ASSESSMENT OF THE TRADITIONAL AND IMPROVED PROCESSING METHODS IN THE REDUCTION OF AFLATOXIN LEVELS IN MAIZE AND MAIZE PRODUCTS

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DECLARATION

I declare that this thesis is my original work and has not been presented for an award in any other institution.

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A56/64623/2010

This thesis has been submitted with my approval as university supervisor

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TABLE OF CONTENTS

LIST OF ACRONYMS

AOAC-Association of the Official Analytical Chemists

CDC- Centre of Disease Control Aand Prevention

CGIAR- Consultative Group for International Agricultural Research

CPMU-Central Planning and Monitoring Unit

DN- Daily Nation

ELISA- Enzyme-linked Immunosorbent Assay

FAO-Food Agricultural Organization

ICRISAT-International Crop Research Institute for the Semi Arid Tropics

IFPRI- International Food Prolicyduction and Research Institute

KARI-Kenya Agricultural Research Institute

KEBS- Kenya Bureau of Standards

PEA-Project Executing Agency

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Aflatoxins are fungal secondary metabolites produced predominantly by certain strains of the *Aspergillus* molds that if ingested in acute or chronic toxicity and a variety of other adverse effects on both humans and animals. The objective of this study was to investigate the effectiveness of traditional processing methods which included decortication by pounding of wet maize with mortar and pestle, alkaline cooking using Magadi soda and maize cob ash and improved methods which included dehulling in mechanical mill, exposure to solar and artificially generated UV radiation in reduction of aflatoxin in maize. Standardization of alkali cooking was done before the actual treatment of the contaminated samples. Naturally contaminated samples of maize from the National Cereals and Produce Board's stores were sampled according to the standard sampling procedure for cereals and pulses to make a composite sample. Aflatoxin analysis before and after each of the treatments was done to check for their effect on reduction of aflatoxin using ELISA (Boratest® Technology) and expressed as total aflatoxins in ppb, dry weight basis. The results were subjected to statistical analysis at the significance levels 5% ($p \leq 0.05$). The success of any method was based on their ability to lower the levels to below the maximum allowable limits as set by the Kenya Bureau of Standards and East Africa Standard, which is 10ppb for total aflatoxin.

The reduction of aflatoxin was found to be highest when maize was boiled in maize cob ash solution and it resulted in loss of aflatoxin from 83.1±0.3 ppb to 7.0±3.9 ppb. Dry decortication reduced aflatoxin from 51.3±15.3 to 9.6±0.8ppb, boiling in Magadi soda led to a drop of aflatoxin from 59.5±3.818ppb to 13.4±0.424ppb, solar irradiation for 18 hours caused a drop of aflatoxin from 60.8±1.8ppb to 13.7±0.1 ppb, UV irradiation for 18 hours caused a reduction from 81.7±0.5ppb to 61.4±4.5 and pounding in Magadi soda resulted in least loss of aflatoxin from 81.5±0.3 to 72.7±0.2ppb. Aflatoxin loss in all the treatments was significant except for dry decortication. Only dry decortication and boiling in maize cob ash solution brought down the aflatoxin levels to below the maximum allowable limit of 10ppb.

CHAPTER ONE: INTRODUCTION

1.1 Background Information on Aflatoxin Contamination

Aflatoxins are potent toxic, carcinogenic, mutagenic, immunosuppressive agents, produced as secondary metabolites mainly by the fungus *Aspergillus flavus* and *Aspergillus parasiticus* (Reddy et al., 2000).The fungus invades several crop species and under certain environmental conditions produces aflatoxins in standing crop and harvested crop like maize and groundnuts (Lava et al., 2011). In maize particularly, it can occur in the standing crops during the late milk stage when there is high precipitation in the environment or when there is drought stress (Williams et al., 2004). It can also accumulate post-harvest when maize is stored with improper drying and storage in non-ventilated storage or in adverse storage conditions which allow moisture reintroduction either through leakages or damp storage conditions, through insect infestation and high temperatures (Moss et al., 2002, Dowd, 2003 and Hell et al., 2011). *Aspergillus parasiticus* produces four major aflatoxins: B1, B2, G1 and G2, in which the toxicity is in the order of B1 > G1 > B2 > G2 (Abbas et al, 2004 and Domer et al., 2004). Aflatoxins are chemically stable at processing temperatures and are not destroyed completely by boiling water, autoclaving and a variety of food and feed processing procedures (Feuell, 1966).

Maize is the main dietary staple of Kenyans, and it is also one of the crops most susceptible to infection by *Aspergillus flavus* contamination. Kenya is one of the world's hotspots for aflatoxin as shown by the survey done by the International Food Policy Research Institute. From 2004 to 2006, about 200 Kenyans have died after consuming aflatoxin contaminated maize (IFPRI, 2010).

In Kenya, occasional epidemics of aflatoxicosis receive media attention, but awareness of the probability and effects of chronic exposure are low (IFPRI, 2010). The research by KARI and

CIMMYT from the year 2009 with support of ACDI/VOCA showed that high risk and low risk areas are the Eastern and Southwestern Kenya respectively. Highest level of Aflatoxin contamination in a farmer's field was found to be 1455ppb, in Mbooni East in Eastern Kenya (IFPRI, 2010).

In large parts of Eastern and Coastal provinces in the year 2010, hundreds of thousands of bags of maize were condemned for containing more than the tolerable levels of aflatoxin. They were bought by government at low prices for destruction because of contamination (Jacob and Ngetich, 2010).

This massive contamination resulted in the declaration of at least 2.3 million bags of maize unfit for human and livestock consumption and trade, a large loss of revenue to the dismay of the millions of small-scale farmers that depend on the crop for food and income (CGIAR, 2010).

1.2. Problem Statement

There have been frequent contamination of grain with aflatoxin, particularly maize in Kenya, in the past and some outbreaks have resulted in loss of lives (Imungi, 2011). Since the main form of exposure is through diet, the common food preparation methods used before consumption are not capable of reducing aflatoxins to safe levels. These methods are mainly physical separation, washing, fermentation and heating in the presence or absence of water. Boiling or roasting of contaminated maize or preparation of maize pastes from milled products as is usually the practice in many households do not reduce aflatoxins. In fact, fermentation leads to increase in aflatoxin. Consumption of these products by humans and animals lead to aflatoxicoses since aflatoxins are stable at temperatures used during cooking. Animals fed on aflatoxin contaminated products also lead to subsequent exposure to aflatoxins when their products are consumed and therefore are continually exposed to sub-

lethal doses becoming chronic toxicity. Where there has been acute toxicity, death has been reported.

In Kenya maize is the staple food. It is also used industrially for manufacture of animal feed. Maize is the major source of income for the small farmers who constitute the majority and hence loss of maize through aflatoxin is an economic blow to these farmers.

Most small-scale holders do not apply the expert technology of storage and when the crop is contaminated with aflatoxin, they are not well equipped with detoxifying methods. In fact they have been used rarely in Kenya as these methods of detoxification such as ammoniation or ozonization are expensive and not easily available. Also, some of these modern methods like ozonation have been found to decrease the nutritive value of treated meal (Dollear et *al.,* 1968) while the fate of aflatoxin in ammoniated commodities has not been clearly elucidated (Alex, 1966).

1.3 Justification of The Study

In Kenya, aflatoxin has become a chronic occurrence especially in the parts of Eastern and the Coastal regions. Farmers have lost their income from maize, which they depend on for their livelihood because of the contamination (IFPRI 2009, Daily Nation, 2010). It is almost correct to assume that each year; crops will be contaminated with aflatoxin leading to exposure of many households to the toxin through consumption of the staple meals as most people in these regions depend on maize as a staple food with few or no alternatives. Sometimes lives are lost to the aflatoxicosis. They have no alternative food in most cases and consumption of contaminated maize is sometimes a choice to die with.

Efforts have been directed to reduction of the contamination in the post-harvest chain of the crop especially in storage. Contamination may occur despite the efforts directed at prevention

because the handlers are mainly small scale farmers who are not knowledgeable in the dangers associated with consumption of contaminated maize. This problem is aggravated by the fact that most of the maize produced is for domestic consumption. The main aim of the decontamination is to inactivate, destroy or remove the toxin without any change in the nutritive value and food/feed acceptability of the product (Farid et al., 2008).

Due to above reason dry dehulling, Magadi soda decortication, ash decortication and solar radiation could be the most cost effective, easy to use and easily available local methods of reducing aflatoxin contamination if their effectiveness is proven. UV radiation could be exploited for use commercially by incorporating it to the commercial drying system. The small scale holders could be educated on the importance of these methods in reducing aflatoxin if incorporated in their food processing at home and save the lives that could otherwise have been affected or lost through aflatoxicoses.

1.4 Objectives

1.4.1 Main Objective

To assess the effectiveness of traditional and improved processing methods in the reduction of aflatoxin levels in maize and maize products

1.4.2 Specific Objectives

1. Determine the effectiveness of the solar and UV irradiation in the reduction of aflatoxin in maize
2. Determine the effectiveness of cooking in Magadi soda and maize cob ash in the reduction of aflatoxin in decorticated maize kernels
3. Determine the effectiveness of the wet and dry decortications in the reduction of aflatoxin in maize.

CHAPTER TWO: LITERATURE REVIEW

2.1 General Information on Aflatoxin

Aspergillus flavus, a species of mold, is responsible for aflatoxin accumulation in the sub-Saharan African food supply (IFPRI, 2011). Research shows that in areas where aflatoxin is a persistent and serious problem, there is a very high occurrence of one of the most toxic strains of *A. flavus* in the world, the S strain (Claudia et al., 2007).

In grain, the aflatoxin production was thought to be largely a storage problem. However recent studies have shown that aflatoxin was found in corn in the field at all stages of development, from the late milk stage until harvest (Anderson, *2010*).

The *Aspergillus flavus* fungus present in the soil and air can infect maize at any stage from pre-harvest to postharvest storage. Pre-harvest contamination occurs in the field due to growth cracks, mechanical injury and damage by pests to the plant parts or seeds lead to infestation by fungi. Toxins are produced under high temperatures, drought, and terminal water stress prior to harvest (Lava et al., 2011).

Postharvest contamination occurs when fungi continue to grow and produce aflatoxins under high moisture and warm temperatures. This process is enhanced if drying is delayed. Damage by pests can also facilitate mold invasion and toxin production during storage (Lava et al., 2011).

Aflatoxin are chemically stable at processing temperatures and are not destroyed completely by boiling water, autoclaving and a variety of food and feed processing procedures (Feuell, 1966). Studies have shown that sunlight can be very effective in destruction of the aflatoxin in oil (Shantha and screenivasarmuthy 1975, 1977). Aflatoxins are unstable in the presence of

bases (Imungi, 2011) as they cause hydrolysis of the lactone ring in aflatoxin B1 (Joan and Alfredo, 2005)

2.2 Chemical Structures of Aflatoxins

Figure 1 shows the chemical structures of the aflatoxins B_1, B_2 G_1 and G_2. These letters representing them are given because of their colour when exposed to ultra violet light (Farid et al., 2000).

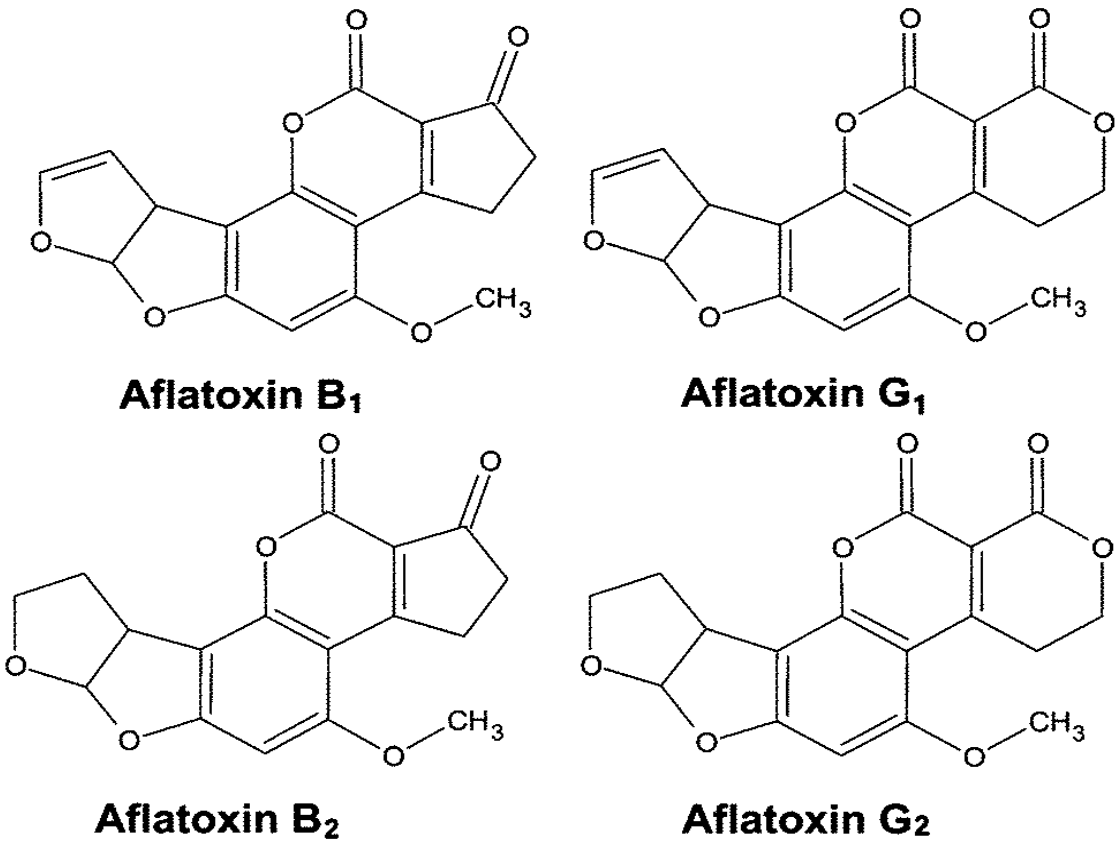

Figure 1 Structures of aflatoxins B_1, B_2, G_1 and G_2

2.3 Properties of Aflatoxin

2.3.1 Physical Properties of Aflatoxin B1, B2, G1 and G2

Aflatoxin B1 (AFB1) is normally predominant in amount in cultures as well as in food products.

a. Pure AFB1 is pale-white to yellow crystalline, odorless solid.

b. Aflatoxins are soluble in methanol, chloroform, acetone, acetonitrile.

c. The aflatoxins fluoresce strongly in ultraviolet light (ca. 365 nm); B1 and B2 produce a blue fluorescence where as G1 and G2 produce green fluorescence(Farid et al., 2000).

Table 1 shows the physical and chemical properties of aflatoxin B1, B2, G1 and G2 (O'Neil et al., 2001)

Table 1: Properties of aflatoxins

Aflatoxin	Molecular formula	Molecular weight	Melting point (^{o}C)
B_1	$C_{17}H_{12}O_6$	312	268-269
B_2	$C_{17}H_{14}O_6$	314	286-289
G_1	$C_{17}H_{12}O_7$	328	244-246
G_2	$C_{17}H_{14}O_7$	330	237-240

2.3.2 Chemical Reactions of Aflatoxins

The reaction of aflatoxins to various physical conditions and reagents has been studied extensively because of the possible application of such reactions to the detoxification of aflatoxin contaminated material.

a. **Effect of heat:** Aflatoxins in dry state are very stable to heat up to the melting point which is very high as it ranges from 237- 289^{o}C (O'Neil et al., 2001). When roasting peanuts, the toxicity of aflatoxin B1 is reduced by 70%, and that of aflatoxin B2 by 45%. Thus, heat treatment cannot be considered as a satisfactory means to eliminate them. However, in the presence of moisture and at elevated temperatures, there is destruction of aflatoxin over a period of time. Although the reaction products have not been examined in detail, it seems likely that such treatment leads to opening of the lactone ring with the possibility of decarboxylation at elevated temperatures (Farid et al, 2000).

b. **Effect of alkalis on aflatoxin**: In alkali solution hydrolysis of the lactone moiety occurs. This hydrolysis appears to be reversible, since it has been shown that recyclization occurs following acidification of a basic solution containing aflatoxin (Alex et al., 1966). At higher temperatures (ca. 100^{o}C), ring opening followed by decarboxylation occurs and reaction may proceed further leading to the loss of the methoxy group from the aromatic ring (Farid et al., 2000).

c. **Effect of acids on aflatoxin:**In the presence of mineral acids, aflatoxin B1 and G1 are converted into aflatoxin B2A and G2A due to acid-catalyzed addition of water across the double bond in the furan ring. In the presence of acetic anhydride and hydrochloric acid the reaction proceeds further to give the acetoxy derivative. Similar adducts of aflatoxin B1 and G1 are formed with formic acid-thionyl chloride, acetic acid-thionyl chloride and trifluoroacetic acid (Farid et al., 2000).

d. **Effect of oxidizing agents:**Many oxidizing agents, such as sodium hypochlorite, potassium permanganate, chlorine, hydrogen peroxide, ozone and sodium perborate react with aflatoxin and change the aflatoxin molecule in some way as indicated by

the loss of fluorescence. The mechanisms of these reactions are uncertain and the reaction products remain unidentified in most cases (Farid et al., 2000).

e. **Reduction:** Hydrogenation of aflatoxin B1 and G1 yields aflatoxin B2 and G2 respectively. Further reduction of aflatoxin B1 by 3 moles of hydrogen yields tetrahydroxyaflatoxin. Reduction of aflatoxin B1 and B2 with sodium borohydride yields aflatoxin RB1 and RB2 respectively. These arise as a result of opening of the lactone ring followed by reduction of the acid group and reduction of the keto group in the cyclopentene ring (Farid et al., 2000).

2.4 Aflatoxin Poisoning

Diet is the major way through which humans as well as animals are exposed to aflatoxins. Apart from this, exposure to aflatoxin can occur through ingestion of contaminated milk containing Aflatoxin M1 (metabolite of AFB1) (Bommakanti and Farid, 2000).

The adverse effects of aflatoxins on animals can be categorized into two general forms;

1) Acute toxicity

2) Chronic toxicity

2.4.1 Acute Toxicity

Acute toxicity is caused when large doses of aflatoxins are ingested (Bomakanti, 2000). At high enough exposure levels, aflatoxins can cause acute toxicity, and potentially death, in mammals, birds and fish, as well as in humans (Richard, 2013). Acute toxicity is common in livestock. The principal target organ for aflatoxins is the liver. After the invasion of aflatoxins into the liver, lipids infiltrate hepatocytes and leads to necrosis or liver cell death. This is mainly because aflatoxin metabolites react negatively with different cell proteins, which leads to inhibition of carbohydrate and lipid metabolism and protein synthesis. In correlation with

the decrease in liver function, there is a derangement of the blood clotting mechanism, icterus (jaundice) and a decrease in essential serum proteins synthesized by the liver. Other general signs of aflatoxicosis are edema of the lower extremities, abdominal pain, and vomiting (Bomakanti, 2000). Although the liver is the principal organ affected, high levels of aflatoxin have also been found in the lungs, kidneys, brains and hearts of individuals dying of acute aflatoxicosis. LD_{50} (lethal dose) values for animals vary between 0.5 and 10 mg/kg body weight (Richard, 2013)

2.4.2 Chronic Toxicity

This is due to long term exposure of moderate to low aflatoxin concentration. The symptoms in animals include decrease in growth rate, lowered milk or egg production, and immuno suppression. There is some observed carcinogenecity, mainly related to aflatoxin B1. Liver damage is apparent due to the yellow color which is the characteristic of jaundice and the gall bladder becomes swollen. Immuno-suppression is due to the reactivity of aflatoxins with T-cells, decrease in Vitamin K activities, and a decrease in phagocytic activity in macrophages. These immunosuppressive effects of aflatoxins predispose the animals to many secondary infections due to other fungi, bacteria and viruses (Robens et al., 1992 and Mclean, 1995).

The incidence of chronic aflatoxicosis in humans is unknown and is almost impossible to estimate because the symptoms are so difficult to recognise. However, human liver cancer is quite common in parts of the world where aflatoxin contamination of food is likely and there may be a link, although this remains unproven (Richard, 2013).

2.5 Aflatoxin Exposure from Maize in Kenya

In developing nations, many people are exposed to aflatoxin through food grown at home. Widespread subsistence farming systems, lack of irrigation, and inadequate drying and storage facilities impede the prevention and detection of aflatoxin in crops (IFPRI, 2011). As

a result, an estimated 4.5 billion people living in developing countries may be chronically exposed to aflatoxin through their diet (CDC, 2004) and at risk of serious health problems. In Kenya, acute aflatoxin poisoning results in liver failure and death in up to 40% of cases. The first reported aflatoxicosis outbreak in Kenya occurred in 1981 (Ngindu et al., 1982)

Multiple aflatoxicosis outbreaks have also been documented since 2004, resulting in nearly 500 acute illnesses and 200 deaths (CDC 2004:Azziz-Baumgartner et al., 2005). Most reported aflatoxicosis outbreaks have occurred among people living in rural, subsistence farming communities in Kenya's Eastern province and were usually associated with consuming homegrown maize (Azziz-Baumgartner et al., 2005, Mwanda et al., 2005, Daniel et al., 2011). In May, 2006, an outbreak of acute aflatoxicosis was reported in a region of Kenya where aflatoxin contamination of homegrown maize has been a recurrent problem (CDC 2012).

Research was done in the year 2010 by CIMMYT and KARI along the entire value chain in Eastern and south western Kenya in the farms just before harvest and post-harvest at the stores, markets and Posho mills which showed that aflatoxin contamination exceeding 10ppb was higher on the south western Kenya than on the eastern part. The contamination levels increased further on both parts by almost double fold after storage. The amount of aflatoxin contamination was as high as 1700 ppb in Eastern and 600ppb in south western (IFPRI, 2011). Cross sectional study done to check on human exposure to aflatoxin in year 2007 in Kenya by Ellen et al. showed that aflatoxin exposure varied by geographical location. From highest to the lowest were Eastern, Coast, Nairobi, Central, North Eastern, Western, Rift Valley and Nyanza. It also showed that aflatoxin levels were higher in urban participants than in rural (Ellen et al., 2013). This therefore means that during outbreak of aflatoxicoses everyone in the country is at risk.

2.5.1 Efforts to Curb Aflatoxin Poisoning in Kenya

As it is realized that absolute safety is never achieved, many countries have attempted to limit exposure to aflatoxins by imposing regulatory limits on commodities intended for use as food and feed (Isman et al., 2009). In Kenya the regulatory body which sets the limits is the Kenya Bureau of Standards and by extension the East African Maize Standard which has set the limit to 10ppb (KS EAS 2:2000). Another effort to reduce aflatoxin exposure in Kenya has been through mopping of maize in the affected areas by the government so that clean maize is distributed like in the year 2010. The problem has been the willingness of the farmers to surrender their maize because it was being purchased at lower price than the aflatoxin free maize.

2.6. Detoxification of Aflatoxin

Aflatoxin-contaminated commodities can be detoxified by a variety of methods based to some extent on economics and the physical and chemical characteristics of the substance being treated. Processes for detoxifying aflatoxin must take into consideration the usual non homogeneity of the contamination. Aflatoxin often is localized in only a small proportion of the contaminated product which may prove an asset in some inactivation methods but poses a difficulty in others (Ciegler et al., 1966).

Numerous strategies for detoxification of aflatoxin have been proposed and some have been extensively researched on. These include physical methods of separation, thermal inactivation, irradiation, solvent extraction, adsorption from solution, biological detoxification by microbial inactivation and fermentation (Denli et al., 2006 and Anderson 2010). Chemical methods of detoxification are also practiced as a major strategy for effective detoxification.

2.6.1 Physical Methods

Physical separation techniques, such as sieving and electronic and hand sorting, have been investigated as possible means for segregating contaminated peanuts and other nut meats. The need for both electronic sorting and hand picking is emphasized, because it is indicated that either method alone is inadequate (Dickens and Whitaker, 1975). Physical separation procedures have proven most successful with peanuts and other nutmeats, since aflatoxin-contaminated kernels are usually damaged, shriveled, or discolored (Natarajan et al., 1975a). Hence, a combination of sieving and electronic sorting should serve to eliminate most of the undesired kernels and leave the remaining nuts virtually free of aflatoxin.

The use of these techniques to segregate contaminated kernels of corn is much more difficult than for peanuts; contaminated corn kernels can appear sound, thus obviating any electronic sorting process. Brekke et al., (1975) found that physical separation methods were generally ineffective for lowering the aflatoxin content of naturally contaminated corn used in the experimental work. Dry cleaning, wet cleaning, density separation, and preferential fragmentation of the grain were evaluated in laboratory tests. Aflatoxin contaminated corn was buoyant in these liquids and could be separated from the sound material, with a significant reduction in overall level of aflatoxin in the non buoyant corn. The need for both electronic sorting and hand picking is emphasized in a recent publication that indicates either method alone could be inadequate (Dickens and Whitaker, 1975). However; physical separation methods are generally ineffective.

Heat treatment of aflatoxins can lead to detoxification. Experiment done by boiling aflatoxin infested maize showed that aflatoxin reduced to a considerable extent (Reddy et al., 2002). Toasting and boiling of the corn during pinole preparation showed that more aflatoxin was

lost in toasting than in boiling (Mendez-Albores et al., 2004). Dry heat has very small change in reduction of aflatoxins.

Other detoxification methods are through the use of radiations. Use of gamma radiation, UV radiations can cause significant reduction of aflatoxin.

2.6.2 Biological Methods

Biological detoxification of mycotoxins works mainly via two major processes, sorption and enzymatic degradation, both of which can be achieved by biological systems (Halazs et al., 2011 and Yan Ni et al., 2007). Live microorganisms can absorb either by attaching the mycotoxin to their cell wall components or by active internalization and accumulation. Dead microorganisms too can absorb mycotoxins and this phenomenon can be exploited in the creation of bio filters for fluid decontamination or probiotics to bind and remove the mycotoxin from the intestine. Enzymatic degradation can be performed by either extra or intracellular enzymes. The degradation can be complete, the final product being CO_2 and water. Alternatively, enzymatic modification can alter, reduce or completely eradicate toxicity (Magan, 2004). Several bacterial species, such as Bacillus, Lactobacilli, Pseudomonas, *Ralstonia* and *Burkholderia species* have shown the ability to inhibit fungal growth and production of aflatoxins by *Aspergillus species* under laboratory conditions. In the year 1960, Ciegler et al., (1966) and Lillehoj et al., (1967), screened over 1000 microorganisms for the ability to degrade aflatoxins and found that only one bacterium, *Flavobacterium aurantiacum* B-184, was able to irreversibly remove aflatoxin from solutions.

2.6.3 Chemical Methods

Chemical methods of detoxification of aflatoxin include oxidation, bleaching, ammoniation, sulphitation among others. Efforts have been made to prove the efficacy of these methods in several research experiments.

Several studies have shown that alkali treatment, using inorganic or organic bases, is an effective and economically feasible method of degrading aflatoxins like the conditions used in making tortillas otherwise called nixtamalization (Hamed, 2006). Alkalis cause the hydrolysis of the lactone ring in AFB; however, evidence suggests that the hydrolyzed lactone ring can close again under acidic conditions and regenerate back to AFB (Mendez-albores et al., 2004).

Treatment with ammonia in gaseous phase, in solution or substances capable of releasing it, achieved optimum results when used with peanut, cotton and corn meals (Piva et al., 1995). Detoxification correlated to quantity used, reaction time, temperature and pressure levels and combination with formaldehyde (Frayssinet et al., 1972)

Sodium bisulfate is less efficient than ammonia (Piva et al., 1995). Main reaction product is sulfonate, called 15α-sodium sulfonate (Hagler et al., 1983, Yagen et al., 1989).

Formaldehyde is efficacious in attacking and neutralizing the AFB1 molecule (Piva et al., 1995). Studies have showed its enhanced efficacy in association with ammonia (Frayssinet et al., 1972) and Calcium Hydroxide (Codifer et al., 1976).

Other substances that seem active versus aflatoxin include some oxidants like Sodium hypochlorite, potassium permanganate, hydrogen peroxide and sodium borate (Piva et al, 1995).

2.7 Methods of Processing and Preparation of Maize in Kenya

Maize food products can be processed at home on a small scale as well as on a larger industrial scale, transforming the raw material into food products. The aim of processing is to provide convenience and extended shelf life. Other products should be consumed immediately after production as they quickly undergo spoilage. Methods of processing and preparation of maize can be local or industrial.

2.7.1 Local Methods

1) **Alkaline cooking**: This is done in Kenya by Kalenjin tribes of Rift Valley province, parts of Nyanza and Western provinces. It involves boiling of the maize kernels in alkaline solutions derived from wood ash or Magadi soda where the pericarp loosens and dissolves. The kernels are then washed to remove the residual ash and the pericarps. The product can be cooked while wet with or without pulses or vegetable. It can also be dried for future use and in rare cases, is milled to flour. This process is usually meant to increase the palatability, convenience and cooking time. Though the practicing communities may not be aware, it reduces phytates and increases the availability of niacin (Muindi et al., 2006).

2) **Wet pounding**: This is done by steeping or soaking of maize kernels in water or alkaline solution to mellow the pericarp. The kernels are then pound using mortar and pestle where the pericarp comes out by impact (Mutungi et al., 2008). During the process, the kernels can be de-germinated, broken into grits and floury particles. The product is dried to ease the separation process which starts with winnowing to remove floury particles and the pericarp which is used as animal feed. The grits are separated from the whole and larger kernels by screening. This can be milled to flour, stored or sold. The main purpose of this process is to increase, palatability and cooking time.

When pounding is done in the presence of traditional leafy vegetable, it increases the nutritional value by introduction of iron and vitamins.

3) **Milling**: milling is done to whole or preprocessed maize. Local milling is done using two stones or manual grinder which yields grits and flour. The end product depends on the end use of the product. The grits is cooked in a way resembling rice. The finer flour can be mixed with other cereal flours for use in the preparation of pastes which can be fermented to make sour porridge while fresh flour is used for making pastes like *ugali* and porridge depending on the consistency.

4) Cooking: In Kenya, methods of preparation for consumption vary with communities. Some mix it with beans and then boil. After boiling, it is either salted or eaten directly or stewed with vegetables to improve the flavor. Sometimes the maize could be toasted.

2.7.2 Industrial Maize Processing

The maize kernel is transformed into valuable foods and industrial products by two processes, dry milling and wet milling. The first yields grits, meal and flours as primary products. The second yields starch and valuable derived products (Jeffry and Maria, 2013).

1) **Dry milling:** Industrial dry milling includes particle size reduction of clean whole maize with or without screening separation, retaining all or some of the original maize germ and fiber (Brubacher, 2002). Depending to a large extent on particle size, products are classified into flaking grits, coarse grits, regular grits, corn meal, cones and corn flour by means of meshes ranging from 3.5 to 60. Their chemical composition has been well established and their uses are extensive, including brewing, manufacturing of snack foods and breakfast cereals and many others (Jeffry and Maria, 2013).

Because of the high-fat content, whole or partially de-germinated maize products are not particularly shelf stable. Therefore, de-germination of maize is done and this involves mechanical separation and processing, resulting in dry shelf-stable products with a majority of both germ and fiber removed. Much of the particle size reduction and separation is accomplished with equipment similar to that employed in wheat flour milling, including hammer mills, stone mills, roller mills, screeners, sifters, specific gravity separators, and aspirators. Specialized equipment, such as de-germinators and de-hullers or peelers, may be employed in maize processing. Generally, whole, partially de-germinated, and de-germinated maize products require additional processing before consumption. These processing steps may be accomplished in a large-scale industrial setting, small-scale local processor, or in the home. These secondary processes may include addition of other ingredients along with thermal processing, including boiling, drying, frying, or baking, all of which can affect the nutritional attributes of the finished product (Jeffry and Maria, 2013).

2) **Wet milling**: In this process clean maize is soaked in water under carefully controlled conditions to soften the kernels. This is followed by milling and separation of the components by screening, centrifugation and washing to produce starch from the endosperm, oil from the germ and other valuable byproducts such as maize gluten meal and feed. Maize is separated into relatively pure chemical compound classes of starch, protein, oil, and fiber. The products and co-products obtained from the process are not typically directly used by the consumer and often require further industrial processing before consumption (Jeffry and Maria, 2013).

   The starch is used as a raw material for a wide range of food and non-food products. It has industrial applications such as production of alcohol and food sweeteners by

either acid or enzymatic hydrolysis. The latter is done with bacterial and fungal alpha-amylase, glucoamylase, beta-amylase and pullulanase. Saccharides of various molecular weights are liberated yielding sweeteners of different functional properties. These include liquid or crystalline dextrose, high-fructose maize syrups, regular maize syrups and maltodextrins, which have many applications in foods (Jeffry and Maria, 2013).

The prevalence of large-scale, industrial processors in Kenya contributed to preferences, particularly in urban areas, for dent types. The removal of the germ and pericarp makes refined meal look whiter, last longer, and taste sweeter than whole meal. Hammer-milled, whole meal remains the primary staple food in the grain self-sufficient rural areas (Melinda and Thom, 2003).

CHAPTER THREE: MATERIALS AND METHODS

3.1. Collection of Samples

Bagged maize which had been mopped up from farmers after it was confirmed was aflatoxin positive was randomly sampled from the National Cereals and Produce Board stores (harvest of the year 2010) by spiking randomly using sack-type spear to 90kg bulk sample(KEBS 2008-KS EAS 79:1999). This sample was then mixed thoroughly to homogenize it using sample divider cum homogenizer which mixes the sample. The initial moisture content of the samples was analyzed using the oven drying method as per the AOAC method (AOAC, 1999). The bulk sample was then divided into six parts each of 15kgs for the six treatments i.e. wet decortications, dry decortications, boiling in Magadi soda, boiling in maize cob ash, solar irradiation and UV irradiation. The samples were then kept in the dark during the experiment period.

3.2. Methods of Processing and Preparation

3.2.1 Decortication by Boiling in Magadi Soda Solution

1) Pretrial process

The process was standardized by a pretrial process, where progressive weights of Magadi soda of 25g, 50g, 75g and 100g were admixed with 250g of maize and 750ml of water and boiled till the testa dissolved readily. The time in minutes needed for effective decortications in each of the weights of Magadi soda was recorded. Corresponding pH's of the solutions at each of these weights of the Magadi soda was also measured at the room temperature. To ensure the optimization of the process, the least weight Magadi soda that can do decortications at the shortest time possible was taken which would then be used in the actual treatment of the contaminated sample.

2) **Treatment of the contaminated sample**

The contaminated maize to be boiled in Magadi soda was hand sorted to remove rotten diseased and discolored maize then passed through the sieve to remove any foreign matter using 4mm diameter sieve. Before boiling in the Magadi soda solution, the initial aflatoxin content of the sample was analyzed and then 250g was weighed, washed in water to remove surface dirt and then mixed with the weight of the Magadi soda which resulted in the least time as in the pretrial process. Three quarter liter (750mls) of water was added to the mixture and then brought to a heat source to boil for the length of time corresponding to the weight that brought the least time of decortication. When it was ready, the mixture was washed serially four times in water where 500ml of water was used in each washing while being rubbed gently between the palms to remove the dissolved and loose pericarp. From the cleaned sample, 50g was taken for aflatoxin analysis. This process was done twice.

For the control experiment of this treatment, the aflatoxin-contaminated sample was boiled in750ml of tap water (without Magadi soda) for the length of time that the sample was boiled and then washed serially four times in water where 500ml water was used in each washing while being rubbed gently between the palms just as for the sample which was boiled in Magadi soda. This was also done twice.

3.2.2 Decortication by Boiling in Maize Cob Ash

1) Pretrial process

The standardization of this treatment was done through a pretrial process, where 50g, 75g, 150g and 200g of ash were dissolved in 1500 milliliters of water then 500g of maize grains was weighed and boiled in the respective solution where the effectiveness of decortication was observed by taking a cooked grain and rubbing against the thumb and the index finger to feel the ease with which the pericarp comes off. The time needed for effective decortications

was recorded in minutes and corresponding pH's of these solutions at every weight of ash was taken. To ensure the optimization of the process, the least weight maize cob ash that can do decortications at the shortest time possible was taken which would then be used in the actual treatment of the contaminated sample. The process was carried out thrice.

2) Treatment of the sample

The contaminated sample was hand sorted and then passed through a 4mm meshed sieve to remove foreign matter. After the analysis of the initial aflatoxin content, 500g of this sample was admixed with the least amount of ash that resulted in the shortest time of decortication as was obtained during the standardization step above. When the sample was ready, it was decorticated by rubbing off the pericarp between the palms and then rinsed serially in 4 liters of water with 1litre in each rinse until the cleaning water became clear. The final aflatoxin was then measured.

Five hundred grams of the control sample was cooked in 1500ml of pure water for the length of time same as the one used for the ash-cooked sample and then washed 4 times in 1 liter of water per wash as in figure 2 below. The final aflatoxin content of the sample was then measured.

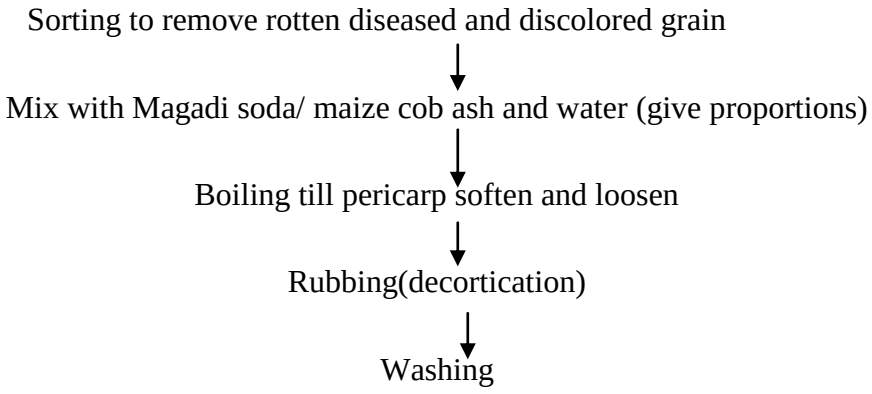

Figure 2: Flow chart for decortication by boiling in Magadi soda and maize cob ash

3.2.3 Machine Decortications

Two kilograms of contaminated maize was cleaned by screening through 4mm sieve. The initial aflatoxin content of the sample was measured before taking it for de-hulling using maize de-huller at the Estate Posho mill in Rabai road, Buruburu Estate where the locals mill their grains. The de-hulled sample was then passed through sample divider and homogenizer. Hundred grams of this sample was ground and tested for aflatoxin. The process was carried out twice.

3.2.4 Solar Irradiation

Moisture content of contaminated maize sample each 2Kgs, was analyzed and then spread on a black plastic sheet of $1M^2$ to one layer thick on the open sunlight for 24 hours which was done in three phases of 6 hours a day at the National Cereals and Produce Board's open field Doonholm, Outering Road. In each day, the sample was exposed to the sunlight for 6 hours, from 10am to 4.00pm preferably when the sun is bright. Aflatoxin content for the samples was taken after every 6 hours. Moisture content was also analyzed after each of the phases.

This exposure to the bright sunlight was carried out on 8^{th}, 9^{th} and 10^{th} February 2012 when there was plenty of sunshine while the second experiment was carried out on19^{th}, 20^{th}, and 21^{st} of March 2012 of which it was partly cloudy. This was carried out to check how the aflatoxin reduction occurred in partial sunshine and in bright sunshine.

The strength of the solar radiations for each of these days was obtained as average values of radiation per day from the solarimeter stationed at Jomo Kenyatta International Airport courtesy of Kenya Meteorological Department Headquarters, Nairobi. This was to ascertain the relationship between the strength of the solar radiations [affected by the cloud cover (insulation)] and the time of exposure with the loss of aflatoxin.

3.2.5 Wet Pounding Using Mortar and Pestle

About two and half kilograms maize sample was weighed accurately and sorted to remove extraneous matter, diseased, rotten and discolored grains. After taking the initial level of aflatoxin, 2kgs of the maize was weighed and placed in wooden pestle where it was sprinkled with 250ml of water containing 150g of Magadi soda without giving time to soak. The conditioned maize was then pound on a mortar using a pestle where the pericarp chipped off through abrasion and impact. The decorticated maize was then dried briefly in cold air in the absence of light so as to allow easy separation of the pericarp from the decorticated grains during winnowing. The final moisture and the aflatoxin content of the sample after the treatment were analyzed. The flow chart of each of the steps in wet pounding using pestle and mortar is shown in the Figure 3.

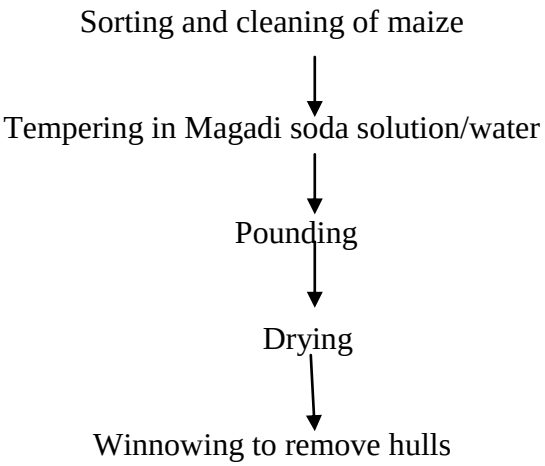

Figure 3: Flow chart for maize processing using mortar and pestle (wet pounding)

3.2.6 UV Irradiation

Two hundred grams of sorted contaminated maize sample in which the initial aflatoxin levels had been determined was spread on a black polythene sheet at one layer thick. It was then exposed to the ultra violet radiations from the UV lamp at wavelength of 346nm for 18 hours. This was achieved through daily exposure for 6 hours for three days where 50g was picked

for aflatoxin analysis and moisture determination. The remaining sample was stored in the dark before the next exposure.

3.3 Analytical Methods

3.3.1 Determination of Moisture Content

The moisture content was analyzed using oven drying method. Empty dish and the lid were dried in the oven (model) at 105°C for 3 h and then transferred to a desiccator to cool. The empty dish and lid were then weighed. 3 g of sample was weighed to the dish and spread to the uniformity. The dish with sample was placed in the oven to dry for 3h at 105°C. After drying, the dish was transferred with partially covered lid to the desiccator to cool. The dish and its dried sample were then reweighed. Moisture content was expressed as percentage weight loss.

Moisture (%) = (W1-W2)/W1 x 100

Where: W1 = weight (g) of sample before drying

W2 = weight (g) of sample after drying

3.3.2 Measurement of pH

The pH of the maize cob ash and Magadi soda solutions used for cooking the kernels was measured in a calibrated digital pH meter, model-PHS-3B at the room temperature.

3.3.4 Aflatoxin Analysis

Aflatoxin testing for the samples was done using Enzyme Linked Immunosorbent Assays (ELISA) as outlined in the training manual courtesy of Boratest® Technology.

a) **Sample preparation and extraction**: Fifty grams of sample was ground until became fine using commercial blender which grinds and mixes the sample to uniformity. From the ground sample, 10g was weighed on a piece of aluminum foil and then transferred into 50ml beaker where it was mixed with 50ml of methanol water. Ten milliliters of hexane was added then mixed thoroughly for 30 minutes using a magnetic stirrer. Ten milliliters of the mixture was centrifuged at 1500rpm (speed 8) using a Digisystem Laboratory Inc. centrifuge for 10 minutes. From the lower methanol –water layer, 3ml was extracted and mixed thoroughly on a vortex. Four hundred microlitres (400µl) sample extract was pipetted into a mixture of 1600µl phosphate buffered saline (PBS) and 2000µl methanol: PBS (10:90) in a mixing vial. This was used for ELISA step 3.3.4(b)

b) ELISA analysis: The required number of ELISA micro-strips, enzyme conjugate and enzyme substrate were removed from the refrigerator and placed at room temperature for 30 minutes.

Aflatoxin standards (0ppb, 5.6ppb, 15.7ppb and 50) were prepared by serial dilution of the calibrated standard as follows:

Five disposable test tubes were removed and marked as N, S1, S2, S3 and S4

Table 2 shows the volume of the diluents and the volume of the aflatoxin standards to be placed in each of the marked tubes

Table 2: The respective dilutions for the aflatoxin standard

Tube Number	Volume of diluents (methanol: PBS, 10:90)	Volume of aflatoxin solution to be added
N	1000µl	10µl of provided aflatoxin standard
S1	2000µl	20µl of solution in tube N
S2	1000µl	500µl of solution in tube S1
S3	1000µl	500µl of solution in tube S2
S4	1000µl	Nothing

Enzyme conjugate was prepared by diluting 10µl enzyme conjugate concentrate in 10ml of PBS and mixed gently at 3000rpm on a vortex mixer- Model- VM-1000- Digisystem Laboratory Instrument Inc. ELISA micro-strips were washed two times with distilled water and semi-dried. Fifty microliters (50µl) of the standards prepared in step one above was transferred in duplicates and sample extracts in designated wells where 50µl of the diluted enzyme conjugate was added to all these wells.

ELISA micro-strips were then incubated for 60 minutes at room temperature in the dark and thereafter emptied; washed three times and semi-dried. Hundred microliters of substrate solution was added to each well. Color was allowed develop for 8 minutes after which the color reaction was stopped by adding 100µl of stopping solution to each well.

Using an ELISA reader Stat fax 3200-spectrophotometer model, the absorbance (intensity of color) was read in all micro-plate wells and the aflatoxin levels obtained as was displayed on the screen. Levels of aflatoxin were calculated in parts per billion (ppb) of the sample.

3.4 Statistical Analysis of Data

Data showing the final aflatoxin content for maize exposed to sunlight, UV light, Magadi Soda and maize cob ash were subjected to one way ANOVA using Genstat® Discovery 13thEdition at 95% confidence interval ($P \leq 0.05$). Variable means for measurements showing significant differences in the ANOVA were compared using the LSD. Values were judged to be significantly different by LSD if $P < 0.05$.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1. Cooking in Magadi Soda (Pretrial)

The change in the pH of the solution with the increase in the concentration of Magadi soda is shown in Table 3. Table 3 also shows how the concentration of dissolved Magadi soda affects the amount of time needed for effective decortication.

Table 3: The relationship between the concentration of Magadi soda, pH change and the time needed for effective decortications

Weight of Magadi Soda(g/l)	*pH of solution	*Time taken for effective decortications(mins)
33.3	10.2 ± 0.3^{a}	50.5 ± 0.7^{a}
66.7	10.1 ± 0.1^{a}	42.0 ± 1.4^{b}
100.0	10.0 ± 0.1^{a}	33.0 ± 1.4^{c}
133.3	9.9 ± 0.2^{a}	33.5 ± 0.7^{c}

Figures with the same superscript in the pH column are not significantly different (p=0.539) and for time column (p<0.001)

***(mean ± SD), N=3**

Increasing the concentration of the Magadi soda did not cause significant change in the pH of the solution (p=0.539). The highest pH attained at the lowest concentration of the Magadi soda of 33.3g/liter did not differ significantly from the lowest pH attained with the highest concentration of 133.3g/ litre. When concentration of Magadi soda is increased, its pH seemed to reduce. The reduction is however not statistically significant, because of the common ion effect according to Le Chatelier's, principle (Harwood et al., 2007). This shifts the equilibrium toward the reactants and they continue to be less and less soluble.

Nevertheless, Magadi soda solution exhibits alkaline properties and that is why it has found its use in the softening of the pericarp of cereal and legume grains such as sorghum, millet, maize and beans so that they can be cooked quickly (Muindi et al., 2006).

From Table 3, when the concentration of Magadi soda was increased from 33.3 g/litre to 100g/litre, the time needed to soften the pericarp reduced significantly from 50.5±0.7 to 33.3±1.4 minutes. Concentration of Magadi soda at 100g/litre of water was therefore found to be the optimum for effective decortications of 250g of maize as it resulted in the least time. This ratio combination would be recommended for decortication.

4.2. Decortication Using Maize Cob Ash Infusion (Pretrial)

Maize cob ash was found to be strongly alkaline when dissolved in water as it exhibited pH above 11 from the first suspension containing 33.3g/l. The pH continued to rise as more ash was added to the suspension. The pH of the ash suspension containing 33.3g of maize cob ash/liter of water was 11.6±0.7. There was no significant change in alkalinity when the amount of ash was increased to 50g/l. However, when the amount of ash was doubled to 100g, the pH increased significantly to 12.0±0.0 and increased further to 12.5±0.1 when the ash was raised to 133.3g/litre but this time not significantly.

The time needed for effective decortication decreased significantly ($p<0.001$) as the amount of ash was increased. The time needed for effective decortication was 49.7±1.5minutes with 50g of ash per litre. When the amount of ash was increased to 75g, the time needed for effective decortication reduced to 39.3 ±2.1 minutes even though the pH did not change. As the amount of ash was increased to 150g, the time needed for effective decortication reduced to 25.3±0.6 minutes. When the amount of ash dissolved is increased to 200g, the time needed for effective decortication is reduced to 19.0 ±1.0 minutes. When using maize cob ash, the

lesser the amount of ash in the solution, the longer the time needed for effective decortication and vice versa.

The pH changes in relation to the amounts of maize cob ash dissolved in 1litre of water and the time of boiling to effect easy removal of the pericarps are shown in Table 4.

Table 4: The relationship between the amounts of maize cob ash in water, the pH values, the time for effective decortication and the color of decorticated kernel

Weight of maize cob ash in g/l of water	*pH of solution	*Time for effective decortications(minutes)	Texture and color of the decorticated kernels
33.3	11.6 ± 0.7^{a}	49.7 ± 1.5^{a}	Soft and darkly colored orange
50.0	11.7 ± 0.1^{a}	39.3 ± 2.1^{b}	Slightly soft and slightly orange
100.0	12.0 ± 0.0^{b}	25.3 ± 0.6^{c}	Hard and colored bright yellow
133.3	12.5 ± 0.1^{c}	19.0 ± 1.0^{d}	Hard, bright and creamish yellow

Figures with the same superscript in a column are not significantly different ($p \leq 0.001$)

***(mean ± SD), N=3**

Table 4 also shows the color of the decorticated maize associated with different levels of ash. When maize kernels are cooked in alkaline solutions, a yellowish orange color develops. The intensity of the color developed is affected by the concentration and the length of cooking. In this experiment, when less ash was used the time required to decorticate the maize increased. This resulted in the softening of the kernel due to overcooking and hence more penetration of the ash into it. Increased penetration of ash into the kernel makes the ash harder to rinse off. This leads to undesirable change in the flavor (soapy) and darkening of the endosperm. Using higher amount of ash on the other hand resulted in brightly colored and hard kernel because

the cooking time is short and does not cause undesirable softening of the kernel. This results in less penetration of ash into the endosperm making it easier to rinse off.

Using 133.3g of ash/liter of water resulted in the shortest time needed for effective decortication i.e. 19 ± 1.0 minutes and has the highest pH value of 12.5 ± 0.1 which is strongly alkaline. This is the most optimum point in decortication using maize cob ash solution. This weight of ash and cooking time combinations recommended as a standard procedure. Higher ratios of ash to water more than133.3g effects higher pH can be considered uneconomical in the use of ash. In addition to this, the suspension becomes too thick to boil and sticks onto the pan as was noted during the experiment. Lower levels of ash changes the palatability and the resulting color of the decorticated kernels.

4.3 Effect of Cooking in Maize Cob Ash and Magadi Soda Infusions on Aflatoxin

The effect of cooking with Magadi soda and maize cob ash infusion on aflatoxin levels are shown in Table 5 in comparison with the control samples which were boiled in pure water.

Table 5: The effect of boiling contaminated maize in the Maize cob ash and Magadi soda solution on the final aflatoxin levels

		Control (water)		Alkaline cooked	
Cooking Medium	***Initial Aflatoxin(ppb)**	***Final Aflatoxin(ppb)**	**Percent Drop**	***Final Aflatoxin(ppb)**	**Percent drop**
Magadi Soda(100g/l)	59.5 ± 3.8^{a}	44.1 ± 0.3^{b}	25.9	13.4 ± 0.4^{c}	77.5
Maize cob ash(133.3g/l)	83.1 ± 0.3^{a}	40.2 ± 0.3^{b}	51.6	7.0 ± 3.9^{c}	91.6

*Figures with the same superscript in Magadi soda and maize cob ash cooking row are not significantly different ($p<0.001$), LSD=7.155 and7.183 respectively.
***Mean ± SD (N≥3)**

Boiling of the contaminated grains in Magadi soda led to a significant decrease in the level of aflatoxin from 59.5±3.8ppb to 13.4±0.4ppb. This translates to a loss of 77.5%. On the other hand, the loss of aflatoxin in the control sample was from 59.5±3.8ppb to 44.1±0.3ppb, which is 25.9%.

Boiling of the sample in the maize cob ash solution led to a significant decrease in the amount of aflatoxin from 83.1±0.3ppb to 7.0±3.9ppb which is a loss of 91.6%. Boiling of the same sample in water (control) led to decrease of aflatoxin from 83.1±0.3ppb to 40.2±0.3ppb which is a loss of 51.6%.

When maize kernels were cooked in Magadi soda and maize cob ash solutions to effect decortication, the loss of aflatoxin was probably partly due to alkali degradation and leaching of the aflatoxins into the wash waters because even in the absence of the two alkalis, there was significant aflatoxin loss as shown by the control samples. Boiling of contaminated maize reduces aflatoxin to a considerable extent (Reddy et al, 2002). The difference in the aflatoxin loss between the samples boiled in water (control) and the alkali cooked samples can be accounted for by the action of these alkalis on aflatoxin. Alkalis are capable of disrupting the lactone ring of the aflatoxins when exposed to it (Joan and Alfredo, 2005). The aflatoxin lost during the cooking process could also be attributed to high temperatures used. High temperatures (ca. 100^{o}C) lead to ring opening followed by decarboxylation and reaction may proceed further, leading to the loss of the methoxy group from the aromatic ring (Farid et al, 2000).

Use of other alkalis like wood ash and calcium hydroxide in the nixtamalization of maize for tortillas by Mexicans also led to decrease of aflatoxins significantly (Albores- Mendez et al 2004, Hamed, 2006). The difference however between the Mexican corn nixtamalization and the traditional process as is practiced by some Kenyan communities is that in the latter, there

is no steeping of maize in the alkali. Boiling is done directly because steeping led to deep discoloration of the endosperm which was undesirable as it increased residual alkali in the endosperm and hence alteration of the kernel flavor when cooked (Hamed, 2006).

When Magadi soda and maize cob ash were compared in their ability to bring down aflatoxin content in the contaminated samples during the alkali cooking, maize cob ash was proven to be more effective than Magadi soda. Maize cob ash reduced aflatoxin levels to below the maximum allowable limit of 10ppb (KS EAS 2:2000) from 83.1ppb to 7ppb. Magadi soda reduced the levels of aflatoxin to 13.4ppb which is very close to the tolerance level of 10ppb. During cooking for consumption, it is possible that the levels would fall below the tolerance.

4.4. Solar Irradiation

Tables 6a and 6b show the relationship between the solar irradiation and the time of exposure on the level of aflatoxin during bright sunshine and partial sunshine.

Table 6a: Relationship between time of exposure to the sunshine and the aflatoxin level during bright sunshine

Time(hrs) of exposure	Average solarimeter reading (MJ/M^2)	*Aflatoxin content(ppb)	Percentage drop in aflatoxin
0	0	62.1 ± 1.0^a	0
6	27.4	16.7 ± 0.4^b	73.1
12	26.2	13.6 ± 0.1^c	78.1
18	26	13.8 ± 0.6^c	77.8

Figures with the same superscript in the final aflatoxin column are not significantly different ($p<0.01$, LSD=3.501)

***(mean ± SD), N=3**

NB: the initial aflatoxin content of each subsequent phase is the final aflatoxin content of previous phase. Initial aflatoxin at time zero (0) is the aflatoxin content during the beginning of the experiment.

During the process, in the first test when there was bright sunshine, the exposure to the sunlight for the first 6 hours at a radiation of 27.4MJ/M^2 reduced the total aflatoxin content from 62.1±1.0 to 16.7±0.4 ppb which is 73.1% reduction. Additional exposure to sunlight for another 6 hours at a radiation of 26.2MJ/M^2 caused further drop of aflatoxin content from 16.7 ± 0.4ppb to 13.6±0.1ppb, (78.1%) reduction. Further exposure for additional 6 hours did not cause further change.

Table 6b: Relationship between time of exposure and the aflatoxin level during partial sunshine

Time (hrs) of exposure	Average solarimeter reading (MJ/M^2)	*Aflatoxin content(ppb)	Percentage drop in aflatoxin
0	0	59.5 ± 0.1^a	0
6	23.3	16.3 ± 0.4^b	72.6
12	23.5	16.3 ± 0.6^b	72.6
18	26.1	13.6 ± 0.8^c	77.1

Figures with the same superscript in the final aflatoxin column are not significantly different ($p<0.001$)

***(mean ± SD), N=3**

In the second experiment which was done in partially cloudy weather, the first 6 hours of exposure at a radiation value of 23.3 MJ/M^2 caused the total aflatoxins to drop significantly from 59.5±0.1ppb to16.3±0.4ppb which is a percentage drop of 72.6%. Further exposure to radiation of 23.53 MJ/M^2 for further 6 hours did not cause significant drop in the level of aflatoxin at $p<0.001$. However when the amount of radiation is increased to 26.1 MJ/M^2, the aflatoxin levels dropped significantly from 16.3±0.6 ppb to13.6±0.8 ppb which is a drop of 77.1%.

The two experiments carried out in bright and partial sunshine proved that the drop of aflatoxin in the contaminated maize was being affected by the strength radiations from the sun which was affected by the cloud cover. Where the radiations were stronger, the reduction of aflatoxin was higher on exposure to the sunlight (Table 6a) as compared to where strength of radiations was lower (Table 6b). It was also proven that the loss of aflatoxin in whole maize was significant in the first 6 hours and thereafter, further exposure caused very small or no change in aflatoxin levels. Though it reduced, aflatoxin in maize kernels could not be destroyed completely by the sunlight.

In whole grains, the destruction of aflatoxin by the sunlight could be limited by the size of the grains since a similar experiment done using ground contaminated animal feed showed that aflatoxin was almost completely destroyed when exposed to the sunlight. In this experiment however, the strength of the solar radiation was not measured (Gowda et al., 2005). The reason why aflatoxin could not be destroyed completely in whole kernels could be because in whole maize grain, aflatoxin can be found in the inner parts of the kernels. Fractions of the contaminated maize kernels showed that aflatoxin was present in each one of them though in different levels. The bran had the highest aflatoxin levels as compared to the endosperm (Miren et al., 2008).Considering its size, it therefore becomes very difficult for the sun's radiations to penetrate deeper parts of the kernel where aflatoxin could be embedded and hence the constant level of aflatoxin despite increased time of exposure to the sunlight.

4.5 Effect of Artificially Generated UV Radiation

Table 7 shows the change of aflatoxin levels (dry weight basis) with time during the exposure to UV radiation at a wavelength of 346nm and a constant energy of $1.166 * 10^{-19} MJ/M^2$. The initial aflatoxin level is at the aflatoxin at zero (0) hour of exposure.

Table 7: Change of aflatoxin levels with time of exposure to UV radiation

Time of exposure (hrs.)	*Final mean aflatoxin content(ppb)	Percentage drop in aflatoxin
0	81.7 ± 0.5^{a}	0
6	64.3 ± 3.3^{b}	21.3
12	62.2 ± 3.4^{b}	23.9
18	61.4 ± 4.5^{b}	24.8

***(Mean ± SD), N=3**

Figures with the same superscript in the final aflatoxin column are not significantly different (p=0.01), LSD=9.08

Exposure of the UV light to the contaminated maize in the first 6 hours caused a slight decrease in the level of aflatoxin from 87.7 ppb to 64.3 ppb which is a decrease of 21.3%. Further exposure for more than 6 hours did not cause a significant change in aflatoxin levels ($p<0.01$). The total loss of aflatoxin during the 18 hours is 20.3 ppb. UV light was proven to reduce aflatoxin to a very small extent in contaminated whole maize kernels leaving it far above the recommended safe levels of 10ppb. The change in aflatoxin is only significant for the first 6 hours of exposure and thereafter, there is no further change. The possible reason for this could be that during the first 6 hours, the aflatoxin which was found topically had been destroyed so that even if the exposure to the UV light continued, there would be no significant change. These could be attributed to the opaque nature of the kernels where only the topical aflatoxin was removed. The results of this experiment agree with a test done on peanut which showed that when peanut meal was exposed to 8 hours of UV radiation, there was no change in the aflatoxin levels while peanut oil (transparent) exposed to the same radiation for 2 hours showed a 45 % decrease in aflatoxin levels (Samarajeewa et al., 1990). Santha and Screenvasamurthy (1977) also found out that UV light removed 85% of the aflatoxin in peanut oil after 8 hours of exposure and 30-40% in 2 hours (Santha and Screenvasamurthy, 1977).

4.6 Dry Mechanical Dehulling and Wet Dehulling In Mortar and Pestle

Table 8shows how wet dehulling by pounding using Magadi soda solution, pure water (control) and machine dehulling using convectional Posho mill affect the level of aflatoxin in de-hulled maize. Aflatoxin levels are expressed on dry weight basis.

Table 8: Effect of wet and dry dehulling on aflatoxin levels

Decortication type	*Initial aflatoxin level (ppb)	*Final aflatoxin level(ppb)		Percentage drop in aflatoxin
		Control	Magadi/machine	
Wet	81.5 ± 0.3^{a}	80.2 ± 0.2^{b}	72.7 ± 0.2^{c}	10.8
Dry	51.3 ± 15.3^{a}	n.a	9.6 ± 0.8^{a}	81.3

Figures of the same superscript in a row are not significantly different for wet decortication and dry decortication ($p<0.001$, LSD=0.7576 and $p=0.063$, LSD=47.17)

***(Mean ± SD), N=3**

Decortication of contaminated maize containing 81.5 ppb of aflatoxin in the presence of Magadi soda reduced the aflatoxin level by 10.8 %. On the other hand, doing decortication using pure water (control) reduces aflatoxin by 1.6% which shows that pounding maize with Magadi soda solution at room temperature affects reduction of aflatoxin to small extent. This is a small change compared to boiling in Magadi soda to remove the pericarp, which proves that heat catalyzes the breakdown of aflatoxin. Though the final aflatoxin level in both cases are statistically different from the initial one, this change is still far above the minimum level of aflatoxin as set by the East African Maize Standard which is 10 ppb. This agrees with the results by Mutungi which showed that pounding of the contaminated maize soaked in Magadi did not reduce aflatoxin by a significant level (Mutungi, 2006). The aflatoxin reduction is however slightly greater with the use of Magadi soda than in control which showed that there was alkaline deactivation of aflatoxin.

Another reason for the slight drop in the aflatoxin levels in this treatment could be attributed to the fact that since the process involved the use of water, diffusion of aflatoxin to inner parts of the kernel was most likely since aflatoxin is soluble in water. At the end of wet decortication, while some will have been removed with the bran, much would have also diffused to the decorticated kernels and especially since the process leads to cracking and breaking of the kernels due to impact.

Machine decortication of contaminated maize with aflatoxin level of 51.3ppb reduced the toxin level by 81.3% to 9.6 ppb which is below the minimum tolerance level set by the East African Standard which is 10ppb. Statistically, this change is insignificant because of the large variance of the initial aflatoxin levels which brought about a very large standard deviation in the final aflatoxin of the decorticated samples which is due to the non-homogeneity in aflatoxin distribution in the infested maize kernels (Whitaker, 2003). Aflatoxins are usually concentrated in small pockets of which when one of the grains is picked from this pocket, considering the size, it can bring a very large difference.

The drop of the aflatoxin levels in the contaminated maize during dry dehulling agrees with the findings of similar researches done before. Dehulling maize grains and wheat could reduce aflatoxin contamination by up to 92% and 95% respectively (Schroder et al., 1968, Siwela et al., 2005). In both cases, artificially contaminated maize was used during the research as opposed to naturally contaminated as one used in this experiment, which could be the reason for the slight difference in the drop of aflatoxin levels between these findings and theirs. In artificially contaminated maize, all aflatoxins would almost be found topically and hence easily removed by dehulling. The drop in aflatoxin in maize kernels during decortication is dependent on how deep the aflatoxin has diffused because aflatoxin that has diffused deep into the endosperm would be very difficult to be reduced by removing the outer

coat of the maize. The depth of the contamination in naturally contaminated grains is dependent on the stage of maturity during infestation of the mold, presence of cracks or any form of mechanical damage; insect infestation and the length of exposure of the grains to the mold. The advantage of dry decortication is that aflatoxins neither have a chance to diffuse to other grains nor be re-enumerated to the grains because no moisture is introduced to support mold growth. The effect of laboratory and industrial debranning in reducing mycotoxin content is extremely variable in different fractions depending on the length of time it is done (Federicia et al., 2013). This applies to both dry and wet milling.

4.7 Summary and Comparison of The of The Methods of Processing and Preparation

Figure 4 shows the effect of the processing methods in reduction of aflatoxin expressed as percentages of the initial aflatoxin levels.

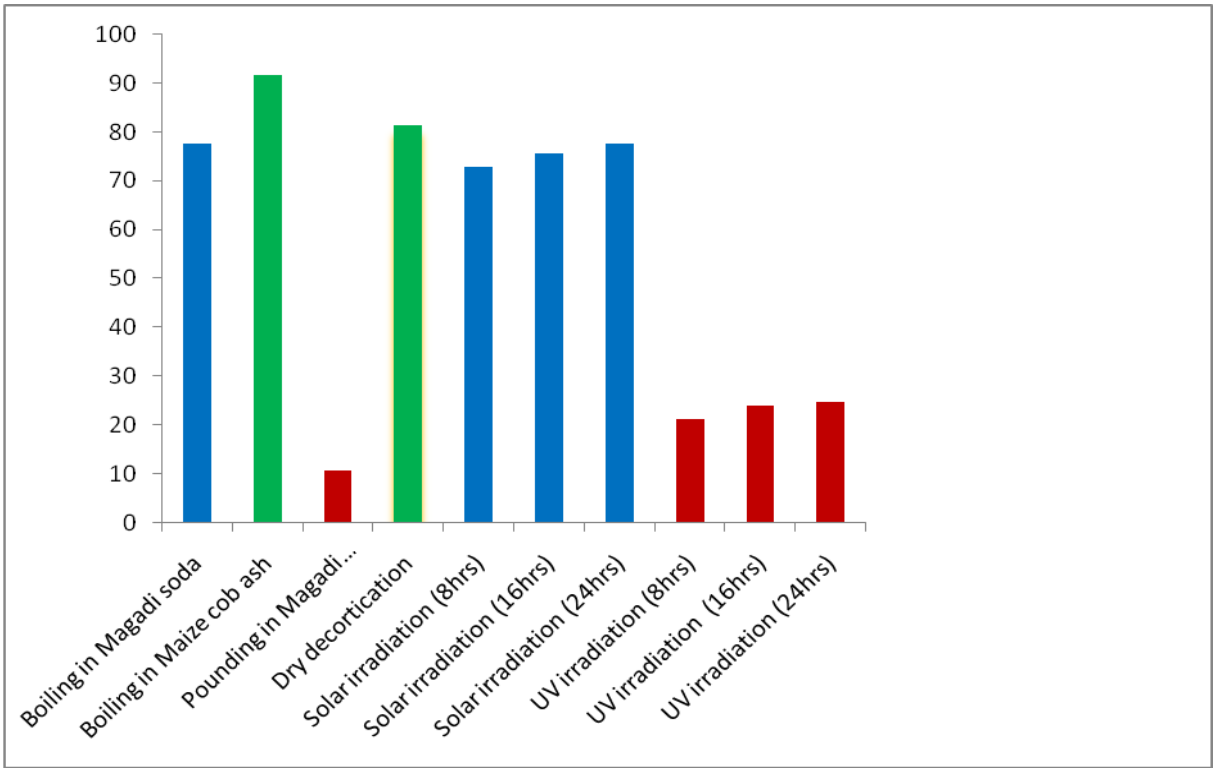

Figure 4: Percentage loss in the aflatoxin levels for all the treatments given

Key

- final aflatoxin<10ppb

- final aflatoxin between 11ppb and 20ppb

- final aflatoxin> 20ppb

Boiling of the contaminated grains in Magadi soda led to a decrease in the amount of aflatoxin by 77.5%. Boiling of the sample in the maize cob ash solution led to a decrease in the amount of aflatoxin by 91.6%. When these two local alkalis were compared in their ability to bring down aflatoxin content in the contaminated sample, maize cob ash did better. This could be accounted to the stronger alkalinity of the ash as was demonstrated earlier in Table 4.

Pounding of the contaminated maize in the presence of Magadi soda during the preparation of *Muthokoi* reduced aflatoxin by 10.8% loss. This agrees with the Mutungi's results that pounding of the contaminated maize soaked in Magadi did not reduce aflatoxin to significant level (Mutungi, 2006). The large difference in the ability to reduce aflatoxin when Magadi soda solution is used to de-hull by pounding and boiling showed that the reduction of aflatoxin in alkaline solutions was largely catalyzed by the presence of heat.

Dry dehulling of the contaminated maize in the convectional mills dropped the level of aflatoxin by 81.3% which makes it better than wet dehulling with or without Magadi soda. During dry dehulling all the aflatoxins which are found at the peripherals are removed with bran and part of the aleurone layer. These aflatoxins do not have a chance to diffuse to other parts of the kernel. The remaining percentage could be found deeper in the endosperm and at the germ as this process does not degerminate. In wet dehulling, water provides a medium for dissolution and diffusion of aflatoxin to other grains and other parts of the grain.

Solar irradiation for the first 6 hours of exposure led to 72.9% drop in aflatoxin. Continued exposure for further 6 hours led to10.3% drop. Exposure for 8 hours more to sunlight caused 7.4% drop. Solar irradiation for a total of 24 hours led to 77.5% loss of aflatoxin. These small changes of aflatoxin level when exposed to solar radiation for longer time of more than 6 hours can be attributed to the fact that aflatoxin exposure to the sunlight was limited by the

degree of penetration of the solar radiation into the grain because of its size. After 6 hours, almost all the aflatoxin in the peripheral parts of the kernel had been detoxified. Aflatoxin that is deeply embedded in the grain is less exposed or not exposed at all to the sunlight. This can be supported by the experiment done using ground animal feed samples, where the aflatoxin reduction was drastic and almost completely destroyed when exposed for 14 hours (Gowda et al., 2005). The exposure of the aflatoxin contaminated maize to UV radiation for 18 hours reduced aflatoxin by 24.8%. The exposure to the first 6 hours led to a drop of aflatoxin levels by 21.3%. Exposure for additional 8 hours led to further drop 3.3%. Further exposure of the sample for another 8 hours led to further drop of aflatoxin 1.3%.

It is therefore clear that the loss of aflatoxin in infested maize kernels due to radiation is greater in the first 6 hours of exposure. Though the aflatoxin loss could increase with the time of exposure, subsequent two 6 hour phases caused a very small change. Sunlight is better than UV radiation in the detoxification of aflatoxin for equivalent length of exposure.

From the chart, the aflatoxin loss was very high where contaminated maize was boiled in maize cob ash solution. The least reduction was noticed with the exposure to the UV light. Exposing the maize to sunlight for 18 hours has the same effect as nixtamalization using Magadi soda.

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

When maize was exposed to solar and UV radiations, none of the two was capable of reducing the aflatoxin levels to maximum allowable limits. However solar radiation reduced aflatoxin to levels low enough that subsequent food preparation for consumption could reduce to safe levels. UV radiation caused only a slight change leaving very high levels of aflatoxin. For both UV and solar irradiation, reduction of aflatoxin was significant only in the first 6 hours of exposure. Further exposure brought a small change.

When the kernels were cooked with Magadi soda and maize cob ash, only maize cob ash was effective in reducing the aflatoxin levels to below the maximum allowable limits by KEBS. Although Magadi soda did not reduce the aflatoxins to maximum allowed, the final levels were low enough that it could be reduced to safe levels during preparation of maize for consumption.

When maize is decorticated by wet pounding and machine decortication, only dry machine decortication reduced aflatoxins to below the maximum allowed limits. Wet pounding had very low impact on reduction of aflatoxin and thus it remained high above the maximum allowed limit.

5.2 Recommendations

Communities should be trained on exposure to the sun, of maize kernels suspected to be contaminated with aflatoxin before preparation for consumption. For maize which would be milled to meal, decortication before milling is recommended to reduce aflatoxin levels. Such machines are now available locally in many Posho mills

In industrial set up, reduction of aflatoxin levels can be done by establishment of Hazard Analysis and Critical Control Points where sorting and cleaning can be done before milling.

During milling to flour, decortication can be done to remove the aflatoxins which will majorly be concentrated in bran fraction. In domestic set up, after preliminary sorting and cleaning, the grains can be sun dried at least for a day and then debranned or decorticated (depending on the use).

Since the use of UV radiation seemed ineffective in reducing aflatoxins, a process which could have been employed in a commercial set up, the government and other grain handling organizations will have to explore other forms of radiation like ozonization in dealing with aflatoxin contaminated maize.

Cooking with alkali as had been done by other experiments modifies the proteins so that they acquire the characteristics of gluten. Such decorticated maize is used for mashing or drying into flour for tortillas in South/Central America. Consumption of tortillas should be introduced to expand the foods from maize and to augment *chapatis*

Studies have to be done to confirm the fate of aflatoxins when two or more of these treatments are combined in contaminated maize.

Exposing maize to solar and UV radiations does not reduce the aflatoxin levels to maximum allowable limits. This has led to growing aflatoxin concerns in maize and maize products. There is thus a continued need for multidisciplinary and comprehensive policy on traditional and improved approach to reduction of aflatoxin levels. Maize cob ash and solar radiation methods should be incorporated into aflatoxin reduction policies as they were found to reduce aflatoxin levels to below the maximum allowable limits. The policies should also be incorporated in a regional approach to containing aflatoxin levels in maize, such as the Partnership for Aflatoxin Control in Africa (PACA).

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APPENDICES

Composition of Buffers and other solutions

Phosphate Buffered Saline (PBS)

NaCl	6.79g
Ba_2HPO_4	1.47g
KH_2PO_4	0.43g
Distilled water	1000ml

Washing solution (NaCl – Tween)

Nacl	8.5g
Tween 2	200µl
Distilled Water	1000ml

Methanol: water 50:50

Add 100ml methanol to 100ml of distilled water.

Methanol; PBS 10:90

10ml of methanol is added to 90ml of PBS

Stopping solution

Sulphuric acid

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