, 2016).In this method, white sorghum is sterilized and later inoculated with the atoxigenic fungi before being broadcasted on the farm. It has been used on maize and groundnut farms (Cotty, Antilla, & Wakelyn, 2007; Cotty & Bhatnagar, 1994). Given the complexity of production of the product and application of this strategy, there is great need for information, education and supervision on

its usage. There is also need for collaboration among various stake holders including government and international organizations in addition to promotion for its demand (Felicia & Khlangwiset, 2010).

The government through agricultural extension officers and in collaboration with non-state actors also continues to educate farmers on Good Agricultural Practices (GAP). Some of these practices include irrigation, mulching, crop rotation, maintenance of optimal plant density on farm among others (Waliyar, et al., 2005a; Waliyar, Moses, Sudini, & Samuel, 2013). Water activity (a_w) above 0.70 at degrees celsius has been reported to be unsafe since that would be ideal for fungal growth and possible aflatoxin production. Irrigated farms prevent plants from suffering from drought and heat stress thus minimizing pre-harvest aflatoxin contamination as has been shown in maize. Excessive weeds on farm also deplete the available moisture on farm (FAO, 2004; Marete, et al., 2019). Some of the post-harvest practices that are currently being used in Kenya as aflatoxin management strategies include storage and drying devices like metal silos, Purdue Improved Crop storage and hermetic bags (ACDI/VOCA, 2019; ACDI/VOCA, 2015). A multi-month on farm study conducted in Kenya in 2016 reported reduction in aflatoxin levels in dry maize stored in triple layered hermetic bags (Ng'ang'a, Mutungi, Imanthiu, & Affognon, 2016).

Summary of literature review and gaps

Several studies have been conducted to assess the prevalence of aflatoxin in several parts of the country. However, only one study has been conducted in Busia (Mutegi C. , 2010). The communities in the study area rely on maize, millet, sorghum and cassava, which are also prone to aflatoxin contamination (Bandyopadhyay, Kumar, & Leslie, 2007; Kang'ethe E. , et al., 2017; Mutegi C. , 2010). While aflatoxin is ubiquitous, there are regulatory limits for both human and

animal food and feeds that have been legislated. Aflatoxin mitigation strategies that are currently being used are described. Many of these strategies however have been tried on maize and most of these are not indigenous to the study population. (Price & Jorgensen, 2006; Fandohan P. , et al., 2005; Mutungi, Lamuka, Arimi, Gathumbi, & Onyango, The fate of aflatoxins during processing of maize into muthokoi: a traditional Kenyan food, 2008). The literature review reveals an information gap relating to food consumption patterns of aflatoxin prone foods in the study area hence possibility of aflatoxin exposure. This study therefore endeavored to establish the consumption patterns of aflatoxin prone foods in study households, map out the predominant food storage and food preparation practices and explore their effect on aflatoxin levels in food. These findings will help fill the lacuna of information on an issue that is of great public health significance in Kenya.

CHAPTER THREE: PROBLEM STATEMENT

3.1 Introduction

Aflatoxin contamination of staple foods remains a significant public health challenge. Aflatoxin exposure and its association with mortality (Centers for Disease Control and Prevention, 2004), growth impairment (Gong Y. , et al., 2002), hepatomegaly (YunYun & Wilson, 2012), impaired immunity (Jiang 2005) and liver cancer (Groopman, Kensler, & Wild, 2008) earlier addressed constitute a public health burden.

In 2018, Kenya had a 1.02 Human Immuno-Deficiency Virus (HIV) incidence per 1000 uninfected people and 25,000 persons died from an HIV related- illness (UNAIDS, 2018). As reported above, aflatoxin has been suspected to impair immunity thus exposure to this toxin by HIV persons poses a greater risk to attack by other HIV related illnesses hence increasing the probability of the country's and indeed the county's disease burden.

Additionally, aflatoxin B1 has been classified as a Group 1 human carcinogen by the International Agency for Research on Cancer (IARC (IARC Working Group Report No. 9, 2015). Liver cancer in Kenya is currently rated tenth with a 2.8% prevalence (IARC, 2018). It can therefore not be ignored that ingestion of aflatoxin contaminated food could be one of the causes of liver cancer. For example, an estimated 8-27% of liver cancer cases in Nigeria were as a result of exposure to aflatoxin (Wu & Khlangwiset, 2010). Kenya has had a number of aflatoxicosis outbreaks, most of which have been in the Eastern part of Kenya and have been attributed to consumption of contaminated maize (Azziz-Baumgartner, et al., 2005). Based on the findings from the aflatoxin sero-survey conducted in Kenya in 2010, 78% of the sampled population were exposed to aflatoxin, with 100% of those from Busia testing positive for the toxin (Yard, et al., 2013). This is

a major cause for concern because Busia has one of the highest HIV prevalence of 7.7% among the 47 counties of Kenya (National Aids Control Council, 2018).

Aflatoxicosis affects livestock health and production too. Intake of high levels of aflatoxin by livestock causes acute toxicosis and death while chronic exposure causes liver damage, decreased appetite, decrease reproductive function, gastrointestinal dysfunction (IFPRI, 2013). Poor conversion ratios and reduction in body weight in animals intentionally fed on aflatoxin has been reported (Khlangwiset, Shephard, & Felicia, 2011). This causes economic loses for livestock farmers.

3.2 Study justification

While aflatoxin is ubiquitous, the regulations on aflatoxins are not protective to especially the rural population who rely on their own food production. This subsistence food is not inspected because enforcement is limited (Shephard, 2008). Several studies that have been conducted on the prevalence of aflatoxin contamination of various types of food in some parts of Kenya have reported contamination levels above regulatory limits in various grains (Mutegi C. , 2010; Mutiga S. , Hoffman, Harvey, Milgroom, & Nelson, 2015). In the Mutegi study which was conducted in Busia and Homabay, 7.54% of the groundnut samples were contaminated with aflatoxin and were considered unfit for human consumption. Given the differences in tolerance levels between humans and animals, 2.1% of the contaminated groundnuts were still unfit for animal consumption because they exceeded 100ppb which is the US FDA allowable limit. Mutiga et al (2015) also conducted their study in Western Kenya in maize. Of the 48% samples that had detectable levels of aflatoxin, 15% had levels above allowable limits.

The risk factors which were associated with the 2004 aflatoxicosis outbreak in Kitui and Makueni districts are similar to those in Busia county (Strosnider, et al., 2006; Azziz-Baumgartner, et al., 2005). These include but are not limited to pre and post- harvest food handling practices and varying weather conditions. However, evidence shows that some local food preparation methods can lead to reduction of aflatoxin in food commodities (Njapau, Muzungaile, & Changa, 1998; Mutungi C. , Lamuka, Arimi, Gathumbi, & Onyango, 2008). The efficacy of aflatoxin decontamination of some of these traditional food preparation methods remains untested in these communities. This study therefore sought to investigate the prevalence of aflatoxin in communities' staples and assess risk factors associated with contamination and also assess the efficacy of some of the food handling and preparation methods on aflatoxin mitigation.

3.3 Theoretical Framework

This study was designed to assess the extent of aflatoxin contamination in selected susceptible foods and to establish the predominant food sources, storage and preparation methods that are associated with aflatoxin contamination. The study also aimed at evaluating the effect of food preparation methods on the toxin levels in the commonly consumed foods. Finally, the study also aimed at establishing the dietary practices of households in the study sites.

Agriculture is the backbone of Busia County's economy with over 80% of the county's population solely depending on the sector for their livelihoods. The communities in these study sites however face a myriad of challenges. These range from erratic climate conditions, high cost of farm inputs, poor quality planting materials to over-reliance on a few food crops (Busia County , 2014). These coupled with small land holding practices, limited knowledge and skills on effective agricultural, livestock, and fishing practices, negative attitudes and stereotypes on land-use, have made it hard to ensure food security.

3.3.1 Influence of food storage and preparation methods on Aflatoxin contamination

In developing countries, it is estimated that 30% of the foods consumed are perishable (FAO, 1993c). Increase in population has led to increase in demand for food supplies. This has prompted increased production of food commodities. Surplus grains require quality storage. Proper storage increases the shelf-life of food. Losses are bound to be high if grains are not well managed. As defined by FAO, losses refer to the total modification or decrease of food quantity or quality which makes it unfit for human consumption (FAO, 2021).

Moisture content, environmental temperature and sanitation are the most important factors that deserve attention with regards to aflatoxin contamination. It has been noted that the maintenance of safe levels would be effective in the control of contamination (Torres, Barros, Palacios, Chulze, & Battilanic, 2014). The recommended moisture levels for white maize are 13.5%, 11.5% for maize meal, 15% millet and 13.5% for sorghum for storage (FAO, 1993c). *Aspergillus flavus*, has been observed to thrive in 70% of grains with moisture content levels greater than 18% with a positive correlation between the rate of aflatoxin production and rate of infection (Mora & Lacey, 1997; Kaaya, Kyamuhangire, & Kyamanywa, 2006).

Storage is a risk factor of aflatoxin contamination in food. During the 2004 aflatoxicosis outbreak investigations, storage of damp maize had a 3.5 likelihood of contamination while storage of maize inside the main house rather than in outside granary storage had a 12 times likelihood of contamination (Centers for Disease Control and Prevention, 2004). Moisture and temperature influence the growth of toxigenic fungi in stored commodities and the level of contamination can increase 10-fold in a 3-day period when field harvested maize is stored with high moisture content (Waliyar, et al., 2005a). Contamination also increases with storage period (Hell, et al., 2008).

Some food preparation methods such as sorting, washing, crushing and dehulling may reduce aflatoxin levels (Fandohan P. , Zoumenou, Hounhouigan, Marasas, & Wingfield, 2005; Njapau, Muzungaile, & Changa, 1998). Traditional methods of cooking food with softening salts have also shown reduction in levels of aflatoxin. Mutungi et al (2008) found that magadi, an alkaline mineral salt is also used in cooking, and it reduced levels of aflatoxin in muthokoi (dehulled maize) (Mutungi C. , Lamuka, Arimi, Gathumbi, & Onyango, 2008). Dry roasting of peanuts has also been shown to reduce aflatoxin levels by up to 80% (Mutegi C. , 2010) . The study investigated storage and preparation methods of the commonly consumed foods and the effect of these on aflatoxin levels.

3.3.2 Effect of local decontamination methods on aflatoxin levels in foods

While there exist several types of aflatoxin, the most common are B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_2) AFG_2 (G_2) and M_1 (AFM_1), with AFB_1 being the most toxic (Olaru, Vasile, & Ivanescu, 2008). Aflatoxin M_1 and M_2 are metabolites of B_1 and B_2 which are found in milk (Bahout & El-Abbassy, 2004). While aflatoxin has been shown to be relatively stable, it is observed to be unstable to extremes of pH (<3 or >10) and unstable in the presence of oxidizing agent. It has also been observed that it can melt at various degrees (O'Neil M. , Smith, Heckelman, & Budavari, 2001) with some degradation happening in methanolic solution, a process which is accelerated with light and heat (Wogan, 1966). Table 3 below shows the various ranges of melting points for aflatoxin B_1, B_2, G_1, G_2 to M_1.

Table 3: Melting points of aflatoxins

Aflatoxin	Melting point (°C)
B_1	268-269 (decomposition)
	crystals from $CHCI_3$
B_2	286-289 (decomposition)
	crystals from $CHCI_3$ pentane
G_1	244-246 (decomposition)
	crystals from $CHCI_3$ methanol
G_2	237-240 (decomposition)
	crystals ethyl acetate
M_1	299 (decomposition)
	crystals from methanol

Source: (O'Neil M. , Smith, Heckelman, & Budavari, 2001)

Aflatoxin contamination of food poses an exceptional challenge to food safety. Risks associated with ingestion of contaminated food can be reduced through some decontamination procedures. Effectiveness of a decontamination procedure should be hinged on chemical stability of the mycotoxin, nature of the process, type and interaction with the food matrix and multiple mycotoxins if present (Park D. , 2002). In light of the aforementioned characteristics of aflatoxins, an ideal decontamination must ensure that it inactivates, destroys or removes the toxin, does not leave toxic residues in the food or feed, the food or feed retains its nutritive value and it does not alter the acceptability of the food and that, if possible, destroys the fungal spores (Park D. , 1993).

Best decontamination processes are those that are approved by regulatory agencies, cost effective and reduce mycotoxins concentration to acceptable levels (Park D. , Effect of Processing on Aflatoxin, 2002). Ammoniation has so far been approved in the United States in the states for

decontamination of aflatoxin in cottonseed and corn products meant for animal feed while in Africa, this is routinely used in Senegal and Sudan (Park D. , 1993).

The study aimed at assessing the extent to which aflatoxin is inactivated, destroyed or removed by various local decontamination procedures practiced in the study area during traditional food preparation methods. Options for utilization of food were also be assessed. Effective decontamination procedures were determined and will be promoted for use at household and community level.

3.3.3 Effect of social and environmental factors on aflatoxin contamination and mitigation in foods

Social and environmental factors have the potential to influence the capacity of individuals and households to take measures that mitigate against aflatoxin contamination of foods during production, storage and access. The conceptual framework depicts the pathways through which this happen (Figure 2).

Environmental factors such as humidity and geographical location variedly affect aflatoxin contamination (Strosnider, et al., 2006). The higher the moisture content in grains the higher the risk of contamination (Hell & Mutegi, 2011) and the longer the storage period the more prone the foods will be to contamination (Kaaya & Kyamuhangire, 2006). Post- harvest handling practices such as timely harvesting, culling of damaged maize, drying of kernels to moisture content levels of 13% and storing the harvest in well ventilated stores and containers reduces the risks of aflatoxin contamination (Waliyar, et al., 2005a; Cotty & Lee, 2007).

The social environment includes culture which encompasses material culture for example type of granaries for storage as well as community knowledge, attitudes and values. The social

environment helps shape human behavior. Knowledge on food production, storage, preparation methods and dietary patterns is cultural information that is passed on from one generation to another (Alesina & Giuliano, 2015). The higher the awareness levels on aflatoxin contamination of foods and the risk factors associated with contamination, the more likely households will be to consciously take steps to mitigate the problem. Conversely, the lower the awareness levels on the aflatoxin problem, the less likely these household members will be to take steps to mitigate it.

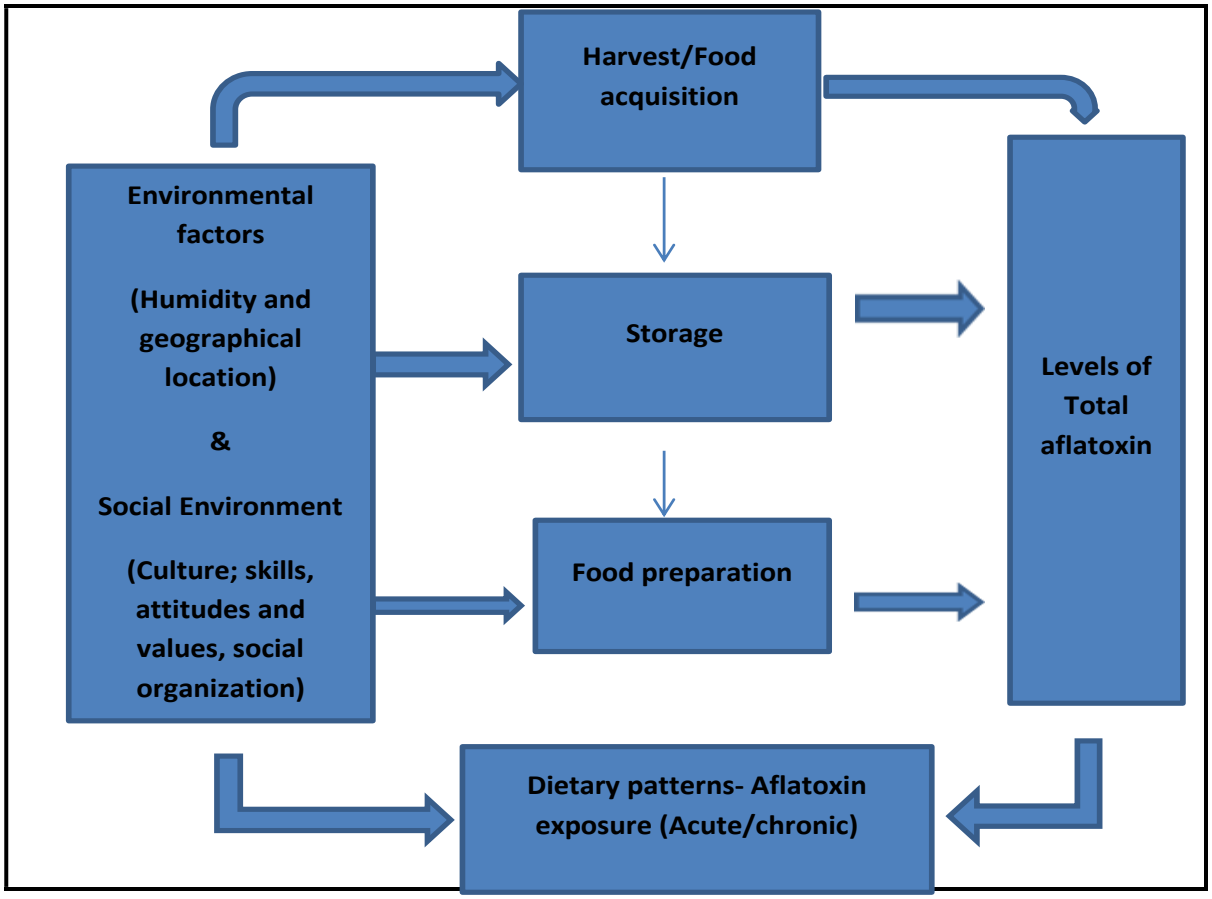


Figure 2: Conceptual framework

Awareness of storage related risk factors such as storage of damp grains and usage of inside storage rather than outside granary storage and prolonged storage period would help household members to take intentional steps towards aflatoxin mitigation. Also, the level of awareness that some food preparation methods would help mitigate aflatoxin would influence a people's way of doing things. Fandohan (2005) and Mutungi (2008) established that some traditional methods of cooking also reduce aflatoxin to varied levels.

Both environmental and social settings may affect practices and dietary patterns of households. This study investigated the extent to which the social, cultural and environmental settings influence specific household practices and consequently the total aflatoxin content of foods.

3.4 Study rationale

Data on the prevalence of aflatoxins in staple foods are essential to understand their impact on health and to map out effective mitigation strategies (IARC Working Group Report No. 9, 2015). In order to identify susceptible edible crops that are responsible for exposure to toxins in specific populations, it is critical to establish knowledge that is specific to that given region.

Results from an aflatoxin sero-survey conducted in Kenya in 2010 with the aim of assessing aflatoxin exposure in a subset of the population, showed detectable levels of aflatoxin B1 lysine adducts in 78% of the samples. Notably, in this sero-survey were the regional differences in exposure, but all participants from Busia County had detectable aflatoxin levels (Yard, et al., 2013). This is of great public health concern since aflatoxin has been reported to impair the immune system and hence reduces resistance to environmental stressors consequently increasing susceptibility to diseases (Jiang, 2005). Additionally, chronic exposure to aflatoxin has been

associated with negative health effects like stunting in children and hepatocellular carcinoma (Gong, et al., 2004; Groopman, Kensler, & Wild, 2008).

Busia County is predisposed to the risk of contaminated foods as it is the main point of entry between Kenya and Uganda, accounting for the bulk of trade in maize, millet, groundnuts and sorghum between the two countries. Aflatoxin contamination of maize and groundnuts has been shown to be high in Uganda (Kaaya & Warren, 2005).

While the likelihood of contamination of many food commodities with aflatoxin remains high, research efforts addressing the aflatoxin problems in Kenya have focused mainly on maize (the staple food) following outbreaks of aflatoxicosis in the eastern parts of the country (Muriuki & Siboe, 1995). Establishing the dietary patterns of households in the study area helped enhance our conceptual understanding of dietary practices of the communities in the study area and provided a basis for dietary recommendation that would help reduce the risk of exposure to aflatoxin, thus reducing aflatoxin exposure related health events. The study findings will help guide public health and agricultural interventions.

Since there are several interventions that have been suggested that can help mitigate aflatoxins in cereals, selected interventions were evaluated with a view to inform and design of sustainable, culturally acceptable and economically feasible interventions for aflatoxin decontamination. Additionally, while it is not possible to prescribe a single set of physical or chemical method for use by the community in all foods due to the differences in food constituents, findings from the decontamination methods are documented and through laboratory analysis the study investigated methods with the highest impact on aflatoxin decontamination.

3.5 Key objective and research questions

Overall objective:

This study was designed to determine the most commonly consumed foods among the study households and determine their consumption patterns; to determine the prevalence of aflatoxin in these cereals (maize, millet, sorghum), and groundnuts and cassava; identify, and describe the factors associated with aflatoxin contamination of the cereals; and assess community members' knowledge and awareness of aflatoxin for purposes of creating a body of evidence that would help improve the awareness of aflatoxin among the community members and county leadership and help guide agricultural and public health interventions.

3.5.1 Specific objectives of the study

1. To determine the consumption patterns of maize, sorghum, millet, groundnuts and cassava in the study households.
2. To determine the prevalence of aflatoxin in the main cereals (maize, sorghum, millet,), groundnuts and cassava consumed in households within Budalang'i, Nambale and Teso- South sub-counties in Busia County.
3. To describe factors associated with aflatoxin contamination of the cereals within the study area.
4. To evaluate the impact of selected preparation methods on aflatoxin contamination levels in main cereals consumed in the study area.

3.5.2 Research questions

3. What are the consumption patterns of maize, sorghum, millet, groundnuts and cassava by the residents in the study county?
4. To what extent are the main cereals (maize, sorghum, groundnuts and millet) and cassava that are consumed by the abantu, nilotes and nilo-hamite communities in Busia County contaminated with aflatoxin?
5. What are the key risk factors associated with aflatoxin contamination of maize, millet, sorghum, groundnuts and cassava in the study area?
6. What are the most effective aflatoxin decontamination methods that can be adopted by communities in Busia?

SECTION 2

This section comprises of one chapter, chapter four which provides a detailed description of study methodology. This chapter provides a description of the study site and study population, including demographic and socio-economic profile of study participants. The chapter then systematically discusses sampling procedures for both quantitative and qualitative data collection and data management and analysis. Information on the study design and description of the variables under investigation are also presented. The community entry procedures including all ethical issues are provided herein.

CHAPTER FOUR: METHODOLOGY

4.1 Study site

The study was conducted in Busia County, one of the four counties in the Western region of Kenya. Busia County lies approximately 431 km, to the west of Nairobi, Kenya's capital city. The County has two border crossing points into Uganda (Busia and Malaba towns). It is approximately 1,695 km², 10% of which is covered by Lake Victoria. Of the total area, Busia has 924km ^{2} of arable land**.**

The altitude in the county is undulating and rises from about 1,130m above sea level at the shores of Lake Victoria to a maximum of about 1,500m in the Samia and North Teso Hills. Busia County is characterized by an average temperature of 22°C and varying humidity between 40-89% in a day. This county is sub-divided into seven sub-counties namely Budalang'i, Funyula,

Matayos, Nambale, Butula, Teso South and Teso North **(Figure 3).**

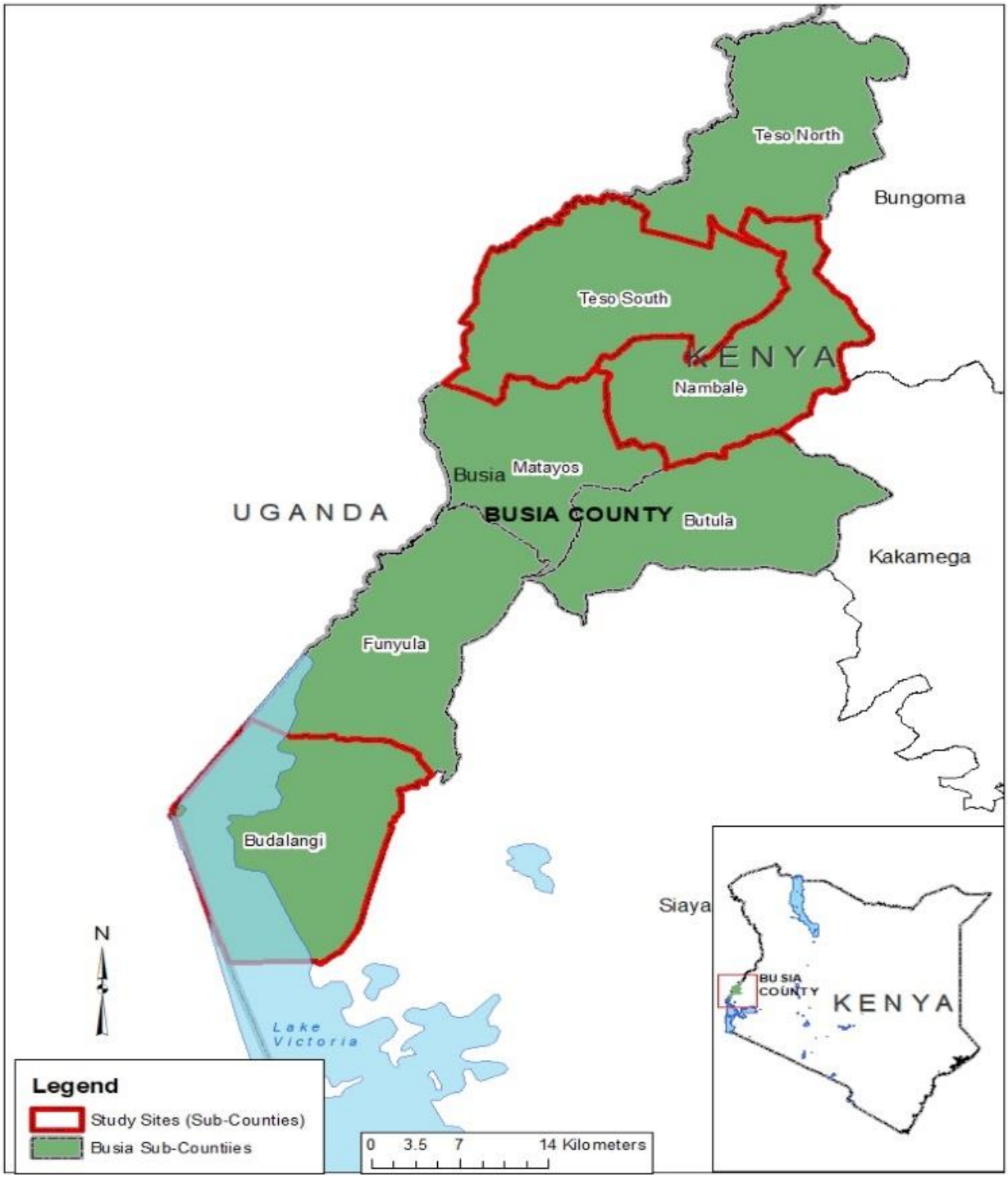


Figure 3: Illustration of Busia County and study sites

Governance and Administration

Upon the promulgation of the constitution in 2010, Busia became one of the 47 newly formed county governments. Busia County is made up of seven administrative sub – Counties namely Teso North, Teso South, Funyula, Nambale, Matayos, Budalang'i and Butula and 35 electoral wards. These sub - Counties are further divided into 10 divisions, 60 locations, 181 sub-locations and villages (Busia County, 2018).

Busia County has its headquarters in Busia town and is governed by a Governor. The Governorship of the county is comprised of the office of the Governor, Deputy Governor and County Secretary. The governorship spearheads policy formulation, promotes the rule of law and mobilizes resources. Additionally, this office is mandated with the coordination and supervision of public service delivery and response to critical community needs during disaster occurrences. The County Assembly represents the legislative arm of government and its core functions are to develop legislation, perform oversight and representation. Additionally, there are 10 departments in the county namely, Health and Sanitation, Public Service Management, Trade, Cooperatives and Industry, Education and Vocational Training, Water, Irrigation, Environment and Natural Resources, Youth, Sports, Tourism, Culture and Social Services, Finance, Economic Planning and ICT, Education and vocational training, Agriculture, Livestock Production, Veterinary and Fisheries and Lands, Housing and Urban Development (Busia County Government, 2020).

The agriculture and animal resources department consist of Agriculture, Livestock Production, Veterinary and Fisheries directorates which are charged with the responsibility of managing agriculture related activities. Agricultural sector is the mainstay for the County's economic growth and also provides for more than 60% of the informal employment in the rural areas.

The department of Trade, Cooperatives and Industry has Trade, Co-operatives, the Cooperative Enterprise Development Fund and Weights and Measures as its directorates which facilitate and promote trade and co-operative development through fair trade practices. This department implements programmes targeting diversification, innovation, value addition, business information sharing, market linkages and trade infrastructure support.

The department of Health and Sanitation comprises of three directorates namely: Administration and Support services, Curative Health Services and Preventive & Health Promotion Services. It implements its mandate through three programmes namely; General administration and support services, Curative health services, Preventive and health promotion services which have been sub programmed into Referral services, Referral (Hospital) services, Public health systems and Primary health care (Busia County , 2014).

Infrastructure

The main urban areas in Busia County are Busia Township, Malaba, Nambale, Bumala, Port Victoria. Adungosi, Butula, Amkura, Lukolis, Funyula and Angurai. These areas act as shopping areas, transport nodes, cross border centers, and residential areas. Among some of the challenges faced in the county are the lack of urban policy and spatial/integrated urban plans to guide urban growth, lack of proper sewerage systems, lack of storm water drainage systems, lack of amenities like slaughter houses, stadiums, cemetery, library and land for expansion within the peri urban and agricultural rural set up and built up market areas to serve the huge cross border traders creating huge population of hawkers and substandard stalls to meet the huge demand (Nabulindo, 2019).

Busia County has a total road network of approximately 1,600 kilometers (km) of which 169.64 km is tarmacked, 591.91 km are of gravel surface and 838.55 km earth surface. Some of the roads

are however impassable during rainy seasons because they lack appropriate drainage. There is only 11 km of railway that crosses into Uganda and one railway station in Malaba Town. There is no functional airport or airstrip. The County has two ports at the Lake Victoria shores. The Sio Port in Samia Sub -County and Port Victoria in Budalang'i (Budalang'i) Sub - County serve as fish landing ports (Busia County, 2018).

Trade

Busia county is a major trading location and accounts for substantive trade between the two East African countries, Kenya and Uganda. The principal economic activities in the urban areas are cross border cereal trade, fish trade, hospitality, sell of fruits across the border, operation of motor vehicle garages, motor bike and bicycle transport operations commonly known as "bodaboda", wholesale and retail shop operations. The primary economic activities in rural Busia County are cash crop and subsistence farming, fish farming, trade in farm produce and artisanship. Most people in Busia County earn their living from farming, producing maize, cassava, sugarcane, millet, sorghum and rearing livestock and poultry (Nabulindo, 2019).

A significant portion of these trade activities are conducted informally. Informal Cross Border Trade (ICBT) at the Busia and Malaba border points pre-date back to the pre-colonial and post-colonial state boundaries reflect longstanding indigenous patterns. ICBT has been reported as not an anomaly but as an integral to the formal market channel (Little, 2007). Indeed, the volumes of informal maize grain traded in second quarter of 2017 comprised of a third of the total traded volumes in Kenya, with main sources of exports of maize and sorghum being Uganda (FSNWG, 2017).

Land ownership and agricultural practices

Land in Busia County is held on leasehold and freehold tenures with most leasehold tenures being in Busia Town. Majority of the population own less than a hectare because of continued sub-division to meet the need of the growing population. Land in Busia County is predominantly ancestral (91.7%) and most of it is acquired through inheritance (84.6%). Unfortunately, most beneficiaries of land inheritance are male children thus 82.6% of land is male owned. Most farmers use polypropylene bags, wooden granaries for storage and keep the farm produce in their houses. The only few modern stores such as silos owned by the National Cereals and Produce Board (NCPB) are in Malaba Town, far from the study area (Busia County, 2018).

4.2 Population of Busia County

The population of Busia County is 893,681with a population density of 527 people per Km^2 and annual growth rate of 2.9 %. Females comprise 52% of the population. Of the seven sub-counties, Teso South is the third most densely populated followed by Nambale and then Budalang'i at 555, 469 and 447 persons per square kilometers respectively. There are 198,152 households with an average household size of 4.5 (KNBS, 2019). The age distribution is as follows: - 0-14 years (47.9 %), 15-64 years (48.4 %), 65+ years (3.7 %). The labour force in Busia County in 2015 was estimated at 400,017 with about 71 per cent of these being engaged on family farms (Busia County, 2018) while the county's poverty gap in 2016 was estimated at 16.8% compared to the nation estimate of 12.2% during the same period (KNBS, 2016).

This population was chosen following findings from a previous CDC aflatoxin sero-survey that showed 100% exposure in all samples from the region (Yard, et al., 2013) and cognizant of the fact that residents' dietary staples were foods prone to aflatoxin contamination.

4.3 Study design and sample selection procedures

This study utilized an integrated convergent mixed methods design. Qualitative and quantitative data were collected in parallel, analyzed separately and then triangulated at results and discussion levels. The study was classified as Arm 1 and Arm 2. Arm 1 comprised of the cross-sectional household survey which entailed administering a household questionnaire to respondents who were responsible for food preparation in the households and collection of food samples for aflatoxin testing while Arm 2 comprised of qualitative methods including focus group discussions (FGDs) and key informant interviews (KIIs).

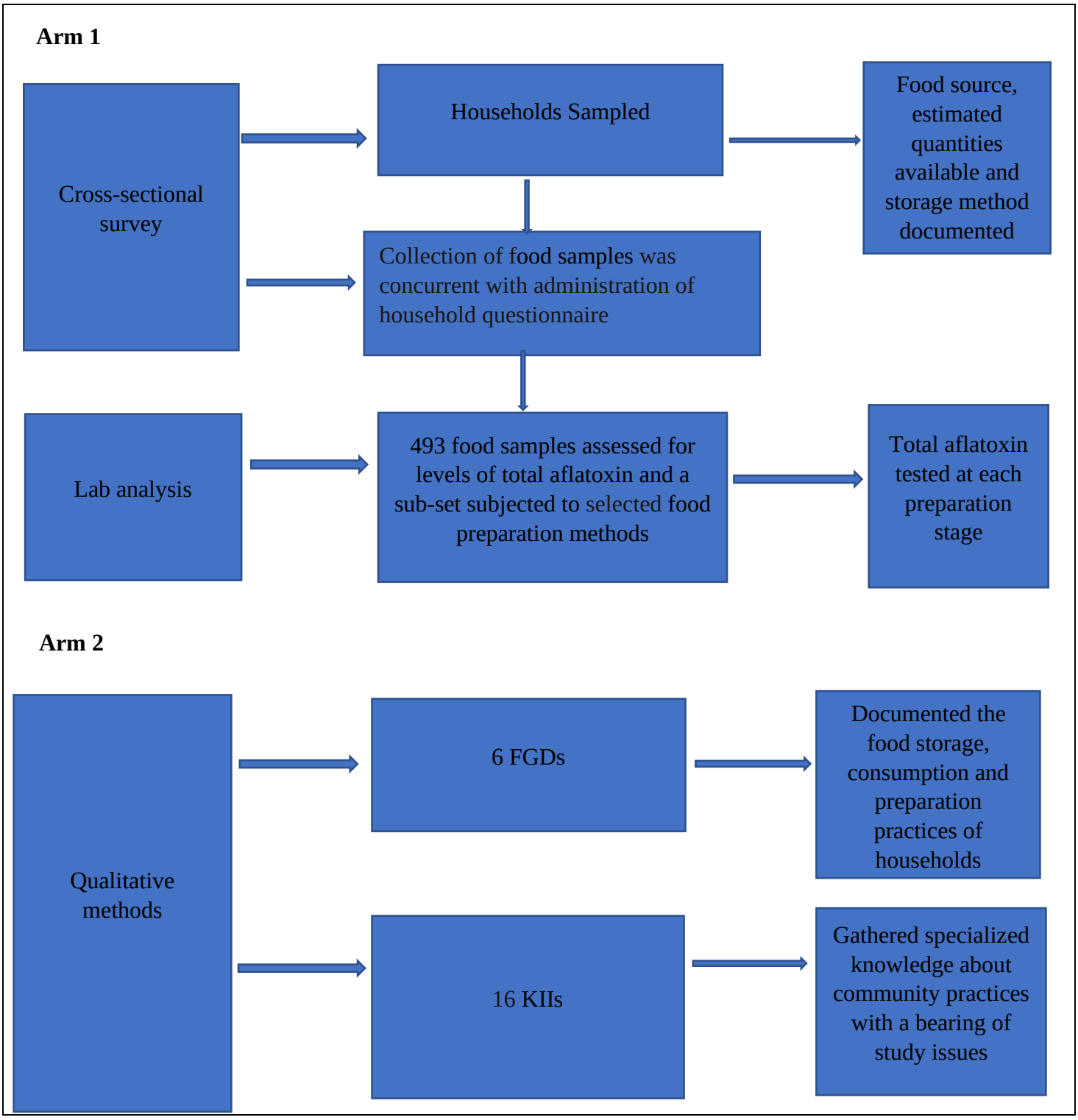


Figure 4: Data collection procedure

Source: Author

Sampling Procedure

The sampling method used to determine study sites was a 3 stage cluster sampling design using Chromy's sequential sampling (Chromy, 1979) in SAS, 9.4 (Statistical Analysis Software Institute, Cary, NC). The sampling frame, Busia County comprises 7 sub-counties namely Teso North, Teso South, Nambale, Butula, Matayos, Funyula and Budalang'i. Teso North and Teso South sub-counties are predominantly inhabited by nilo-hamites, Nambale, Butula and Matayos sub-counties are predominantly inhabited by abantu and Funyula and Budalang'i sub-counties are mainly occupied by both nilotes and abantu. First, 3 sub-counties namely Nambale, Budalang'i and Teso-South were randomly selected from the 7 sub-counties. Second, 4 locations namely Bukhayo East in Nambale sub county, Budalang'i Central in Budalang'i sub-county and Amukura and Ochude both in Teso-South sub-county were randomly selected. Lastly, 4 of 70 sub-locations namely, Okiludu and Amukura located in Ochude and Amukura locations respectively, Buyofu in Bukhayo East location and Magombe East in Budalang'i Central location were also randomly selected. The smallest sub-areas for which population data was available was the sub-location (Kenya National Bureau of Statistics -KNBS, 2013). Locations, sub-locations, villages and households were sampled based on probability proportional to the size (PPS).

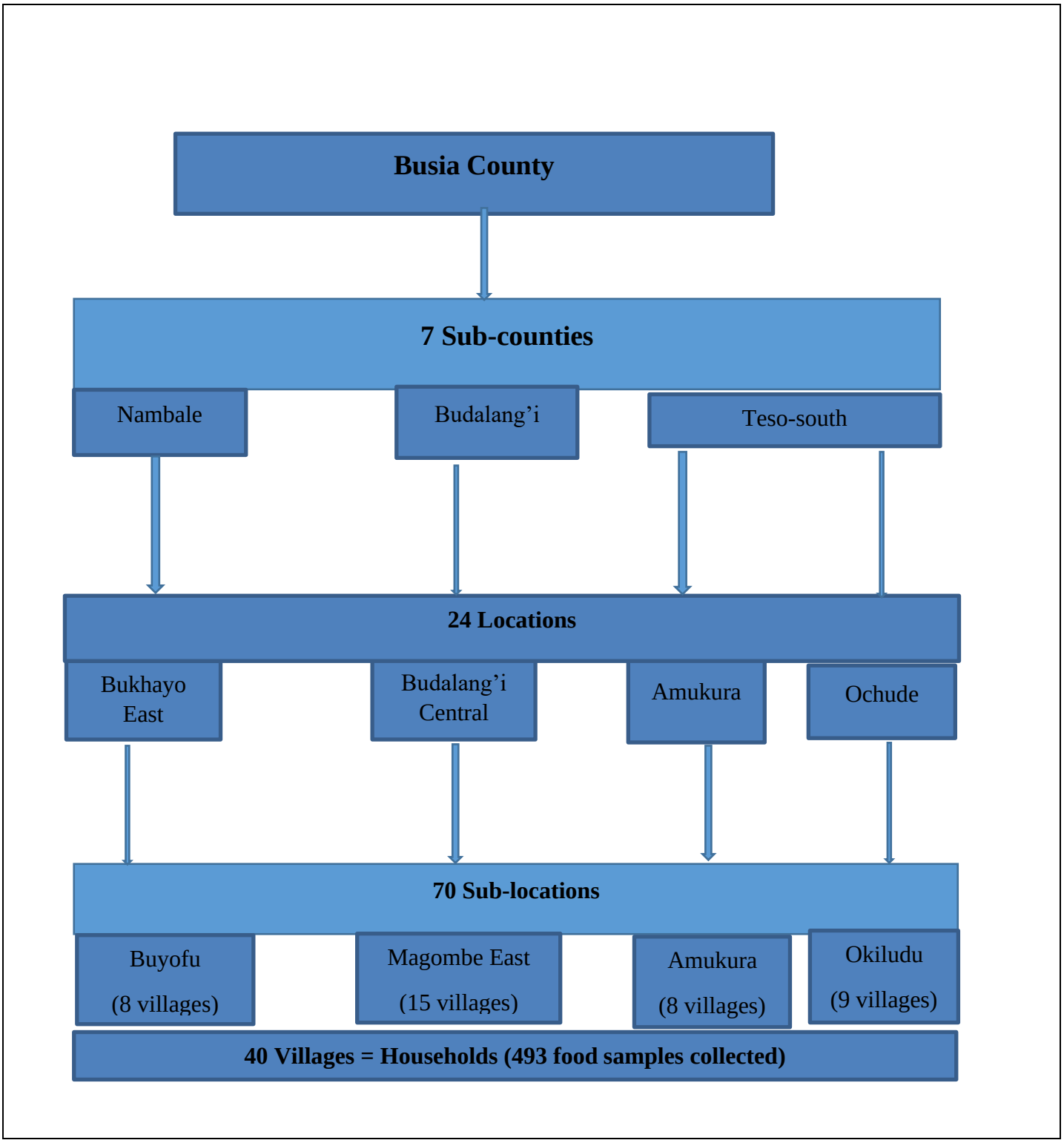


Figure 5: Household food sampling procedure

4.3.1 Arm 1: Household survey

The household survey was conducted in 40 villages located in 4 sub-locations in 3 sampled sub-counties of Busia County.

Sample size calculation

The sample size was determined based on reported aflatoxin prevalence of 7.5% in groundnuts in Busia and 17.5% and 44.9% in groundnuts and maize respectively in Uganda (Kaaya & Warren, A review of past and present research on aflatoxin in uganda, 2005; Mutegi C. , 2010). Since Maize had a 2.5-fold higher prevalence of contamination compared to groundnuts, 19% prevalence was assumed.

A 19% frequency of outcome factor was hypothesized, 95% confidence limits and a design effect of 2.

Sample size (n) = $[DEFF*Np(1-p)]/ [(d^2/Z^2_{1-\alpha/2})*(N-1)+p*(1-p)]$ (Sullivan, 2003)

Where,

N= Total population (893,681)

P = Probability of exposure/contamination in the population (19%)

DEFF = Design effect for cluster survey (2)

d = Absolute precision on either side of the proportion (95%)

$Z_{(1-\alpha/2)} = 1.96$

The calculated sample size of 472 food samples was obtained.

The total sample was distributed among the sub-locations based on probability proportional to the population size (PPS) respectively using Chromy's sequential sampling (Chromy, 1979) using SAS, version 15 (Statistical Analysis Software Institute, Cary, NC). To get the sampling interval,

the total number of households were divided by the sample size allocated to each sub-location as shown in (Table 4).

Table 4: Sampling and sample distribution by sub-location

Sub-county	Ward	Location	Sub-location	Number of villages	Households	Number of samples	K (sampling interval)
Teso south	Amukura	Amukura	Amukura	8	500	500/2719 x 472 = **87**	500/87 = 6
	Chakol	Ochude	Okiludu	9	815	815/2719 x 472 = **141**	815/141 = 6
Nambale	Nambale	Bukhayo East	Buyofu	8	782	782/2719 x 472 = **136**	782/136 = 6
Budalang'i	Budalangi	Budalang'i Central	Magombe East	15	622	622/2719 x 472 = **108**	622/108 = 6

Household selection and enrolment

The primary unit of sampling at the village level was the household (HH) defined as all people who eat from the same pot and are answerable to the same household head (KNBS, 2019). Typically, households function as a basic social and economic unit of a group of people. Members of a household (although not necessarily related by blood or marriage) living together in the same house or compound and shared sleeping and eating arrangements and were cared for as a unit. A polygamous situation was considered to constitute one household if they ate from the same pot.

The sampling interval of 6 households was arrived at by dividing the total number of households by the sample size to get an interval. In order to identify the first households to be sampled in each village, four teams started off from a central landmark identified by the team with guidance from the village elder, and community health volunteers. The team moved simultaneously to the four ordinal directions sampling households. Vacant households at the time of visit or potential respondents who declined to participate were replaced with the next closest household.

Inclusion criteria for households were: 1) Consent (written or thumbprint) by respondent to participate in the study; 2) presence of a respondent (≥18 years) who had information on food sources and food preparation practices at the time of the survey and 3) with respondent whose weekly household diet comprised of ≥4 meals prone to aflatoxin contamination (maize, groundnuts, sorghum, millet or cassava). A household tracking log was used to track the number of households covered per day for each sub-area and to plan consecutive schedule.

Household data and food sample collection procedures

At the household, geographical coordinates captured using Open Data Kits (ODK) and written informed consent obtained.

Data were collected using pre-tested structured questionnaires. The household survey questionnaire was loaded on the ODK. The questionnaires modules were organized in the following 4 sections (1) socio- demographic profile; (2) food source and storage practices; (3) food consumption; (4) food availability and food preparation methods. Paper based 24-hour food recall questionnaire, food frequency questionnaire and dietary diversity tools were used to gather additional data on the respondents' diets. Dietary Diversity is considered as one of the indicators of food security by the Food and Agricultural Organization of the United Nations (FAO)(Hoddinott 1999). Guidelines developed by USAID's Food and Nutrition Technical Assistance project (FANTA) and adopted by the Food and Agricultural Organization were used (Swindale & Bilinsky, 2006). Dietary diversity data, defined as the number of different foods consumed by household members over a seven-day period, was also collected to measure a household's food access. Food access is defined as the ability to acquire a sufficient quality and quantity of food to meet all household members' nutritional requirements for productive lives (FAO, 2019).

A household questionnaire was used to collect the social demographic profile of the respondent, the household food source and storage practices, information on food consumption and food preparation methods. Additionally, information was collected from a sub-set of community members to gather more in-depth information on the community food sources, dietary, storage and food preparation practices in order to give a complete picture and possible explanation to the lab results that would be obtained.

From each household, at least one representative food sample weighing 250 – 500g was collected and tested for levels of total aflatoxin. In order to minimize the skewness of aflatoxin distribution in the food samples, collected multiple grab samples were taken from top, middle and bottom of food storage bag or container then mixed to get a homogeneous sample of the whole as recommended Whitaker et al (Whitaker, Slate, Doko, Maestroni, & Cannavan, 2011). As a measure to control moisture content, properly sealed paper bags were used for sample packaging in the field. At the end of the household survey, all samples were packaged in carton boxes and transported by road to the lab at the School of Biological Science, Chiromo Campus- University of Nairobi.

To assess the effects of different processing techniques. Four main food preparation techniques were used. The boiling technique was used across the 3 food preparation procedures under assessment. For the first sample, water was added to a 20gof flour sample and gradually brought to a boil. The heat temperature was maintained at 100°C for a duration of 10 minutes. A liquid sample was then obtained and aflatoxin levels were tested in the extract. The second procedure involved use of softening salt solution. The softening salt solution was prepared by adding 20 ml of water to 5g of burnt banana peels ash and sieved to get the extract which was then added to a water portion 5 times its amount to provide an alkaline solution in which ground food samples

were boiled at 100°C for a duration of 10 minutes. For the third preparation method, 20g flour meant for porridge preparation was mixed with 5ml of water and was let to set for a period of either 24 or 48 hours. . FGD participants described the process as taking an amount of flour and adding water to it then stirring it to form a thick paste-like consistency. The final stage involved bringing water to a boiling then adding the fermented mixture. This mix was stirred consistently and left to boil at a steady temperature of 100°C. Samples were run in duplicates with a control.

Data collection tools

The household survey questionnaire was loaded on to tablets (Samsung tab 3 lite with android) installed with Open Data Kit (ODK) application. Field teams used pre-tested structured questionnaires to gather information on socio demographic characteristics, common household foods, food sources, and storage and food preparation methods.

Additionally, a dietary diversity tool, which is a measure of a household's food access was used to collect information on household dietary diversity (World Food Programme, 2012) (Appendix 5). A series of questions which would elicit yes, or no responses were asked to the respondent, who was also the person responsible for food preparation. The tool had a list of 21 food groups which were then grouped to 12 in line with the FAO guidelines. The following are the food groups used:-

(i) Cereals: Ugali, bread, rice noodles, biscuits, or any other foods made from millet, sorghum, maize, rice, wheat, or [any other locally available grain] cereals,

(ii) Roots and tubers: potatoes, yams, manioc, cassava or any other foods made from roots or tubers,

(iii) Vegetables

(iv) Fruits,

(v) Meat, poultry and offal: Beef, pork, lamb, goat, rabbit wild game, chicken, duck, or other birds, liver, kidney, heart, or other organ meats,

(vi) Eggs,

(vii) Fish and seafood: fresh or dried fish or shellfish,

(viii) Pulses, legumes and nuts: Foods made from beans, peas, lentils, or nuts,

(ix) Milk and milk products: Cheese, yogurt, milk or other milk products,

(x) Oils and fats: Foods made with oil, fat, or butter,

(xi) Sugar or honey and

(xii) Any other foods, such as condiments, coffee, tea.

A 24-hour food recall interview was used to capture information about all foods and beverages consumed by the respondent, who is the person responsible for preparing meals for the household, in the past 24-hours. Respondents were instructed to include all foods eaten or drank a day before the survey day, consumed within the home or away from home. This data was be used to describe the community's food consumption patterns and to examine the relationship between diet and health variables such as possible aflatoxin exposure (National Cancer Institute, 2020). Respondents were asked to indicate whether the previous day was typical of their usual food intake. The time at which each food item reported was also recorded. Probes were regularly used to prompt for any additional foods consumed.

A paper form Food Frequency Questionnaire (FFQ) was developed and used for dietary intake assessment of study households. The objective of collecting food frequency data was to assess the habitual dietary intake of all household members. The FFQ tool was adapted to capture participants' frequency of consumption of foods prone to aflatoxin contamination: maize, groundnuts, sorghum, millet and cassava. The respondent was asked the frequency of consumption

of the food items under investigation. Responses targeted four levels of frequency: daily, weekly, monthly or never.

4.3.2 Arm 2: Qualitative study

Focus Group Discussions (FGDs) were conducted by trained moderators and note takers while the KIIs were led by the Principal Investigator (PI). Both the FGDs and KIIs were recorded using an Olympus Voice recorder. Informational data was then transcribed as a first step of data analysis.

Focus Group Discussions (FGDs)

Focus group discussions (FGDs) are defined as informal but structured interactions among 8 to 12 participants. The FGDs were conducted in all three sub-counties that participated in the household survey. The FGDs comprised of women drawn from six villages namely Bukuyu Idokho, Khuriaka, Elwnikha 'A', Buyofu 'A', Lelesi and Okiludu. Participants were divided into two age categories aged $18 \leq 34$ years and above 35 years in order to gather age cohort related perspectives and experiences and allow for easier communication among peers. This homogenous composition of the groups was designed to ensure more confidence during interaction among participants and foster ease of sharing thoughts. Six Focus Group Discussions (FGDs) and 16 Key Informant Interviews (KIIs) were also conducted in this study. Table 5 shows distribution of the participants.

Table 5: Geographical locations of Focus Group Participants in various sub-counties

Sub-county	Sub-location	Village	Women Age-group (yrs)	No. of participants
Budalang'i	Magombe East	Buyuku Idokho	18 ≤ 34	8
Budalang'i	Magombe East	Khuriaka	35 ≤	12
Nambale	Buyofu	Elwanikha 'A'	18 ≤ 34	10
Nambale	Buyofu	Buyofu 'A'	35 ≤	11
Teso South	Amukura	Lelesi	35 ≤ above	10
Teso South	Okiludu	Okiludu	18 ≤ 34	9
Total				**60**

Information on the planned study and description of eligible participants was shared with the chairpersons of the Community Health Units of the four sub-locations in the 3 sub-counties. The Community Health Unit (CHU) chairpersons helped identify and invite the participants. Eligibility criteria for participants included (1) those residing in the sampled sub-county (2) were women (3) aged ≥18 years. At the start of discussions, each participant was allocated a numerical identifier. Each FGD comprised of 8-12 participants and lasted for between 1 and 2 hours. Written informed consent to participate in the FGD was sought and the participants appended a signature or thumb print on the consent form **(Appendix 6).**

The FGDs were held either in classrooms of schools in the area or community halls. All FGDs were conducted in Kiswahili and only one comprising the older group (aged ≥35years) was conducted in Lelesi village, in Teso-South required the services of a translator.

The FGDs were designed to gauge the community's knowledge on aflatoxin, causes of aflatoxin contamination and consequences of food ingestion of aflatoxin contaminated food. These discussions were guided by questions, which were open-ended in order to elicit discussions which in effect allowed for in-depth perspectives on the research questions to be obtained. Information

on the common foods consumed in communities, the major food sources, the various methods used for food storage within the community, and information on the common food preparation methods for the dietary staples were captured. The information aimed at capturing the cultural diversity, current and historical aspects of food sourcing and preparation practices of the study communities (**Discussion guide, appendix 7**).

Key Informant Interviews (KIIs)

Key informants were selected purposively. Respondents were chosen based on their role in the community, specialized knowledge about community life and willingness to participate in in-depth interviews. Inclusion in the study was designed to elicit deeper insights about the community deriving from the expertise and unique expertise. The key informants included village elders, women group leaders, Community Health Volunteers (CHVs), Non- Governmental Organization representative and county government officials.

The KII discussion guides **(see Appendix 8 & 9)** were tailored for each category of key informant. The guides entailed, in addition to the introduction of the interviewer and the research study questions that would help elicit more information on knowledge of food contamination by aflatoxin, the most commonly contaminated foods, the causes of contamination, aflatoxin decontamination practices and challenges encountered, and the role of government in mitigation of aflatoxin challenge. In addition to collecting informant's demographics (age, gender, education level, occupation, role in community), questions revolved around awareness of aflatoxicosis, knowledge about aflatoxin contamination and decontamination, commonly consumed foods in the county and common sources of grains. Food security and safety issues were also explored. Probes were used in the course of the interviews to help provoke more information from the respondents. Consent to participate was sought days ahead of the commencement of the exercise.

4.3.3 Ethical Consideration

Ethical approval for the study was obtained from the Kenyatta National Hospital-University of Nairobi Ethical Review Committee (Ref: KNH-ERC/A/114). In addition, a research permit was obtained from the National Commission of Science Technology and Innovation (NACOSTI) # NACOSTI/P/17/23914/20543 to conduct research in Busia County.

Consenting Process

The consent form was used as a guide for the verbal explanation of the study. The study was explained to the potential participant verbally, describing the purpose, procedures, risks, benefits and alternatives to participation. Potential participant's comprehension of the study was assessed using open-ended questions which were posed by the investigator to gauge the understanding of the research and the risks and benefits involved. Participants, witnesses and research assistants signed and dated the consent form and a copy of the consent form was shared with the participant. As part of the training process, all project personnel were mandated to review and sign a confidentiality statement. All data was kept confidential, as permitted by law. Laboratory samples were coded with a unique identification number for the purpose of tracking laboratory information. Access to participant data was limited to relevant persons. Electronic copies of the data were kept in databases, which were only accessible to relevant University of study staff and were password protected. The informed consent form was paper based thus was stored under lock and key at until final analyses and reports were prepared. They will be destroyed according to prevailing regulations ethics regulations in Kenya.

4.4 Key Variables

Based on the study objectives, the socio-demographic characteristics of the study population were documented. This included the household members' sex, age, ethnicity, education level and number of household members. Below are the independent and dependent variables studied.

Independent variables

1. Geographical location: Budalang'i, Nambale and Teso-South sub-location
2. Food sources: homegrown, market or gifts/donations
3. Food consumption pattern
4. Food storage containers: - sisal bags, nylon bags, buckets, granaries
5. Food preparation methods: boiling, fermentation and boiling with softening salts

Dependent variable

Levels of aflatoxin in five staple foods; groundnuts, maize, millet, cassava and sorghum

4.5 Study preparatory activities

Prior to implementation of this study, a reconnaissance visit was conducted by the PI with the aim of meeting and briefing relevant government authorities of the planned study and familiarizing with the study locations and terrain of the sampled sub-locations in Teso South, Budalang'i and Nambale. Briefing meetings were held with the County Director of Health, County Commissioner, and County Director of Education. Additionally, planning meetings were also held with County Monitoring and Evaluation team members, public health officers and the local area chiefs in the study areas. A suitable training facility and operation center for the study period was also identified.

4.6 Recruitment and training of study staff

The research team comprising of Ministry of Health personnel and university graduates with prior experience in data collection and conducting community surveys were identified, recruited and trained. The team of 10 research assistants was taken through a 5-day training which was conducted from May 28th to June 1st, 2018. Areas covered during the training included a comprehensive introduction to and description of the study. Specifically, the public health importance of food safety and food security, ethics in field research, the study background, objectives and study approach (Mixed methods) were covered. The team was trained on how to sample households and recruit potential study participants who met the eligibility criteria. The RAs were also trained on rapport building and how to obtain written informed consent. The content of the data collection tools (household questionnaire and FGD guide) was also reviewed and discussed thoroughly, both in English and in Kiswahili. The team's comprehension of all questions in the tools was reviewed in order to reduce the variation of the understanding and to avoid distortion of the intended content. The food sampling technique was also demonstrated and the need for obtaining a homogenous sample emphasized. Finally, "in-house" role-plays of gaining informed consent and administration of questionnaire and entry of the visit log were practiced.

4.7 Pre-testing/Piloting

In order to provide hands on training for the data collection team and identify potential problem areas and challenges in administration of the study, field pre-tests for both household survey and Focus Group discussion were conducted. The research team working in pairs administered the questionnaire in 10 households. Pilot testing was conducted in Magombe West sub-location in Budalang'i, an area not sampled to be part of the study but with similar characteristics as one of the sampled sub-locations. The data collection team and field supervisor got the opportunity to

work together and to synergize skills of interviewing, data recording and uploading onto the web-based platform (cloud) that was to support the data storage under overall oversight of the PI. As part of the pretest, the team also conducted 1 FGD. The efficiency of the electronic equipment was also tested. A feedback session was held and the various issues observed in the field were discussed. Individuals were given feedback based on performance of the pre-test with a view to strengthen consenting, interview procedure, recording and sample collection and packaging.

4.8 Data management and quality control

4.8.1 Data management

Data entry and storage

During the study, all paper-based tools were compiled by the field supervisor and stored in a safe location. Additionally, to guard against inadvertent loss or damage all data collected was transmitted to a cloud server and backed up daily. Electronic copies of data were maintained by the investigator on secure computers. All data was entered onto password-protected relational databases in Microsoft Excel (Microsoft, USA). To ensure accuracy, all questionnaire data entry was double-checked by a data manager. Access to the data by study identification number was only available to study investigators, data entry personnel, and laboratory personnel. All stored records were locked in a file cabinet for the duration of the analysis (approximately one year) and are archived in a locked facility until upon publication of the data. Additionally, all participant information was kept confidential and each participant was assigned a unique alphanumeric identifier that was used throughout the study. The names and household locations were stored along with study identification numbers in a separate electronic database. No names or household locations were provided to the laboratory team.

4.8.2 Quality control

The Principal Investigator provided oversight of the data collection processes on a daily basis. The questionnaires were validated and checked for accuracy during the pre-testing exercise that happened before the questionnaires were programmed in ODK on the tablets. The investigator accompanied the interviewers on 10% of the household visits, at the beginning of the study, to monitor quality of data collection. Validation rules and checks were programmed within the ODK to ensure data collected was accurate and valid. Data were entered in the ODK and uploaded at the end of each household interview session to the cloud. In-flow of this data was monitored real time by a data manager who had accessibility credentials from Nairobi. Cleaning codes was done continually during the pilot phase on the data to identify errors and inconsistencies. Errors in coding or missing information was reviewed and re-training of study personnel was provided, where it was deemed necessary.

4.9 Data analysis

Key components that were analyzed quantitatively were social demographic data, dietary diversity data, food frequency, 24 hr food recall, levels of aflatoxins in food and effect of food preparation on aflatoxin levels. Both descriptive and statistical analysis of data were performed.

4.9.1 Descriptive data analysis

Frequencies and cross tabulations were used to give counts, percentages, means and medians in the descriptive analysis of demographic characteristics of study respondents and food samples collected. The measure of dietary diversity was based on counting the number of food groups consumed in the past 24 hrs. Each food group had an equal weight of one. The response categories were either “Yes” or “No” and scored with either “1” or “0” respectively. A count of food groups consumed was recorded. Household Dietary Diversity Scores (HDDS) of the three sub-counties,

defined as the sum of a number of food groups consumed over the study's reference period were computed. These scores reflected the economic ability of a household to consume a variety of foods (Swindale and Bilinsky 2006).

Dietary diversity

Dietary diversity is a measure of household access.

Household food consumption is a function of food access and availability, socio-economic status, environment and culture (National Research Council, 2013). Dietary diversity is a qualitative measure of food consumption and is a measure of food access to a variety of food and is a proxy of nutrient adequacy and healthy diet (Swindale & Bilinsky, 2006; FAO, 2011; Hoddinott & Yohannes, 2002). Dietary diversity reflects the economic ability of a household to access a variety of foods. Household dietary diversity is achieved when household members access to adequate nutrients. In order to determine the consumption pattern of aflatoxin prone foods in study households, foods eaten and drank a day before the survey day by respondents and any member of their households, whether outside or at home were recorded. The 21 food groups captured were then categorized into 12 original food groups based on FAO recommendations for ease of comparability with the FANTA methods by (Swindale & Bilinsky, 2006).

The Household Dietary Diversity Score (HDDS12) was calculated for each household following FANTA version 2 methods (Swindale & Bilinsky, 2006) as shown in the below tabulation.

HDDS (0-12) = Total number of food groups consumed by members of the household. Values for A through V will be either "0" or "1".
Sum (A + B + C + D + E + F + G + H + I + J + K + ……)

The percentage of households consuming the various food group items was calculated using the following formula:

$$\mathrm{N} = \frac{Number\ of\ households\ that\ consumed\ food\ y}{Total\ number\ of\ respondents}\ X\ 100$$

Where y is the food group of interest.

The food samples of interest were grouped by sub-county. For each sub-county the percentage of samples in each category was computed. In order to know which food groups were predominantly consumed at different levels of the scores, the average HDDS indicator was also calculated for the sample population using the below formula.

$$\text{Average HDDS} = \frac{\text{Sum (HDDS)}}{\text{Total Number of Households}}$$

HDDS target was set by taking the average diversity of the 33 percent of households with the highest diversity (upper tercile of diversity). This allowed for comparison across the sub-counties. Frequency tables were used to show the variations in consumption patterns for the aflatoxin prone foods by sub-county.

Data was exported from ODK to ACCESS and analyzed using Statistical Analysis System (SAS) version 9.4. Foods sampled were categorized as aflatoxin contaminated and considered not fit for human consumption if they had above 10 parts per billion (ppb) (the East African regulatory limit

for aflatoxins in grains) and having detectable aflatoxin if they had >0.1 ppb (the lowest detectable level and negative if less than 0.1ppb. Frequencies of various types of food samples above this limit by food sources, sub location and storage vessels were calculated.

Analysis for levels of total aflatoxin

Samples were transported to the University of Nairobi, Mycotoxin laboratory for testing. ELISA method (Helica Biosystems' Total aflatoxin kits LOT No. AF102815, CAT No. 941AFL01M-96) was used for sample testing. Samples were ground to a fine powder to achieve effective distribution. A 20g portion of the sample was weighed using a Sartorius CP423 S weighing balance then aflatoxin extracted using methanol (70%) in a ratio 5:1 (w/v). The extract was mixed with Horseradish Peroxide (HRP) conjugated aflatoxin B_1 and added to an antibody-coated microwell. The extract was filtered through 125mm Whatman filter paper. A microplate reader with an absorbance filter of 450nm (OD_{450}) was used to optically measure the microwell optical densities (ODs), which were then compared to the OD's of the kit standards to determine and interpretative results. Levels of aflatoxin by the reader were multiplied by 5 to obtain the total amount of aflatoxin per gram of sample (ppb). Samples readings below 20ppb were actual values within the curve and for those reading above 20ppb, further dilution was done in order to get actual values and used the multiplier factor to determine the actual aflatoxin levels.

Using recipes obtained from focus group discussions on preparation methods for various foods, four food samples were prepared in the laboratory for standardization. In order to determine the effect of the food preparation method on aflatoxin, samples were taken at various stages of food preparation to analyze for total aflatoxin.

Effect of food preparation on aflatoxin levels

The primary hypothesis, H_o: $\mu_0=\mu_1$ was tested versus the alternative hypothesis H_1: $\mu_0\neq\mu_1$, where μ_0 and μ_1 are the means of the within-food preparation changes from baseline [the value at the end of each food preparation stage minus the value at baseline] in total aflatoxin.

4.9.2 Statistical data analysis

Bivariate analysis was performed to determine the association between a food sample and levels of contamination (above 10ppb) as the outcome of interest whilemultiple logistical regression models were employed to assess food type, source, storage and demographic characteristics associated with high levels (10ppb<) of aflatoxin.. Results presented herein are based on weighted data to account for the survey sampling design. The weights are used to correct for unequal probability of selection, produce results that are a representative of the population from which the sample was collected and to adjust for survey non-response. SAS procedures accounted for multi-stage stratified sampling designs producing reliable standard errors and confidence intervals. Odds ratios and 95% confidence intervals (CIs) were computed with a P-value of < 0.05 considered significant. For hot-spot analysis, focused analysis was used in order to identify statistically significant hot and cold spot clusters of aflatoxin contamination within the study area. A hot spot is defined as a region that had aflatoxin levels higher relative to its surroundings. Thematic maps were used to present the data. Hotspots featured in the top threshold range, when high contaminated samples are surrounded by other samples with high values. Results of all the food samples collected were used. Aflatoxin contamination incident levels and household geocode data were fed into the Getis- Ord Gi* tool within ArcGIS software ((Environmental Systems Research Institute (ESRI), 2011) which were aggregated into weighted features (Getis & Ord, 1992; Environmental Systems Research Institute (ESRI), 2011). The tool identified an appropriate scale

of analysis and the statistical significance reported in the output features. This was automatically adjusted for multiple testing and spatial dependence using the False Discovery Rate (FDR) correction methods.

4.9.3 Qualitative data analysis

A qualitative data management and analysis software (NVivo® Version 10, Burlington, M.A) was used to manage the coding and the analysis of the qualitative data. Since the FGDs and KIIs were mainly in Kiswahili and English, the recorders integrated the field notes into the transcripts and then combined data transcripts and translations daily. Themes were generated deductively. A codebook organized by theme was developed using the moderators guide and the FGD transcripts. Two independent investigators (MO, AO) used the software to code the transcripts and to ensure the methodological rigor in the results, using triangulation strategy (Corbin & Strauss, 2015).

To illustrate the quantitative findings, some verbatim quotes from the participants have been used. The results are reported by age cohorts since there were some differences in some responses but with no regional differences. The quotes are labeled in terms of participants' specific age group affiliation (labelled either as 18 ≤ 34 or 35 and above) and their numerical identifier (P1, P2 etc) shown as follows (P1, 18 ≤ 34; P2, 35 and above). An ellipsis (…) indicates omitted words or sentences. Findings are presented according to the analytical typologies

4.10 Study limitations

This study was conducted in only 3 of 7 sub-counties of Busia County which were randomly selected. This was occasioned by financial constraints. The study was however premised on the assumption that the 3 sub-counties were representative of the main ethnic compositions, dietary habits, and food sourcing and food preparation practices of residents of Busia County by using a

robust sampling strategy. Dietary diversity data were collected at only one point in the agricultural cycle, thus dietary diversity could not be associated with seasonality. This being an integrated convergent mixed methods design, only single point data collected during the household survey, and no temporal dimensions were collected thus could not simultaneously assess contamination and outcome. It was therefore not possible to report conclusively a true cause and effect relationship. In addition, only readily available samples were collected during a time of food shortage, and prevalence of different food sources may be different at different points within the year. Some food types had very small sample numbers to compute any association with source or storage. This could have been because sampling was done in June, a period of food shortage, just before harvest season. Associational analysis was restricted to food samples like maize that had reasonable numbers.

SECTION 3

This section comprises three chapters. The first chapter, chapter five, presents the household survey results triangulated with findings from the qualitative study. In this chapter, household food diversity and dietary patterns are described. Household sources of key staple foods consumed namely maize, millet, sorghum, groundnuts and cassava are identified and the study communities' ability to access these foods reported. The various post-harvest handling and storage practices of the five common foods are also identified and the reasons behind the practices of choice explained. The prevalence of aflatoxin in the five foods is defined and factors associated with the contamination described. Finally, a map showing both the high risk and low risk locations based on the aflatoxin tests of the food samples is presented. Chapter six is the discussion in which these study findings are compared to other similar studies conducted elsewhere. Lastly, in chapter seven the study conclusions are stated, and the recommendations specified.

CHAPTER FIVE: RESULTS

5.1 Demographic characteristics of study participants

A total of 469 households that met the inclusion criteria were surveyed from three sub-counties of Busia County: (23% in Budalang'i, 29% in Nambale and 48% in Teso South). Among the respondents, the median age was 43 years (range, 20-93 years), and majority (97%) were females (n=457). Of all participants, 61% had completed pre-primary school education, whereas 1.5% had completed college education (Table 6).

Table 6: Demographic characteristics of study participants in Busia County, 2018

Characteristics		Budalang'i n (%)	Nambale n (%)	Teso-South n (%)	Total N (%)
		108	136	225	469
Sex	Female	101(93.5)	134(98.5)	222(98.7)	457(97.4)
	Male	7(6.5)	2(1.5)	3(1.3)	12(2.6)
Age	Median	50.5	40	42	43
	Range	[21, 90]	[20, 85]	[20, 93]	[20, 93]
Ethnicity	Bantu	105(97.2)	114(83.8)	36(16)	255(54.4)
	Nilotes	3(2.8)	4(2.9)	36(16)	43(9.2)
	Nilo-Hamites	0(0)	18(13.2)	153(68)	171(36.5)
Education Level*	Pre-primary	73(67.6)	74(54.4)	140(62.2)	287(61.2)
	Primary	21(19.4)	44(32.4)	62(27.6)	127(27.1)
	Secondary	4(3.7)	13(9.6)	19(8.4)	36(7.7)
	College and above	1(0.9)	4(2.9)	2(0.9)	7(1.5)
	Refused to respond	9(8.3)	1(0.7)	2(0.9)	12(2.6)
Household Members	Median	5	6	6	6
	Range	[1, 10]	[2, 13]	[1, 13]	[1, 13]

** Level of education completed by the respondent.*

Of 67 residents who consented, 60 females took part in the FGDs while seven, four females and three males were Key Informants. Over half (55%) of the FGD participants were aged ≥35 years (Table 7). The focus group discussion sessions ranged between 80 and 240 minutes with a median of 145 minutes per session.

Table 7: Demographics of the Focus Group Discussion participants and Key Informants respondents in Busia County

Sub-County	Sub-location	Village	Age-group	No. of participants
Budalang'i	Magombe East	Buyuku Idokho	18 ≤ 34	8
Budalang'i	Magombe East	Khuriaka	35 and above	12
Nambale	Buyofu	Buyofu A	18 ≤ 34	10
Nambale	Buyofu	Elwanikha A	35 and above	11
Teso South	Amukura	Alelesi	35 and above	10
Teso South	Okiludu	Okiludu	18 ≤ 34	9
			Total	**60**
Key Informant	**Sector**	**Designation**	**Gender**	
KI 1	Health	Community Health Volunteer	Female	
KI 2	Health	Community Health Unit Chair	Male	
KI 3	NGO - FHI360	County Nutrition Coordinator	Female	
KI 4	Ministry of Agriculture	County Agri-Nutrition Coordinator	Male	
KI 5	Agriculture	Food technologist	Female	
KI 6	Health	Director of Health	Female	
KI 7	Health	Public Health Officer	Male	
KI 8	Community	Village elder – Nambale	Male	
KI 9	Community	Village elder – Nambale	Male	
KI 10	Community	Village elder - Budalang'i	Male	
KI 11	Community	Village elder - Budalang'i	Male	
KI 12	Community	Village elder – Teso South	Male	
KI 13	Community	Village elder – Teso South	Male	
KI 14	Community	Women group leader – Nambale	Female	
KI 15	Community	Women group leader - Budalang'i	Female	
KI 16	Community	Women group leader – Teso South	Female	

5.1.1 Determination of household food consumption patterns and dietary diversity

Respondents to the household dietary diversity were persons responsible for food sourcing and preparation who in the study were mostly women. Budalang'i, Nambale and Teso-South sub-county HDDS targets were 11.2, 12 and 12 respectively. Figure 6 below shows the proportion of households achieving the HDDS target. Budalang'i recorded the least percentage (10%) of such

households while Teso-South had the highest percentage (53%) achieving the target while Nambale recorded slightly under half (45%). Low HDDS for Budalang'i was attributed to sporadic floods and limited financial resources. *"…lack of money and floods around here" (KI 10 – Village Elder Budalang'i).*

One village elder from Nambale's attributed the challenges to food accessibility to limited work force and limited household finances to procure seed.

> "*Farmers also lack seeds to plant and lack of enough finances to source for people to help in cultivating farms especially when children are in school because when they are not in school, they become handy in terms of labor (KI 14 Village Elder Nambale)*

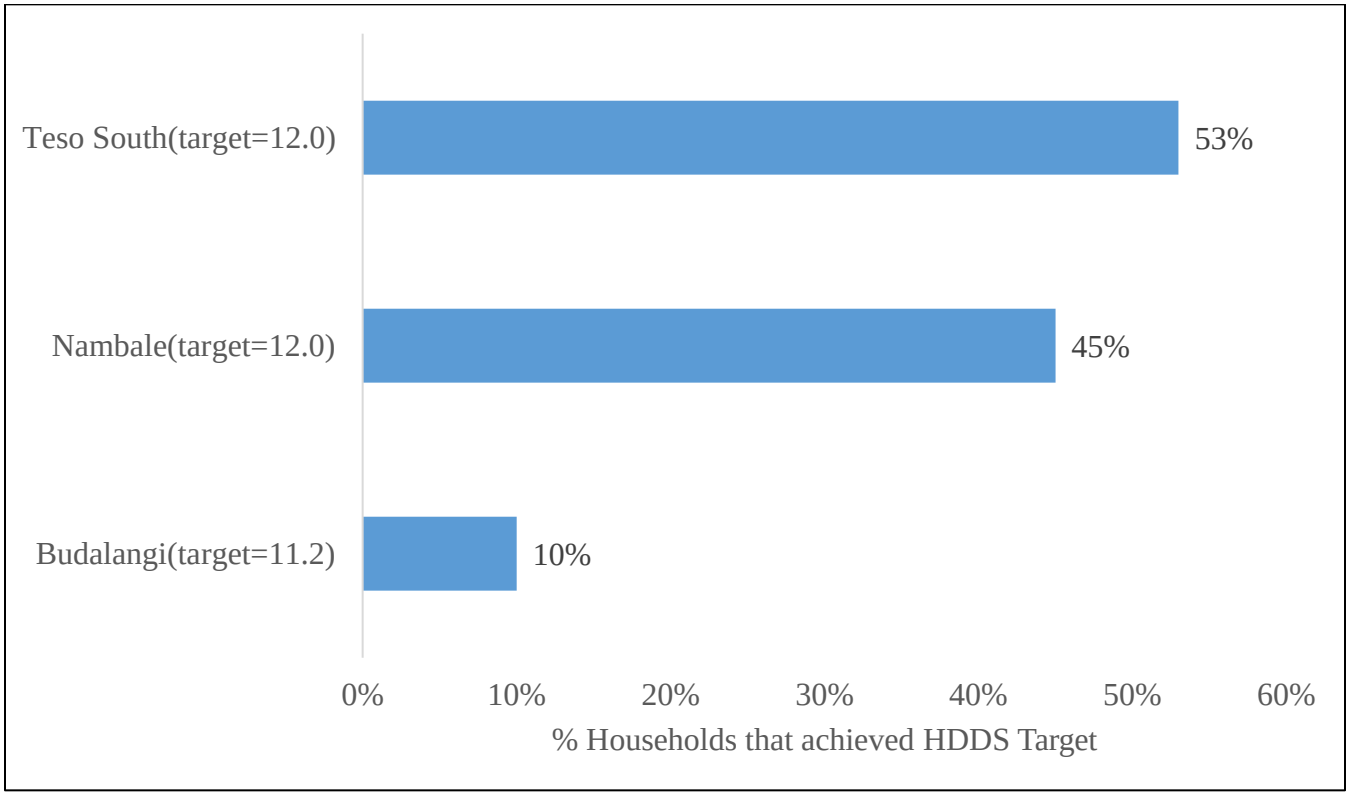


Figure 6: Proportion of households able to access food in the 3 sub-counties in June

All respondents, (n=440) reported consuming foods from group1 (foods made from grains and prone to aflatoxin contamination). Consumption of dark green leafy vegetables (96%) and vitamin A rich fruits (mangoes pawpaws and guavas) (97%) also received a high rating in Budalang'i (88%, 89%), Nambale (99%, 97%) and Teso South (99%, 100%) respectively indicative of possible ease of access or affordability but intake of dark green leafy vegetables was lowest in Budalang'i sub-county (88%). FGD participants corroborated these findings as they indicated that seasonal fruits like guavas and mangoes were commonly consumed because they were readily available and cheap. Majority of the respondents (93%) consumed foods from group 2 (white roots and tubers) and pulses. While least consumed foods across all three sub-counties were nuts and seeds (49%), households in Budalang'i Sub-county had the least access to all foods from all food groups. Intake of food from animal sources (meat, poultry, offal, milk and milk product) were not as high as intake of foods made from grains. Generally, intake of foods from all food groups was lowest in Budalang'i (Table 8).

"Ugali" which is stiff porridge made from maize or a blend mixture of maize, sorghum and or cassava is a staple in almost every household. It was reported to be the most popular food by both FGD participants and Key informants from Teso South and Budalang'i. This was the same trend across sub-counties.

> *"…the food that we like most is ugali…. Even if somebody has eaten other things like bananas or cassava, if they eat ugali they feel as if they have eaten" (P2,* 35 and above*).*

Table 8: Household dietary diversity in Busia County, Kenya

Food group	Nambale Households % (n)	Budalang'i Households % (n)	Teso-South Households % (n)	Total Households % (n)
Food Group1 (Food from Grains)	100 (n=122)	100 (n=104)	100 (n=214)	100 (n=440)
Food Group2 (White Roots, Tubers & plantains)	93 (n=122)	81 (n=104)	97 (n=214)	92 (n=440)
Food Group3 Pulses	95 (n=122)	83 (n=104)	98 (n=214)	93 (n=440)
Food Group4 Nuts & Seeds	46 (n=122)	23 (n=104)	63 (n=210)	49 (n=436)
Food Group5 Milk & Milk Products	89 (n=122)	62 (n=104)	96 (n=214)	86 (n=440)
Food Group6 Organ Meat	57 (n=122)	30 (n=104)	74 (n=214)	59 (n=440)
Food Group7 Meat and Poultry	93 (n=121)	74 (n=104)	95 (n=213)	89 (n=438)
Food Group8 Fish & Seafood	93 (n=122)	83 (n=104)	99 (n=212)	93 (n=438)
Food Group9 Eggs	90 (n=122)	65 (n=104)	94 (n=212)	86 (n=438)
Food Group10 Dark green Leafy Vegetables	99 (n=122)	88 (n=104)	99 (n=212)	96 (n=438)
Food Group11 Vitamin-A rich veges/roots/tubers	92 (n=122)	73 (n=104)	96 (n=212)	89 (n=438)
Food Group12 Vitamin-A rich fruits	97 (n=122)	89 (n=104)	100 (n=211)	97 (n=437)
Food Group13 Other veges	98 (n=122)	93 (n=104)	100 (n=211)	98 (n=437)
Food Group14 Other fruits	84 (n=122)	71 (n=104)	91 (n=211)	84 (n=437)
Food Group15 Insects and other small protein foods	70 (n=122)	33 (n=103)	74 (n=210)	63 (n=435)
Food Group16 Other Oils & Fats	83 (n=120)	71 (n=80)	77 (n=206)	77 (n=406)
Food Group17 Savoury & Fried Snacks	92 (n=122)	59 (n=83)	93 (n=209)	86 (n=414
Food Group18 Sweets	59 (n=122)	18 (n=83)	74 (n=209)	58 (n=414)
Food Group19 Sugar-Sweetened Beverages	86 (n=122)	41 (n=83)	90 (n=209)	79 (n=414)
Food Group20 Condiments & Seasonings	100 (n=122)	95 (n=83)	100 (n=207)	99 (n=412)
Food Group21 Other Beverages & Foods	98 (n=122)	95 (n=83)	99 (n=204)	98 (n=409)

Some respondents reported using blended flours for 'ugali' and porridge preparation. A blend of maize and sorghum was most popular (83%) followed by maize and cassava (78%) as shown in table 9. This mixture was perceived to be more energizing as reported by most of the elderly

participants. Indeed, it was also noted that ugali prepared from blended flours was preferred by the older persons in Teso South.

> *"I enjoy eating ugali mixed with cassava and millet, yes that "ugali" makes me have a lot of energy" (P6, 35≤ above).*

> *"…older people in our community prefer cassava ugali to maize ugali" (KI 13 – Village Elder Teso South).*

While older respondents from Budalang'i sub-county noted that sorghum consumption had reduced significantly when compared to intake in the last two decades, they noted increased maize intake and attributed this shift to the cost difference. Maize was reported to be cheaper than sorghum and millet and was more easily accessible.

Table 9: Common food items mixed with maize to make posho/ugali (stiff porridge)

Main food item	Other ingredients	Budalang'i n (%)	Nambale n (%)	Teso – South n (%)	Total N (%)
Maize for ugali (stiff porridge)	Sorghum	28(58.3)	21(60)	34(89.5)	**83(68.6)**
	Millet	17(35.4)	7(20)	8(21.1)	**32(26.5)**
	Cassava	30 (62.5)	27(77.1)	21(55.3)	**78(64.5)**
	Other	1(2.1)	0(0)	0(0)	**1(0.8)**

While the most consumed foods were grains and legumes, vegetables and '*Omena*'-rastrineobolaargantea fish were popular accompaniments to "Ugali". Participants in Budalang'i noted that they easily sourced their '*omena*' from the lake at no cost.

Infants and young children's diets typically differed from diets consumed by adults in the household. This was attributed to guidance received from health care workers at the post-natal

clinics as reported by mothers from Budalang'i. Infants' gruels were prepared using special flour mixtures to ensure better nutrition.

> *"when we go to the hospital, like we mothers who attend post-natal clinic, we are advised by the health worker to fry groundnuts and soya then dry them after which we mix with millet and abit of rice, sorghum and cassava. We then grind and make flour for porridge for the child. The child is to be fed frequently, daily" (P4, 18 ≤ 34)*

However, it was noted that while children below five years in many households feed on the same food as the rest of the household members, during regular mealtimes, they were given additional foods in between these meals. These foods comprised mainly millet porridge and bananas.

Household food choice was reported by some key informants to be influenced by personal preferences, the age of the household members and the household's financial capability. While, one village elder from Budalang'i reported that many residents in Budalang'i had no choice but to eat what was available at the time, another key informant, a women group leader from Teso South reported that household size coupled with economic capability was a determinant of food choice. Households with more members opted for cheaper foods.

> *"… if we have many people in the household cheaper foods are bought so it can be enough for everybody (KI 16 Women Group Leader Teso South).*

There were mixed responses from participants on who made the decision on the choice of food for the household. While some participants reported that men were responsible since they were *"head of households"* or "*bread winners*", majority of participants, both male and female, noted that women were the decision makers.

"The woman, because men spend a lot of time away from home working, women spend more time at home" (KI 12 – Village Elder Teso South).

"The woman because the woman understands the needs of all family members" (KI 13 – Village Elder Teso South).

Study participants reported a change of dietary habits when compared to the past 2 decades. FGD participants from Teso South observed an increase in the consumption of maize meal while participants from Budalang'i noted an increase in rice consumption.

"Many people are now eating maize. The young generation are no longer taking cassava as much but have resorted to eating maize and cabbage or kales (P8, 35 and above*).*

5.1.1.1 Frequency in consumption of aflatoxin prone foods

Assessment of the community's dietary intake of the aflatoxin prone food groups, focused on (i) Ugali or any other foods made from millet, sorghum, maize, rice (ii) cassava (viii) any foods made from beans, peas, lentils, or nuts the participants reported the frequency of consumption of these foods. Four levels of frequencies: daily, weekly, monthly and never were used. Meat, fish and fruits were also included in the food frequency tool. These findings would help assess relative risk of chronic exposure to aflatoxin.

All respondents in the three sub-counties of Nambale (100%), Budalang'I (100%) and Teso-South (100%), reported consuming foods made from grains. Only a negligible percentage of population reported having never consumed maize. However, daily maize consumption was higher in Nambale and Budalang'i. The highest daily consumption of sorghum was recorded in Budalang'i (36.7%) and least in Teso- South (19.1%). Cassava was most consumed in Nambale (28.8%) and least in Budalang'i (21.4%). Millet was mostly (21.5%) consumed in Teso-South. Fruits intake

was notably second highest after maize. High fruit intake was consistent in all the 3 sub-counties. Most popular fruits listed by respondents and FGD participants were mangoes, guavas and avocadoes. Notably, groundnuts, millet, sorghum, cassava, meat and fish were consumed mostly on a weekly basis compared to maize daily consumption. Sorghum and cassava intake were comparable across all sub-counties. The food that received least ratings for daily consumption was groundnuts as shown in figures 7a, b and c below. Key informants from Teso South attributed scarcity of groundnut to low production because groundnuts were rarely planted in the village due to "*soil that is not suitable for groundnut growth*" while an informant from Budalang'i noted that they got groundnuts from the market "*though they were not affordable thus not largely consumed"*. Millet too was not so frequently consumed because of low production and its high market cost.

> *"Millet is mostly used by household with children to make their porridge and mostly bought because it is planted in small scale" (KI 10 – Village Elder Budalang'i).*

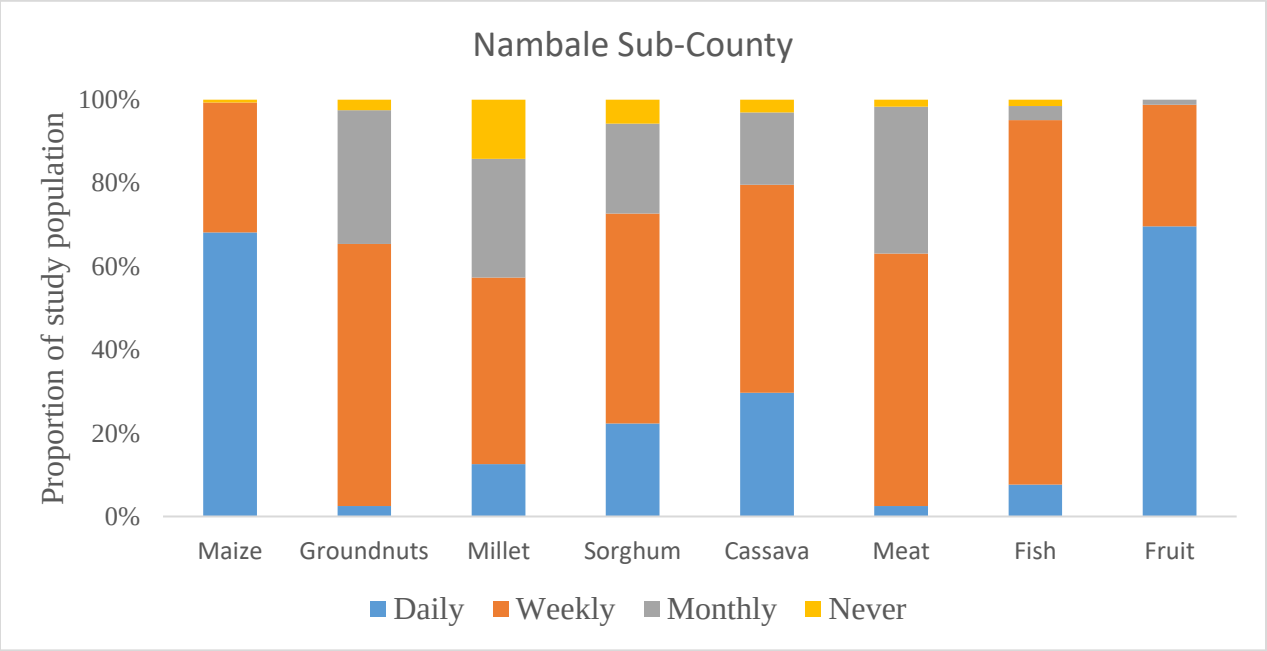
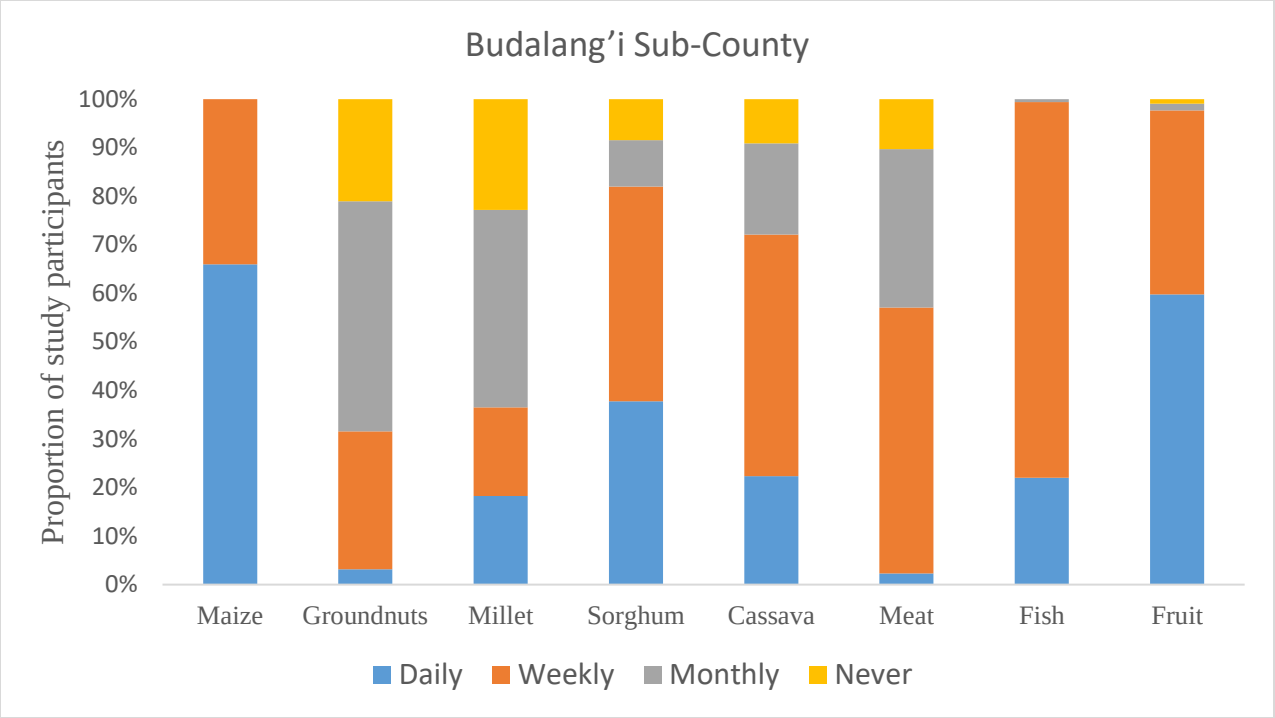
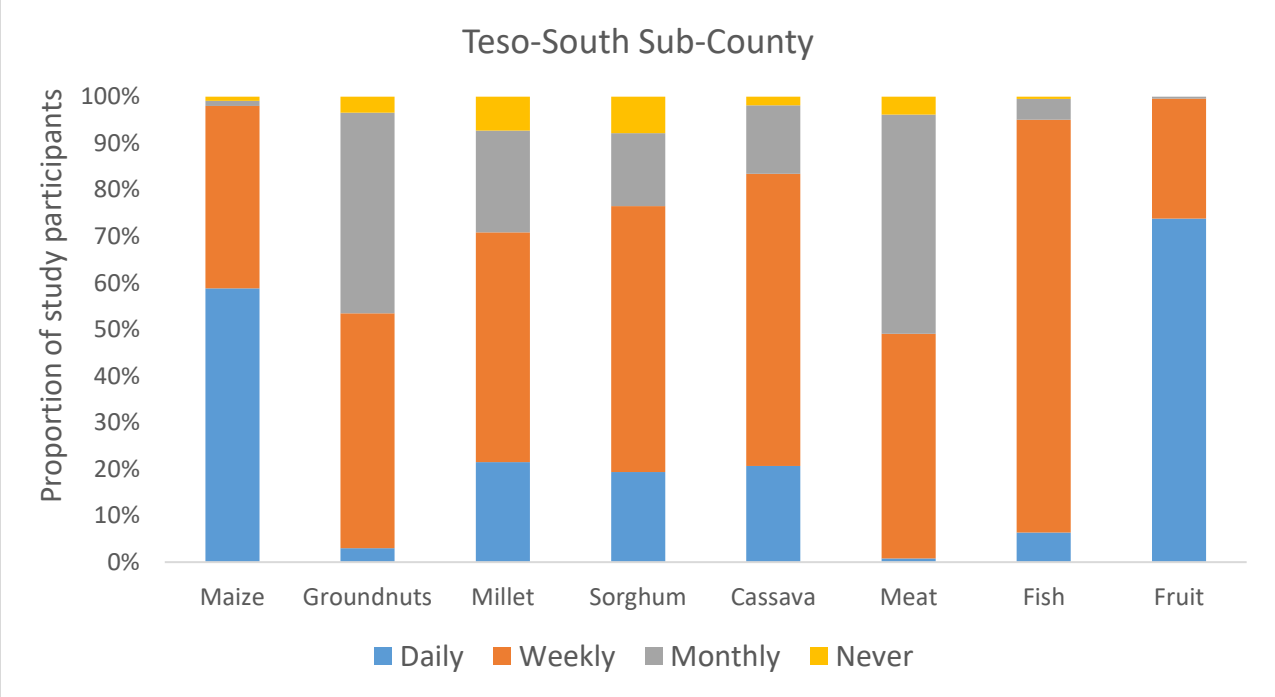


Figure 7a: Frequency of consumption of aflatoxin prone foods and major proteins by household in Nambale Sub-County, Busia County



b 1: Frequency of consumption of aflatoxin prone foods and major proteins by household in Budalang'i Sub-County, Busia County



c 1: Frequency of consumption of aflatoxin prone foods and major proteins by household in Teso-South Sub-County, Busia County

5.3 Food availability

Food availability as one of the food security metrics presumes sufficient quantities of food of appropriate nature and quality supplied through domestic production or imports including food aid (FAO, 2006). Household food stocks of maize, millet, sorghum, groundnuts and cassava at the time of data collection were determined. As shown in figure 8 below, almost three quarters (73%) of households in all the 3 sub-counties had maize. While over three quarters (81.5%) of respondents in Budalang'i had maize, respondents of Nambale recorded the least possession (66.9%) of this food item.

The average quantity of maize per household was 21.6kgs with a range of 1 - 360kgs. Less than a quarter of the households had millet (16.6%) and groundnuts (7.7%) in all three sub-counties. The highest quantities of millet and groundnuts owned by households were 180kgs and 80kgs respectively. These millet and groundnut quantities were both in Teso-South sub-counties. Although most households had at least one of the targeted foods, the quantities were not much. Maize, which was most common among the households, recorded the highest quantities (range 1-360kgs, median 6 kgs) while groundnuts recorded the least (range 1-80kgs, median 2.5kgs) at the time of the study as shown in table 10.

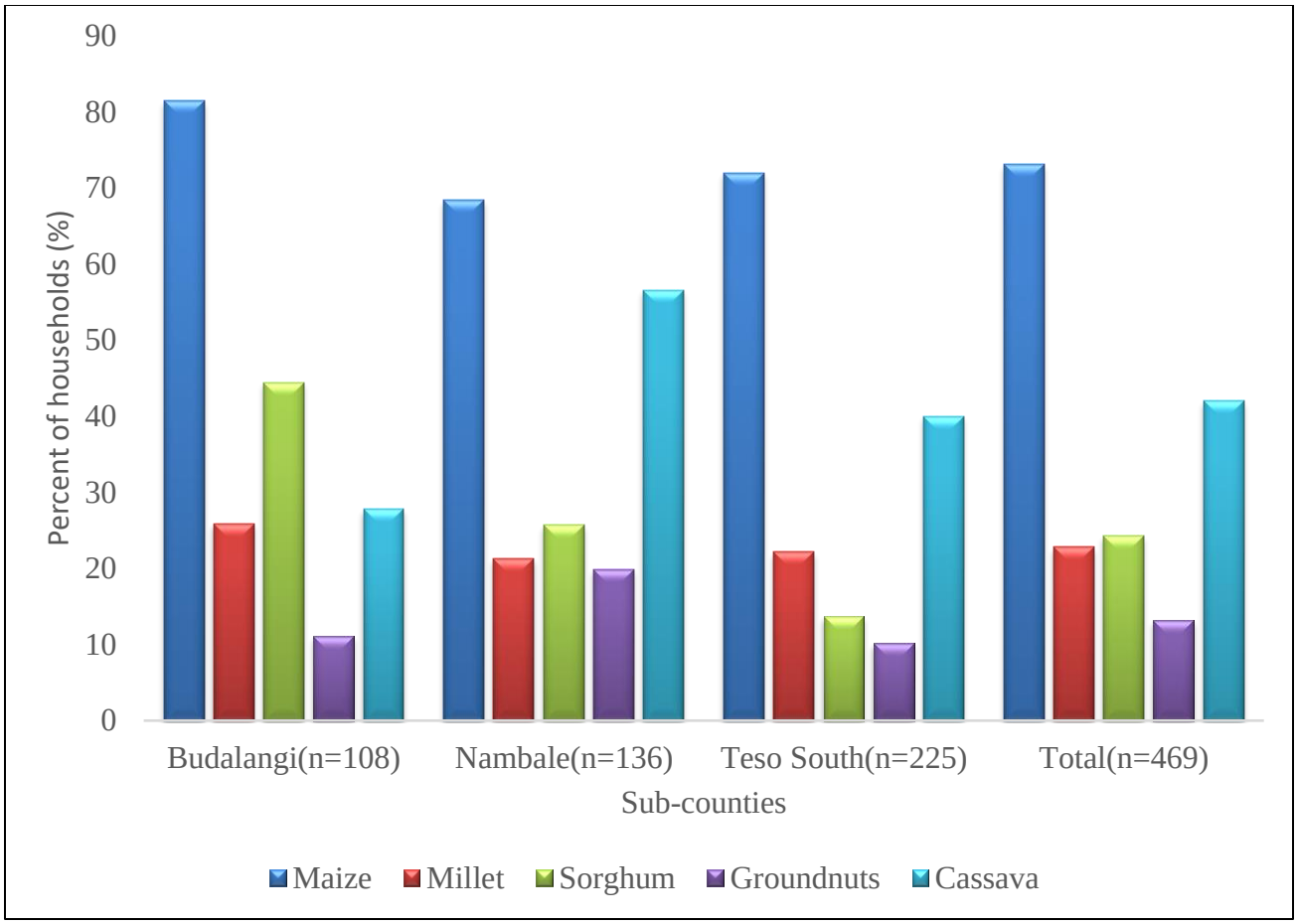


Figure 8: Proportion of households with aflatoxin prone foods by sub - county

Table 10: Quantities of targeted foods in study households

Food Item		Budalang'i Quantity (Kgs)	Nambale Quantity (Kgs)	Teso South Quantity (Kgs)	Total Quantity (Kgs)
Maize	N*(%)	88 (81.5)	91(66.9)	162(72)	341(72.7)
	Mean (SD)	22.5(52.1)	28.2(51.5)	17.5(31.1)	21.6(43.3)
	Median	4.5	10	6	6
	Range (min,max)	(1,360)	(1,360)	(1,240)	(1,360)
Millet	N*(%)	18(16.7)	14(10.3)	46(20.4)	78(16.6)
	Mean (SD)	8.8(13.1)	4.7(6.5)	24.1(40.1)	17.1(32.5)
	Median	3	2	8	4
	Range (min,max)	(1,40)	(1,20)	(1,180)	(1,180)
Sorghum	N*(%)	42(38.9)	24(17.6)	30(13.3)	96(20.5)
	Mean (SD)	24.8(62.6)	12.3(31.8)	13.2(32.7)	22.4(48.8)
	Median	8	4.5	4	5.5
	Range (min,max)	(91,270)	(1,160)	(1,180)	(1,270)
Groundnuts	N*(%)	1(0.9)	15(11.0)	20(8.9)	36(7.7)
	Mean (SD)	1	3.2(2.8)	14.0(20.9)	9.2(16.5)
	Median	1	2	4.5	2.5
	Range (min,max)	(1,1)	(1,10)	(1,80)	(1,80)
Cassava	N*(%)	20(18.5)	71(56.6)	89(39.5)	180(38.4)
	Mean (SD)	6.6(9.2)	18.9(21.3)	18.5(22.2)	17.3(21.0)
	Median	4	12	10	10
	Range (min,max)	(1,40)	(1,120)	(1,120)	(1,120)

***Number of households**

Food security has been defined as a state when all members of a household at all times have both physical and economic access to sufficient food to meet their dietary needs for a productive life (USAID, 1992). Food security is however not equitable to nutrition security defined as the intake of a wide range of foods which provide the essential needed nutrients (FAO, 2012). Respondents' opinion on food security varied; there are those who felt Busia was food secure and those who thought otherwise. Those who perceived the county as food secure attributed it to the practice of reliable small-scale farming. However, one key informant has a contrary opinion as he said *"…we*

are not 100% food secure but we cannot be compared to other counties. I would say may be 80% we are food secure…" (KI 4).

This sentiment was reinforced by some FGD participants who reported periods of food shortage in Budalang'i.

> *"Often when it floods in this area (Budalang'i sub-county), our foods are swept away in the shamba…" (P7,* 18 ≤ 34*).*

In Teso South and Nambale, food shortage was occasioned by adverse weather conditions it was noted that *"There are times when we have long spells of drought, we experience some shortages of some types of foods, so it does affect us" (KI 2).*

Teso-South was perceived as more food secure when compared to Nambale and Budalang'i sub-counties. This was attributed to their fertile soils and diverse food products. Household economic capability was reported by FGD participants as being one of determinants of food availability.

> *"…you find that, economic status of the household is also very determinant. I might not be able to cultivate my food but I have the capacity to purchase" (KI 4).*

The respondents shared that sometimes during droughts, they had to purchase foods, mainly maize and groundnuts, from other regions like Uganda without having the knowledge on how the food was handled or prepared. The trend was the same across the 3 sub-counties. This however predisposed them to dealing with middlemen and unscrupulous traders as indicated by a village elder from Nambale sub-county

> *"Sometimes conmen take advantage of people while they try to source for this food from across the border in Uganda especially when middlemen are engaged in these*

transactions. There are also added taxes while purchasing these foods from Uganda." (KI Village Elder Nambale).

5.2 Determination of prevalence of Aflatoxin in food samples

A total of 493 food samples which comprised of maize (230, 47%), cassava (99, 20%), millet (43, 9%), sorghum (41, 8%), groundnut (32, 6%), and composite samples (a blend of one or more of the foods (48, 10%) were collected.

All samples had detectable levels of aflatoxin. The levels of contamination ranged from 1.0 to 1584ppb in maize, 0.3 to 740ppb in sorghum, 0.5 to 15ppb in cassava, 0.5 to 12 ppb in millet and 0.1 to 2.8 in groundnuts. The proportion of maize contaminated with aflatoxin >10ppb from Budalang'i, Nambale and Teso South was 3%, 22% and 37% respectively. Median aflatoxin levels in maize from Nambale was 231.7ppb (n=59), Budalang'i 3.5ppb (n=63) and Teso-South 228.5ppb (n=108) (Table 11).

Table 11: Distribution of aflatoxin levels in food samples from study households by food type and sub county, Busia 2018

Site	Food type	% >10ppb	Median (ppb)	Range (ppb)
Budalang'i	Cassava (n= 7)	0	0.5	0.5 – 7.5
	Groundnuts (n=0)	-	-	-
	Maize (n= 63)	2.9	3.5	1.0 – 1584
	Millet (n= 10)	1	1.5	0.5 – 12
	Sorghum (n= 23)	1.7	2.0	0.5 – 740
Nambale	Cassava (n= 47)	2.1	2.6	1.0 – 15
	Groundnuts (n= 25)	0	0.8	0.1 – 2.8
	Maize (n= 59)	22	231.7	3.0 – 1456
	Millet (n= 7)	0	0.6	1.0 – 2.0
	Sorghum (n= 4)	0	1.3	2 – 3.5
Teso-South	Cassava (n= 45)	0	0.6	1.0 – 3.5
	Groundnuts (n= 7)	0	0.7	0.5 – 2.1
	Maize (n= 108)	37	228.5	3.5 – 1432
	Millet (n= 26)	0	0.6	1.5 – 2.5
	Sorghum (n= 14)	0	1.2	2.0 – 5.5

Of the 41 sorghum samples collected, only 4 (10%) had levels of aflatoxin >10ppb. All 4 samples were from Budalang'i. Notably, only one millet (n=43) and one cassava (n=99) sample had aflatoxin contamination above 10ppb, while none of the groundnut samples (n=32) had aflatoxin contamination above 10ppb in all the three sub-counties.

All 48 composite/blended samples had detectable levels of aflatoxin. However, only 3 blends: Maize and cassava blend (67%, n=3); cassava and sorghum blend (11%, n=13); and maize and sorghum blend (33%, n= 3) had contamination levels above 10ppb. All the blended samples with levels >10ppb were Nambale and Teso-South sub-counties (Table 12).

Table 12: Distribution of aflatoxin levels in composite/blended food samples, Busia 2018

Sub-county	**Composite/blended sample**	**% samples >10ppb**	**Median**	**Range**
Budalang'i	cassava and sorghum flour (n=2)	0	2.0	2.0 – 2.0 [a]
	maize and sorghum flour (n=7)	0	3.5	1.0 – 8.0
	maize and cassava flour (n=0)	-	--	--
	maize, sorghum and cassava flour (n=2)	0	5.5	2.0 – 9.0
	sorghum and millet flour (n=0)	-	--	--
	cassava and millet flour (n=1)	0	1.5	1.5 – 1.5
	maize and millet flour (n=1)	0	1.0	1.0 – 1.0
	maize, millet and cassava mixed flour (n=2)	0	1.5	1.5 - 1.5
Nambale	cassava and sorghum flour (n=4)	0	0.5	1.8 – 2.5
	maize and sorghum flour (n=0)	0	--	-
	maize and cassava flour (n=3)	67	317.3	330 – 644.0
	maize, sorghum and cassava flour (n=2)	100	1030.3	743 – 1472.0
	sorghum and millet flour (n=0)	-	--	-
	cassava and millet flour (n=0)	-	--	-
	maize and millet flour (n=1)	0	3.0	3.0 – 3.0
	maize, millet and cassava mixed flour (n=0)	-	--	
Teso-South	cassava and sorghum flour (n=13)	11	60.5	3.5 – 195.0
	maize and sorghum flour (n=3)	33	98.6	1.5 – 172.0
	maize and cassava flour (n=2)	0	0.4	1.8 – 2.0
	maize, sorghum and cassava flour (n=0)	-	--	-
	sorghum and millet flour (n=4)	0	0.9	1.8 – 2.5
	cassava and millet flour (n=1)	0	0.5	0.5 - 0.5
	maize and millet flour (n=0)	-	--	-
	maize, millet and cassava mixed flour (n=0)	-	--	-

[a]Lower limit of detection is 0.1ppb

Food is considered safe, if it is free of contaminants and does not cause harm to the consumer. While most participants reported knowledge of grain spoilage which they described as grains which were discolored or molded and pest infested, they demonstrated limited knowledge of aflatoxin. One of the key informants, a Ministry of Agriculture officer, attributed the lack of knowledge of aflatoxin by community members to the lack of sensitization through campaigns by the ministry and also not having suffered from the aflatoxin scourge.

> *"… you know the fact that we have not done campaigns and may be farmers have not been able to see the effects of aflatoxin. Since it might be chronic because you know when it is acute, people will tend to be more cautious on how to live so they act on this…"* (*KI 4).*

5.2.1 Hot spot analysis

Figures 9a, b, c and d represent results of the hotspot analysis. Red spots on our maps indicate statistically significant hot spot clusters with high aflatoxin contamination values ($p<0.05$) while the grey spots indicate clusters that were not statistically significant ($p>0.05$). The blue spots represent cold spots clusters that are statistically significant (low levels of contamination). Hotspots were observed only in 2 of the 3 sub-counties; Teso- South (Okiludu and Amukura sub-locations) and Nambale (Buyofu sub-location). Most of these hotspots were in Okiludu sub-location, in villages closer to the border with Otimong' sub-location of Teso South.

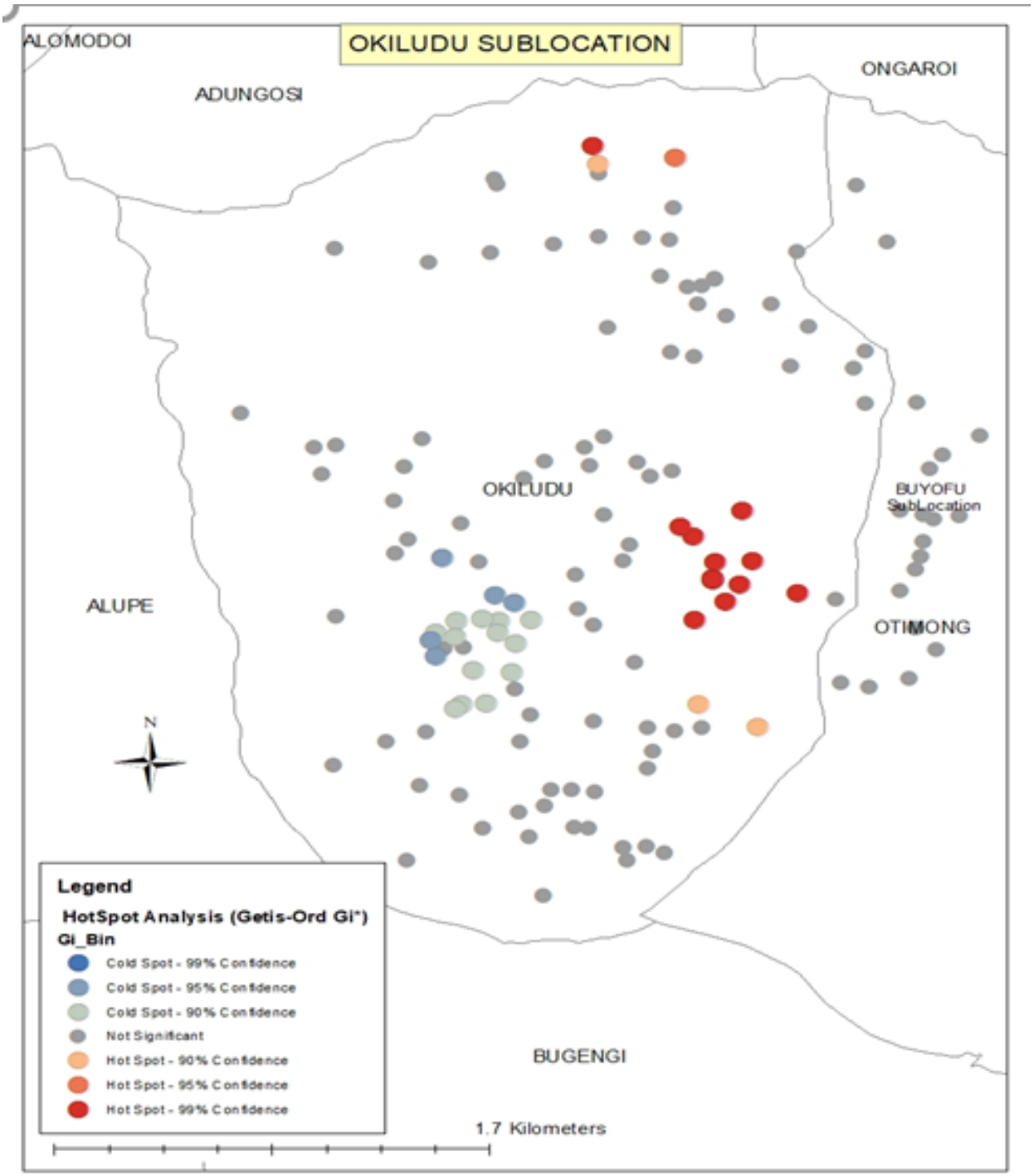
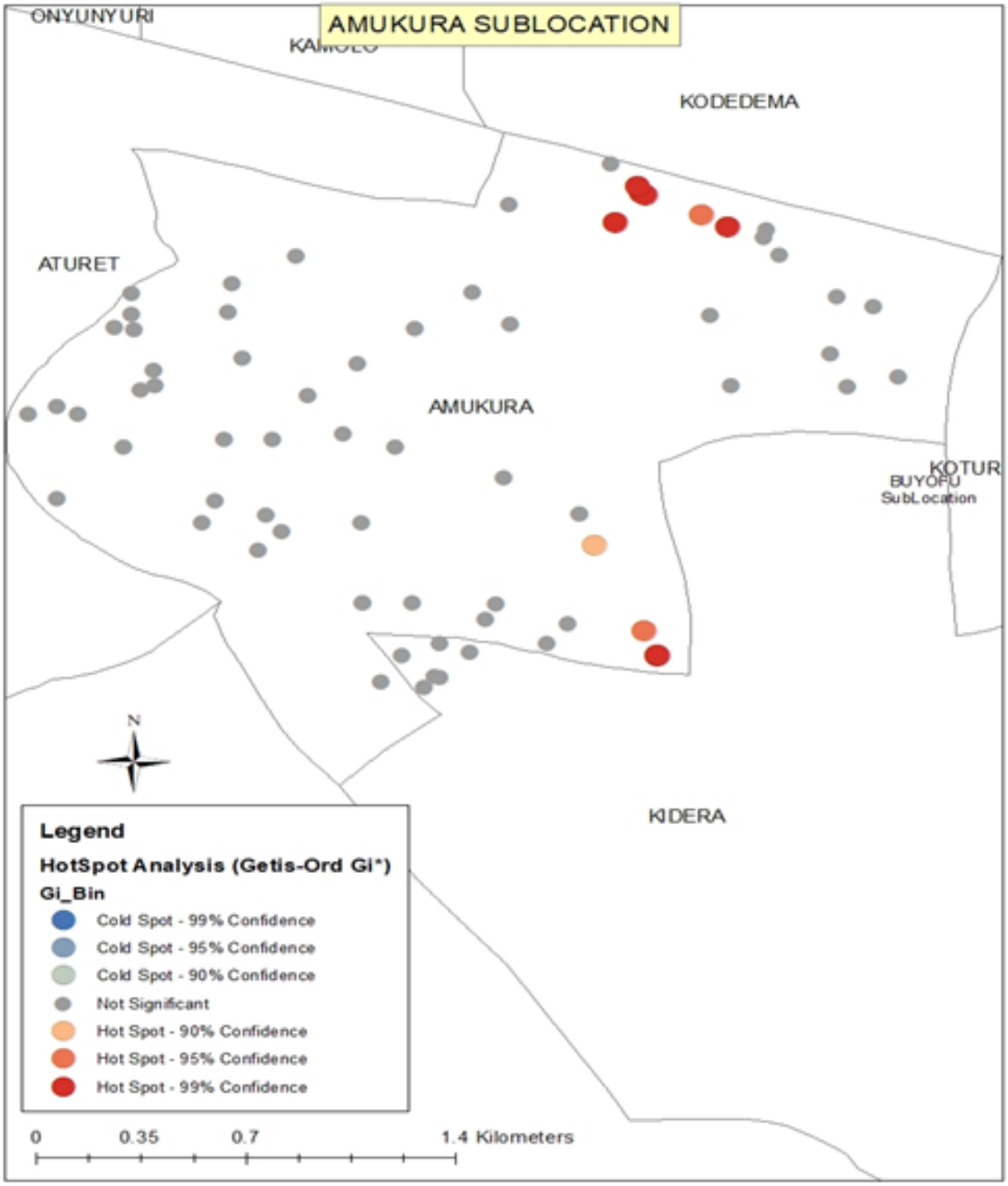
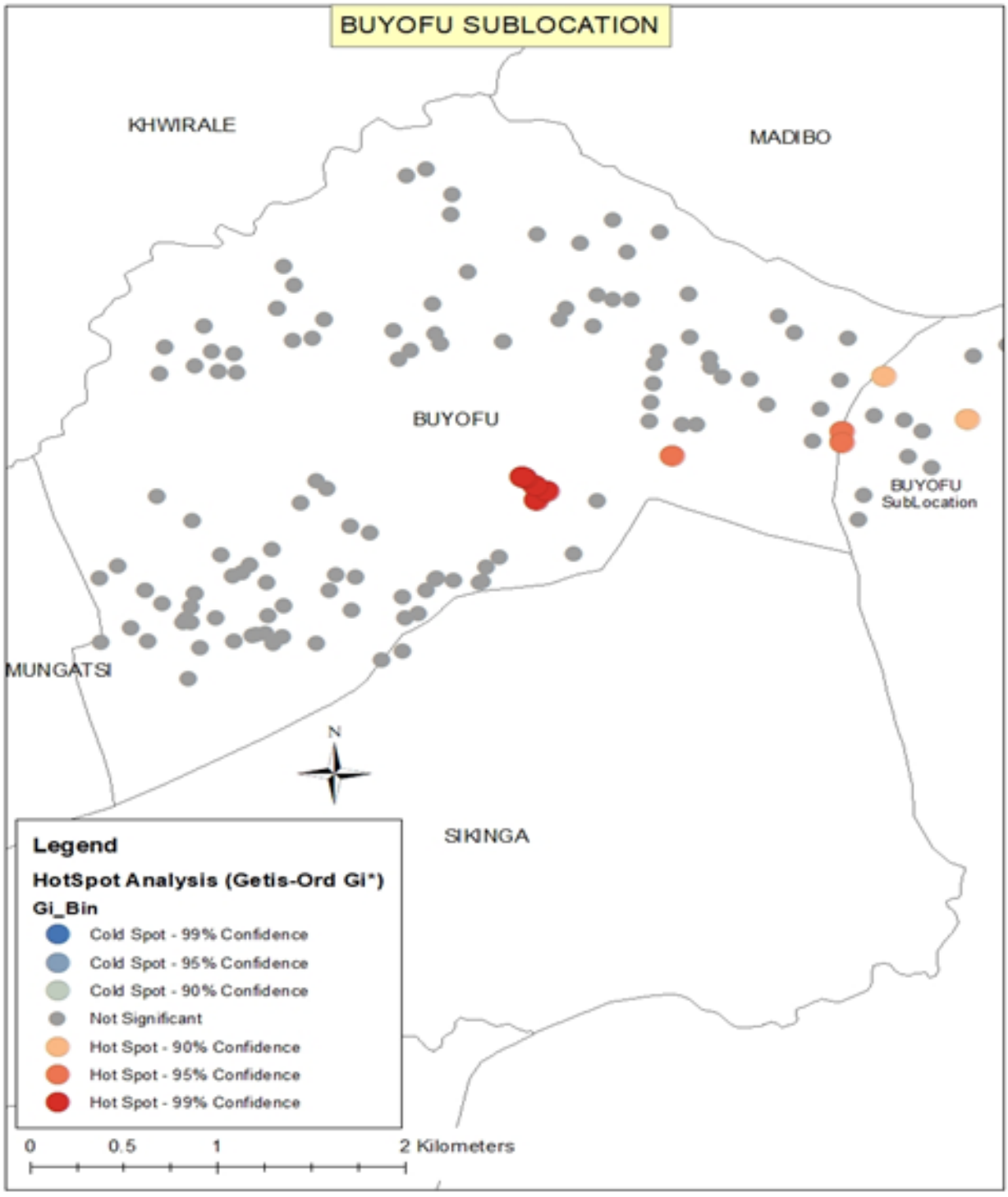


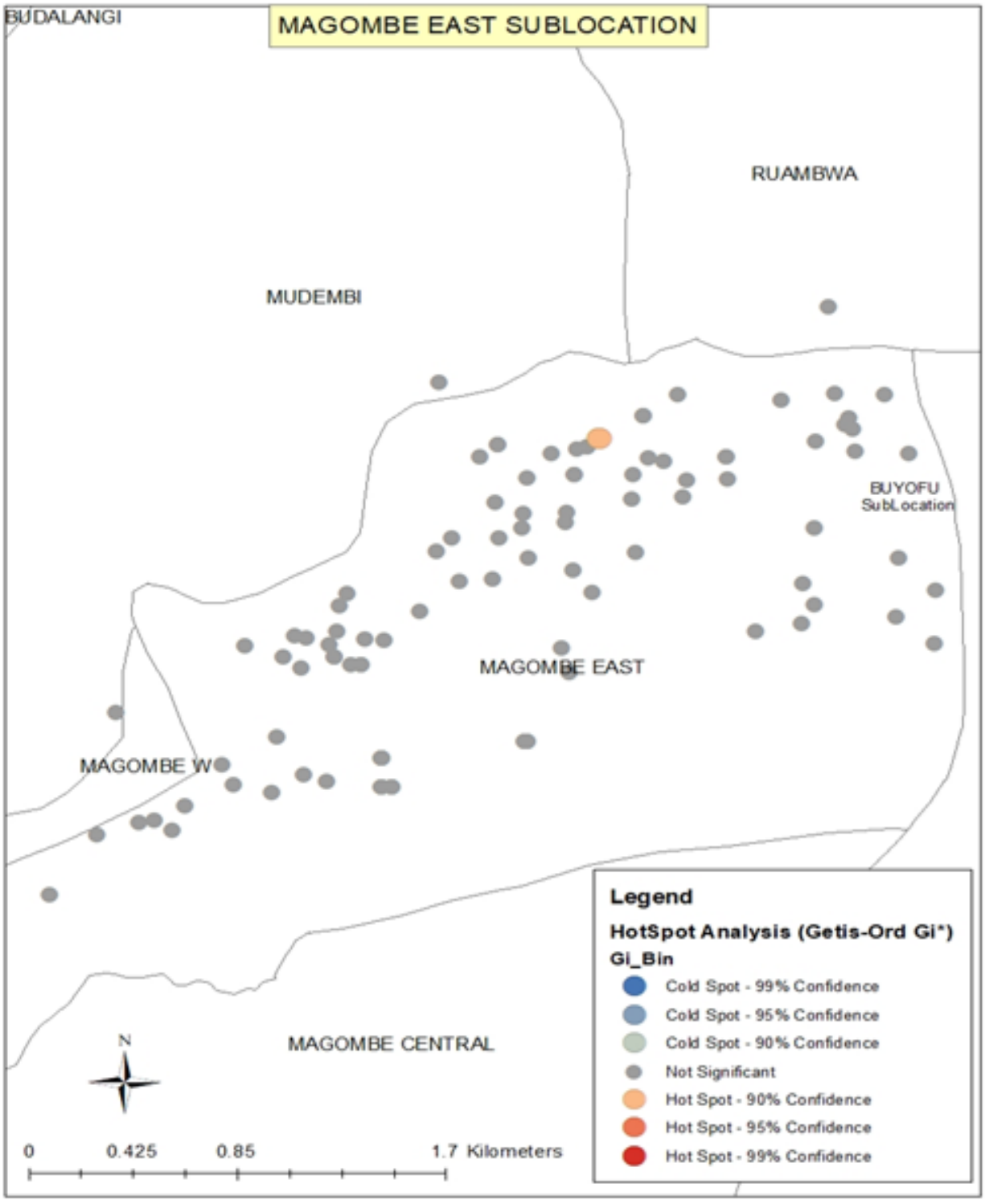
Figure 9a: Aflatoxin hotspots Okiludu sub-location of Teso-South Sub-County, Busia County in June 2018



b 2: Aflatoxin hotspots Amukura sub-location of Teso-South Sub-County, Busia County in June 2018



c 2: Aflatoxin hotspots Buyofu sub-location of Nambale Sub-County, Busia County in June 2018



d 1: Aflatoxin hotspots Magombe East Sub-location of Budalang'i Sub-County, Busia County in June 2018

5.3 Description of factors associated with aflatoxin contamination

When assessing food source and storage factors associated with aflatoxin contamination, analysis was limited to (i) samples collected from households whose respondents provided information on food source and storage practices and (ii) samples that had a proportion with aflatoxin levels above 10ppb. Cassava, millet and groundnut samples were excluded from this analysis because they did not meet the inclusion criteria for this analysis.

Source and storage

While many members in the community practice subsistence farming, their stocks are normally not enough to last through to the next harvest. There was consensus among respondents that there are usually periods of food shortage, which are due to poor weather conditions and natural calamities like floods as is in the case of Budalang'i sub-county.

> *"There are times when we have long spells of drought, we experience some shortages of some types of foods, so it does affect us" (KI 2).*

> *"Often when it floods in this area (Budalang'i sub-county), our foods are swept away in the shamba,…" (P7,* 18 ≤ 34 *).*

To meet the short fall, grains are purchased from the local markets like Bumwayo, Busagwa, Nyadorera, Port, and Vihayo. Most of the grains at the local markets are imports from the neighboring county of Uasin Gishu or neighboring country of Uganda- across the border.

> *"… you will harvest after three months; it will then take you for another one month. So, when it is finished, you will be forced to get from those who sell them. They bring them to the market from Kitale." (P3, 18 ≤ 34).*

Of the 203 maize samples collected, 138 (68%) were bought from the local markets, 59 (29%) were grown by the household and 6 (3%) had be acquired as gifted by relatives. Home-grown samples had the least percent of samples (27.35%) with aflatoxin levels >10ppb. Home-grown source had 1.185 times less likely (OR1.185, 0.554, 2.534) to have higher levels of aflatoxin (>10ppb) compared to market sourced though there was no statistical significant association between the source of maize and the level of aflatoxin. Samples from "Other" sources had the most percent of samples with aflatoxin of more than 10ppb. Contamination at the market was attributed to poor storage practices by the middlemen and retailers.

> *"At the retailers and middlemen, here contamination mainly occurs during storage. Food is not stored in the right temperature conditions. It is sometimes stored on the floor and in humid conditions causing Aflatoxin contamination when in the hands of afore mentioned players"* (KI 5)

Most popularly used storage vessel for both maize and sorghum was the nylon sacks (71% and 89% respectively). Maize stored in a nylon sacks had 35.73% of samples with more than 10ppb while maize stored in a bucket had 25.65%. The odds of maize stored in nylon sacks was seemingly higher than that stored in buckets though not significant (p=0.2398). The odds of aflatoxin contamination in sorghum stored in buckets was about 13 times higher than he odds of that stored in nylon and this was significant (p= 0.0096) (Table 13).

Table 13: Association between the risk of aflatoxin contamination, source and storage Busia 2018

Food type	Source	≤10ppb n (%)	>10ppb n (%)	OR (95% CI)	*P* – Value
Maize	Home-grown (n=59)	45 (72.65)	14(27.35)	Ref	.0760
	Market (n=138)	99 (69.15)	39 (30.85)	1.185(0.554, 2.534)	
	Other* (n=6) *	1(5.95)	5(94.05)	41.977 (1.708, >999)	
Sorghum	Home-grown (n=14)	13 (94.83)	1(5.17)	1.129(0.550, 2.316)	.6821
	Market (n=13)	12 (94.2)	1(5.80)		
	Storage				
Maize	Nylon sack (n=137)	96 (64.27)	41(35.73)	1.611(0.642, 4.042)	
	In bucket (n=55)	39 (74.35)	16(25.65)	Ref	.2398
Sorghum	In nylon sack (n=25)	24(97.06)	1(2.94)	Ref	.0096
	In Bucket (n=3)	2 (72.03)	1 (27.97)	12.815(2.566 63.992)	

*These were described as either having been received from a relative or neighbor

The parameter estimates from the multiple logistic regression model and associated Odds Ratios are presented in table 14.

Table 14: Estimates from multiple logistic regression model relating to the levels of aflatoxin with descriptive variables of food type, source, storage method and demographic characteristics of study participant

Variable	Category	Total	>10ppb n (%)	≤10ppb n(%)	adjusted odds ratio (95% CI)	*p-value*
Food type	Maize	230	71 (30.87)	159 (69.13)	6.462(0.924−45.222)	0.0568
	Sorghum	41	4 (9.76)	37 (90.24)		
Source	Home-grown	118	17 (14.41)	101 (85.59)	1.345(0.664−2.723)	0.3298
	Market source	224	8 (25.8)	22 (70.8)		
Storage	In nylon sack	264	48 (18.18)	216 (81.82)	0.973(0.456 − 2.073)	0.1103
	In bucket	73	17 (23.29)	56 (76.71)		
Respondents' age (yrs)	20-39	188	42 (22.34)	146 (77.66)	2.130(0.588− 7.719)	0.5267
	40- 59	178	21 (11.8)	157 (88.2)		
	60+	111	21 (18.92)	90 (81.08)	1.743(0.469−6.476)	0.8756
Education	Pre-primary	296	56 (18.92)	240 (81.08)	1.021(0.221− 4.711)	0.7948
	Primary	127	20 (15.75)	107 (84.24)		

Aflatoxin contamination in maize was seemingly higher than in sorghum though not statistically significant (p=0.0568).

5.3.1 Household Food sources

The food source system in the study area is diverse. Knowledge of a household's food source could help inform decisions to maximize quality and nutritional value. Respondents were asked about the sources of their tubers and cereal based food. Main food sources reported were either home-grown or the local market. While many community members practiced subsistence farming, their stocks are normally not enough to last through to the next harvest. Of all respondents (n= 341) who had maize, over half (64%) bought the grains from the local market. The same was noted across the 3 sub-counties. However, Budalang'i recorded the highest percentage (70.4%) of market

procured maize. FGD participants and Key informants from Budalang'i corroborated this finding, stating that their crop had been washed away by floods and as such resorted to purchasing their grains from local markets like Bumwayo, Busagwa, Nyadorera, Port, and Vihayo. Most of the grains at the local markets were reported to be imports from the neighboring county of Uasin Gishu or neighboring country of Uganda- across the border.

> *"... you will harvest after three months; it will then take you for another one month. So, when it is finished, you will be forced to get from those who sell them. They bring them to the market from Kitale." (P3, 18 ≤ 34).*

Another, source of household grains as reported by some FGD participants was the government and the Kenya Red Cross during calamities such as floods, in Budalang'i, as was reported by some participants.

> *"...so sometimes the red - cross brings us relief food…. But they normally bring us supplies such as beans, rice, but we do not know where they get them from. They provide them door to door" (P7,* 18 ≤ 34*).*

Notably, over half of all millet (62.8%), groundnuts (88.9%), sorghum (63.5%) and cassava (76.7%) available was homegrown as shown in figures 10 a, b, c, d and e below.

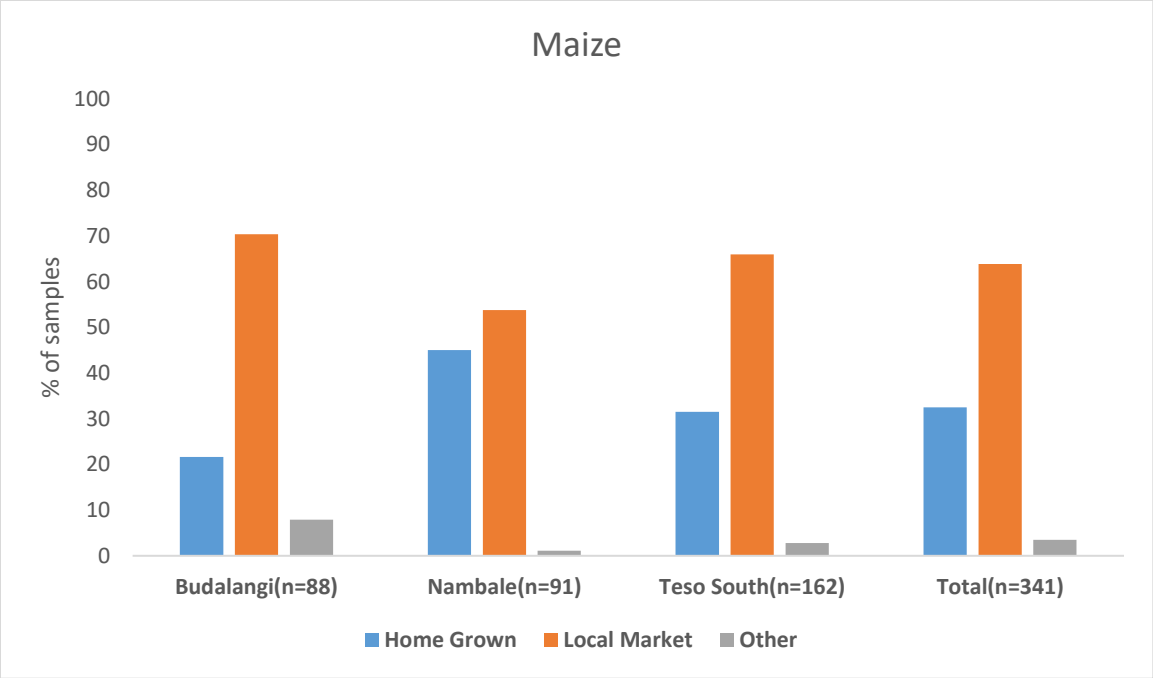
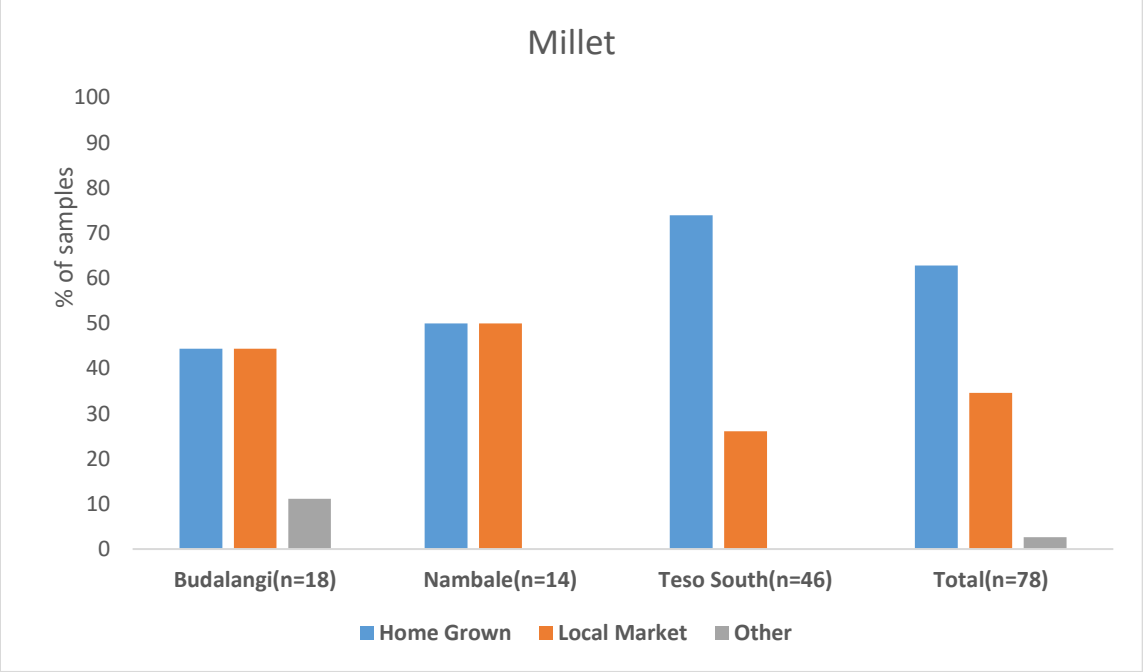
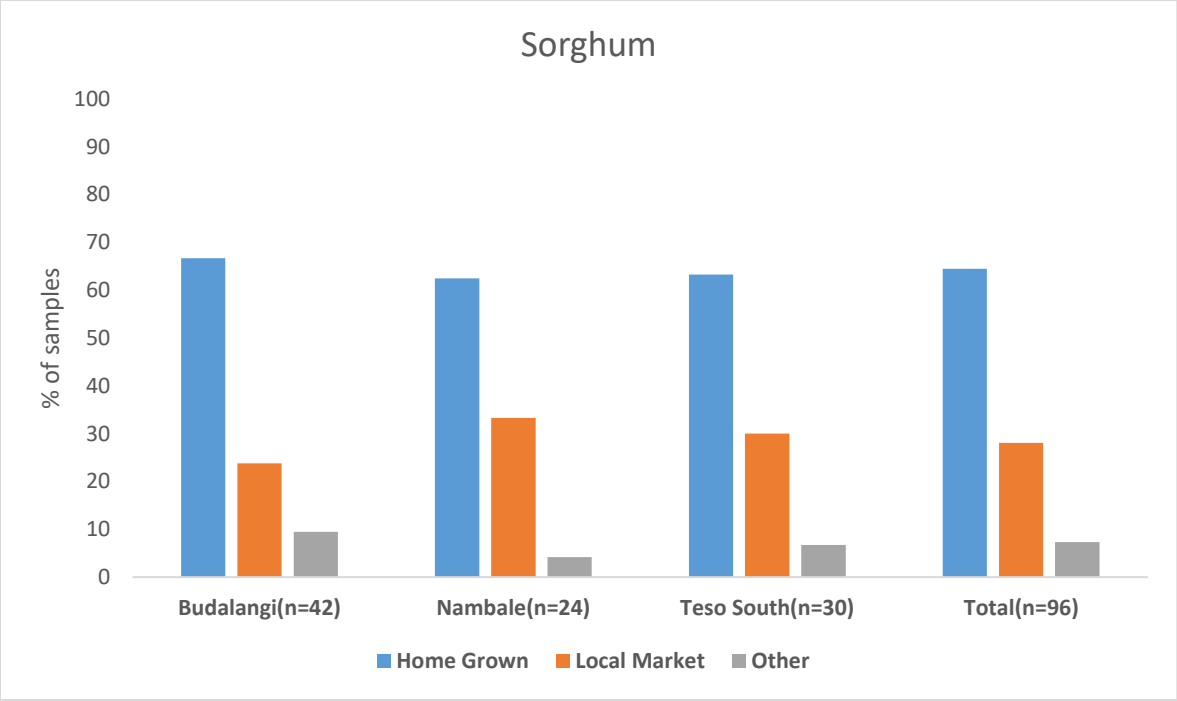


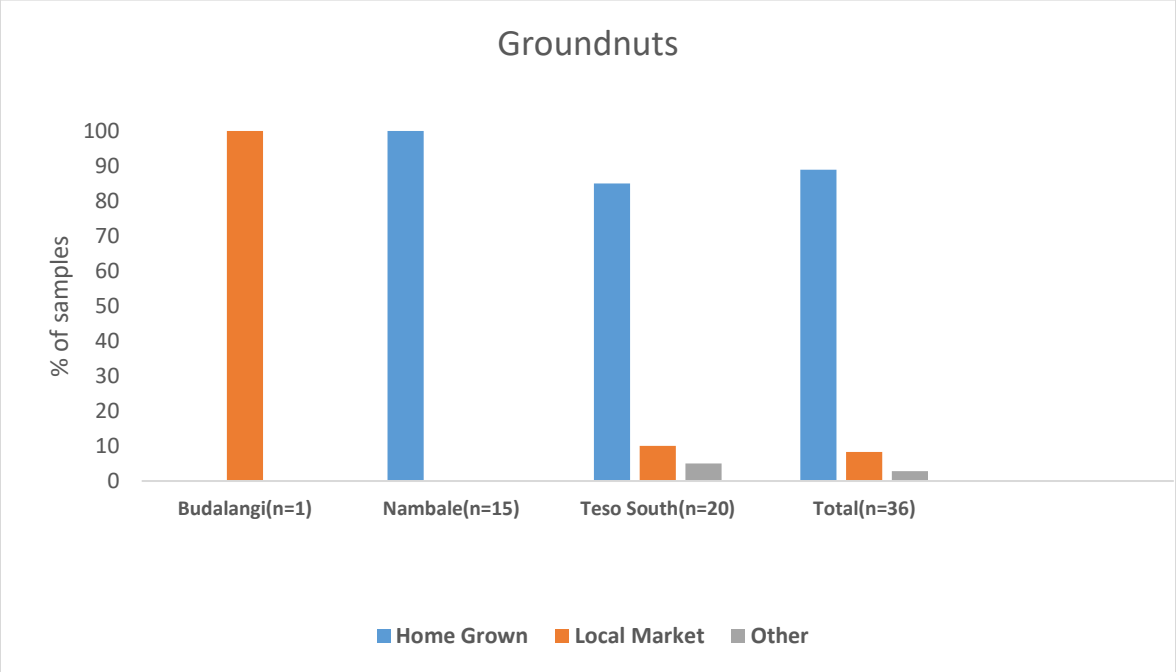
Figure 10a: Household maize by source in June, 2018



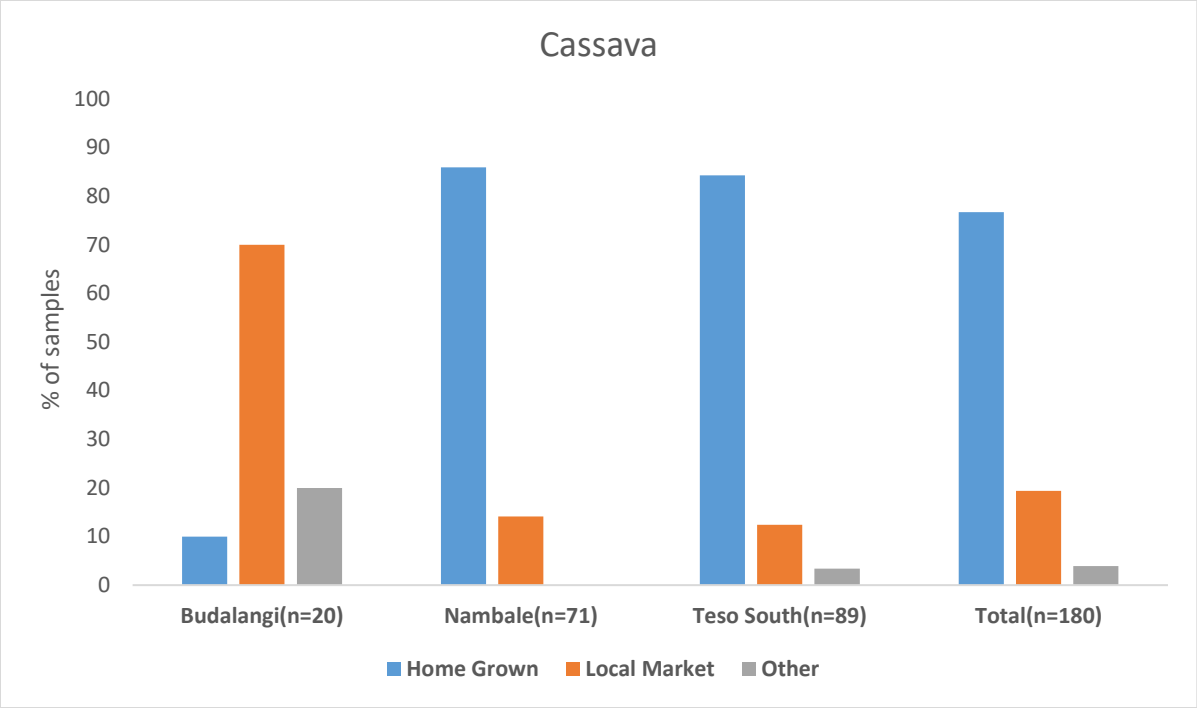
b 3: Household millet by source and in June, 2018



c 3: Household sorghum by source and in June, 2018



d 2: Household groundnuts by source and in June, 2018



e 1: Household cassava by source and in June, 2018

5.3.2 Household food drying practices

One risk factor that predisposes grains to fungi invasion is high moisture content. Proper drying is a necessary precautionary measure in the control of aflatoxin in grains and is also a requirement for grains meant for long-term storage as it helps reduce quality and quantity loses. When grains are harvested, they contain high levels of moisture which must be reduced to safe levels. Sun drying, use of mechanical driers and use of solar driers are the most common field drying methods. The various drying systems available farmers vary from thin layer drying in the sun to mechanized systems. Traditional methods include drying in the field before harvesting, drying in shallow layers and exposing to sun and wind on a surface that prevents moisture or drying in a structure that has open sides to allow air flow (Figure 11).

Only respondents with homegrown grains and cassava were asked how they dried the food items. Of the respondents who had home-grown maize, millet, sorghum, groundnuts and cassava,

majority (89.2%, 79.6%, 78.7%, 78.1% and 92.8%) respectively reported to have sun -dried them but not directly on the ground thus preventing moisture and contaminants like aflatoxin as shown in table 15. These findings were corroborated by FGD participants who reported drying their unthreshed sorghum and maize cobs in direct sunlight on either bare ground or on sacks spread on the ground. A key informant also noted that some community members dried grains along highways on tarmac roads and on rocks.

> "*... the first thing I realized when I came here, for grains, if you go along the road, if you are travelling, you will find they are just dried on the tarmac (highway) yes they are aired, so that they dry. ... (appears uneasy to express her views"(KI 3).*

Table 15: Proportion of respondents practicing different food drying practices by sub-county.

Food type	Drying practice	Budalang'I n (%)	Nambale n (%)	Teso-South n (%)	Total N (%)
Maize	Sun dry directly on ground	6 (31.6)	0 (0)	4 (7.8)	**10 (9.0)**
	Sun dry not directly on ground	13 (68.4)	41(100)	45 (88.2)	**99 (89.2)**
	Other	0 (0)	0 (0)	2(3.9)	**2 (1.8)**
Millet	Sun dry directly on ground	1(12.5)	0 (0)	7 (20.6)	**8 (16.3)**
	Sun dry not directly on ground	7 (87.5)	7(100)	25 (73.5)	**39 (79.6)**
	Other	0 (0)	0 (0)	2 (5.9)	**2 (4.1)**
Sorghum	Sun dry directly on ground	9 (32.1)	1(7.1)	2 (10.5)	**12 (19.7)**
	Sun dry not directly on ground	19 (67.9)	12 (85.7)	17(89.5)	**48 (78.7)**
	Other	0 (0)	1 (7.1)	0 (0)	**1 (1.6)**
Groundnuts	Sun dry directly on ground	-	3 (20.0)	3 (17.7)	**6 (18.8)**
	Sun dry not directly on ground	-	12 (80)	13 (76.5)	**25 (78.1)**
	Other	-	0 (0)	1(5.9)	**1(3.1)**
Cassava	Sun dry directly on ground	1(50)	0 (0)	7(9.3)	**8 (5.8)**
	Sun dry not directly on ground	1(50)	61(100)	66 (88.0)	**128 (92.8)**
	Other	0 (0)	0 (0)	2 (2.7)	**2 (1.5)**

Figure 11: Some of the drying practices observed in Busia County in June, 2018

FGD participants from Nambale sub-county described differences of maize drying practices over time. Previously, maize was left to dry on stocks while in the field and only brought to the homestead for storage. However, this practice has changed significantly since maize is harvested before completely drying and dried in the household. Respondents reported increased cases of crop theft in the fields. Additionally, use of ash was reported as a common drying practice in the past as noted by the FGD participants.

> *"In the past, when our parents harvested maize,… they would take cow dung and beans' pods and burn to ash, (muherekha), then mix with maize before keeping in sacks, it was not easy for such maize to get spoilt. (P11, 18 ≤ 34)*

Almost all respondents (97%) with groundnuts had homegrown groundnuts at the time of the study. All were from Teso South and Nambale sub-counties. These respondents indicated to have

sun-dried the groundnuts. FGD participants reported that groundnuts were dried while in pods and stored in pods. Most participants who had market sourced groundnuts were from Budalang'i. It is likely that market purchased groundnuts was not subjected to further drying since they were presumed dry at purchase.

Of 180 respondents who had cassava, 160 reported to have homegrown cassava. Through the FGDs, it was noted that after harvest, cassava was peeled then kept in order to form mold before scrapping off the mold, crushing to smaller pieces then drying.

5.3.4 Household food storage practices

Post-harvest practices encompass shelling, drying, preservation techniques and grain storage methods. Proper storage protects grains from pest infestation, mold proliferation and guarantees long-term storage and prevents losses which would otherwise expose farmers to vulnerability. In an ideal storage facility, relative humidity, that is, percentage of water vapor in the air between grains, should not exceed 65 %, temperature should not be between 15˚C - 34˚C as molds develop between 15˚C - 30˚C and most storage insects thrive between 25˚C - 34˚C while the moisture content of the dry grain should range from 6 to 15 percent depending on the grain (Taruvinga, Mejia, & Alvarez, 2014).

Participants were asked about the current and previous post-harvest practices with a special focus on grains. Almost all participants reported that food preservation and storage practices varied by food type.

> *"Every plant has its uniqueness, for instance when you harvest maize, you will shell them, dry them and prepare them for storage. But when its sorghum, you will harvest them and prepare for storage but you will not mix them with maize. You will put it aside. So, the one*

that has a lot of pressure is maize, harvesting, shelling and drying. But for millet, after harvesting, you will only remove the chaffs then you keep" (P9, 18 ≤ 34*).*

"Nowadays they put them in sacks, after harvesting maize, you shell them, then you dry in the sun, when it has dried you place it in the sacks in the house, and you can air it weekly. (G6, P3).

Very few participants reported usage of granaries for storage of maize (2%), millet (6 %) and sorghum (3%). Notably, no respondents reported storing cassava and groundnuts in granaries or in any outside storage facility (Table 16). Residents attributed this low usage of granaries to insecurity and expenses of putting up granaries.

"I store in the house. If you keep outside, it is stolen…" (P8, *35 and above*)

"Some people have granaries, but I saw my father having a house for keeping the maize, it is normally roofed halfway so that the air can circulate freely. Even the maize was kept there, … (P9, 35 and above*)*(Figure 12).

However, some older participants still believe in the importance of granaries and continue to use them.

"I store my millet in the granary. But maize is put in the sacks. Every grain should have its own granary." (P4, 35 and above).

Table 16: Household food storage methods in Busia County

Food	Location of storage	Budalang'i n (%)	Nambale n (%)	Teso South n (%)	Total N (%)
Maize	In an outside granary	1(1.1)	3(3.3)	1(0.6)	**5(1.5)**
	Inside your house	86(97.7)	86(94.5)	161(99.4)	**333(97.7)**
	Other	1(1.1)	2(2.2)	0(0)	**3(0.9)**
Millet	In an outside granary	1(5.6)	0(0)	4(8.7)	**5(6.4)**
	Inside the house	17(94.4)	14(100)	42(91.3)	**73(93.6)**
	Other	0 (0)	0(0)	0(0)	**0(0)**
Sorghum	In an outside granary	2(4.8)	0(0)	1(3.3)	**3(3.1)**
	Inside your house	40(95.2)	24(100)	29(96.7)	**93(96.9)**
	Other	0(0)	0 (0)	0 (0)	0 (0)
Groundnuts	Inside your house	1(100)	15(100)	20(100)	**36(100)**
	In an outside granary	0 (0)	0 (0)	0 (0)	**0 (0)**
	Other	0 (0)	0 (0)	0 (0)	**0 (0)**
Cassava	Inside your house	20(100)	71(100)	89(100)	**180(100)**
	In an outside granary	0 (0)	0 (0)	0 (0)	**0 (0)**
	Other	0 (0)	0 (0)	0 (0)	**0 (0)**

Figure 12: Local granaries (Edula – iteso) and (Esiaki – luhyia Kihayo) observed in Teso-South and Nambale sub-counties, 2018

Before storage, the discussions revealed use of commercially obtained pesticides for pest control in maize and sorghum. Millet on the other hand was just storage in sacks but with no pesticides because community members believe that these grains were not easily attacked by weevils locally known as "*osama*" or "*embungi*" since they had small surface area. Pesticides were also not used on homegrown groundnuts as they were stored while in pods. Pesticides were also not added to cassava. However, ash was reported as a preservative but among residents with "limited financial capability" and "older community members". Grains meant for seed were stored on kitchen ceilings above the fireplace where, the seeds were exposed to smoke and soot. This practice helps with continued drying and safekeeping from pest infestation.

5.4 Evaluation of effect of food preparation methods on aflatoxin levels

Boiling of food with water or with solution with an alkaline mineral and fermenting flour for porridge preparation were the most popular preparation methods reported in the FGDs. Most of the older participants from Teso-South and Budalang'i reported the use of local softening salts.

The salt, locally called '*Balang*' by the iteso and '*Munyu* or *muherekha*' by the luhyia was made from either banana peel ashes or pods from beans.

They expressed that the solution flavored and softened food. This locally made softening salt was compared to an alkaline mineral salt commonly referred to as "*Magadi*" and commercially obtained. *Magadi* was however believed by the older FGD participants to cause ailments like bone pains and cancer.

"Long time ago there used not to be diseases like cancer." (P2, *35 and above).*

Figure 13, 14, 15 and 16 below show the effect of various food treatments on aflatoxin levels. A reduction of the levels of aflatoxin in 3 food samples was observed when they were subjected to the boiling process with plain water (n=2, 55-56%), boiling with softening salts (n=2, 72-91%) and 24 hr fermentation (n=3, 38-55%) and fermentation then boiling (36-68%). There was however an increase of aflatoxin in cassava and sorghum sample after a 24 hour and 48-hour treatment of the cassava and sorghum sample.

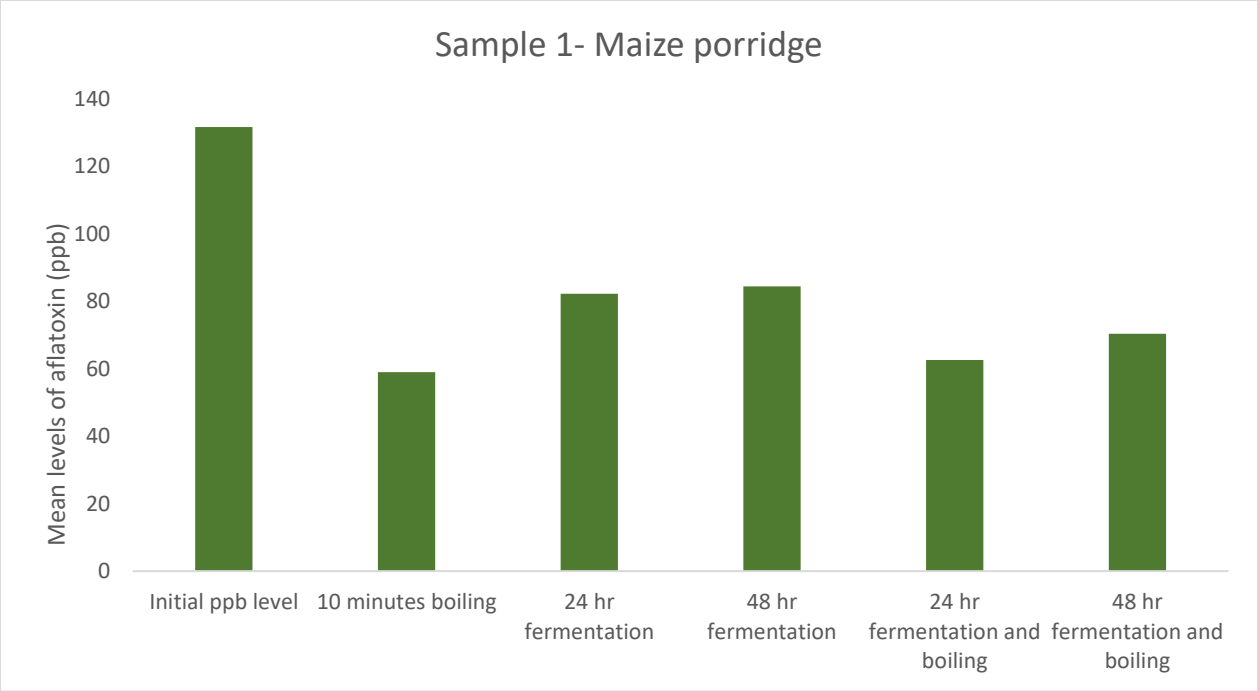


Figure 13: Effect of 4 food preparation methods on level of aflatoxin in maize porridge

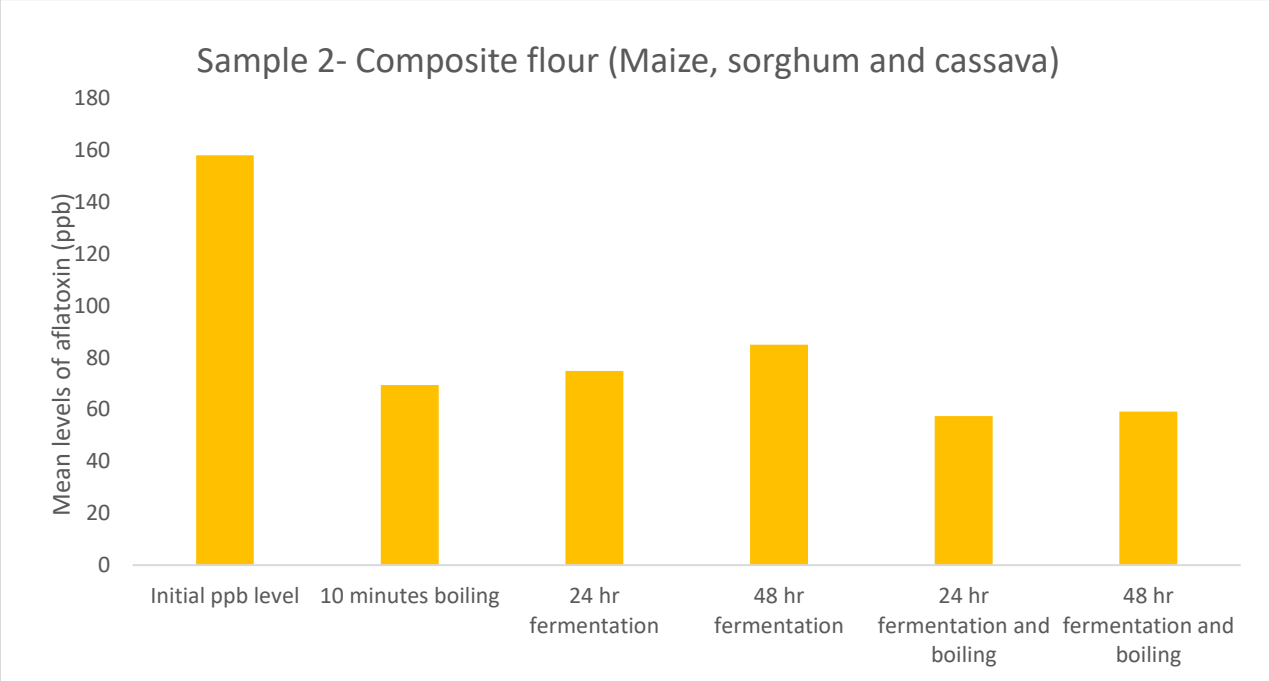


Figure 14: Effect of 4 food preparation methods on level of aflatoxin in maize, sorghum and cassava porridge

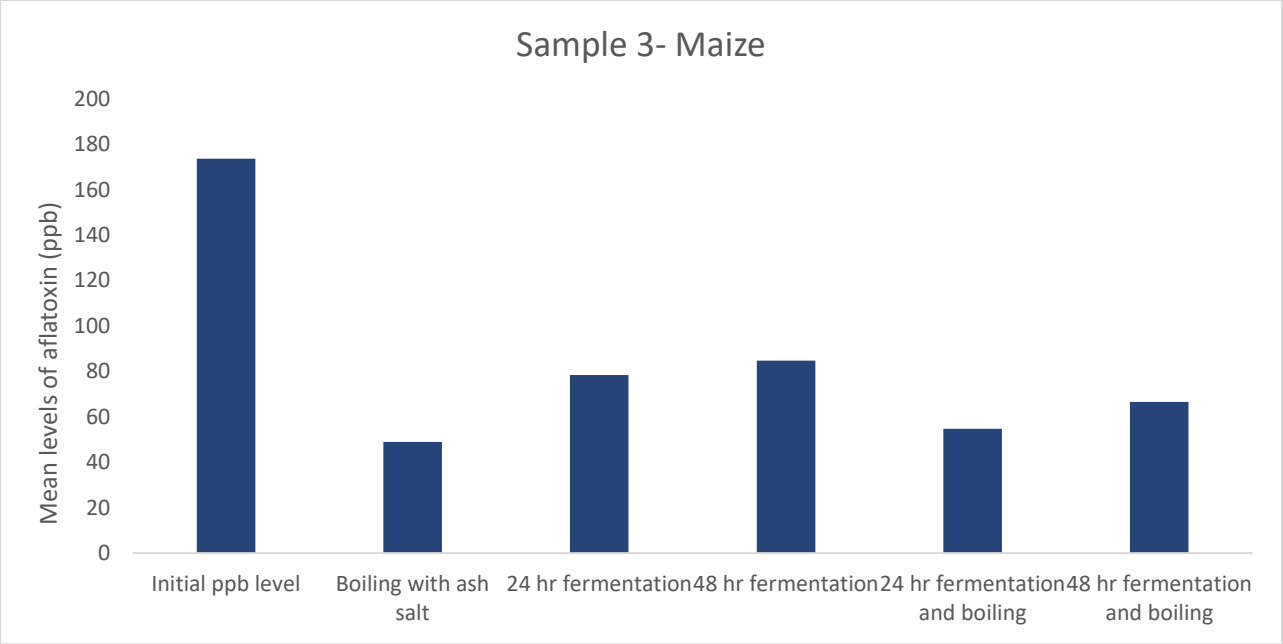


Figure 15: Effect of 4 food preparation methods on level of aflatoxin in maize

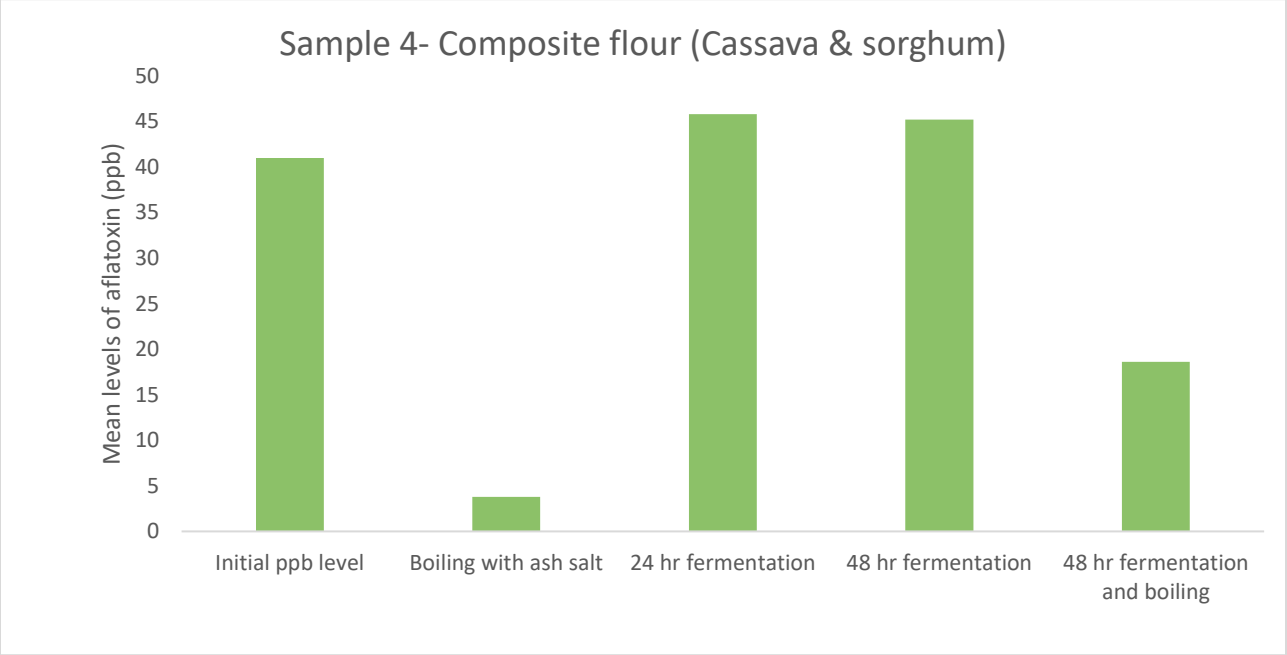
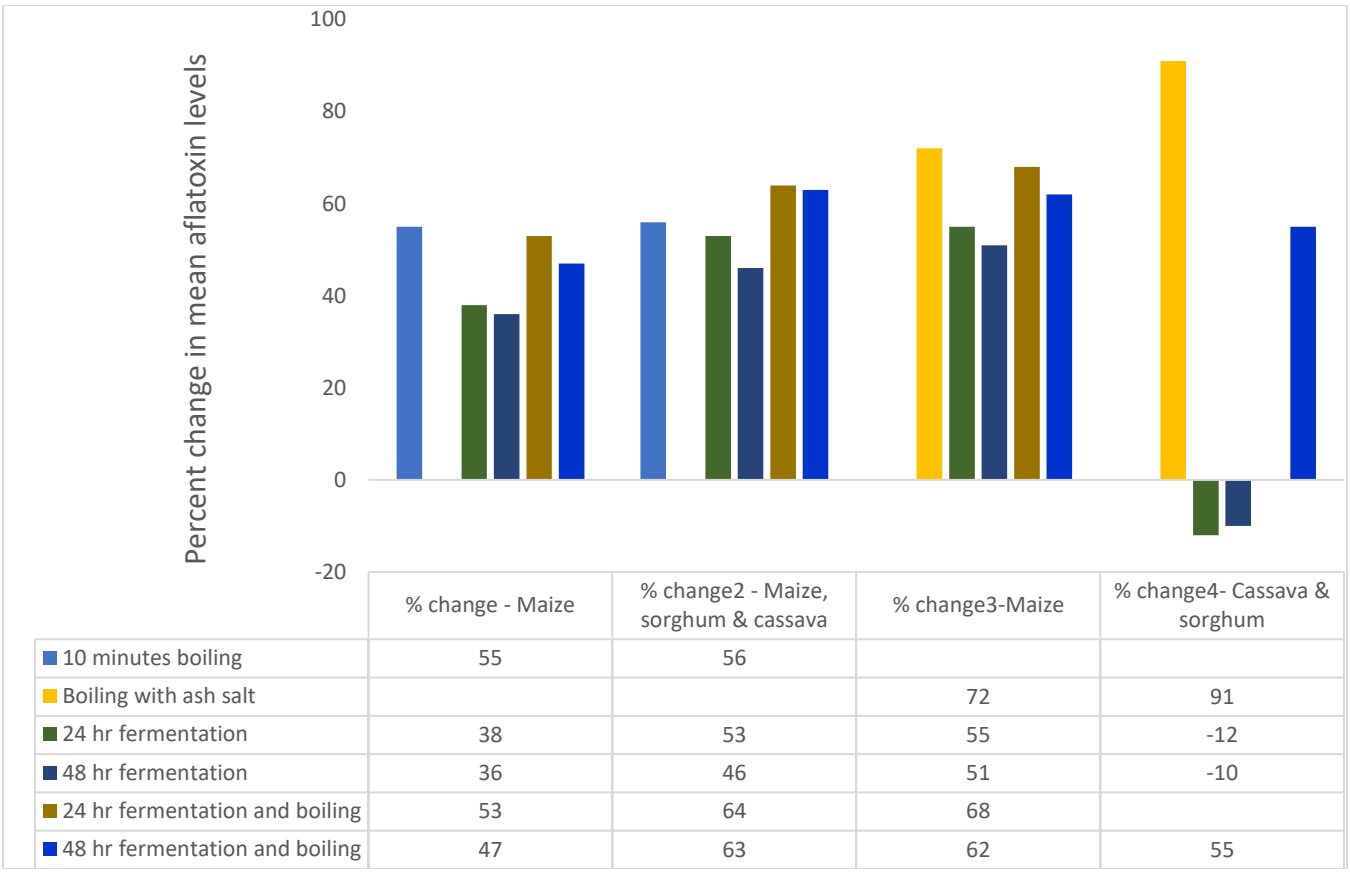


Figure 16: Effect of 4 food preparation on levels of aflatoxin in cassava and sorghum mixture



	% change - Maize	% change2 - Maize, sorghum & cassava	% change3-Maize	% change4- Cassava & sorghum
10 minutes boiling	55	56		
Boiling with ash salt			72	91
24 hr fermentation	38	53	55	-12
48 hr fermentation	36	46	51	-10
24 hr fermentation and boiling	53	64	68	
48 hr fermentation and boiling	47	63	62	55

Figure 17: Percentage effect of food preparation on aflatoxin in three types of food samples

CHAPTER SIX: DISCUSSION

The aflatoxin problem in Kenya is longstanding. Current research efforts addressing the aflatoxin challenges in Kenya have focused on regions that have previously reported aflatoxicosis outbreaks and mainly on maize grain yet the prevalence of aflatoxin in dietary staples in Busia County remains unclear. Determination of prevalence of aflatoxin in the dietary staples and identifying novel post-harvest handling and food preparation practices and methods that are culturally acceptable may inform aflatoxin mitigation efforts and subsequently reduce exposure. This discussion is framed around the four research questions that sought to answer the extent to which foods that are commonly consumed in the study area are contaminated with aflatoxin, consumption patterns of these aflatoxin prone foods, risk factors associated with contamination and feasible aflatoxin decontamination methods adaptable by communities**.** In this study, the prevalence of aflatoxin in maize, sorghum, millet, groundnuts and cassava consumed by the study population was determined, the consumption patterns of these cereals explored and factors associated with aflatoxin contamination of the cereals described. The impact of selected preparation methods on aflatoxin contamination levels in these cereals was also evaluated.

6.1 Consumption patterns of aflatoxin prone foods in Busia County

Understanding the household food consumption is key to assessing consumption patterns of aflatoxin prone foods, which in turn increases the ability to assess effects of aflatoxin on human health. This assessment can be used as a basis for comprehending diet - disease relations and help guide dietary advise to households, nutritionists, health care providers and policy makers on the possible effect of aflatoxin on infants, young children and adult health (Nettleton, Polak, Tracy, Burke, & Jacobs, 2009; Schulze, MartÃnez-GonzÃ¡lez, Fung, Lichtenstein, & Forouhi, 2018).

Maize, sorghum, and millet are the main grains consumed in the study community. All respondents, irrespective of ethnic affiliation reported consuming foods prone to aflatoxin contamination with daily maize consumption being highest among all grains. Groundnuts, millet, sorghum and cassava were consumed mostly on a weekly basis compared to maize which was more on a daily basis. This finding is comparable to a study that was conducted in Tanzania that also reported high consumption of cereals (Ochieng, Afari-Sefa, Philipo, & Dubois, 2017). The frequency of consumption of maize products is also corroborated by a report by ACDI/VOCA that indicated that maize is a staple in Kenya with a daily consumption rate of 258g/person (ACDI/VOCA, 2019) and is consistent with other studies in the region (Nabuuma, Ekesa, & Kennedy, 2018). Frequency of consumption of these aflatoxin prone foods poses a potential health challenge to young children who are more vulnerable to aflatoxin because the high daily intake (Armstrong, Zaleski, Konkel, & Parkerton, 2002). When children are chronically exposed, they may suffer disproportionately from the long-term effects of aflatoxin exposure since they have more time for outcomes such as liver cancer to appear compared to adults (Wild & Jos, 2003). Some studies conducted in Kenya have shown extensive chronic exposure in different populations. Wangia et al (2019) reported aflatoxin exposure in child aged 6-12 years while Yard et al (2013) reported exposure among adults (Wangia, Githanga, Wang, & Anzala, 2019; Yard, et al., 2013). Findings reported from a sero-survey in Uganda by Zitomer et al (2020) also indicated widespread burden of aflatoxin exposure throughout Uganda and the number of cereal based meals consumed per day were statistically significant predictors of aflatoxin exposure (Zitomer, et al., 2020).

"*Ugali*" - stiff porridge made from maize or a mixture of maize, sorghum and or cassava was perceived to be the most important food among this study population. This is possibly because maize is readily available by all ethnic groups because it thrives well in many parts of Busia county

and is also imported from neighboring Uganda and Kitale county, a situation that has led to bumper stocks at the market but also led to local commercial farmers complaining about a drop in maize prices (The East African , 2019).

Food access presumes the accessibility for one to have the necessary food resource for a nourishing diet which is determined by monetary resource and the access rights to produce food. Not surprising, the main factor reported in this study as a determinant of household food access is production capability which is dependent on access to productive land, financial capability for purchase of seed and hire of manpower and seasonality. Indeed, dietary diversity was lowest in Budalang'i Sub County. This could indicate limited crop production among the abantu and nilotes of Budalang'i or limited capacity to access varied foods. Household dietary diversity score was also lowest in Budalang'i. This could be because of the intermittent floods that make the farmlands not usable as was reported by FGD participants and key informants. It is possible that dietary diversity in Budalang'i was also influenced by low levels of education. Indeed, Budalang'i is one of the sub-counties in Busia County with the highest number of residents with no formal education, majority of household respondents in this study had only a pre-primary education (KNBS & SID, 2013). This finding is consistent with a study conducted in Morogoro municipality Tanzania that reported positive outcome on nutritional knowledge among households as being linked to higher levels of education (Pillai, Kinabo, & Krawinkel, 2016). Additionally, Budalang'i had a more elderly population (median age of 51 years) which might have been less energetic to work on the farms for better production.

FGD participants and Key Informants revealed that infants and young children under 2 years of age consumed different diets from those consumed by adults as family diets were either modified or enriched and were also supplemented with porridge and fruit. This observation is similar to

findings reported from studies conducted in Kitui, Vihiga, Isiolo, Marsabit and Turkana counties where porridge, milk, rice, potatoes and ugali formed the culturally core infant and young children food in all the study sites and in Bukoba, in Tanzania infants and young children's diets were also influenced by societal staple diets and agricultural activities (Pelto & Thuita, 2015; Nabuuma, Ekesa, & Kennedy, 2018). In the current study, provision of children's special diets was probably an outcome of the nutritional education provided to young mothers and caregivers at the health facilities to promote the child's healthy growth and development. Indeed, a study implemented in Tanzania whose objective was to quantify short-term effects on intervention integrating dietary diversification, food safety and hygiene on child growth, reported improvement in child diets after caregivers underwent training on the consequences of exposure to aflatoxins and how best to minimize contamination (Seetha, et al., 2020). While it is not feasible to avoid consuming aflatoxin contaminated food, some interventions have been explored.

Chemoprevention and dietary change are some other interventions that have been shown to reduce exposure to aflatoxin. An interventional study conducted in Tanzania among mothers of children aged 6 to 23 months who had started complementary foods, reported a reduction in aflatoxin levels in urine samples by 64% after aflatoxin free diversified food was introduced in the intervention group of the study population (Seetha, et al., 2020). This intervention is however not feasible as households cannot be conditioned to always procure the food from a given source. Also, it would be difficult for households to identify aflatoxin free foods from contaminated foods without testing them.

Awuor et al (2016) reported efficacy of use of Air Classified Calcium Silicate **(**ACCS100), a calcium montmorillonite clay added to human food reduced the bioavailability of aflatoxins by adsorbing the aflatoxins in the gastrointestinal tract among 50 healthy adult volunteers (Awuor, et

al., 2016). The study was a double blind, cross-over clinical trial implemented in Kenya's Makueni County, in a community where most of the people are small-scale, subsistence farmers, with maize as the staple crop. However, although the intervention was effective, acceptable and palatable, the authors suggested further evaluation among vulnerable populations in aflatoxin prone areas. This intervention to would require importation of ACCS100 which is currently not locally available which makes it not feasible. Other clinical trials that have shown promise were conducted in China using oltipraz, an anti-schistosomal drug. In these clinical trials, the investigators observed increased level of glutathione *S*-transferase-mediated conjugation of aflatoxin 8,9 epoxide but also inhibited the enzyme that activates aflatoxin to the reactive epoxide (Kensler, Groopman, Sutter, Curphey, & Roebuck, 1999; Wang, et al., 1999). These drugs have not been evaluated locally and have not been authorized for use in humans exposed to aflatoxins by the Pharmacy and Poisons Board in Kenya.

Chlorophyllins have also been hypothesized as effective agents of binding carcinogens like AFB_1 thus reducing their bioavailability by impeding their absorption. In a 4- month clinical trial conducted in China showed a 55% reduction of aflatoxin in median urinary of aflatoxin N^7 guanine adducts when 100mg of chlorophyllin was consumed at each meal when compared to the placebo (Egner, et al., 2001). However, while results of these clinical trials were found effective, the investigators advised on careful evaluation of use of these agents in humans including on the long-term effect on the enzyme modulation and potential interferences with the uptake of essential nutrients in the diets (Phillips, et al., 2008).

6.2 Prevalence of aflatoxin in cereals and cassava in Busia County

Data on the prevalence of aflatoxins in staple foods are essential to understand their impact on health and on effective mitigation (IARC Working Group Report No. 9, 2015). Region specific

knowledge on prevalence of aflatoxin enables the identification of susceptible edible crops that are responsible for exposure to toxins in specific populations.

This study showed that aflatoxin contamination is prevalent in staples consumed by the Bantu, Nilotes and Nilo-Hamite communities in Busia County. The occurrence of aflatoxin was generally wide spread as all food samples analyzed in this study had detectable levels of aflatoxin. This was not surprising as mycotoxins are natural contaminants which have a wide occurrence in different kinds of matrices (Bosco & Mollea, 2012; Dors, et al., 2011). It is evident that the conditions that influence fungal growth and aflatoxin production such as humidity, fluctuating daily temperatures and daily net evaporation after harvest, exist in the study community (Busia County, 2018). *Aspergillus* has been reported to grow under the combination of 0.995-0.85 a_w and temperature of 15-25◦C and maximum aflatoxin production was observed at 30◦C (Marin, Companys, Sanchis, Ramos, & Magan, 1998). The study site is characterized by an average temperature of 22°C and varying humidity between 40-89% in a day which makes it a suitable location for aflatoxin growth. Studies have also shown many mycotoxigenic fungi grow and produce more mycotoxins under environmental stress (Kim, et al., 2005; Mannaa & Kim, 2017).

The prevalence of aflatoxin observed in this study is consistent with findings from a number of other studies previously conducted in various regions in Kenya (Wagacha & Muthomi, 2008; Nabwire, et al., 2020; Mutiga S. , Hoffman, Harvey, Milgroom, & Nelson, 2015; Kang'ethe E. , et al., 2017; Mutegi C. , 2010; Sirma A. , et al., 2016; Lewis, et al., 2005). Nabwire et al (2020) recently reported prevalence of aflatoxin in maize samples collected from households in both Siaya, Western Kenya and Makueni in Eastern Kenya. Kang'ethe et al (2017) also reported occurrence of aflatoxin in maize and sorghum from a household survey conducted in Eastern and Rift valley regions of Kenya. Similar occurrence was also report by Mutiga et al (2015) in maize

samples collected from hummer mills and farmers' storage sheds in Bungoma, Western Kenya. Mutegi (2010) reported prevalence in groundnuts in Busia and Homabay, Western Kenya. Millet and sorghum are no exception as Sirma et al (2016) reported occurrence of aflatoxin in millet, sorghum and maize samples from four ecological zones in Kenya. Occurrence of aflatoxin has also been reported across the globe in different food items. Occurrence in rice, maize and groundnuts has been observed in China, Egypt, India, Pakistan and United States among other countries (Lutfullah & Hussain, 2012; Lai, Liu, Ruan, Zhang, & Liu, 2015; Chala, Mohammed, Ayalew, & Skinnes, 2013; Robens & Cardwell, 2003). These findings are indicative of how pervasive aflatoxin contamination is both locally and globally.

In this current study, the proportion of maize contaminated with aflatoxin above threshold (10ppb) from Budalang'i, Nambale and Teso - South sub-counties of the current study was 3%, 22% and 37% respectively while 0% of the groundnut samples had levels above the KEBS allowable limit. This was indicative of higher likelihood of risk of exposure to aflatoxin through ingestion of maize among the nilo-hamites of Teso South Sub-County. Aflatoxin contamination in maize was seemingly higher than in sorghum though not statistically significant ($p=0.0568$) possibly due to the small sample size. These findings of aflatoxin contamination are comparable to those reported by Kiarie et al (2016) where 95% of maize samples and 100% of sorghum samples from Nairobi's (Korogocho and Dagoretti) had detectable levels of aflatoxin and 16% and 11% of maize and sorghum samples were above the KEBS threshold (Kiarie, Dominguez-Salas, Kang'ethe, Grace, & Lindahl, 2016).

Similar observations have also been reported in the region. Findings from Burundi and Eastern Democratic Republic of Congo revealed extensive aflatoxin contamination in varied crop samples collected with 60% of them above the European Union allowable limit of 4µ/kg, while Osuret and

team reported contamination in all their groundnut samples which were all above 20 µ/kg (Udomkun, et al., 2018; Osuret, et al., 2016). However, while all groundnut samples in this study had detectable levels of aflatoxin, none had levels above the KEBS allowable limit (10ppb), unlike the Ugandan study conducted by Osuret and team that had 100% of groundnut samples with aflatoxin levels above 20ppb. This could indicate the likelihood of exposure to aflatoxin by communities in Busia County from ingestion of imported groundnuts from neighboring Uganda. In 2018 alone, Kenya imported 6,750 metric tonnes of groundnuts from Uganda (Apedia Agri Xchange, 2019). It is possible that this volume was more than what is documented because of the unregulated cross-border trade. It is highly likely that the unregulated imports ended up in the local Busia Kenya local markets and were consumed locally as was reported during the focus group discussions. The safety of the imported grounds could not be confirmed as they were not subjected to any quality checks by government authorities. Buyers only visually inspect the product before purchase.

This study also noted relative severity of contamination across the various foods commonly consumed within the community. This observation is consistent with findings reported from other studies undertaken in Kenya and in West Africa. Bandyopadhyay et al (2007) reported maize as being more prone to aflatoxin colonization when compared to groundnuts, sorghum and millet in West Africa, while Sirma et al (2016) in their study in Kenya also observed AFB_1 as being more prevalent in maize (76%) compared to sorghum (60%) (Bandyopadhyay, Kumar, & Leslie, 2007; Sirma A. , et al., 2016). However, while this current study tested for total aflatoxin and observed higher prevalence in sorghum compared to millet, the Sirma study that specifically analyzed for AFB_1 found higher prevalence of AFB_1 in millet (64%) compared to sorghum (60%). Additionally, the incidence of aflatoxin in maize samples in this study (100%) was much higher than those

reported in Bungoma (45%), Homa Bay (66%) and Rachuonyo (93%) (Mutiga S. , Hoffman, Harvey, Milgroom, & Nelson, 2015). Prevalence of aflatoxin in the foods also differed by geography. Median aflatoxin levels in maize across sub-counties differed (Nambale was 231.7ppb, Teso-South 228.5ppb and Budalang'i 3.5ppb). This difference could however be because of difference in seasonality or ecological conditions. This finding suggests that consumption of maize posed the greatest public health risk given the daily consumption and resulting bioaccumilation.

In this study, contamination varied widely among the maize samples (range 1.0 -1584ppb). These levels in maize are much higher when compared to another study conducted in 3 sub-counties in Nandi county which reported aflatoxin levels in maize (range 0.17-5.3ppb) (Sirma, et al., 2015). However, the aflatoxin range in the maize samples are still much narrower when compared to levels reported in Eastern Kenya, in some of the regions where aflatoxicosis outbreaks were reported (1ppb to 46,400ppb). Also, 55% of maize from markets in Kitui, Machakos and Thika, had aflatoxin levels greater than 20ppb (Daniel J. H., et al., 2011; Lewis, et al., 2005). The highest level of aflatoxin in millet in the current study was 12ppb against 6.4ppb in Nandi while sorghum was 15-fold higher at 740ppb compared to 48.36ppb in the Sirma study (Sirma A. , et al., 2016). This would suggest that while millet is the source of the least exposure to aflatoxin, maize is the main source of exposure followed by sorghum in Busia but also that residents of Busia are more exposed to higher levels of aflatoxin compared to residents in Laboret, Kilibwoni and Chepkongony sub-locations of Nandi. Indeed, other studies have shown that maize intake is a significant contributor to aflatoxin exposure (Kamala, et al., 2018; Lewis, et al., 2005). Kamala et al (2018) reported results of an aflatoxicosis outbreak in Tanzania that occurred in 2016 in which 20 of the 68 cases identified died. Similarly, Lewis et al (2005) responded to aflatoxicosis

outbreaks in Eastern Kenya in 2004 and 2005 (Centers for Disease Control and Prevention, 2004; Lewis, et al., 2005). In both outbreaks, maize was established as the potential source of exposure.

Surprisingly, while all the groundnut samples had detectable levels of aflatoxin, none were above the Kenya Bureau of Standards (KEBS) (10ppb) and all were within the European Union (EU) regulatory limit of 4 ppb. This finding differs from those reported by Mutegi et al (2009) where 63.7% of groundnuts from Busia had detectable levels of aflatoxin while 7.54%were contaminated based on the KEBS standards and Osuret et al (2016) who reported all their groundnut samples as being above 20 µ/kg (Mutegi, Ngugi, Hendriks, & Jones, 2009; Osuret, et al., 2016). It is possible that the post-harvest management practices of groundnuts have improved in these study sites. It is also possible that production has declined leading to a lower likelihood of post-harvest contamination.

Aflatoxin was detected in all maize, sorghum, millet, cassava and groundnuts samples. These findings are indicative of pervasive contamination of the county's dietary staples during the time of this study. This is probably because of the limited knowledge and awareness of aflatoxins observed among most study participants and across all study sites hence no intentional aflatoxin mitigation measures practiced by residents. These low levels of awareness differ from those reported by Ndwiga and Marechera (2014) in a study they conducted in Eastern Kenya, an area where aflatoxicosis is endemic. In their survey which targeted farmers randomly selected from 4 counties, they reported over 90% of participants having heard about aflatoxins (Ndwiga & Marechera, 2014). However, findings of this study corroborate those reported by by Kang'ethe and Langa and Sabra et al (2012) on the lower levels of knowledge about aflatoxin among women in Kenya and Malaysia (Kang'ethe & Lang'a, 2009; Sabra, et al., 2012). Kang'ethe and Langa compared the levels of knowledge between men and women in Eldoret, Machakos and Nyeri and

found that only 40% of the women participants were knowledgeable while Sabra et al (2012) investigated determinants of adults' knowledge on fungal and aflatoxin contamination of diets. While the referenced Sabra study was a self-administered questionnaire survey with only aflatoxin related statements measured with only a three-point scale, that is, 0 = don't know, 1 = not sure and 2 = know, the Kang'ethe and Langa's study was a survey using questionnaires administered at the household level semi quantitative data collection techniques, the current study applied mixed methods to gather data. The extensive contamination indicates that residents of Busia County irrespective of their ethnicity are chronically exposed to aflatoxin through their dietary staple and are predisposed to effects of chronic aflatoxin exposure. Participants showed limited knowledge on the aflatoxin pathways to human. Community members use the spoilt grains in feeding chicken, making animal feed and local brew. This finding suggests that residents of Busia County might be exposed to aflatoxins either directly through consumption of spoilt grains or indirectly through animal products like eggs and milk. These findings are comparable to those from Nandi and Makueni counties (Kang'the, et al., 2017). Studies in Kenya's Eldoret, Machakos and Nyeri by Kang'ethe & Langa (2009) reported contamination of animal feed and milk while Senerewa and colleagues in their cross-sectional study found high aflatoxin contamination of dairy feed and milk in Kwale, Isolo, Tharaka Nithi, Kisii and Bungoma counties in Kenya (Kang'ethe & Lang'a, 2009; Njugi, Nyang'au, Maribel, & Ahend, 2018; Senerwa, et al., 2016). Kang'ethe & Langa also reported only 68% of the participants did not know how animals got aflatoxin and only 33% had heard of aflatoxin in milk. Alternative uses of contaminated grains predispose humans to further exposure to aflatoxin by ingestion of metabolites M_1 found in milk and eggs (Bahout & El-Abbassy, 2004). Whereas the tolerance levels to aflatoxins varies among various species, the conversion ratio between ingested aflatoxin in the grain AFB_1 and AFM_1 is estimated at 1-3%

(Barbieri, Bergamini, Ori, & Pesca, 1994; Lanza, Washburn, Wyatt, & Marks, 1982). While these findings should be interpreted with caution, this lack of awareness could have contributed to the extensive aflatoxin exposure that was reported in the aflatoxin serology from Busia County in 2007, an indicator of chronic exposure in the region (Yard, et al., 2013).

This could also be a possible explanation of the reported 100% exposure to aflatoxin in a sero-survey among humans from this region of Kenya in 2007 (Yard, et al., 2013). Chronic aflatoxin exposure in humans has been linked to hepatocellular carcinoma (Yan & Wu, 2010; Ross, et al., 1992), impaired immunity (Jiang 2005) and stunted growth among children (Gong Y. , et al., 2002; Khlangwiset, Shephard, & Wu, 2011). Aflatoxin ingestion has also been associated with decreased micronutrient levels in children and can impair child growth (Khlangwiset, Shephard, & Wu, 2011). Aflatoxin exposure has also been found to be significantly correlated with wasting in children under 3 years of age in Kisumu, Kenya (Ohingo, 2010) and to stunting, (Turner, et al., 2007; Gong Y. , et al., 2002). Young infants may be exposed through breastmilk through the excretion in breast milk of aflatoxin metabolite - aflatoxin M_1 (AFM_1) (Lamplugh et al. 1988; Wild et al. 1991; Jonsyn et al. 1995; Tchana et al. 2010). While exclusive breastfeeding is recommended for the first 6 months of a baby's life, in Kenya, only 61% are exclusively breastfed while 15% are breastfed and given complementary foods (KNBS, 2014) which can contain aflatoxins (Ohingo, 2010). However, while AFM_1 is less toxic than AFB_1 the impact of M1 exposure in neonates and young infants is unknown (Gong, et al., Postweaning Exposure to Aflatoxin Results in Impaired Child Growth: A Longitudinal Study in Benin, West Africa, 2004). Aflatoxin B_1 is lipophilic therefore it can have significant effect on faltering in fetal growth (Turner, et al., 2007). The foetus can be exposed to aflatoxin in utero through maternal food intake. While these communities have

not experienced any documented aflatoxicosis outbreak, they are at risk of negative health effects associated with aflatoxin exposure.

6.3 Key risk factors for aflatoxin contamination of specific foods

To determine appropriate preventive measure, and ensure food safety, a clear understanding of risk factors for aflatoxin contamination in local settings is necessary. Potential preventive measures should be identified and considered to guide corrective food safety actions within the community. Risk factors have varied impact on levels of contamination.

Market as a source of maize was established as a risk factor for maize in this study. While this study was a household survey, almost two-thirds (68%) of the maize samples had been sourced from the local markets. Majority (68%) of the maize samples were bought from the local markets, whereas 29% were home- grown and 3% had been gifted by relatives. Market sourced maize had a more likelihood (OR1.185) of having levels of aflatoxin above regulatory limits (10ppb) compared to homegrown maize, however, this association was not statistically significant. These findings are similar to those reported by Mutiga *et al* (2015) who observed significantly less contamination among home grown maize when compared to purchased maize from Nyanza region (Mutiga S. , Hoffman, Harvey, Milgroom, & Nelson, 2015). Though many participants reported to have home grown maize, they admitted that they deplete their stocks before the next harvest which necessitates them to purchase grains from the local market. Residents continue to be exposed to aflatoxin from market sourced maize.

Participants demonstrated limited awareness of grain contamination by aflatoxin but had high awareness levels of causes of grain spoilage. Whereas study participants could visually identify spoilt grains, they were not aware that seemingly clean grains could be colonized by aflatoxin. Participants associated spoilage with discoloration and bitter taste of flour contrary to findings

reported by various investigators who have shown that aflatoxin detection was only possible by using laboratory methods (Wacoo, Wendiro, Vuzi, & Hawumba, 2014). While discoloration of grains would be an indicator of fungal growth, not all fungi produce toxins (Hill, et al., 2007). Contamination of maize in the market possibly resulted from poor post-harvest handling practices at source or exposure to humid conditions in storage or storage in poorly aerated containers and spaces at the stores in the market or prolonged storage periods, aspects that were noted by some study informants. Conditions that affect toxin production have been reported to include fungal strains and the genera of fungi most implicated is *Aspergillus* (Ciegler A. , 1978). They attributed the spoilage to lack of proper drying facilities, storage of grains while damp or storage of warm grains in air- tight containers. These findings are consistent with findings by Hell and Mutegi who also reported that the higher the moisture content in the grains the higher the chances of aflatoxin colonization (Hell & Mutegi, 2011). Hill et al (2007) also demonstrated that the basic requirements for aflatoxin production was optimum temperature of 33℃ and water activity (a_w) of 0.99 (Hill, et al., 2007). This would suggest that most contaminated maize could have been more of a grain management issue at the market. Indeed, high prevalence of aflatoxin in market samples has been reported in Burundi and Eastern Kenya (Udomkun, et al., 2018; Lewis, et al., 2005). In cross sectional survey conducted in Eastern and Central Kenya in 65 markets among 243 maize vendors select markets, Lewis et al found 55% of the maize products with aflatoxin levels greater than the then Kenyan regulatory limit of 20ppb, 35% had levels >100 ppb and 7% with levels >1000 ppb. Similarly, Udomkun et al reported 51% of crop samples from 26 local markets in Burundi and Eastern Democratic Republic of Congo had levels above the European Union maximum tolerable limits of 4 ppb. These findings differ with those reported during the 2004 aflatoxicosis outbreak investigations in Kitui, Eastern Kenya which found homegrown maize as the primary risk factor

for developing aflatoxicosis (Azziz-Baumgartner, et al., 2005). Maize stored in nylon sacks were more prone to contamination with levels more than 10ppb compared to maize stored in open buckets. In an interventional study conducted in Guinea by Turner and colleagues, education on hand sorting of groundnuts, use of natural fiber mats for drying, properly under sun and use of natural fiber bags for storage on wooden pallets in addition to application of insecticides on the floor of the storage facility resulted in a reduction of aflatoxin and ultimately a reduction of exposure to aflatoxin (Turner, et al., 2005). This interventional study also showed that the combine use of these interventions could prevent aflatoxin accumulating even after 5 months of storage. While the referenced study focused on groundnut farmers, the same situation would apply to the management of all the other aflatoxin prone grains. It is therefore likely that the market vendors in the local markets in the current study did not practice all if not some of these practices in this package. These findings would also suggest that there is need to educate the market vendors on the recommended post-harvest handling practices. It is noteworthy that in a cross sectionally study conducted in four villages in Ejura Sekyedumase district of Ghana, aflatoxin exposure was positively impacted by farmers' knowledge of aflatoxin risk (Jolly, et al., 2006). Infact, knowledge of health risks and benefits of health practices has been reported as one of the five core sets of socio- cognitive theory that is a pre-condition for change (Bandura, 2004).

6.4 Current local, effective, and adaptable aflatoxin decontamination methods

While there are varied efficacious methods for aflatoxin decontamination, it is important to identify the most efficacious methods that are currently utilized within the communities, are affordable, accepted and adaptable.

Thermal treatment, fermentation and use of alkaline solutions were investigated. While AFB_1 has been reported to be stable to dry heat up to 260°C (Ciegler & Vesonder, 1983), temperature of

300◦C has been observed to degrade AFB_1 (Fischbach & Campbell, 1965), more so in the presence of moisture that is critical to the enhancement of degradation of AFB_1. Results of this study have shown that boiling maize at a temperature of 100◦C for 10 minutes can reduce levels of total aflatoxin by 55%. Higher decontamination efficacy has been reported from another study that used pressurized cooking method on peanuts and recovered between 90-100% AB_1 (Dhanshetty, Elliott, & Banerjee, 2020). However, in this mentioned study, the investigators added sodium chloride and citric acid. While the efficacy was high in their study, and the investigators purported that addition of citric acid and sodium chloride would not interfere with the organoleptic properties of the food, uncertainty remains on the acceptance of the final product by the community as this was a study conducted in the lab.

Boiling maize in alkaline solution (ash salt) also recorded marked reduction of aflatoxin by 72%. This finding is consistent with that from another study in Kenya in which magadi, an alkaline mineral salt, was used in cooking dehulled maize (locally known as *muthokoi*) and noted aflatoxin reduction of between 22-78% (Mutungi, Lamuka, Arimi, Gathumbi, & Onyango, 2008). In Benin, West Africa preparation methods of mawe, makume, ogi, akassa, and owo, maize-based foods which involved sorting, winnowing, washing and dehulling showed a significant reduction of aflatoxin in maize (Fandohan P. , et al., 2005). Nixtamalization, the traditional alkaline treatment in Latin America, a process in which maize is soaked and cooked in an alkaline solution, has also been reported to reduce levels of aflatoxin B_1 by 94% (Elías-Orozco, 2002; Price & Jorgensen, 2006). However, Price and Jorgensen point out that the tortilla manufacturing process may not be as effective in aflatoxin destruction as initially hypothesized as acidifying process prior to analysis caused reformation of much of the original aflatoxin.

In the current study, a combination of fermentation and boiling had a more improved effect on aflatoxin reduction in maize, sorghum and cassava than fermentation only. Additionally, fermentation of maize fluor for 24hrs resulted in a 38% reduction of aflatoxin but upon further fermentation, to 48 hours, only a 36% reduction was observed. Reduced efficacy of fermentation on aflatoxin reduction has also been reported in other studies (Fandohan P. , Zoumenou, Hounhouigan, Marasas, & Wingfield, 2005; Kpodo, Sorensen, & Jakobsen, 1996). In their study conducted in Accra Ghana under laboratory conditions, Kpodo and colleagues observed and explained persistence of aflatoxin during the fermentation process as a result of reduced pH during fermentation combined with acids produced by organisms in the fermented product, which likely created an acid condition that resulted in a reformation of aflatoxin as opposed to a reduction of the same. This same study also reported 80% aflatoxin reduction of aflatoxin when fermented maize was boiled for a period of 3 hours.

Study participants reported practice of hand sorting as an exercise of physical removal of spoilt grains. The positive effect of this practice has been validated by an experiment that Matumba et al (2015) conducted with the objective of assessing effectiveness of hand sorting, flotation/washing, dehulling and combinations thereof on the decontamination of mycotoxin-contaminated white maize. These investigators reported greatest effect on mycotoxin removal with hand sorting that only left less than 6 percent of aflatoxin B1 compared to flotation (Matumba, Poucke, Ediage, Jacobs, & saeger, 2015). These investigators however recommended hand sorting as a method that should be used as a last option for aflatoxin decontamination as aflatoxin is invisible to the human eye. Another interventional study which aimed to evaluate the effectiveness of how to identify and remove moldy groundnuts to reduce aflatoxin B_1 contamination was conducted in Rural Gambia among women. This study reported a 42.9% reduction of aflatoxin B_1 contamination based on

median aflatoxin levels at baseline (Xu, et al., 2017). Another study conducted in the United States that sort to determine the efficacy of electronic color sorting and subsequent hand picking to remove aflatoxin contaminated kernels from commercial lots of shelled groundnuts, reported greater efficacy of selection of contaminated kernels using careful hand sorting when compared to electronic color sorting (Dickens JW and Whitaker, 1975). Although some contamination may persist after hand sorting, physical removal represents a low-cost acceptable alternative for domestic use. The advantage with sorting is that it does not interfere with the nutritional properties of the grains and does not produce any toxin degradation products.

Participants had limited knowledge on aflatoxin decontamination methods. Participants reported using processes such as additional drying of already contaminated grains, washing contaminated grain then drying or mixing contaminated grains with seemingly non-contaminated grains. Washing contaminated grains or solar drying have been demonstrated not to be efficacious in aflatoxin removal or decontamination because aflatoxins are very slightly soluble in water and melts at very high temperatures (O'Neil M. , Smith, Heckelman, & Budavari, 2001). Participants embraced drying practices however the manner in which this was done varied among participants. Drying grains directly on the ground was reported and observed in the current study. Participants were unaware of the danger they exposed the grain to as aflatoxin has been isolated in the soil.

Strengths and limitations

The main strength of the current study is the use of both quantitative and qualitative data which allowed for triangulation. Qualitative findings helped corroborate and elaborate some of the results of the household survey thus provided a more comprehensive understanding of the issues under investigation. Another strength was the use of an objective measurement for assessing the levels of aflatoxin contamination in food. While aflatoxin contamination in food is heterogeneous in

nature and can result in unreliable estimates of contamination and exposure, a food sampling protocol was used to ensure uniformity of getting homogenous food samples. This study had some limitations. First, dietary diversity data were collected at only one point in the agricultural cycle, thus dietary diversity could not be associated with seasonality. Additionally, this being a cross sectional study, only single point data was collected, and no temporal dimensions were collected thus could not simultaneously assess contamination and outcome. It was therefore not possible to report conclusively a true cause and effect relationship. Second, some food types had very small sample numbers to compute any association with source or storage. This could have been because sampling was done in June, a period of food shortage, just before harvest season. Associational analysis was restricted to food samples like maize that had reasonable numbers. Third, while data gathered from the FGDs and KIIs provided the general overview of the market storage conditions, we were unable to link specific market sourced samples to the various market storage conditions associated with them thus levels of aflatoxin contamination in market sourced grains could not be correlated with storage conditions at the store. Fourth, during the evaluation of the impact of food preparation techniques only few samples were analyzed. However, these samples were prepared and analyzed under controlled laboratory conditions which are replicable. Lastly, while the findings are not generalizable, they are indicators of the general levels of awareness of food safety situation and specifically of aflatoxin prevalence in the county. In addition, there might also be some implicit and unarticulated knowledge, beliefs and practices that are not reflected in these results.

CHAPTER SEVEN: CONCLUSION AND RECOMMENDATIONS

7.1 Study conclusions

Food safety is a necessary condition to attainment of food security. The importance of dietary staples like maize and sorghum in nutrition needs of both young children and adults cannot be overemphasized however their value is threatened by their susceptibility to aflatoxin contamination. Despite their importance in this study community, the prevalence of aflatoxins in these foods had not been documented. This study set to determine the levels of aflatoxin to determine the prevalence of aflatoxin in the main maize, sorghum, millet, groundnuts and cassava consumed in households within Budalang'i, Nambale and Teso - South sub-counties, to determine the consumption patterns of maize, sorghum, millet, groundnuts and cassava in the study households, describe risk factors associated with aflatoxin contamination of the cereals within the study area and to evaluate the impact of selected preparation methods on aflatoxin contamination levels in main cereals consumed in the study area.

Consumption patterns of maize, sorghum, millet, groundnuts and cassava: Findings showed high consumption of foods made from grains with daily maize consumption being highest followed by sorghum. Groundnuts, millet, sorghum and cassava were consumed mostly on a weekly basis compared to daily maize consumption. Based on frequency of consumption of maize and high aflatoxin levels in maize, ingestion of maize poses the greatest public health risk when compared to consumption of groundnuts, millet, sorghum and cassava. Limited knowledge and awareness of the various pathways to human exposure to aflatoxin was also apparent as appropriate disposal methods of aflatoxin contaminated food were not known and contaminated food was being feed to domestic poultry and animals. This indicates that residents of Busia County continue to be at risk of chronic exposure to this carcinogenic toxin. Chronic exposure to aflatoxins is not well

understood but is a known risk factor for liver cancer and is suspected to be a factor in immune dysfunction.

Prevalence of aflatoxin in community diets: It is now evident that aflatoxin is ubiquitous and is prevalent in maize, sorghum, groundnuts, millet and cassava that are consumed in Busia County. While the severity of this contamination varied across the various food types, maize purchased from the local markets was observed to be most susceptible to contamination. Most of the contaminated maize had aflatoxin levels above Kenya Bureau of Standards regulatory limit (10ppb). Qualitative findings revealed limited knowledge and awareness of aflatoxin and aflatoxin pathways to humans among the study participants. There is need for urgent public health intervention. Additionally, consumption of millet in this study area posed the least chances of exposure to high levels of aflatoxin.

Risk factors associated with aflatoxin contamination: Findings revealed limited awareness among community members on the various sources of aflatoxin contamination. Market sourced maize had a higher likelihood of contamination compared to homegrown maize indicating that contamination might have occurred at the market-place. Incidentally, most grains are sold through informal marketing systems which are rarely monitored for aflatoxin by the local regulatory authorities in Busia County. The county continues to experience transitory food insecurity. Additionally, maize stored in nylon sack has higher likelihood of contamination when compared to open buckets. There is need for market-based interventions including government interventions such as regulations, inspections, and disposal mechanisms.

Impact of selected preparation methods on aflatoxin contamination levels: Results of this study showed that aflatoxin reduction in grains is possible using traditional food processing techniques. Boiling maize in alkaline solution (ash salt) showed marked reduction of aflatoxin and

a combination of fermentation and boiling had a positive effect on aflatoxin reduction in maize, sorghum and cassava. This confirms the hypothesis that indeed some local food preparation methods have an impact on the levels of aflatoxin in community foods.

7.2 Recommendations

1. Aflatoxin are an environmental health hazard and food safety challenge in the study community. Aflatoxin is ubiquitous and there is now compelling evidence of the prevalence of aflatoxin in the community diets thus there is need for urgent mitigation of aflatoxin in Busia County. This being a community that practices subsistence farming, consumption of unmonitored food is in no doubt. There is need to reduce these levels to below the Kenya maximum tolerable levels. While it is not feasible to monitor all household foods, it is possible to educate the household members on proper food handling practices that help mitigate aflatoxin contamination. It is therefore imperative to create awareness among farmers and retailers on pre and post-harvest handling practices and causes of aflatoxin occurrence and health implications, with the objective of encouraging voluntary compliance to public health regulations and improved food handling practices. Farmers have to be educated on how to identify visibly damaged and moldy grains, shown how to successfully determine fully dried grains and how to store the grains. Education on proper disposal or alternative use of contaminated grains would also be needed in order to protect residents from being exposed to this carcinogenic toxin. This would therefore require agricultural extension workers to provide the information at both farm and market levels.
2. Market sourced maize had higher levels of aflatoxin. These grains are either surplus stocks from local farmers or imported from other counties and countries. While it would be

desirable to have intercounty collaborative efforts focusing on food safety surveillance it might not be feasible given the various modes of grain acquisition and transport. However, it is possible to regulate formal cross-border trade of these grains. Monitoring and testing of grains coming in from across the border is needed. This is possible with the use of rapid test kits and establishment of a laboratory testing for mycotoxins at or close to the border for expeditious testing and results dissemination to traders. This would also help inform decision makers on what trade related policies and actions to formulate and enforce. Additionally, there is need to create awareness among the market vendors on the recommended post-harvest handling practices by public health officers and agricultural extension officers at the market places or during informal meetings convened by the local government administration also refered to as '*barazas'*.

3. Results of this study showed high frequency of consumption of these aflatoxin prone foods and relative severity of contamination in the various food types. There is increasing body of evidence showing chronic exposure to aflatoxin is associated with negative health effects. While several dietary and chemo prevention interventions have been evaluated. Also, encouraging community members to consume more of millet, sorghum and cassava-based foods and less of maize where feasible. Community education remains the most practical intervention at subsistence or farm level.
4. Findings have further demonstrated the potential risk factors of aflatoxin contamination and exposure. To address the aflatoxin food safety challenge in the County, knowledge on current situation and trends of occurrence in the food chain is vital. This knowledge needs to be continually up-dated thus systematic food sample collection, analysis and interpretation of data and dissemination is advised to inform policy decisions. In line with

this, having food sample testing laboratory within the county is necessary for timely testing and response to any food safety challenges. Additionally, surveillance of populations exposed to aflatoxin levels beyond KEBS acceptable limits is warranted.

5. It is the obligatory function of the local government to assess health needs and to assure and maintain appropriate requisite personal, educational and environmental health services, provision of access to necessary services and solutions to health problems. The county government MOH and MOA should consider using these findings to guide risk communication on aflatoxin exposure and associated health risks. This can be done throught the community strategy with the help of public health officers and community health workers.

Future research

1. For comprehensive public health interventions to be advocated and implemented by the county government and other stakeholders, a holistic county aflatoxin landscape is necessary. There is need for further research to document prevalence of contamination of animal source products such as eggs, milk and pork in Busia.
2. A longitudinal study that would collect samples at different times of the year from the same sites in addition to socioeconomic, temporal, and biophysical data to assess for other determinants is highly recommended.
3. Maximum Tolerable Limits that are based on dietary consumption patterns have been set at 10ppb by Kenya Bureau of Standards. Therefore, an aflatoxin sero-survey and a health impact assessment of this population is recommended given their high frequency of consumption of these aflatoxin prone foods. The sero-survey will help determine the extent of aflatoxin exposure across the county and

help identify the populations most at risk for chronic low dose exposure or an acute outbreak in order to target public health interventions. The health impact assessment would be highly encouraged among the vulnerable populations like infants and young children, particularly during the first 1,000 days, and persons suffering from suppressed immune systems or co-infections from HIV/AIDS who are more adversely impacted by aflatoxins.

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Appendices

Appendix 1: KNH_UON Ethical Approval

UNIVERSITY OF NAIROBI
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Ref. No.KNH/ERC/R/38

March 18, 2019

Abigael Obura
Reg. NO.H80/92361/2012
School of Public Health
College of Health Sciences
University of Nairobi

Dear Abigael

Re: Approval of Annual Renewal – Evaluation of efficacy of food storage and preparation on aflatoxin mitigation: A study of the Abantu, Nilo-hamites and Nilotes of Busia, Kenya (P720/11/2015)

Refer to your communication dated February 14, 2019.

Upon review of your communication, the KNH-UON ERC hereby grants you annual extension approval for ethics research protocol **P720/11/2015**.

The approval dates are 29th March 2019 – 28th March 2020.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN- ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

Protect to discover

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Yours sincerely,

PROF M.L. CHINDIA
SECRETARY, KNH-UON ERC

c.c. The Principal, College of Health Sciences, UoN
The Director CS, KNH
The Chairperson, KNH-UoN ERC

Appendix 2: NACOSTI Research Authorization

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

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NACOSTI, Upper Kabete
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NAIROBI-KENYA

Ref: No. **NACOSTI/P/17/23914/20543**

Date: **18th January, 2018**

Abigael Obura Awuor
University of Nairobi
P.O. Box 30197-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Evaluation of efficacy of food storage and preparation on aflatoxin mitigation: A study of the Abantu, Nilo-hamites and nilotes of Busia Kenya,"*** I am pleased to inform you that you have been authorized to undertake research in **Busia County** for the period ending **18th January, 2019.**

You are advised to report to **the County Commissioner and the County Director of Education, Busia County** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

BONIFACE WANYAMA
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Busia County.

The County Director of Education

Appendix 3A. Consent Form for Respondents (English)

(Flesch-Kincaid Reading Level: 7.0)

*Note: This form will be **translated** into Kiswahili and Luhyia and the translation will be checked for accuracy and verified by study staff from University of Nairobi.

MANAGEMENT OPTIONS FOR FOOD STORAGE AND PREPARATION AGAINST AFLATOXIN: A CASE STUDY OF THE ABANTU AND ATESO OF BUSIA KENYA

English version

Introduction:

Hello, my name is (Interviewer/research assistant). I thank you for accepting to talk with us today. This session will take about one hour. I am working on this study which seeks to establish ways to minimise your exposure to aflatoxin. Aflatoxin comes from a mold that can grow on maize, millet, sorghum, cassava, groundnuts among other cereals. It can make you very sick. We want to see if certain food preparation methods can help reduce aflatoxin in your food and keep you safe from aflatoxin.

Why is this study being done?

We want to gain a basic understanding of Aflatoxin prevalence among Busia County's bantu, nilotes and nilo-hamite communities. We also strive to know if there are any local traditional food preparation methods that can be used to help mitigate aflatoxin contamination thus reduce human aflatoxin exposure. Information we learn from this study will help the communities in Busia County, will be shared with the general community so that people can be encouraged to practice relatively safer food storage and preparation methods thus minimising risk of exposure to aflatoxins. We have chosen your community because there is little to no knowledge of aflatoxin prevalence in this region yet the people here consume very many aflatoxin prone foods. We estimate that 472 households will be visited during this study.

What will happen today?

If you agree to be part of this study, we will ask you a few questions to see if you are able to participate. This includes the following:

- We will ask you to provide us with some food samples today.
    - These will be tested to determine whether your food has aflatoxin
- We will also ask you some questions today. This will take about 45 minutes. You can refuse to answer any questions. The questions will be about:
    - What kind of food you eat every day
    - Where you get you main foods
    - How much maize, millet, sorghum, cassava and groundnuts you eat every day

- How you prepare your grain based meals

If you agree to these terms then we will enrol you in the study.

Benefits of being in the study:

- We will give you transport reimbursement of Kshs 300.
- You will learn more about aflatoxin
- You will learn the better ways of storing your aflatoxin prone foods to avoid aflatoxin contamination
- You will learn better ways of preparing your aflatoxin prone foods to reduce levels of contamination
- You will be helping us learn about ways to protect communities from aflatoxin exposure which will be a benefit to the country at large.

Risks:

There are no risks involved with this study. In the event that the study finds high prevalence of aflatoxin contamination in Busia County, the food data will not be able to be traced back to an individual's household because it is only alphanumerical identifiers that will have been used during data collection.

Privacy:

We will keep the information about you private to the extent allowed by law. We will record your address so that the study workers can find you easily during the study. Only the study team, University of Nairobi, regulatory agencies, and the ethics committee can see your information. All the information will be kept in secured computer files. Information will be in summarized in reports. No one will be able to identify you or your household. All personal information that can identify you will be destroyed and will not be used in any publication.

Voluntary:

You are free to choose whether or not to be in this study. You are also free to say no to any part of this study. Even if you say yes, you may change your mind at any time. Nothing will happen to you if you decide not to participate, or if you decide to withdraw from the study.

WHO TO CONTACT: If you have questions or concerns about this study, you can call Abigael Obura at 0710 602 752, Faith Thuita at 0722639719. If you have concerns regarding your personal rights in the study, you can contact the Secretary of the Kenyatta National Hospital -University of Nairobi Ethics and Research Committee on email address uonknh_erc@uonbi.ac.ke or call 020 2726300 Ext: 44102

Has the respondent

1. Accepted □
2. Declined □

If declined, why? _____

AGREEMENT:

I agree to be in this study. The risks and benefits of this study have been explained to me. I have had a chance to ask questions. All my questions were answered. I can choose to be in this study. I can drop out of the study at any time. I will receive a copy of this form.

I have read or had this form read to me. By signing below, I consent to join this study.

Name and signature of participant	Study representative	Date (mm/dd/yyyy)

The study volunteer cannot read. I verify that this consent form has been accurately and clearly read to the study volunteer.

Fingerprint of participant	Signature of witness	Date (mm/dd/yyyy)

SIGNATURE OF WITNESS: (*if participant is illiterate*)

I have heard the explanation of this study. The procedures, risks, and possible benefits were explained to me. I do not work with the principal investigator or with any other person who works under or with the investigator. I confirm that the participant has voluntarily consented to allow his or her household to participate in this study.

Witness Name (print)	**Thumb Print of Person Being Witnessed**
Witness Signature	

Appendix 3B: Informed consent (Kiswahili translation)

MBINU CHAGUZI ZA KUHIFADHI NA KUUANDA CHAKULA DHIDI YA AFLATOKSINI: MCHANGANUO WA ABANTU, NILOTES AND NILO-HAMITES WA KAUNTI YA BUSIA KENYA

Utangulizi:

Habari, jina langu ni (msaidizi wa utafiti).Ningependa kuashukuru kwanza kabisa kwa kukubali kuongea nasi siku ya leo. Mazungumzo haya yatachukua muda wa takribani saa moja. Nafanya kazi kwa utafiti huu ambao unamadhumuni ya kutafuta mbinu za kupunguza hathari yatokanayo na aflatoksini. Aflatoksini ni kuvu inayoweza kumea kwa vyakula kama vile mahindi, mtama, wimbi, mihogo, njugu. Inaweza kukudhuru afya ukiila. Lengo la utafiti huu ni kujua kama mbinu za kuandalizi wa chakula zinaweza kupunguza kiwango cha aflatoksini kwenye chakula chako hivyo kupunguza madhara kwa mwili wako.

Kwa nini utafiti huu unafanywa?

Tunataka kujua iwapo Aflatoksini ipo katika vyakula vya wa abantu, nilotes na nilo-hamite wa Kaunti hi ya Busia na kwa viwango vipi. Pia tunataka kujua iwapo uandalizi wa chakula wa kitamaduni unaweza kupunguza kiwango cha aflatoksini kwenye chakula chako. Matokeo ya utafiti huu itasaidia wenyeji wa kaunti ya Busia. Wenyeji na wakazi wa kaunti ya Busia kwa jumla watapata habari hii na watahimizwa kutumia mbinu zitakazopatikana kukua na umuhimu sana katika kuzia adhari za sumu hii mwilini. Tumechangua jamii hii kwa utafiti huu kwa sababu kuna habari kidogo sana juu ya uwepo wa aflatoksini katika chakula chenu katika mkoa huu ingawa wenyeji wengi wanakila chakula ambacho kinakabiliwa na shida ya kukua na ukoka huu. Tutatembelia nyumba takriban 472 katika utafiti huu.

Ni nini kitakachotendeka leo?

Iwapo utakubali kushiriki katika utafiti huu, tutakuuliza maswali machache kuamua kama unaweza kushiriki. Maswali in yafuatayo:

- Tutakuomba utupe sampuli ya chakula chako .
    - Sampuli hizi zitapimwa kuamua kwango cha aflatoksini
- Pia, tutakuuliza maswali ambayo hayatazidi dakika arubaini na tano. Unawezakukataa kujibu swali lolote. Haya maswali yatakuwa kuhusu:
    - Aina ya chakula unayokula kila siku
    - Namna unavyopata chakula chako
    - Kiwango cha mahindi, mtama, wimbi, mihogo and njugu unachokila kila siku

- Vile unavyokianda chakula chako

Faida za kuwa katika utafiti:

- Mwishoni mwa utafiti,utapewa shilingi mia tatu ya usafiri.
- Utajifunza mengi kuhusu aflatoksini
- Utajifunza mbinu za uhifadhi wa vyakula ilikuzuia uchafuzi wa aflatoksini
- Utajifunza mbinu za uuandalizi wa hivi vyakula ili kupunguza viwango vya aflatoksini.
- Mutatusaidia kujua mbinu maalumu za kulinda jamii na nchi hii kwa jumla dhidi ya sumu ya aflatoksini

Hatari:

Hakuna hatari zinazotokana na utafiti huu. Iwapo viwango vya aflatoksini vitapatikana kuwa vya juu katika kaunti hii ya Busia, matokeo hayataweza kutambua boma lipi haswa lilikuwa na viwango vipi kwasababu majina yenu hayatatumika.

Ufaragha:

Tutaweka taarifa kukuhusu kuwa siri kwa kiwango kinachokubaliwa na sheria. Tutarekodi jina lako na eneo la nyumbani kwako ili wafanyakazi wa utafiti waweze kukupata kila siku wakati wa utafiti. Pia tunaweza kurekodi taarifa ya jina na mawasiliano ya jirani, au mtu anayeweza kutusaidia kukupata endapo hutakuwepo nyumbani tutakapokuja. Ni kikosi cha utafiti, Chuo Kikuu cha Nairobi, uwakala wa usimamizi, na kamati ya maadili tu wanaoweza kuona taarifa yako. Taarifa zote zitawekwa kwenye faili salama za tarakilishi. Taarifa itafupishwa katika ripoti. Hakuna atakayeweza kukutambua au nyumbani kwako. Taarifa zote binafsi zinazoweza kukutambua zitavurugwa na hazitatumiwa katika chapisho lolote.

Kujitolea:

Una uhuru wa kuchagua kushiriki au kutoshiriki kwenye utafiti. Pia una uhuru wa kukataa sehemu yoyote ya utafiti huu. Hata ukisema ndiyo, unaweza kubadilisha fikra zako wakati wowote. Hakuna kitakachokutendekea ukiamua kutoshiriki, au ukiamua kujiondoa katika utafiti.

WA KUWASILIANA NAYE:

Iwapo una maswali yoyote au masuala kuhusu utafiti huu, unaweza kumpigia simu Abigael Obura kwa 0710 602 752 au Faith Thuita kwa 0722639719. Iwapo una masuala kuhusu haki zako binafsi katika utafiti, unaweza kuwasiliana na Katibu wa Bodi Ukaguzi wa Asasi ya Hospitali Kuu ya Kenyatta –Chuo Kikuu cha Nairobi kwa barua pepe uonknh_erc@uonbi.ac.ke au upige simu kwa 020 2726300 Ext: 44102

Mshiriki:

1. Amekubali □
2. Amekataa □

Kama amekataa, ni kwa nini?_____

UKUBALIANO:

Ninakubali kushiriki katika utafiti huu. Nilifafanuliwa taratibu, hatari, na faida zinazowezekana. Nimekuwa na nafasi ya kuuliza maswali na yote yamejibiwa. Ninaweza kushiriki kwa utafiti huu. Pia ninaweza kujiondoa kwa utafiti huu wakati wowote. Nitapokea nakala ya fomu hii.

Nimesoma au nimesomewa fomu hii. Ninakubali kushiriki.

Jina na sahihi ya mshiriki	**Mwakilishi wa utafiti**	**Tarehe (TT/MM/MM)**

Iwapo mshiriki hajui kusoma na kuandika. Ninathibitisha kuwa mshiriki amekubali kwa kujitolea kushiriki katika utafiti huu.

Alama ya kidole ya mshiriki	**Sahihi ya Shahidi**	**Tarehe (TT/MM/MM)**

SAHIHI YA SHAHIDI: (iwapo mshiriki hajui kusoma na kuandika)

Nimesikia ufafanuzi wa utafiti huu. Nilifafanuliwa taratibu, hatari, na faida zinazowezekana. Sifanyi kazi na mchunguzi mkuu au na mtu mwengine yeyote anayefanya kazi chini ya au na mchunguzi. Ninathibitisha kuwa mshiriki amekubali kwa kujitolea kushiriki katika utafiti huu.

Jina (chapisha):	**Alama ya kidole:**
Sahihi:	

Appendix 3C: Informed Consent (Samia Translation)

Engira endayi echobiha nde odeha ebiahuria hulukukuhu lwa Aflatoksin lutanyasa abiahuria: Ohusoma mubaluhya nde abandu bendimi chindi nga abanyolo nde abateso.

Luhyia version

Ohuchakisa:

Obolasi. Erita riange ni (Interviewer/research assistant). Otyo muno otuberesa ebiha bino olomaloma nawe nyangaino. Hunabukula esaa lala riongane. I am working on this study which seeks to establish ways to minimise your exposure to olukuhu lwa aflatoksin. Olukuhu lwa Aflatoksin luhulanga humadimwa, obule, amabere, emiogo, enjugu nde hubiahuria bindi. Olukukhu oluo lunyala lwahureterara obulwaye. Hwenya obona nikari engira chindi chohudeha ebiahuria chinyala ohendesa olukuhu oluo hulutahuretera obuluaye.

Husina esomo rino riholwa?

Hwenya omanya nikari olukuhu lwa aflatoksin lurimubyahuria byabandu bomukaunti ya Busia. Hwenyaomanya nikari engira chihwehonyranga odeha ebyahuria chihedesanga olukuhu olwo hulungi lutaingira mumibiri kwengwe. Amaeko kahuneka ano kanahonya burimundu yamenya ambina ngwe nde mu kaunti ya Busia. Hwaamua ohwicha ohwekera ano sakira olukuhu lwa aflatoksin si lwamanyihana nde abandu nikari burimubyahuria byomukaunti ino. Hunakendera ambi amadala 472musomo rino.

Sina esinahorekhana nyangaino?

Nofukirira oba musomo rino, hunahuteba amatebo obona nikari onyala ochirira obamusomo rino.

- Hunahuteba otuberesaho ebiahuria bididi.
    - Hunaringa ebiahuria biotuberesha nikali biri nde olukuhu lwa aflatoksin.
- Hunahutega amatebo kandi nyangaino. Kanabukula kama edadika kanne nde chitano. Onyala wahaya ohutuborera siosi siosi. Amatebo kano kanalondana nde:-
    - Ebiahuria bimurichanga burinyanga
    - Imunyolanga ebyahuria bweng'we
    - Murichanga amadimwa, obule, amabere, emiogo nde enjugu chaka chitie burinyanga.
    - Engeri imuteresanga ebyahuria bweng'we

Nikari mbwe ofukirira nde kano mahuwa, mani onyala waaba musomo rino.

Enganga yesomo rino huewe:

- Hunahuberesa esilingi emia chidatu echohuhonga hulukendo.
- Oneeka amangi olondana nde olukuhu lwa aflatoksin
- Oneeka engira ndayi chohubiha nde ohuhendesa olukuhu lwa aflatoksin mu biahuria
- Oneeka engira ndayi chohudeha nde huhendesa olukuhu lwa aflatoksin mu biahuria
- Onatuhonya ohweka engira ndayi chohuhonya abandu bekaunti ino otulana nde amabi kolukuhu lwa aflatoksin.

Ebibi biosi biosi:

Siumao esibi siosi esinyala siahunyola olondana nde esomo rino. Niwichuhana hwanyola olukuhu lwa aflatoksin olungi mubwahuria biao kose mu kaunti ino, hasihunanyala ofunya owao sakira hasihunaba hwehonyere erita riao humakaratasi kefwe.

Privacy:

Kosi kahunalomaloma nde kahunanyola owao si hunaborera omundu undi. Hunaandika iwamenda hubandu bahonya musomo rino banyala banyala bola owao bilai. Abandu bahonya musomo rino, aba University ya Nairobi, nde abaemirisi bamasomo oholwa bilayi bongane nibo abanyala obona amahuwa kao akanabihwa mucomputer chibihirwe bilai. Nihwamala esomo, hunaandika aripoti.

Ohwetusa:

Onyalawaunua obamo kose otaba musomo rino hulwao omwene. Onyala wahaya okaluha mutebo riosisriosi. Ata ndenofukirira, onyala wahaya ochiririra esiha siosi siosi. Siumau esifune sinahunyola.

WINA YONYALA WALOMALOMA NAYE: Nikari orinde amatebo kosikosi onyala wahubira Abigael Obura hunaamba ye simu 0710 602 752 kose Faith Thuita hu 0722639719. Ne nobasa bwe soirwa bilayi nde abachreresa esomo rino, onyala walomaloma nde Secretary wa Esbitali hongo ya Kenyatta -University ya Nairobi Ethics and Research Committee hu email ya uonknh_erc@uonbi.ac.ke kose ohube esimu huu 020 2726300 Ext: 44102

Owenyehana oba musomo

1. Afukirire? □
2. Ahaire? □

Ni kariohayire, husina?

_____

OHUFUKIRIRA:

Fukirira oba musomo rino. Mborerwe amabii nde amalai ke somo rino. Mbere nde obweyango bwoteba amatebo. Bakaluse mumatebo kange kosi. Fukirira oba musomo rino. Nyala ndatulamo esiha siosi siosi. Ndabereswa ekaratasi range riekesa bwe fukirire.

Somere kose basomere kosi akarihukaratasi rino. Ohwandika Hwange huno hwekesa mbwe fukirira oba musomo rino.

Erita nde esain eyeingira musomo	Omwimirisi we somo	Oludalo

Nikari mbwe owingira musomo yakotwa osoma. Njakikisha bwe amahuwa kefomu ino bamusomere bilai mani yengira musomo hulwohwenya hwaye omwene.

Oluala lwowingira musomo	Esain yomwimirisi	Oludalo

ESAIN YOMWIMIRISI: (*nikari owingira musomo siyasoma*)

Mburire amahua kosi kachana nde esomo rino. Mborerwe Kosi akanachiririra, amabii nde enganga. Hasiholanga emirimo nde omuimirisi we somo rino. Nyala ndabola mbwe omundu uno owenya oba musomo rino afukirire hulwo hwenya hwaye omwene.

Erita riomwemeresi (andika)	**Olwala lwomundu yemerwa**
Esain yomwimirisi	

Appendix 4: Household survey tool

UNIVERSITY OF NAIROBI

School of Public Health

Evaluation of Traditional food storage and preparation on aflatoxin mitigation:

A case study of the Abantu, Nilotes and Nilo-hamites of Busia Kenya

QUESTIONNAIRE

Code_____ Questionnaire No._____

Sub-county _____

Household No. _____

Interview date dd/mm/yy _____

Interviewer Name _____ No. _____

SECTION 1: SOCIAL DEMOGRAPHIC PROFILE

1. Informed consent for participation signed?
    1. Yes
    2. No [__]
2. Village _____
3. GPS: Latitude: _____ Longitude: _____

    Altitude: _____
4. How long have you lived in this village? _____ (years)
5. Gender:
    1. Male
    2. Female [-__]
6. Age: _____ (in complete years) If <18 Years, **STOP**, exclude participant.
    1. Don't know
    2. Refused
7. Date of Birth: _____yy _____mm _____dd
    1. Don't know
    2. Refused [-__]
8. Are your household members?
    1. Bantu,
    2. Nilotes
    3. Nilo-hamites? _____ [__]
9. Does your household consume maize, groundnuts, millet, sorghum or cassava at least 4 times a week?
    1. Yes
    2. No If no, **STOP**. Participant to be excluded [__]
10. What is the highest level of education completed?
    1. Pre-primary
    2. Primary
    3. Secondary
    4. College and above
    5. Refused
    6. None

    [__]
11. How many members are in your household? _____

SECTION 2: FOOD SOURCE AND STORAGE PRACTICES

12. How much maize do you have now?
    1. ……. bags
    2. None **If None go to 18** **[-__]**

13. What is the source of the household's maize? (Please tick all those mentioned)

1. Home grown
2. Bought it at local market
3. Combination of the above (Please specify): _____
4. Other (Please specify)_____ **[__]**

14. If the maize was homegrown, which year was it harvested?

1. 2014
2. 2015
3. Combination of this year and last year
4. Other: (Please specify) _____ **[-__]**

15. How was the maize dried?

1. Sun dry directly on ground
2. Sun dry not directly on ground
3. Did not attempt to dry maize
4. Other (Please specify): _____ **[__]**

16. Where is the maize stored?

1. In an outside granary
2. Inside your house
3. Other (Please specify) _____ **[__]**

17. How is the maize stored?
    1. In a nylon sack
    2. In a sisal sack
    3. In a nylon paper bag
    4. In a bucket _____ **[__]**

18. How much millet do you have now?

    1. .....kgs
    2. None **If None go to 25** **[-__]**

19. What is the source of the household's millet? (Please select all that apply)

1. Grew it yourself
2. Bought it at local market
3. Combination of the above (Please specify): _____
4. Other (Please specify)_____ **[__]**

20. If the millet was homegrown, which year was it harvested?

1. 2014
2. 2015
3. Combination of this year and last year
4. Other: _____ [-__]

21. How was the millet dried?

1. Sun dry directly on ground
2. Sun dry not directly on ground
3. Did not attempt to dry maize
4. Other (Please specify): _____ [__]

22. Where is the millet stored?

1. In an outside granary
2. Inside your house
3. Other (Please specify) _____ [__]

23. How is the millet stored?

1. In a nylon sack
2. In a sisal sack
3. In a nylon paper bag
4. In a bucket _____ [__]

24. How long have you had your current supply of millet?

1. <1 week
2. 1 to 3 weeks
3. 4 to 8 weeks
4. >8 weeks [__]

25. How much sorghum do you have now?.....kgs

1. None **If none, go to 31** [__]

26. Where did you get your sorghum from? (Please select all that apply)

1. Grew it yourself
2. Bought it at local market
3. Combination of the above (Please specify): _____
4. Other (Please specify)_____ [__]

27. If the sorghum was homegrown, which year was it harvested?

1. 2014
2. 2015
3. Combination of this year and last year
4. Other: _____ [__]

28. How was the sorghum dried?

1. Sun dry directly on ground
2. Sun dry not directly on ground
3. Did not attempt to dry maize
4. Other (Please specify): _____ [__]

29. Where do you store your sorghum?

1. In an outside granary
2. Inside your house
3. Other (Please specify) _____ [__]

30. How is the sorghum stored?

1. In a nylon sack
2. In a sisal sack
3. In a nylon paper bag
4. In a bucket _____ [__]

31. How much groundnuts do you have now?

1. .....kgs
2. None **If none go to 37** [-__]

32. What is the source of the household's groundnuts? (Please select all that apply)

1. Grew it yourself
2. Bought it at local market
3. Combination of the above (Please specify): _____
4. Other (Please specify)_____ [__]

33. If the groundnuts were homegrown, which year was it harvested?

1. 2014
2. 2015
3. Combination of this year and last year
4. Other: _____ [-__]

34. How were the groundnuts dried?

1. Sun-dry directly on ground
2. Sun-dry not directly on ground
3. Did not attempt to dry maize
4. Other (Please specify): _____ [__]

35. Where do you store your groundnuts?

1. In an outside granary
2. Inside your house
3. Other (Please specify) _____ [__]

36. How are the groundnuts stored?

1. In a nylon sack
2. In a sisal sack
3. In a nylon paper bag
4. In a bucket _____ **[__]**

37. How much cassava do you have now?

1. .....kgs
2. None **If none go to 44**

[__]

38. What is the source of the household's cassava? (Please select all that apply)

1. Grew it yourself
2. Bought it at local market
3. Combination of the above (Please specify): _____
4. Other (Please specify)_____ **[__]**

39. If the cassava was homegrown, which year was it harvested?

1. 2014
2. 2015
3. Combination of this year and last year
4. Other: _____ **[-__]**

40. How was the cassava dried?

1. Sun dry directly on ground
2. Sun dry not directly on ground
3. Did not attempt to dry maize
4. Other (Please specify): _____ **[__]**

41. Where do you store your cassava?

1. In an outside granary
2. Inside your house
3. Other (Please specify) _____ **[__]**

42. How is the cassava stored?

1. In a nylon sack
2. In a sisal sack
3. In a nylon paper bag
4. In a bucket _____ **[__]**

43. How long have you had your current supply of cassava?

1. <1 week
2. 1 to 3 weeks
3. 4 to 8 weeks
4. >8 weeks **[__]**

SECTION 3: FOOD CONSUMPTION

44. In which form is maize mainly consumed in your household? (Tick 2 main ways)
    1. Roasted
    2. Boiled
    3. As porridge
    4. As ugali/Posho
    5. Other _____ **[__]**

45. In which form is millet mainly consumed in your household? (Tick 2 main ways)
    1. As porridge
    2. As ugali/posho
    3. As a brew
    4. Other _____ **[__]**

46. In which form are groundnuts consumed mainly in your household? (Tick 2 main ways)
    1. Roasted
    2. Boiled
    3. As a stew
    4. As a paste
    5. Other _____ **[__]**

47. In which form are cassavas consumed mainly in your household? (Tick 2 main ways)
    1. Roasted
    2. Boiled
    3. As a stew
    4. As a paste
    5. Other _____ **[__]**

48. In which form is sorghum mainly consumed in your household? (Tick 2 main ways)
    3. As porridge
    4. As ugali/posho
    5. Other _____ **[__]**

SECTION 4: FOOD PREPARATION METHODS

49. Is ugali/posho mainly made of plain maize flour?
    1. Yes
    2. No **[__]**

50. If no, what do you mix it with?
    1. Sorghum
    2. Millet
    3. Cassava
    4. Other _____ [__]

51. Is porridge mainly made of plain maize flour?

1. Yes
2. No

[__]

52. If no, what do you mix it with?

1. Sorghum
2. Millet
3. Cassava
4. Other _____

[__]

53. Is the porridge made of plain millet?

1. Yes
2. No

[__]

54. Is the posho/ugali made of plain sorghum?

1. Yes
2. No **[__]**

55. Is the stew mainly made of plain groundnuts?

1. Yes
2. No

[__]

56. If no, what do you mix it with?

1. Onions
2. Egg-plant
3. Tomatoes **[__]**
4. Others (specify)

Appendix 5: Dietary Diversity table

	Food Categories	Description/Examples	Consumed Yes=1 No=0
A	Foods made from grains	Maize, millet, rice, wheat, porridge, sorghum, bread, pasta, other foods made from grains	
B	White roots and tubers and plantains	Irish potatoes, yams, cassava, white sweet potatoes, taro, cooking banana/plantain, other roots or tubers	
C	Pulses (beans, peas, and lentils)	Beans, cowpeas, lentils, soy, pigeon peas, other nuts	
D	Nuts and seeds	Peanuts, other nuts, peanut butter, other nut butters	
E	Milk and milk products	Milk, yogurt, cheese	
F	Organ meat	Liver, kidney, heart, or other organ meats	
G	Meat and poultry	Beef, pork, lamb, goat, chicken, duck	
H	Fish and seafood	Fresh or dried fish or shellfish, canned tuna	
I	Eggs	Eggs from poultry or any other bird	
J	Dark green leafy vegetables	Sukumu wiki, spinach, broccoli, amaranth, cassava leaves, other dark green leafy vegetables	
K	Vitamin A-rich vegetables, roots, and tubers	Pumpkin, carrots, red peppers, squash, yellow/orange sweet potatoes, other orange vegetables	
L	Vitamin A-rich fruits	Ripe mangoes, pawpaw, guava, tree tomato	
M	Other vegetables	Onion, tomatoes, cucumber, radishes, green beans, peas, lettuce	
N	Other fruits	Banana, apple, lemon	
O	Insects and other small protein foods	Insects and other small protein foods	
Q	Other oils and fats	Vegetable oil, butter	
R	Savoury and fried snacks	Mandazi, potato crisps, fried potatoes	
S	Sweets	Honey, jam, cakes, candy, biscuits, pastries	
T	Sugar-sweetened beverages	Soda, fruit juice drinks that aren't 100% fruit juice	
U	Condiments and seasonings	Ingredients used in small quantities for flavour such as salt, garlic, spices, yeast, baking powder, tomato sauce, meat or fish in very small quantities	
V	Other beverages and foods	Tea, unsweetened coffee, clear broth, alcohol	

Appendix 6: Consent to Participate in Focus Group Discussion

INTRODUCTION

Hello, my name is Abigael Obura Awuor. Let me start by thanking you for agreeing to talk with us today. I am working on this study which seeks to establish ways to minimise your exposure to aflatoxin. Aflatoxin comes from a mold that can grow on maize, millet, sorghum, cassava, groundnuts among other cereals. It can make you very sick. We want to see if certain food storage and preparation methods can help reduce aflatoxin in your food and keep you safe from aflatoxin.

The purpose of the group discussion and the nature of the questions have been explained to me. I consent to take part in a focus group about my experiences, including some ways that food storage and preparation methods can mitigate Aflatoxin contamination. I also consent to be audio recorded during this focus group discussion. My participation is voluntary. I understand that I am free to leave the group at any time. If I decide not to participate at any time during the discussion, my decision will in no way affect the way I live in this community and the services I receive from the local administration. None of my experiences or thoughts will be shared with anyone outside of the study team unless all identifying information is removed first. The information that I provide during the focus group will be grouped with answers from other people so that I cannot be identified.

Please Print Your Name _____

Date: _____

Please Sign here _____

Appendix 7: Focus Group for women in the study communities

Date: **Place:**

Facilitator:

Note takers: **No. of participants:**

INTRODUCTION

Hello, my name is Abigael Obura Awuor. Let me start by saying thank you for agreeing to talk with us today. I will moderate today's discussion. I am joined by my colleague_____. She is also going to assist in writing notes today. Our discussion will last about 1½hours and we will primarily discuss your experiences.

I am working on this study which seeks to establish ways to minimise your exposure to aflatoxin. Aflatoxin comes from a mold that can grow on maize, millet, sorghum, cassava, groundnuts among other cereals. It can make you very sick. We want to see if certain food storage and preparation methods can help reduce aflatoxin in your food and keep you safe from aflatoxin.

Why is this study being done?

We want to gain a basic understanding of Aflatoxin prevalence in the abantu, nilote and nilo-hamite communities of Busia County. We also strive to know if there are any traditional local food preparation methods that can be used to help mitigate aflatoxin contamination and reduce human aflatoxin exposure. Information we learn from this study will help the communities in Busia County, to prevent people from becoming sick with aflatoxicosis. We have chosen your community because there is little to no knowledge of aflatoxin prevalence in this region yet the people here consume very many aflatoxin prone foods. We shall conduct a total of 6 FGDs in these communities.As women in the community who help in the acquisition of household foods and prepare meals you are the experts on this topic! This will be private, confidential discussion. Although we will be tape recording this session, your responses are confidential and your identity will never be associated with your responses. We cannot insure that group members will not repeat comments outside of the group, but we ask that you keep what is said in the group confidential and not share anything that is said with others outside of this group.

Before we get started, let's just review the rules for this discussion.

Rules

1. This session will take 1-2 hours. This session will be tape recorded, and we will have a note taker. (Note to facilitator: If you haven't already, please introduce your colleague.)
2. Everyone please speak clearly one at a time.

3. We would like everyone to participate, but if you do not feel comfortable talking about a topic you do not have to.

4. There is no right or wrong answer. You should feel free to express whatever you are thinking.

5. Your participation is anonymous and your answers are completely confidential. You can introduce yourself. We will not use your name in any of our reports or attach your name to your comments.

6. Please do not talk about anyone's private information with others outside of this group.

Introductions (warm up)

Please introduce yourselves and tell us about your household composition.

Notes will be taken extensively and will accurately reflect the content of the discussion, as well as any salient observations of nonverbal behavior, such as facial expressions, hand movements, group dynamics, etc.

Source of food

Question 1: What are the common foods consumed in your household?

a. Probes

i. How many times would you consume a particular food in a week?
ii. Are these foods different from what there were 20 years ago?
iii. If there is any difference, please elaborate.

Question 2: What are your major sources of food?

a. Probes

i. Is all the household food home-grown?
ii. If not home-grown, what are the other sources of food for your households?

Food storage

Question 3: What methods of storage of food do you use?

a. Probes

i. Where do you store the household foods?
ii. What storage containers do you use?
iii. Are there historical differences in storage methods?

iv. Does storage differ by type of food?
v. Does storage differ by season?
vi. Does storage differ by financial well-being of a household?
vii. Are there any health challenges associated with consumption of grains that are not well dried and or stored?
viii. Do we know of anyone who has gotten sick from eating contaminated grains?
ix. What can households do to mitigate these health challenges?

Food preparation

Question 4: What are the common food preparation methods for the foods that are commonly consumed within your households?

a. Probes

- Would you provide a recipe for some of these?
- Would you give us a step-by-step process of how to make the two main meals in your households?
- Does the duration of preparation the same time of meal matter at all?

- Are there any additives that you add to your foods during preparation?
- If any additives are used during food preparation, name them and tell us why they are added.
- Any food preparation methods that are no longer being used but were being used in the past?

Appendix 8: Key Informant Interview Protocol for women group leaders.

Date: **Place:**

Facilitator:

Note taker:

INTRODUCTION

Hello, my name is Abigael Obura Awuor. I am a researcher from the University of Nairobi' School of Public Health and I am conducting a study which seeks to establish ways to minimise exposure to aflatoxin in foods consumed in this community. Aflatoxin comes from a mold that can grow on maize, millet, sorghum, cassava, groundnuts among other cereals.The purpose of this interview is to learn more about the practices in this community on food sourcing, storage and preparation. We want to understand how this affects Aflatoxin prevalence in this community. Group discussions will be held with community members. A questionnaire will also be used with women who consent to participate in the study. This will help us to know if there are traditional local food preparation methods that can be used to help prevent aflatoxin contamination of foods consumed and therefore reduce ingestion of contaminated food.

The study findings shall be shared during creating awareness among stakeholders, including high-ranking government representatives, donors and the private sector with an aim of urging them to consider practicing food sourcing, production and food preparation practices which would include those steps that result in big aflatoxin reduction. A detailed survey report and pamphlets will be printed for professionals working in the field of food safety and nutrition at academic and policy levels and easy to read summaries for the lay people at national and county levels shall be prepared. This will provide a simplified better understanding of the magnitude, causes and some consequences of aflatoxins in Busia county. Communities will select representatives who will help formulate measures of reducing aflatoxin contamination based on the findings. These will later be the focal point for community change of attitudes and practices after the project exits.

Before we get started, do you agree to participate in the study?

Yes ( )

No ( )

Thank you for agreeing to do this interview. Do you agree for this session to be audio recorded for study purposes only?

Yes ( )

No ( )

Guidelines for this discussion.

1. This session will take about 1 hour. This session will be recorded, and we will have a note taker.
2. You should feel free to express whatever you are thinking.
3. Your participation is anonymous and your answers are completely confidential. We will not use your name in any of our reports or attach your name to your comments.

Introduction

1. Tell me about yourself.
    i. How long have you lived this county?
    ii. How long have you been in your current position?

Foods consumed and source

2. What are the main foods consumed in this community (Abantu/Nilotic/Nilo-hamite)?

Probes:

i. How do households get this maize?
ii. How do households get their sorghum?
iii. How do households get their millet?
iv. How do households get their groundnuts?
v. How do households get their cassava?
vi. What challenges do community members encounter in sourcing these foods?

About Aflatoxin contamination

3. Are there any foods in this community that are contaminated with aflatoxins?
    i. Which foods are these?
    ii. How do you think contamination happens?
    iii. To what extent do you think foods in this community/county are contaminated by Aflatoxins?
        a) When does contamination of foods happen?

b) What would you say causes contamination?

c) Do you think the storage practices contribute to contamination?

d) Do households store their foods in outside storage facilities like granaries?

e) Which storage containers do households use for inside storage of foods?

Aflatoxin decontamination

4. What are the current practices that help in the reduction of aflatoxin in this community?

5. What practices are available in this community which can help to reduce contamination of foods with aflatoxin?

Probes:

i. What do you think are the challenges to that your community members encounter in food preparation?

ii. How can community members be empowered to tackle these challenges?

iii. Do you think the county government has any role in prevention of aflatoxin?

iv. What facilities can the county government provide to help prevent aflatoxin contamination?

v. What services can the county government provide to help prevent aflatoxin contamination?

Appendix 9: Key Informant Interview Protocol for public health officials and agricultural extension officers.

Date: **Place:**

Facilitator:

Note taker:

Hello, my name is _____. I am a researcher from the University of Nairobi's School of Public Health and I am working on this study which seeks to establish ways to minimise exposure to aflatoxin of the Abantu, Nilotes and the Nilo-hamities communities of Busia County. Aflatoxin comes from a mold that can grow on maize, millet, sorghum, cassava, groundnuts among other cereals.The purpose of this interview today is to learn more about the motivations and beliefs of these communities' residents on food sourcing, storage and preparation. We want to gain a basic understanding of Aflatoxin prevalence in these communities of Busia County. Focus Group Discussions and household surveys will also be conducted so that we can know if there are any traditional local food preparation methods that can be used to help mitigate aflatoxin contamination and reduce human aflatoxin exposure.

The study findings shall be share during advocacy meeting of stakeholders, including high-ranking government representatives, donors and the private sector with an aim of urging them to consider production changes in industry to include those steps that result in big aflatoxin reduction.. A detailed survey report and pamphlets will be printed for professionals working in the field of food safety and nutrition at academic and policy levels and easy to read summaries for the lay people at national and county levels shall be prepared. This will provide a simplified better understanding of the magnitude, causes and some consequences of aflatoxins in Busia county. Communities will select representatives who will help formulate mitigations based on the findings. These will later be the focal point for community change of attitudes and practices after the project exits.

Before we get started, do you agree to participate in the study?

Yes ( )

No ( )

Thank you for agreeing to do this interview. Do you agree for this session to be recorded for study purposes only?

Yes ( )

No ( )

Guidelines for this discussion

1. This session will take about 1 hour. This session will be recorded, and we will have a note taker.
2. You should feel free to express whatever you are thinking.
3. Your participation is anonymous and your answers are completely confidential. We will not use your name in any of our reports or attach your name to your comments.

Introduction

4. Tell me about yourself.
   i. How long have you lived in this county?
   ii. How long have you been in your current position?

Food source

5. What are the main sources of food with in this county, specifically among the Abantu, nilotes and nilo-hamite communities?

   Probes:
   i. Why would you identify these as the main sources of food for these communities?
   ii. Does the availability of these foods vary during the year?
   iii. What causes the variation of the availability of these food items?
   iv. What determines what types of foods a household consumes?

About Aflatoxin contamination

6. Are the residents of this county exposed to Aflatoxins?
7. Are foods in this county contaminated with aflatoxins?

   Probes:
   i. When does this contamination happen?
   ii. What would you say are the risk factors?

Aflatoxin decontamination

8. What are the current practices that help in aflatoxin mitigation in the communities?
9. What practices are available in the communities in this community?
10. What policies have been enacted in the community to help aflatoxin mitigation?
11. How effective have these policies been?

    Probes:
    i. What do you think are the challenges to enactment?
    ii. How can they be improved for effectiveness?
    iii. How can the county government help?
12. How important do you believe aflatoxin control and mitigation is to these communities?

    a) What are the interests of your constituents in terms of food safety?

Appendix 10: Field Data Collection Protocol

1. Household data collection protocol.

Household survey will be conducted in 472 households.

1.1 Household Survey - Sampling

During the Kenya Population and Housing Census 2009 information, Busia county was divided into 5 constituencies namely Busia, Teso North, Teso South, Budalang'iBudalang'i and Samia. Budalang'i and Samia were later renamed to Budalang'i and Funyula sub-counties respectively while Busia was split into three sub-counties namely Namble, Matayos and Butula. Teso North and Teso South retained their names.

Therefore, from each cluster, we randomly selected one sub-county i.e (Teso South, Nambale and Budalang'i).

The household is the primary unit of sampling for this survey. The study total sample size is 472.

Sampling procedure

The county is sub divided into three natural clusters by ethnicity, that is, Nilo-hamites (Teso North and Teso South), Bantu (Nambale, Butula, Matayos) and Mixed Nilotes and Bantu cluster (Funyula and Budalang'i). A 3 stage cluster sampling design was used. The first stage cluster was the sub-counties in Busia County, second stage was the locations within the division and third stage, the sub-locations within the Location.

To enrol 472 households, there is a stage 1 cluster of sub-counties out of a possible 7, 3(43%); these clusters were randomly selected. The locations and sub-locations were randomly selected based on probability proportional to the size (PPS) of the divisions and locations respectively using Chromy's sequential sampling in SAS (Statistical Analysis Software). The number of households were then selected using a systematic sampling technique with K (sampling interval) equal to 6 as shown in the table below.

Sampled Busia Sub-counties, location, sub-location and sample allocation

Sub-county	Division	Location	Sub-location	2009 Households	Number of samples	K (sampling interval)
Teso south	Amukura	Amukura	Amugura	500	500/2719 x 472 = 87	500/87 = 6
	Chakol	Ochude	Okiludu	815	815/2719 x 472 = 141	815/141 = 6
Nambale	Nambale	Bukhayo East	Buyofu	782	782/2719 x 472 = 136	782/136 = 6
Budalang'i	Budalang'i	Budalang'i Central	Magombe	622	622/2719 x 472 = 108	622/108 = 6

With sub-location maps obtained from the sub-county administration office, we shall go to the center of the sub-location and different teams will move in opposite direction sampling households using the sampling interval. RAs will recruit participants who meet the inclusion criteria, ie, Household members should be eating foods prone to aflatoxin contamination at least 4 times a week. Additionally, there should be a consenting female adult of household who should be able to speak on behalf of the household about how the household's resources are used and distributed between its competing needs.

Household is defined as a group of people who function as a basic social and economic unit. Therefore, RAs should consider members of a household (although not necessarily related by blood or marriage) as those who live together in the same house or compound and share sleeping and eating arrangement, and are cared for as a unit.

At each household, RAs will explain the purpose of study and the importance of the household survey and food sample collection as it fits within the broader context of the research study to the county administration and the potential participants. They will articulate the objectives of the study and share the anticipated risks and benefits to the individual participant and the community.

Each RA will administer a minimum of 5 questionnaires a day.

1.2 Household survey

1. 2.1 Consenting guide

Once at the sampled household:-

Ensure that you have the appropriate consent forms

Be friendly, use pleasant tone of voice, use relaxed body language, incorporating humor, be humble, exercise patience and not patronize in order to build rapport with the interviewee

Introduce yourself and the study

Obtain consent from interviewee.

Inform participants that their participation is voluntary through a statement read to them before the start of the interview. Tell them they could stop the interview process at any time. Tell participants that that they could decline to answer any question(s) that make them feel uncomfortable.

Ensure that consenting participants sign informed consent forms to confirm their willingness to participate in the study.

1.2.2 Data collection

Record geo-references (latitude, longitude and elevation) of the household being surveyed.

Go through the questionnaire systematically, question by question

Clearly mark the answers on the tool (Correct entry on the tablet)

Label all data documentation materials with an identical archival number with that on the food samples (Use the marker pens provided)

At the end of the survey, cross check that all answers have been answered. Save and submit/send the completed questionnaire to the email account provided.

Reimburse the participant with Kshs 300 as compensation of their time. The participant should acknowledge receipt of reimbursement by signing on the form. (Funds will be paid using mpesa).

1.2.3 How to Select a Representative Sample of grains from Household Stores

Grain Collection

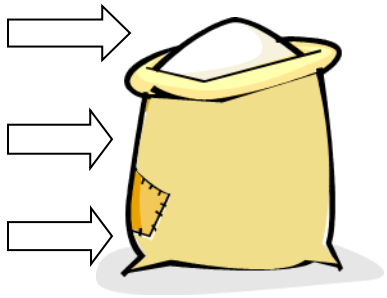
It is important to get a representative sample of maize from each household. A representative sample gives us confidence that the test results that we get truly reflect each household's maize store. The sample that is collected from each household should be 500g in weight.

Definitions:

Representative Sample - a sample of maize that is representative of the household's entire maize store/stock

How to:

Take some maize from the TOP, MIDDLE, and BOTTOM of each bag to create a combined 5 kg sample. Do this even if there is only one bag. Use the bucket available at the household to contain the 5 kg sample.



Then, mix the 5 kg sample with the scooper and scoop out a 1/2 kg sample. Place the 1/2 kg sample in the paper bag.

Or

If less than a bag, tell the interviewee to mix it flour or grain 4 times using her hand then scope a 500g sample.

Label samples to match the household survey.

Seal the sample bag and store in a dry place.

2. Key Informant InterviewsNine Key Informant Interviews will be conducted

We used purposive sampling according to the preselected criteria that was relevant to the research question. We chose 9 participants from the agricultural and health sectors and village leadership levels.

Personnel to be interviewed are:-

- Sub- county agricultural officer (one from each sub-county)
- Sub-county health officers (one from each sub-county)
- Community leaders

3. Mobilization - focus groups

Six FGDs will be conducted - 2 from each sub-county.

We shall use Snowball sampling (chain referral sampling) through village women group leaders

Informants with whom contact will have been established through the organizations with operations at the grass root levels will use their social networks to refer us to other people who could potentially participate in or contribute to the study

Participants in the sampled sub-counties (wards) will be selected because they are in the same age group and of the same gender, that is, FGD will comprise 2 sets of women; 18≤35 years and 36≤50 years.

4. Focus Group Discussions

- Data collection will take a period of 4 days.
- We shall stop the FGDs at that point when data collection will no longer bring additional insights to the research questions (reaches saturation).

4.1 Focus Group Guide Steps

Preparing for the discussion

Getting familiar with the instruments:

Study the discussion guide.

Study the informed consent document.

Practice with partners.

Day of the interview:

1. Use a checklist, verify that you have all the equipment.
2. If the instruments and consent forms exist in more than one language, be sure you have the appropriate ones for that participant.
3. Label all data documentation materials with an identical archival number, including tapes, notebooks, and question guides.
4. Arrive early at the FGD site to set up equipment.
5. Test your recording equipment.

Conducting the Interview

1. Greet the participants in a friendly manner to begin establishing positive rapport.
2. Briefly describe the steps of the FGD process (informed consent, question and answer, their questions, reimbursement).
3. Set the ground rules
4. Obtain informed consent.
5. Turn on the tape recorder and verify that it is working.
6. Verify informed consent orally with the tape recorder on.
7. Conduct the interview according to the interview guide.
8. Give the participants the opportunity to ask questions.
9. Reconfirm the participant's consent while the tape recorder is still on.
10. Turn off the tape recorder and thank the participant.

11. Clarify any factual errors expressed by participants during the interview.
12. Reimburse the participant in accordance with study procedures.

After the Interview

1. Check the tape to see if the interview was recorded. If it was not, expand your notes immediately.
2. Make sure all materials are labeled with the archival number.
3. Debrief with other field staff.
4. Assemble all materials into one envelope. Double-check that you have completed all forms and that all materials are appropriately labeled. Note and explain any missing materials on the archival information sheet.
5. Expand your notes within 24 hours if possible.

4.2 Interview and FGD Checklist

Make arrangements for

❑ Private setting for interview site

❑ Transportation of staff to interview site

❑ Transportation of participant to interview site

❑ Refreshments for participants (if applicable)

What to take to the interview

Equipment

❑ 1 tape recorder (plus 1 extra, if available)

❑ 2 blank 90-minute cassette tapes per interview

❑ Spare batteries

❑ Field notebook and pens

Interview packet

❑ 1 large, heavy-duty envelope

❑ Archival information sheet with archival number

❑ 1 copy of interview guide (in the appropriate language for participant)

❑ 2 informed consent forms (1 for interviewer, 1 for participant, in the appropriate language)

❑ Participant reimbursement (if applicable)

❑ Reimbursement form (if applicable)

What to place in the envelope after the interview

❑ Completed archival information sheet

❑ Signed informed consent form (signed only by interviewer if oral, by participant and interviewer if written)

❑ Labeled interview guide with notes

❑ Field notes

❑ Labeled cassette tapes, re-record tabs punched out

❑ Signed reimbursement form (if applicable)

5. Roles and Responsibilities

Research Assistants:

- Consent study participants
- Conduct household interviews
- Collect the food samples
- Give the supervisor a daily account of the field activities during the field work.

Supervisor:

- Manage teams of RAs
- Introduce survey teams in village
- Accompany team members to spot-check interview. Physically accompany field teams & sit-in on interviews. Strive to see what's happening first hand and do not rely on reports from survey
- Checks all surveys for completeness
- Keep log of interviews completed
- Keep log of samples collected

- Aim to observe all field teams and most RAs
- Make note of common mistakes / problems and regularly communicate them to all field teams
- Give feedback to individual RAs and debrief with team
- Identify RAs who consistently under-perform – have plan of action for consequences / replacement
- Participate in the training sessions

Data manger:

- Developed electronic data collection tool
- Export and review data on daily basis
- Insure data matches field logbooks

Field manager:

- Plans and oversees field work
- Manages all field teams
- Handles logistics and budget
- Primary liaison with research team
- Review questionnaires already checked by supervisor and point out any mistakes that were missed.
- Check for consistency
- General troubleshooting

6. How Quality Control will be implemented during data collection

a) All training will be carried out by the Principal Investigator with the assistance of the field supervisorto ensure standard training.
b) The Research Assistants hired will all be graduates thus have an advantaged level of comprehension of research work.
c) The RAs will be trained to ensure that they have a uniform application of the survey materials and that the rationale of the study and study protocol is explained
d) The field supervisor, will supervise the field activities.
e) Pre-test of the tools will be done at the end of the training to ensure uniform understanding by the RAs. Role play will also be practiced.
f) The training material will be provided to the RAs to use as reference materials.
g) Implementation of the selection of households will be monitored by the field supervisor for accuracy. This will be done on an adhoc basis.
h) RAs will use a pre- programmed tool that already has the skip patterns
i) To avoid loss of data, all completed questionnaires will be sent to database which is password controlled. Only the supervisor and the PI will have access to this account. Data will be transmitted real time. Supervisor and PI will be able to monitor the incoming questionnaires

j) By taking the GPS coordinates we will be able to know the spread of the sampled households

k) We have provided a food sampling protocol to ensure the uniformity of getting homogenous food samples

l) A driver on call to collect the food samples as they are collected and stored in a dry place.

m) The team will hold daily debriefs and hand in their tablets data monitoring and overnight charging.

7. Daily field schedule

a) Every morning, all RAs will be transported to the targeted sub-location to start sampling.

b) RAs will work in pairs thus there will be 4 groups in total.

c) By the end of the study period, each pair is expected to collect data from158 households

d) The qualitative data team is expected to have conducted 6 focus group discussions and transcribe the recordings and save them as both word and PDF documents within 5 days. They will then later join the household survey team to assist with data collection.

e) RAs will have the supervisor's and PI's cell numbers for use if need arises

f) The field supervisor will use a Daily logbook to monitor progress Information on each interviewer will be monitored by the supervisor on an ongoing basis.

Daily logbook

Interviewer	No. of respondents approached	Interviews completed	Household IDs	Incomplete interviews	Refusal rates	Non-contact rates

Appendix 11: Training schedule

EVALUATION OF EFFICACY OF FOOD STORAGE AND PREPARATION ON AFLATOXIN MITIGATION: A STUDY OF THE ABANTU, NILO-HAMITES AND NILOTES OF BUSIA KENYA **PRINCPAL INVESTIGATOR: ABIGAEL O. AWUOR**	
Day 1	
8.00 - 11.00 am	• Introductions • Study background and objectives (Public Health and food safety) • PI's expectations of the research team • Terms of Reference • Questions and Answers
11.00 – 11.30am	**Tea break**
11.30 - 1.00pm	• Ethics in field research o Obtaining informed consent o In-depth review of consent forms (English & Kiswahili)
1.00 – 2.00pm	Lunch break
2.00 – 4.30pm	• Study sites • Study approach (Mixed methods) • Overview of Qualitative vs Quantitative • Qualitative methods:- - Key Informant Interviews (KIIs) - (Who to be interviewed, data to be gathered, how data will be gathered) - Focus Group Discussions (FGDS) o Participants, how they are enrolled, data to be gathered and how o Role of the moderators and recorders o Qualities of a good moderator o Recording and transmitting data • Q&A
Day 2	
8.00 – 1.00pm	• Recap • In-depth review of FGD tools (Both English and translated versions) • Role plays and feedback with both moderators leading either FGDs o Introduction and building rapport o Seeking consent o Facilitation and recording o Probing skills • Debrief

2.00 - 4.30pm	• Quantitative methods:- (Household survey & Sample collection) • Household survey o Sampling procedure for Households o Sampling procedure for respondents (inclusion & exclusion criteria) o Study sites (Magombe, Amugura, Okiludu and Buyofu) o Interviewing techniques (Question by question chronologically) o In-depth review of household questionnaire
Day 3	
8.00 – 1.00pm	• Recap of household survey • In-depth review of translated household questionnaire • Test tries on the questionnaires programmed in the tablets • Role play (Administration & questioning o Introduction and rapport o Obtaining consent o Interview skills o Recording/data entry
2.00 - 4.30 pm	• Food sampling o Obtaining a representative food sample o Labelling the sample o Sample handling • Brief on pilot testing o Team formation o Debrief on procedures o Roles of the supervisor
Day 4	Pilot testing - Qualitative
Day 5	Pilot testing - Quantitative
Day 6	Debrief and final preparation for field work