KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF SCIENCE

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

EFFECT ON AFLATOXIN LEVELS IN THE PROCESSING OF MAIZE INTO KENKEY

A THESIS SUBMITTED TO THE DEPARTMENT OF FOOD SCIENCE
AND TECHNOLOGY IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF
SCIENCE IN FOOD QUALITY MANAGEMENT

BY

FATOLA SAWYERR

JUNE, 2018

CERTIFICATION PAGE

I hereby declare that this submission is my own work toward the Msc. and that, to the best of my knowledge, it contains no material previously published by another person or material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

Fatola Sawyerr …………….. ………………
(PG2584914) Signature Date

Certified by:
Dr. Herman E. Lutterodt (PhD) ……………… ………………
(Supervisor) Signature Date

Faustina D. Wireko-Manu (PhD) ……………… ………………
(Head of Department) Signature Date

ABSTRACT

The study was conducted to determine the decrease or increase in Aflatoxin levels with the various steps in processing of contaminated maize into kenkey which is a popular food consumed by almost all Ghanaians. Maize and maize products are known to be susceptible to contamination by fungi that produce secondary metabolites such as aflatoxins. Aflatoxins have been defined as extremely toxic and carcinogenic compounds which appear to be pervasive in the environment. The incidence and level of aflatoxin contamination in various food commodities have been studied all around the world and continues to be of great concern. A mass of 10 kg of maize was first inoculated and found to be highly contaminated with aflatoxin after which the contaminated maize was sifted to remove all debris and other physical contaminants and the following processing techniques followed: washing, soaking in water (steeping), milling; fermentation and cooking. Aflatoxin levels were determined using high performance liquid chromatography (HPLC).

All the steps involved in the preparation of kenkey showed quite significant reduction in aflatoxin G2, B2 and B1 levels ($p<0.001$) in the maize. There were no signs of aflatoxin G1 in the maize but rather in the water residue used. There was a significant rise in the aflatoxin G1, G2, B1 and B2 levels in the water residues used in the preparation of kenkey. This is matched with the reduced level of the aflatoxin in the kenkey, meaning the process of kenkey preparation significantly reduced aflatoxin levels.

Based on the results obtained, it will be recommended that further work be done on other foods that use these aflatoxin susceptible grains and produce.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENT

All thanks go to the Almighty God for his favour, wisdom, care and protection. To the board of the School of Graduate Studies for offering me admission into the University. I also thank my supervisor, Dr. Herman E. Lutterodt, without his patience and encouragement this thesis would not have been possible. I sincerely appreciate the invaluable academic and personal support I received from him throughout this work. Am also grateful to Cyril, William (Laboratory) and Gifty for their support.

DEDICATION

I dedicate this thesis to my dearest son Leon S. Amouzou, my parents and siblings and all loved ones who supported me with wisdom, criticism and encouragement.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

In Ghana, maize accounts for 74% of the total cereals (maize, rice, sorghum and millet) produced (MOFA, 2005) and is considered the most essential cereal. The cultivation of maize in 2005 took a total area of about 740,000 ha and production was 1,171,000 metric tons (MOFA, 2006). Maize plays a very important role in being used as a food crop for both humans and livestock. It is a rich source of energy, vitamins and small amount of protein. Maize is the main source of carbohydrate in most Ghanaian meals and is grown in almost all parts of the country (Coulter *et al*., 1993).

It also plays a crucial role in ensuring food security in Ghana. Annual production of maize has been in excess of 1,000,000 metric tonnes since 2000, averaging 1,772,300 metric tonnes over the period 2009 to 2012 (MoFA, 2013). Maize is used as a domestic alternative for malt when processed into grits (Mills, 2002). Maize is also used in the preparation of foods, animal feed, silage, adhesives and in the making of oil (Nielsen, 2003).

Several factors influence aflatoxin production in maize. Aflatoxin is ubiquitous and is found in foodstuffs that have been inappropriately stored under "predisposing factors of infection" including hot, humid and unsanitary conditions (Baydar *et al*., 2005).

These factors are classified into 3 categories namely; climatic, agronomic and biotic factors are responsible for the infection of maize with Aspergillus fungi and aflatoxin contamination (Diener *et al*., 1987). The four major species are Aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2). These classifications are based on their fluorescence under ultraviolet light (blue or green) and relative chromatographic mobility (Schoental, 1967; Bennett and Klich, 2003; Turner et al., 2009).

Nationwide studies in Ghana have unveiled that about 37% of stored crops consisting of groundnuts, maize and oil seeds, forming a main part of the diet, may be contaminated with the aflatoxins in quantities that exceed the regulatory limit of 20 ppb of United States (Awuah *et al*., 1996).

More than 5 billion people in developing countries worldwide have been estimated to be at risk of chronic exposure to aflatoxins through contaminated foods (Shephard, 2003; Williams *et al*., 2004). Aflatoxicosis occurs as a result of exposure to aflatoxins through food or feed in both humans and animals. It occurs in two general forms (Bankole *et al*., 2003) the acute primary aflatoxicosisis produced when moderate to high levels of aflatoxins are consumed and chronic aflatoxicosis which results from ingestion of low to moderate levels of aflatoxins (Walderhaug, 1992).

Food processing covers all physical, chemical or biological processes undergone by raw grains in the formation of food products. Numerous studies have been conducted to determine the fate of mycotoxins during food processing all over the world in the last 20 years (Hazel and Patel, 2004). Various food processing methods in Ghana include sorting, trimming, cleaning, cooking, baking, frying, roasting, flaking, and extrusion. These processes have variable effects on mycotoxins (Geetanjali, 2015). The nature of the processing method namely physical, chemical, or thermal plays an important role in that, the processes that make use of the higher temperatures have better effects on mycotoxin dissipation (Geetanjali, (2015).

1.2 PROBLEM STATEMENT AND JUSTIFICATION

Corn-based weaning foods intended for children between the ages of 6 months and 2 years that were sampled in the Ashanti region of Ghana was found to contain high levels of aflatoxins (Kumi, 2011).

Contamination of agricultural crops by aflatoxins causes annual losses of more than $750 million in

Africa. Income losses in the USA due to aflatoxin contamination costs an average of more than US $100 million yearly to US producers (Coulibaly, 2008). Aflatoxin-producing fungi also cause direct economic losses by spoiling grain. Animals fed aflatoxin-contaminated grain have lesser productivity and stunted growth. The market value of commodities contaminated with aflatoxins is lower and often consumed locally because they cannot be exported. Acceptable Levels of mycotoxins in foods in developed countries have been lowered which can result in lowered export earnings by African countries that cannot comply with the stricter regulations. The cost burden of mycotoxins in the United States is projected at between $0.5 million to > $1.5 billion (CAST, 2003).

Therefore, in order to avoid the toxicity, the levels of aflatoxins and similar toxic compounds in foodstuffs have to be monitored closely and kept under control continuously in order to avoid the toxicity concerns. If not, related health effects like acute and chronic intoxications, and even deaths, will still be an issue (Becer and Filazi, 2010]

Methods which would focus on reducing dietary exposure to aflatoxins in contaminated foods are greatly desired as a practical move towards alleviating the harmful effects of this toxin (Williams *et al*., 2004).

This, when done, would help solve the problem of food insecurity and improve upon the foreign exchange earning potential of the Ghanaian local crops as they will be able to then meet international standards.

1.3 OBJECTIVE

The objective of this study was to determine the effect of the different processing steps in kenkey production on aflatoxin levels.

CHAPTER TWO

LITERATURE REVIEW

2.1 Maize Production in Ghana

Maize is the most significant cereal crop produced in Ghana and also the most widely consumed staple food in Ghana with ever-increasing production since 1965 (FAO, 2008; Morris *et al*., 1999). In Ghana, maize is produced predominantly by small scale resource poor farmers under rain-fed conditions (SARI, 1996). Two major reasons that account for low productivity in maize are low soil fertility and low application of external inputs.

Maize is extensively cultivated presently all over the world, and a greater quantity being produced yearly as compared to other grains. More than 150 million hectares of maize were planted worldwide, with a yield of 4970.9kilogram/hectare in 2007. West and Central Africa alone had area of maize plantation to be 8.9 million hectares in the year 2005 with a yield of 10.6 million metric tonnes (IITA, 2007).

In Africa, maize is usually consumed as a starchy base in a broad variety of porridges, pastes, grits, and beer. A thin porridge (‘uji’ in East Africa, ‘ogi’ in Nigeria, ‘koko’ in Ghana) is also commonly eaten particularly as weaning food. Green maize (fresh on the cob) is usually parched, baked, roasted or boiled and eaten (IITA, 2007). It thus plays a crucial role in bridging the hunger gap following the dry season (IITA, 2007). Maize is regarded by most people as a breakfast cereal. on the other hand, when processed it serves as fuel (ethanol) and starch. Starch can also undergo an enzymatic reaction and be converted into products such as sorbitol, dextrine, sorbic and lactic acid. These appear in household items such as beer, ice cream, syrup, shoe polish, glue, fireworks, ink, batteries, mustard, cosmetics, aspirin and paint. Maize, as compared to other cereals is high yielding, easily processed, readily digested, and cheaper. It is an adaptable crop and thus grows across a range of agro-ecological zones (Fening *et al*., 2011).

The focus of maize breeders in Ghana has been to improve upon maize varieties in order to perform well in all the agro-ecologies in Ghana. Varieties of maize in Ghana include: ‘Tsolo’ and C50 variety (Sallah, 1986); Nyankariwana Number 1 and Number 2, both yellow varieties; Synthetic 1, 2, and 3 (Sallah, 1986); composite1, 2 and 3, in addition to La Posta CRI, and Golden Crystal; Composite 4, Dobidi and Okomasa (Sallah, 1998). ‘Obatanpa’ (Sallah, 1998); ‘Akposoe’ and ‘Abontem’; ‘Omankwa’ and ‘Aburohemaa’; ‘Etubi’, ‘Enibi’, ‘Golden Jubilee’ and ‘Aziga’ (Variety release, 2007 & 2010); ‘Mamaba’, ‘Dadaba’ and ‘CIDA-ba’; ‘Etubi’ and ‘Enibi’ (Variety release, 2007 & 2010)

2.2 Climatic Requirements for Maize

In West Africa, the best maize growing areas have minimum rainfall of 1,000-1,300mm per annum which is well-distributed during the growth period (Tweneboah, 2000). If severe reduction in yield is to be avoided certain growth periods like tasseling-to- silking stage are critical since grain formation is initiated during this short period (Tweneboah, 2000). Accessibility of soil moisture during tasseling is important for the production of high yields (Tweneboah, 2000). Avoidance of water deficits just prior to tasseling till completion of grain filling results in high yields (Adjetey, 1994).

Temperature has a strong influence in the development of maize and therefore the time required reaching maturity. Maize, being a warm weather crop is not grown in areas where the mean daily temperature is less than 19 ºC (DARSA, 2003). Germination of maize (including imbibition of water and elongation of embryo) is temperature dependent with minimum temperature for germination being 10 ºC. Maize should emerge within five to six days at 20 ºC. Shoot and radicle elongation occurs at maximum rates around 30^{o}C but elongation ceases at temperatures below 9^{0}C and above 40^{0}C (Adjetey, 1994). During the first few weeks after sowing, soil temperature is a pertinent growth limiting factor in maize growing areas outside the tropics. Pollen shed before silk is receptive or death of tassel and drying of silk for high temperature and low moisture (Adjetey, 1994). Maize requires an average temperature of 13-40°C (Awuku *et al*., 1991), with optimum temperature for growth ranging from 18-21°C (Tweneboah, 2000).

Maize is known to utilize sunlight more effectively than any other crop, and its yield per ha is the highest of all grain crops. The total energy used by one plant at maturity is equivalent to that of 8 293 15 W electric globes in an hour (DARSA, 2003). The aspect of light that influences maize growth significantly is light intensity received during the growth period. Maize is known to require lots of clear sunshine (Adjetey, 1994).

2.3 Storage of Maize

Methods used in reducing the losses suffered after harvesting include using storage structures which are moisture proof and are sufficiently aired. Storage of cereals plays a vital role in evening out the rise and fall in production seasonally and yearly (Kimenju and De Groote, 2010). In storage, once elevated temperatures or moisture conditions are left unchecked, there will be mold and insect growth because their activity produces heat, which speeds up grain deterioration more. Moisture content and temperature control of corn throughout the storage period is the most cost effective way to avert spoilage problems. Farmers measure the benefits as against the costs in selecting a particular storage technique to use. Traditional storage structures along with storage of cereals like maize in the houses by small scale and subsistence farmers results in considerable losses due to pest and mould contamination. Alternatively, air-tight storage technologies which includes, metal silos which are said to have zero storage loss costs are expensive for the individual farmer to afford. Farmers would only store cereals if they know they would recover from the storage costs due to rise in prices (Komen *et al*., 2006; Fackler and Livingston, 2002). Storage structures include the metal silos, granaries, bags, cribs, baskets or earthen pots. Storage of cereals includes the use of metal silos, granaries, bags, cribs, baskets or earthen pots. Farmers also use other strategies to minimize post-harvest losses like the use of traditional knowledge including the use of herbs such as Mexican marigold and hot pepper in storage, selling grain soon after harvest and cleaning or dusting the storage structure with pesticide thoroughly before depositing the maize or acquire the new maize storage technologies (Bett and Nguyo, 2007).

2.4 Food processing methods

Processing techniques offer a means to address the alarm in food safety. Food processing covers all physical, chemical, or biological processes undergone by raw grains in the formation of food products. Food processes techniques include sorting, trimming, cleaning, milling, cooking, baking, frying, roasting, canning, flaking, alkaline cooking, and extrusion.

i. **Sorting, trimming and cleaning:** These processes may lessen mycotoxin concentrations in commodities though these operations may not completely remove all of the contamination. Mycotoxin contaminated kernels can be separated from non-contaminated ones through sorting with percentage dissipation being 74% (Scott *et al*., 1983). Cleaning can also be used to remove contaminated kernels.

ii. **Milling:** Also known as grinding is the process of breaking solid materials into smaller pieces. This milling does not the reduce the level of mycotoxin concentration but it simply redistributes the contamination (Tannahill, 1995) **iii. Cooking:** This is the process of applying heat to prepare food. It is made up of a wide range of methods which depends on the customs and traditions, availability and the affordability of the resources (Kaushik *et al.*, 2009). Numerous studies report on effect of cooking on mycotoxin dissipation.

iv. **Frying:** Frying is the cooking of food in oil or another fat. This takes several forms, from *deep-frying,* where the food is completely immersed in hot oil, to *saut´eing* where food is cooked in a frying pan where there is only a thin coating of oil. Frying is the fastest way to cook, as it is the most efficient way to transfer heat into the food. Frying is considered to be a dry cooking method even though using liquid oil, as it doesn't make use of water and ideally the cooking oil will not be absorbed by the food (Tannahill, 1995).

v. **Roasting:** This is a method of cooking that uses dry heat, whether an open flame, oven, or other heat source. It can enhance flavor through caramelization and Maillard browning on the surface of the food.

vi. **Alkaline cooking (Nixtamalization):** This is the process of grain (maize) preparation in which the grain is soaked and cooked in limewater (an alkaline solution) and hulled. Nixtamalization of Maize makes it easy to be ground, increases its nutritional value, and improves the aroma and flavor of the maize. It also reduces the aflatoxins content in maize. The alkaline solution helps to dissolve the hemicellulose of the maize cell walls, thus loosening the hulls from the kernels and softening the corn.

vii. **Extrusion:** Extrusion cooking is a recent processing technique used widely due to its advantages over traditional methods. It improves the quality of intermediate and final processed products and safety because of the potential to reduce mycotoxin levels in cereals (Castells *et al*., 2005). The rate of mycotoxin dissipation in this processing technique depends of several factors including type of extruder, the type of screw, the die configuration, the initial mycotoxin concentration, the barrel temperature, the screw speed, the moisture content of the raw material, and the use of additives (Castells *et al*., 2005) **viii. Fermentation:** Fermentation is a process of hydrolyzing proteins to amino acids and low molecular weight peptides using enzymes. Starch undergoes an incomplete conversion to simple sugars which are also fermented to lactic acid, alcohol, and carbon dioxide (Pardez-Lopez *et al*., 1991).

2.5 The Genus Aspergillus

Aspergillus is a filamentous and cosmopolitan fungus found everywhere in nature. The genus *Aspergillus* belongs to the Deuteromycetes (Fungi Imperfecti; Hyphomycetes); their teleomorphs can be found in the Ascomycetes. The fungi utilize many commodities as good substrate for growth because they can use large number of enzymes for their development (Hell *et al*., 2000b). The Ascomycete produces sexual spores (ascospores) endogenously in a well-differentiated ascocarp but the Ascomycetes and the Deuteromycetes reproduce vegetatively by conidia (Pelczar *et al.*, 1993). Aflatoxins are only produced by two related species: *Aspergillus flavus* and *Aspergillus parasiticus,* with the latter species producing specifically the G type of aflatoxin (Hell *et al*., 2000b). Among all filamentous fungi, *Aspergillus* is in general the most commonly isolated one in invasive infections. It is the second most commonly recovered fungus in opportunistic mycoses following Candida (Collier *et al*., 1998). Moulds are known to need an energy source in the form of carbohydrates or vegetable oils as well as a source of nitrogen either organic or inorganic, trace elements and available moisture for growth and toxin production. Cereals, mainly oilseeds sufficiently offer all these nutrients and so are considered ideal substrates for growth of fungi and consequently, toxin synthesis (FAO, 1983).

2.6 Aflatoxins

Aflatoxins are mycotoxins produced by the *Aspergillus flavus* and *Aspergillus parasiticus* species of fungus, common post-harvest fungi. The term “aflatoxin” is coined from three words: (i) the “a” that stands for the *Aspergillus genus*; (ii) the “fla” that stands for the species flavus and (iii) the “toxin” that means poison (Bhat *et al*., 2010; Bakirdere *et al*., 2012). A total number of eighteen different types of aflatoxins have been identified so far (Bakirdere *et al*., 2012). The four major species are Aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2). These classifications are based on their fluorescence under ultraviolet light (blue or green) and relative chromatographic mobility (Schoental, 1967; Bennett and Klich, 2003; Turner *et al*., 2009).

They have been recognized as noteworthy contaminants by the agricultural production community since the 1960s and control strategies have mostly eliminated harmful exposures in developed countries (Widstrom *et al*., 2003). A survey which lasted for a decade in Ghana revealed that the prevalence of aflatoxins was 50% with a greater number of the samples having aflatoxin levels that exceed the EU maximum limit (Akrobortu, 2008).

The range of food products that are contaminated by aflatoxins consists of cereals like maize, sorghum, pearl millet, rice and wheat; oilseeds such as groundnut, soybean, sunflower and cotton; spices like chilies, black pepper, coriander, turmeric and zinger; tree nuts such as almonds, pistachio, walnuts and coconut; and milk and milk products (Lopez *et al*., 2002).

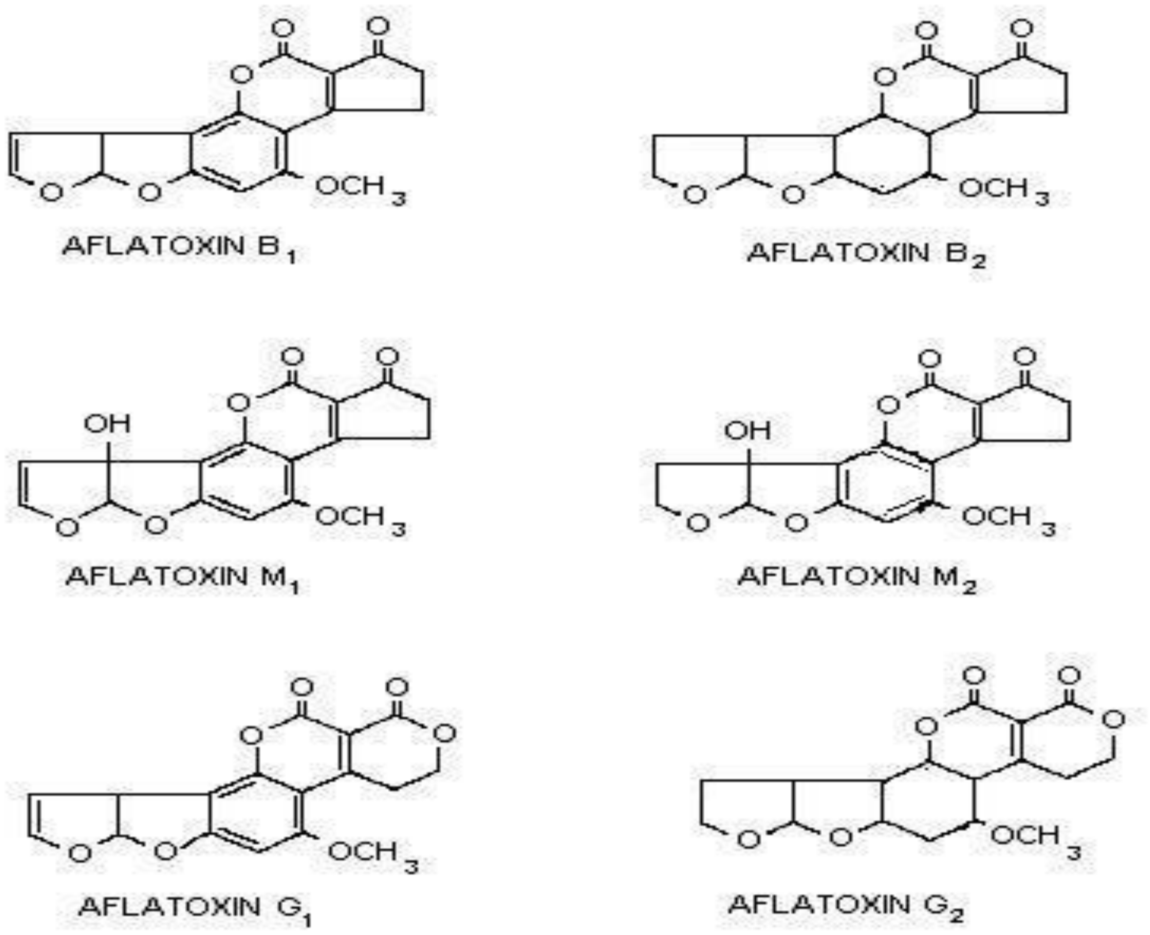


Figure 1. Structures of the major aflatoxins B1, B2, G1, G2, M1, M2, B2A and G2A (Adopted from Reddy, 2012)

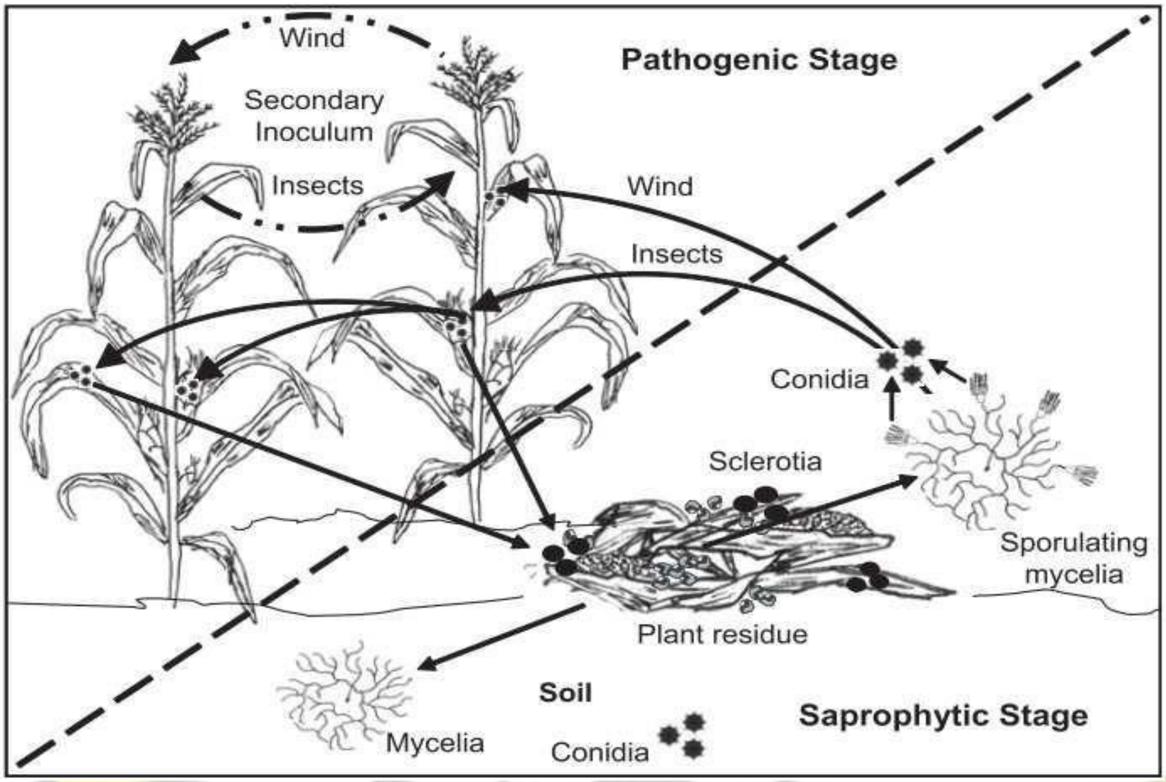


Fig. 2. Life cycle of *A. flavus* in a corn cropping system (Abbas *et al*., 2009)

1.2.5.1 Physicochemical properties of aflatoxins

Aflatoxin	Molecular Formula	Molecular Weight (units)	Melting Point (ºC)
B1	$C_{17} H_{12}O_6$	312	268-269
B2	$C_{17} H_{14}O_6$	314	286-289
G1	C_{17} H12O_7	328	244-246
G2	**$C_{17} H_{14}O_7$**	**330**	**237-240**

Table 1: Physico-chemical Properties of Aflatoxins

Source: (Adapted and Modified from Reddy and Waliyar, 2000).

Aflatoxins, in their pure form are heat stable and their concentration is not affected by normal food processing methods such as cooking or pasteurization (Reddy and Waliyar, 2000).

Aflatoxins have high solubility in solvents such as in methanol, chloroform, acetone and acetonitrile (Reddy and Waliyar, 2000). Because of their instability upon exposure to light, they are stored as chloroform or benzene solutions in the dark and cold (Reddy and Waliyar, 2000). Aflatoxins are also intensely fluorescent when exposed to long wavelength UV light, which enables their detection at very low levels and also provides the practical basis for most methods used for their quantification (Reddy and Waliyar, 2000).

2.7 Factors influencing the production of aflatoxins in maize

The mycotoxin is ubiquitous and is found in foodstuffs that have been inappropriately stored under "predisposing factors of infection" including hot, humid and unsanitary conditions (Baydar *et al*., 2005).

Several factors influence aflatoxin production in maize. They have been classified into 3 categories namely; climatic, agronomic and biotic factors are responsible for the infection of maize with Aspergillus fungi and aflatoxin contamination (Diener *et al.*, 1987)

Climatic factors: *A. flavus* is reported to grow within the temperature range of 10-43ºC and optimal growth rate occurs at a little above 30ºC. Production of aflatoxins occurs over the temperature range 15-37ºC with higher production between 20-30ºC (FAO, 2001). Growth of *A.flavus* was optimal in newly harvested maize with a moisture content of 20-28%, mostly at high temperatures of 20-30ºC (Calderwood and Schroeder, 1968). Storage of commodities at humidities between 75 and 85% makes them prone to fungal attack within the normal storage time (Hell *et al*., 2000b). Mould growth and aflatoxin contamination also depend on the moisture content of the maize. Early harvest of maize at moisture content around 30.3% showed the highest levels of aflatoxins (Kawasugi *et al.*, 1988).

Agronomic factors: Cotty (1994) identified plant stress, irrigation, cropping pattern, variety, date of planting, date of harvesting and storage conditions as agronomic factors that may influence aflatoxin development. Physical and chemical characteristics of husk and grain also render maize susceptible to mould infection (Barry *et al.,* 1986; Cardwell *et al.,* 2000). Planting and harvesting dates may be monitored to lessen aflatoxin contamination (Jones and Duncan, 1981). Field drying followed by mechanical drying is the most effective means of minimising aflatoxin contamination in maize (Tanboon-ek, 1989). *Aspergillus flavus* have been known to stay in the soil in stalks and leaves of harvested maize.

Biotic factors: Insect-attack increases susceptibility of maize for easy fungal infection. Varieties with high resistance to insects and tough kernels are less prone to *A.flavus* infection and aflatoxin contamination. Insects also act as vectors of fungal spores (McMillian *et al.,* 1987) and facilitate the infection process. The rapid multiplication of insects leads to increased moisture content, dust production, *A flavus* infection and aflatoxin contamination (Sinha and Sinha, 1992). development of *A. flavus* in the substrate could be slowed down as a result of competition with other organisms. However, the growth of *A. flavus* has been observed to be fastest at 30ºC.

Aflatoxin contamination in foods has been associated with environmental conditions, poor processing, and lack of proper storage facilities in developing countries (Faromzi, 2006). The vulnerability of food product to fungal attack occurs during the processes of pre-harvest, transportation, storage, and processing of the foods (Bankoje and Adebanjo, 2003; Benneth and Klich, 2003).

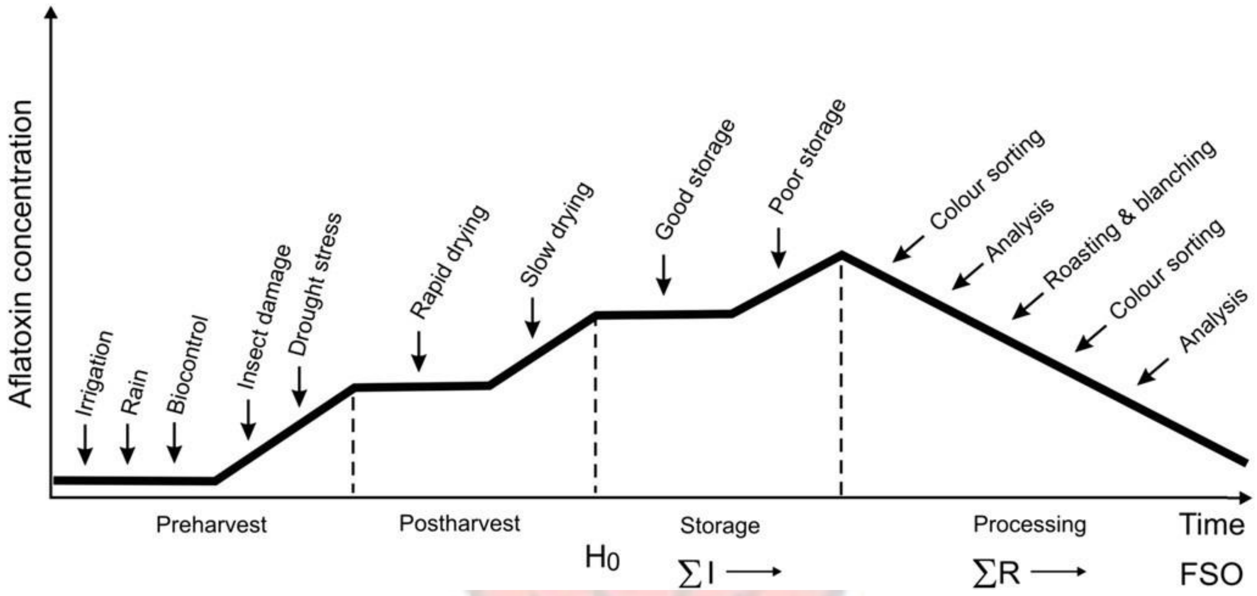


Fig. 3. The time course of aflatoxin formation and reduction in maize, with reference to the Food Safety Objective.

2.8 Analysis of Aflatoxins

In is important to ensure that the methods used for the analysis of mycotoxins, including aflatoxins, in food, feed, and other biological matrices are very accurate with high sensitivity. This will help to meet food safety concerns and official legislated regulations (Shephard, 2009). The fluorescent properties of aflatoxins when exposed to long wavelength UV light facilitates their detection at very low levels thus providing the practical basis for most methods used for their quantification (Reddy and Waliyar, 2000). Commonly used methods include thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), liquid chromatography tandem mass spectrometry (LC/MS) and immuno-chemicals methods, such as enzyme linked immunosorbent assay (ELISA), immuno-sensors, dipsticks and strip-test (Manetta, 2011).

2.8.1 Chromatographic methods

Chromatographic separation of aflatoxins was originally performed by Thin Layer Chromatography. This is because upon identifying aflatoxins as chemical agents, it has been the most generally used separation technique in analysis of aflatoxins in various matrices, including corn, raw groundnuts, cotton seed, eggs (Trucksess *et al.,* 1977), milk (Van Egmond *et al.*, 1978). It has also been considered the AOAC official method for a long period. This procedure is simple and fast and fluorescence spots are observed under a UV light in order to identify aflatoxins. Blue fluorescence color indicates the presence of AFB1 and AFB2 and green for AFG1 and AFG2. Thin Layer Chromatography can also be used for qualitative and semi-quantitative determinations by comparing sample and standard analysed in the same conditions. TLC technique can be employed in laboratories of developing countries instead of other chromatographic methods that are more expensive and sophisticated (Nawaz *et al.*, 1992).

High performance liquid chromatography (HPLC) is now the most commonly used technique in analytical laboratories. This is because of its higher separation power, higher sensitivity and accuracy, the possibility of automating the instrumental analysis. Its fluorescence detection is now the most acknowledged chromatographic method for the determination of aflatoxins. current methods for analysis of aflatoxins rely upon reverse-phase HPLC, with mixtures of methanol, water and acetonitrile for mobile phases (Manetta, 2011). Liquid chromatographic methods for aflatoxins determination comprise both normal and reverse-phase separations (Manetta, 2011). Methods based on HPLC with fluorescence detection are mostly used for instrumental analysis of aflatoxins because of the large diffusion of this configuration in routine laboratories (Manetta, 2011).

2.8.2 Immunological methods

High performance liquid chromatographic (HPLC) methods with fluorescent detection are mainly used in routine aflatoxins analysis (Manetta, 2011). These techniques are often difficult, timeconsuming and very sophisticated, requiring experience of chromatographic techniques. For effective monitoring of aflatoxins in food and feed commodities there is the need for a technique that will be of high sensitivity, specificity, simplicity and fast with high detectability and analytical output. Immunological methods together with a highly sensitive detection of the label can help realize this. Enzyme-linked immunosorbent assay (ELISA) is the best recognized and the most available immunoassay in the rapid detection of aflatoxins, using the 96 well plate microtiter format. The use of immunosensors for aflatoxins involves immobilization of antibodies on the surface of a screen-printed electrode, magnetic beads held on the surface of a screen-printed electrode (Piermarini *et al.*, 2009), on piezoelectric quartz crystal immunosensor with gold nanoparticles (Jin *et al.*, 2009). Determining the aflatoxin content in agricultural products using a homogeneous assay based on fluorescence polarization has been described (Nasir and Jolley, 2002). One set back of this technique is underestimation of the aflatoxin contents, possibly due to low cross-reactivity of the antibody with AFB2, AFG1 and AFG2. The lateral flow device is a simple and fast immunoassay technique. It is a screening test that comes in the form of strip or dipstick (Delmulle *et al.*, 2005). Immuno dipstick or lateral flow immunoassay requires simple and minimal manipulations and little or no instrumentations and has gained increasing attention. Colloidal gold conjugated anti-aflatoxin antibodies are immobilised at the base of the stick for analysis of the aflatoxins.

The current development of biosensors has roused their application to the analysis aflatoxins (Siontorou *et al*., 1998; Carlson *et al.*, 2000; Tombelli *et al.,* 2009; Paniel *et al.*, 2010). The advantages that biosensing techniques provide are: reduced extraction; clean-up analytical steps and global time of analysis (1 min or only few seconds); possibility of online automated analysis; low cost; skilled personnel not required. Sensitivity of biosensing techniques should be enhanced and their stability improved for long-term use.

2.9 Effects of Aflatoxins on human and animal health

Aflatoxins are toxic compounds with mutagenic, carcinogenic, and teratogenic properties (Aycicek *et al*., 2005; Bhat *et al*., 2010; Bakirdere *et al*., 2012). Kwarshiorkor and Reye's syndrome (damage to the liver and kidney), which are endemic diseases in Asia, Africa and Europe are implicated in aflatoxins poisoning (Zöllner and Mayer-Helm, 2006). The order of increasing toxicity of the aflatoxins is AFB1 > AFG1 > AFB2 > AFG2 (Agag, 2004; Espinosa-Calderón *et al*., 2011). The most toxic form is found to be AFB1and it is known to cause damages including toxic hepatitis, hemorrhage, edema, immunosuppression and hepatic carcinoma (Nakai *et al*., 2008). It has been reported to be the most potent natural carcinogen well-known in mammals (Hussain *et al*., 2008; Fallah, 2010)

Aflatoxins have been known to cause primary liver cell carcinoma, immuno suppresion, malnutrition, infertility and growth retardation in humans (Fung and Clark, 2004). They also cause nutrient alterations like vitamin A or D in animal models and thus making them unavailable for the normal body physiology leading to nutritional deficiencies (Peraica *et al*., 1999).

The two broad forms of effects of aflatoxins are acute and chronic toxicity. Acute toxicity comes about as a result of intake of large amount of aflatoxins from greatly contaminated food. This affects liver function and could possibly lead to blood clotting mechanism, jaundice, a decrease in serum proteins that are synthesized by the liver, edema, abdominal pain, vomiting and death of affected person. Aflatoxins cause necrosis of liver cells and death (Chao *et al*., 1991). This similar case was reported in Kenya in 2004 where there were 317 cases and 125 deaths as a result of consumption of maize contaminated with aflatoxins (Probst *et al*., 2010). The S strain of Aspergillus flavus was identified as the causal agent of the outbreak. Studies have indicated that exposure to large doses of aflatoxin causes acute toxicity whereas exposure to small doses for prolonged periods of time is carcinogenic.

Chronic toxicity occurs as a result of long time exposure to low aflatoxin concentration. The main symptoms observed in Chronic aflatoxin toxicity are decreased growth rate which results in stunted growth (Gong *et al*., 2004). Studies in Togo and Benin has shown that children who are underweight as a result of aflatoxins are also at higher risk for infections and diarrhea (Gong *et al*., 2004). Exposure of children to aflatoxin can be through contaminated milk containing Aflatoxin M1 that is a metabolite of AFB1. Aflatoxins cause reduced milk or egg production and immune suppression in domestic animals. This happens due to aflatoxin reactivity with T-cell and a reduction in vitamin K activities including decrease in phagocytic in macrophages (Robens and Richard, 1992). Hepatitis B and Hepatitis C carriers are at high risk of developing cancer as a result of consumption of aflatoxins contaminated food (William *et al*., 2003). Aflatoxins have been associated with immune suppression (Turner *et al*., 2005) and higher prevalence of Hepatocellular cancer reported in Africa (Strosnider *et al*., 2006).

Aflatoxin level (parts per billion)	Limitation / consequence
20	Highest level allowed for humans
50	Highest level allowed for animals
100	Slowed growth of young ones

200-400	Slowed growth of adults
Beyond 400	Liver damage and cancer

Table 2: Aflatoxin ingestion limits and health effects Source: (Kumar *et al.*, 2000)

2.10 Control and Preventive Measures

Crop infestation by aflatoxins is an international food safety concern due to the fact that the compound has high carcinogenicity and mutagenicity in animals and humans (Yin *et al.*, 2008). Management is therefore vital in order to monitor and regulate quantities in food and feed. The European Union and other countries have a maximum level of 2 ng/g for B1 and 4 ng/g for total levels of aflatoxins in crops (van Egmond and Jonker, 2004). Control and prevention can be achieved by pre-harvest and post-harvest handling techniques.

Pre-harvest: The most effective point of control of aflatoxins is before harvesting. This is due to the fact that the crop is first infected by the toxin-producing fungus at this point (Wild and Turner, 2002). Using staple crops that resist colonization by fungus or genetically improved crops that hinder fungal invasions, joined to the elimination of inoculum sources may prevent infection of the crop. Pre-harvest mediations comprise: selecting crops with high resistance to abiotic stresses namely drought, temperature and moisture content; decreasing crop stresses; plant breeding to develop host resistance; using resistant varieties. These processes may not be possible in many high risk regions because of economic reasons.

Post-harvest: Post-harvest activities for instance drying and storage are among the important areas along the maize value chain that is critical to small-scale farmers in Africa. Ineffective storage practices adopted by small-scale farmers leads to some level of grain losses owed to insect infestation, mould growth and discolouration, contamination by aflatoxins, re-wetting and germination of grains is usually encountered. Major storage techniques utilized by farmers in Western Africa vary greatly, but include on-field, open storage, jute bags, polyethylene or polypropylene bags, raised platforms, conical structures with thatched roofs and giant woven baskets (Addo *et al*., 2002). Moreover, drying of maize by farmers is normally done either in storage or while the crop is on the field. Delays in field drying of maize could result in serious grain losses during storage (Kaaya *et al*., 2006). Platform drying, which raises the maize off the ground for longer-term drying, was however, reported to be associated with losses of up to 3.5% in Zambia (Rembold *et al*., 2011).

Post-harvest mediations to control aflatoxins can be accomplished at three stages: drying level, storage level and in food preparation. During food storage, there is a possibility of aflatoxins accruing and so post-harvest control at the farm targets to minimize growth of fungus and aflatoxin production. Aspergillus growth is also influenced by temperature, moisture content and storage time so right temperatures and moisture content can help prevent their survival. Simple food preparation procedures such as sorting, washing, crushing, and grain dehulling, may help to minimise aflatoxin levels (Fandohan *et al.,* 2005; Park, 2002). Prevention of aflatoxins may also be by packing the dried products in polyethylene or propylene bags (Siriacha *et al.,* 1990). In maize, elimination of fungal species is primarily through processes such as nixtamalization in which aflatoxins are eliminated (Méndez and Moreno, 2009). In addition, low concentrations of sodium hydroxide are added to eliminate large amounts of aflatoxins (Carrillo, 2003).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Kenkey production

Ten kilograms (10 kg) of fresh maize was purchased from the market and inoculated to be highly contaminated with aflatoxin.

STEP 1: Contaminated maize was sifted to remove all debris and other physical contaminants. Using the quaternary method, the maize was mixed up and then divided into four portions and 100g of one portion was sampled and coded **NAf001**.

STEP 2: Maize was then washed with twenty Liters (20L) of water. 100 ml of this water was sampled and labelled **NAfw01**. 100g of the maize was sampled and coded **NAf002**.

STEP 3: Maize was again washed for the second time using 20 L of water. 100 ml of this water was also sampled and labelled **NAfw02**. 100g of the maize was sampled and coded **NAf003.**

STEP 4: The washed maize was then soaked in 20 L of water for 56 hours.

After 56 hours, 100ml of the water used to steep was sampled as **NAfw03**. 100g of the maize was also sampled and coded **NAf004.**

STEP 5: Using 20 L, the maize was washed; 100ml of the water used was sampled as **NAfw04.**

The maize was washed the second time; the water used was sampled as **NAfw05** and 100g of the maize was also sampled as **NAf006**.

STEP 6: Using an attrution mill, 5kg of the maize was milled. 500 ml of water was used to knead after the milling and was fermented for 72 hours. 100g of the dough was sampled as **NAf007**.

STEP 7: The dough was further fermented to the 96th hour, sample was labeled as **NAf008**.

STEP 8: Taking 1.5kg of the dough, 2.1 L of water and 15g salt was added, the mixture was then cooked for 40 minutes and left to cool and sampled as **NAf009**

STEP 9: 1 kg of the cooked dough was mixed with 0.5kg of the uncooked dough and folded into corn husk. This was then sampled as **NAf010**.

STEP 10: The dough in the husk was then put on fire with about 1 L of water. Kenkey (400g approximately) was ready after 2 hours. It was sampled as **NAf011** and kenkey water was sample as **NAfw06**.

(All solid samples and liquid samples were 100g and 100ml respectively)

DRY INOCULATED MAIZE

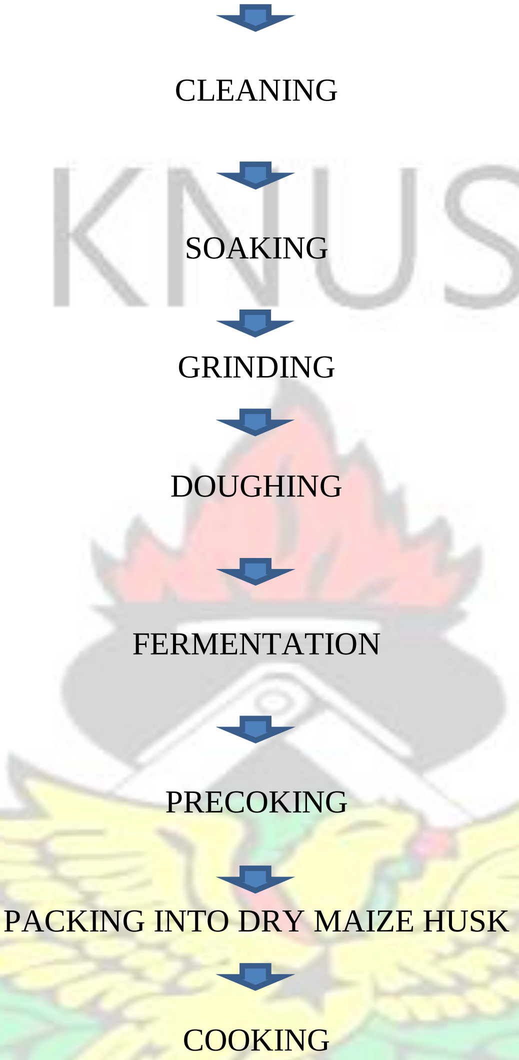


Fig 4. Flow chart of kenkey preparation

3.2 AFLATOXIN ANALYSIS

3.2.1 Extraction and Clean-up

A mixture of ground sample (25g) with 5g of sodium chloride and 125ml of methanol/ deionized water (70:30) was blended at high speed for 2mins and filtered through a fluted filter paper. The extract (15ml) was diluted with 30ml of deionized water and filtered through a1.0 µm microfiber filter. The diluted extract (15ml) was passed through Vicam Aflatest immunoaffinity column (IAC), which was washed twice with 10ml deionized water. Aflatoxin was eluted from the IAC with 1ml HPLC grade methanol and 100ul of the eluent was injected into the HPLC.

3.2.2 HPLC Determination

A Cecil-Adept Binary Pump HPLC coupled with Shimadzu 10AxL fluorescence detector (Ex: 360nm, Em: 435) with Phenomenex Hyper Clone BDS C18 Column (150 x 4.60mm, 5um). The mobile phase used was methanol: water (40:60, v/v) at a flow rate of 1ml/min with column temperature maintained at 40°C. To 1 liter of mobile phase were added 119 mg of potassium bromide and 350ul of 4M nitric acid (required for post column electrochemical derivatisation with Kobra Cell, R-Biopharm Rhone). Aflatoxin Mix (G_1, G_2, B_1, B_2) standards (ng/g) were prepared from Supelco® aflatoxin standard of 2.6ng/µL in methanol. Concentration of B_1 and G_1 were 0.5, 1, 2, 8, 16 ng/g per 100ul injection. Concentration of B_2 and G_2 were 0.15, 0.3, 0.6, 2.4, 4.8 ng/g per 100ul injection. Limit of Detection and Limit of Quantification were established at 0.5ng/g and 1ng/g respectively *(AOAC Official Method 2005.08).*

3.2.3Aflatoxin Calculation

Aflatoxin, ng/g = A x (T/I) x (1/W)

Where A = ng of aflatoxin as eluate injected, T = final test solution eluate volume (ul),
I = volume eluate injected into LC (ul), W = mass (g) of commodity represented by final extract.

NOTE: T = 2000, I = 100, W = 1

(AOAC Official Method 2005.08)

CHAPTER FOUR

RESULTS AND DISCUSION

4.1 RESULTS

4.1.1 Aflatoxin G2, B2 and B1 level in each processing step

The processing went through 10 steps with the level of each aflatoxin determined at each step. Throughout the processing steps it was step 2 (Washing), step 4 (soaking in water), step 6 (Milling/ fermentation) and step 9 (mixing with uncooked dough) that showed significant reductions in aflatoxin levels in the maize ($p<0.0001$).

The levels of aflatoxin G2 determined from step 1-10 were 69.87± 1.37 ng/g, 26.33 ± 1.12 ng/g, 25.23 ± 1.43 ng/g, 2.49 ± 0.01 ng/g, 16.43 ±0.06 ng/g, 0 ± 0 ng/g, 0.83 ± 0.02 ng/g, 4.42 ± 0.13 ng/g, 0.54 ± 0.01ng/g, and 0.66 ± 0.01ng/g respectively. The aflatoxin G2 levels are shown in fig 4.1A.

Aflatoxin B2 levels were 63.36± 1.89, 23 ± 1.78, 16.25 ± 0.14, 4.56 ± 0.44, 10.31 ±1.35, 8.24 ± 0.25, 4.586 ± 0.002, 8.41 ± 0.11, 3.00 ± 0.12, and 3.23 ± 0.15 for step 10 respectively. The aflatoxin B2 levels are represented in the in fig. 4.1B.

The mean aflatoxin B1 levels determined for each processing step is represented in the fig. 4.1C.

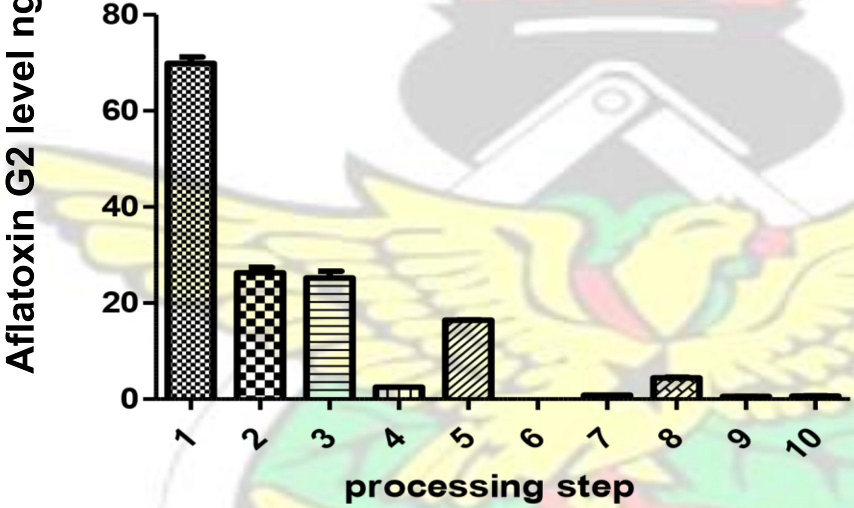


Fig 4.1A: Aflatoxin G2 level in each processing step. Results are means ± SD. Step 1- Sifting; Step 2- Washing; Step 3- Washing; Step 4- Soaking in water; Step 5- Washing; Step 6- Milling/fermentation for 72 h; Step 7- Fermentation to 96 h; Step 8- Cooking for 40 min; Step 9-Mixing with uncooked dough; Step 10-cooking of kenkey.

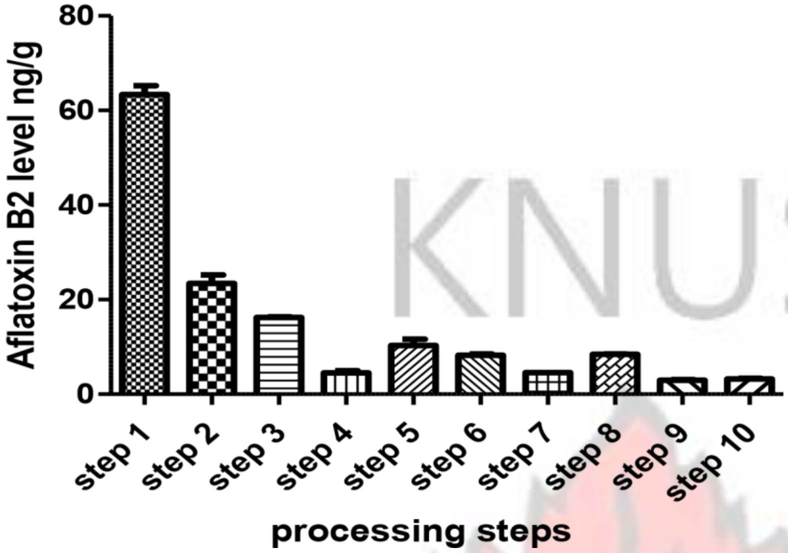


Fig 4.1B: Aflatoxin B2 level in each processing step. Results are means ± SD. Step 1- Sifting; Step 2- Washing; Step 3- Washing; Step 4- Soaking in water; Step 5- Washing; Step 6- Milling/fermentation for 72 h; Step 7- Fermentation to 96 h; Step 8- Cooking for 40 min; Step 9-Mixing with uncooked dough; Step 10-cooking of kenkey.

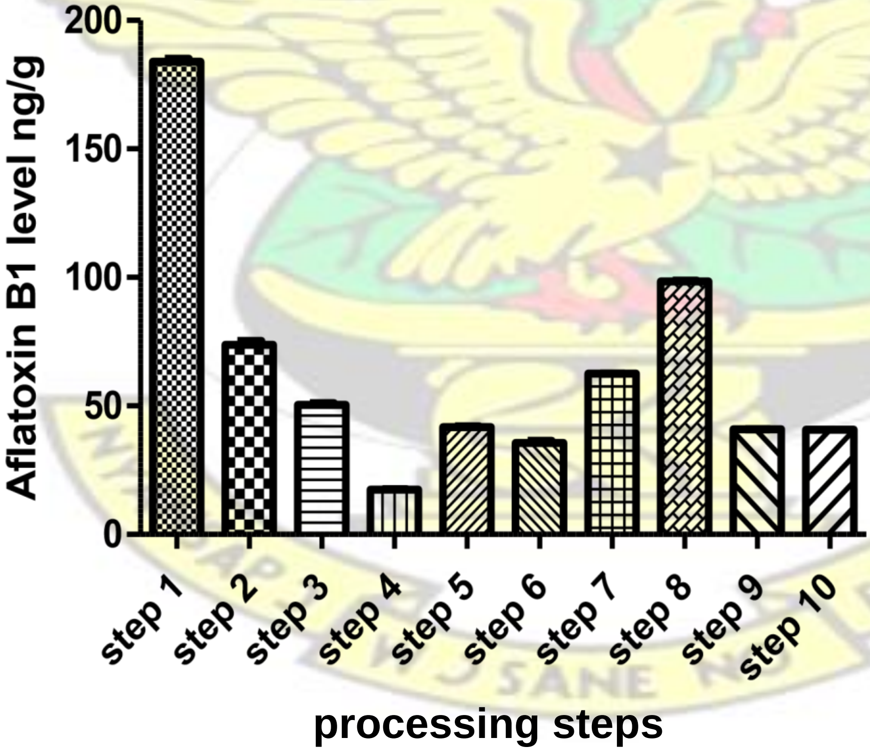


Fig 4.1C: Aflatoxin B1 level in each processing step. Results are means ± SD. Step 1- Sifting; Step 2- Washing; Step 3- Washing; Step 4- Soaking in water; Step 5- Washing; Step 6- Milling/fermentation for 72 h; Step 7- Fermentation to 96 h; Step 8- Cooking for 40 min; Step 9-Mixing with uncooked dough; Step 10-cooking of kenkey.

4.1.2 Total Aflatoxin level in each processing step

The mean total aflatoxin levels determined are represented in the fig. 4.4 below. Throughout the processing steps it was step 2 (Washing), step 4 (soaking in water), step 6 (Milling/ fermentation and step 9 (mixing with uncooked dough) that showed a significant reduction in aflatoxin levels in the maize with a one-way analysis of variance with P-value<0.0001.

The mean total aflatoxin level plus/minus the standard deviation is as follows; 317.12 ± 4.38, 123.49 ± 1.34, 91.88 ± 2.20, 24.41 ± 0.55, 68.48 ± 1.78, 43.85 ± 0.52, 67.96 ± 0.08, 111.26 ± 0.54, 44.38 ± 0.22, and 44.63 ± 0.11 for processing step 1-10 respectively.

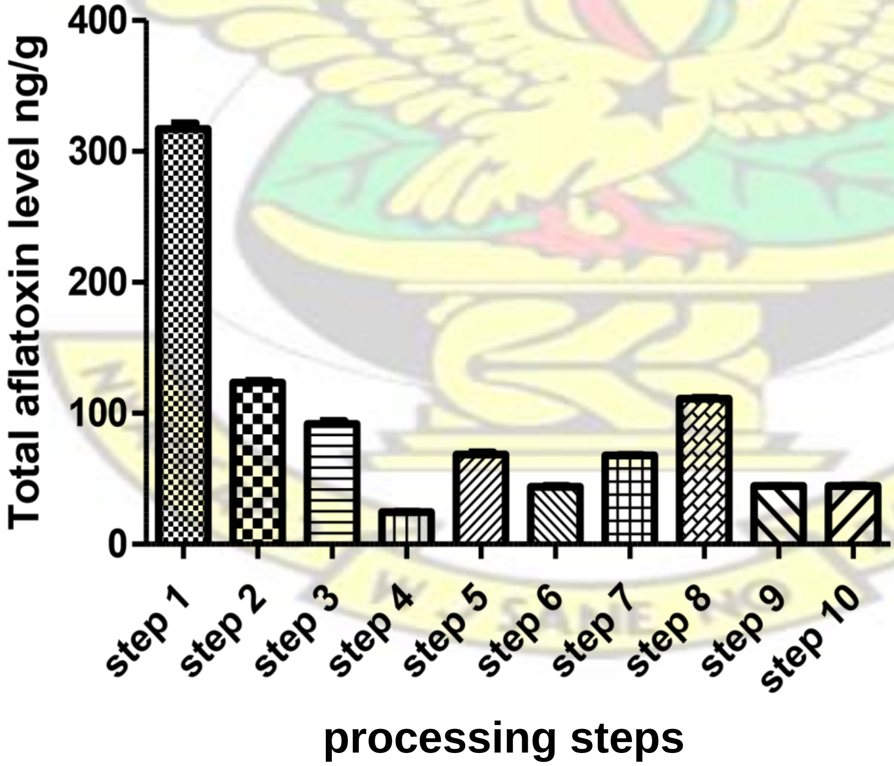


Fig 4.2: Total aflatoxin level in each processing step. Results are means ± SD. Step 1- Sifting; Step 2- Washing; Step 3- Washing; Step 4- Soaking in water; Step 5- Washing; Step 6- Milling/fermentation for 72 h; Step 7- Fermentation to 96 h; Step 8- Cooking for 40 min; Step 9-Mixing with uncooked dough; Step 10- cooking of kenkey.

4.1.3 Aflatoxin G2, B2 and B1 in water

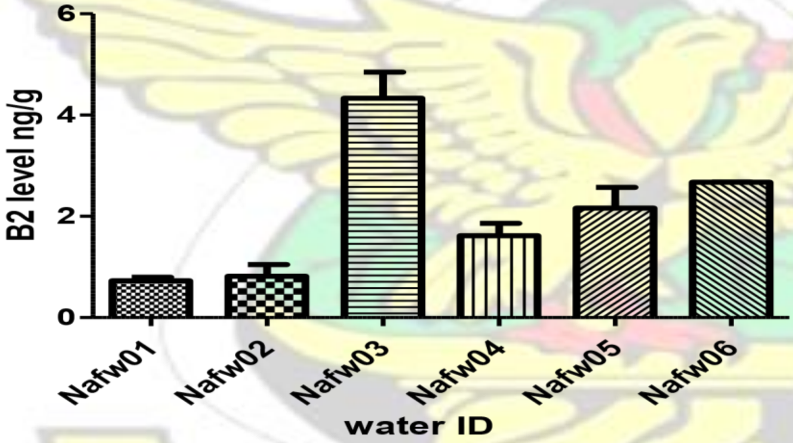
The aflatoxins that leached into the washing water were also measured. Generally, there was a marked increase in all measured aflatoxin levels in step 2; washing, step 3; washing and soaking in water. The aflatoxin levels then decreased in step 5; washing after which there was an increase again. Only aflatoxin G1 levels reduced thereafter but aflatoxin B2 and B1 increased significantly. Significance was determined at $p\text{-value}<0.0001$.

The aflatoxin G2 levels determined during the processing is represented in the figure 4.3A. The mean G2 level for Nafw01 to Nafw06 is given as 0.19 ± 0.10 ng/g, 0.69 ± 0.13 ng/g 2.53 ± 0.44 ng/g, 1.24 ± 1.10ng/g, 2.65 ± 0.18ng/g, 0.39 ± 0.01 ng/g and 1.28 ± 0.01 ng/g respectively.

The figure 4.3B shows the mean aflatoxin B2 level in water used during the processing. The mean B2 level for Nafw01 to Nafw06 is given as 0.73 ± 0.07 ng/g, 0.82 ± 0.23 ng/g, 4.33 ± 0.51 ng/g, 1.62 ± 0.23ng/g, 2.16 ± 0.4 ng/g, and 2.67 ± 0.01 ng/g respectively.

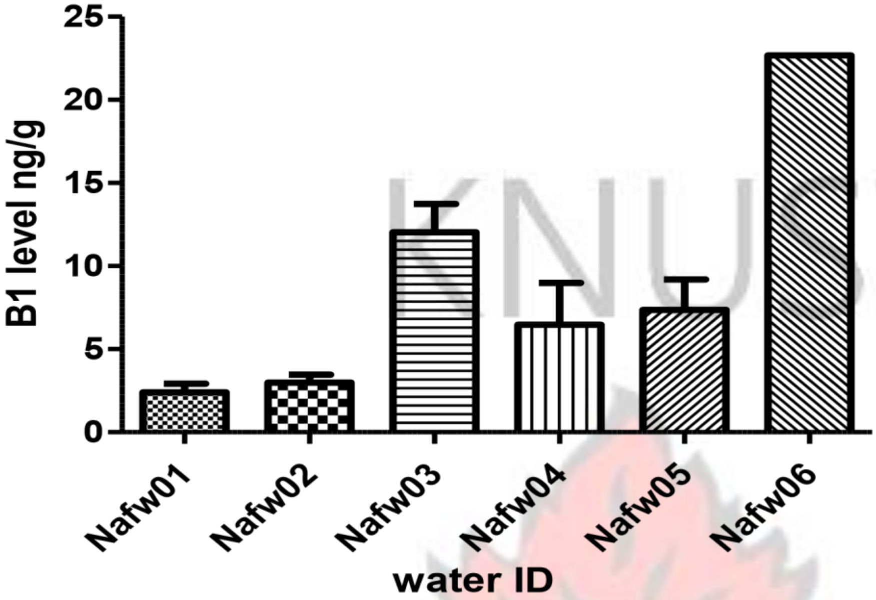
The aflatoxin B1 level from the water used in the processing of the maize is presented in figure 4.3C. The level of B1 in the water samples Nafw01 to Nafw06 were found to be 2.40 ± 0.53 ng/g, 2.99 ± 0.48 ng/g, 12.03 ± 1.71 ng/g, 6.46 ± 2.53 ng/g, 7.36 ± 1.83 ng/g, and 22.67 ± 0.02 ng/g.

Fig 4.3A: Aflatoxin G2 level in water in processing steps. Results are means ± SD. Nafw01- step 2 washing; Nafw02- step 3 washing; Nafw03- step 4 soaking in water; Nafw04- step 5 washing; Nafw05- step 5 second washing; Nafw06- step 10 stock from cooked kenkey



B2 in water

Fig 4.3B: Aflatoxin B2 level in water in processing steps. Results are means ± SD. Nafw01- step 2 washing; Nafw02- step 3 washing; Nafw03- step 4 soaking in water; Nafw04- step 5 washing; Nafw05- step 5 second washing; Nafw06- step 10 stock from cooked kenkey



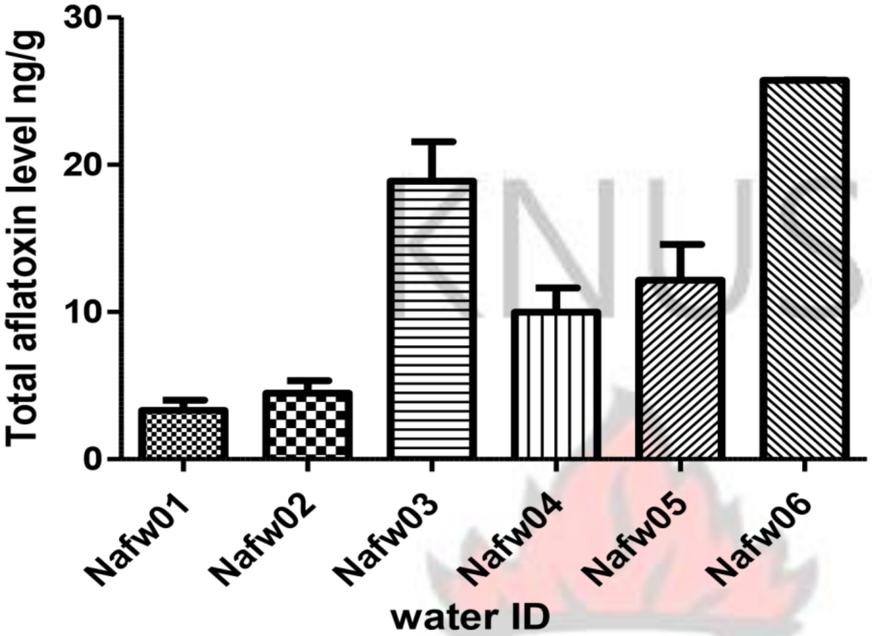
B1 in water

Fig 4.3C: Aflatoxin B1 level in water in processing steps. Results are means ± SD. Nafw01- step 2 washing; Nafw02- step 3 washing; Nafw03- step 4 soaking in water; Nafw04- step 5 washing; Nafw05- step 5 second washing ; Nafw06- step 10 stock from cooked kenkey

4.1.4 Total aflatoxin level in water

The total aflatoxin level of sampled water that was used for the processing the maize is presented in the figure 4.4 below. The aflatoxin levels increased significantly in the washing water in all with exception of step 5 washing.

Concentrations of 3.32 ± 0.70 ng/g, 4.50 ± 0.85 ng/g, 18.89 ± 0.85 ng/g, 9.99 ± 1.64 ng/g, 12.17 ± 2.43 ng/g, and 25.73 ± 0.03 ng/g were determined for water samples Nafw01 to Nafw06 respectively. The mean concentrations were found to be significantly different with pvalue<0.0001 from a one-way analysis of variance analysis.

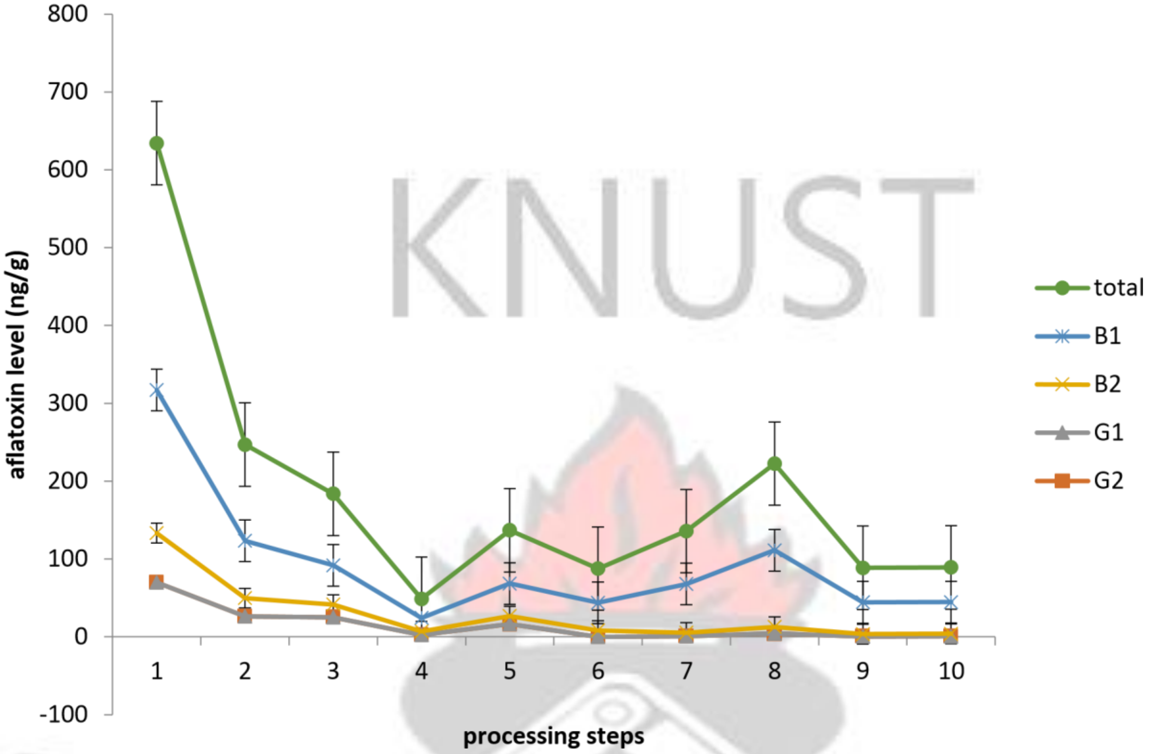


Total aflatoxin level in water

Fig 4.4: Total aflatoxin level in water in processing steps. Results are means ± SD. Nafw01- step 2 washing; Nafw02- step 3 washing; Nafw03- step 4 soaking in water; Nafw04- step 5 washing; Nafw05- step 5 second washing; Nafw06- step 10 stock from cooked kenkey

4.1.5 Levels of G2, G1, B2, B1 and total aflatoxin in the maize at each processing stage

G 1 was not detected in the maize during the processing. The level of each aflatoxin was highest in processing step 1 and then decreased as the processing went on to the lowest level at step 4. After processing step 4, the level of the various aflatoxins fluctuated to the last processing step. This is illustrated in the figure below.

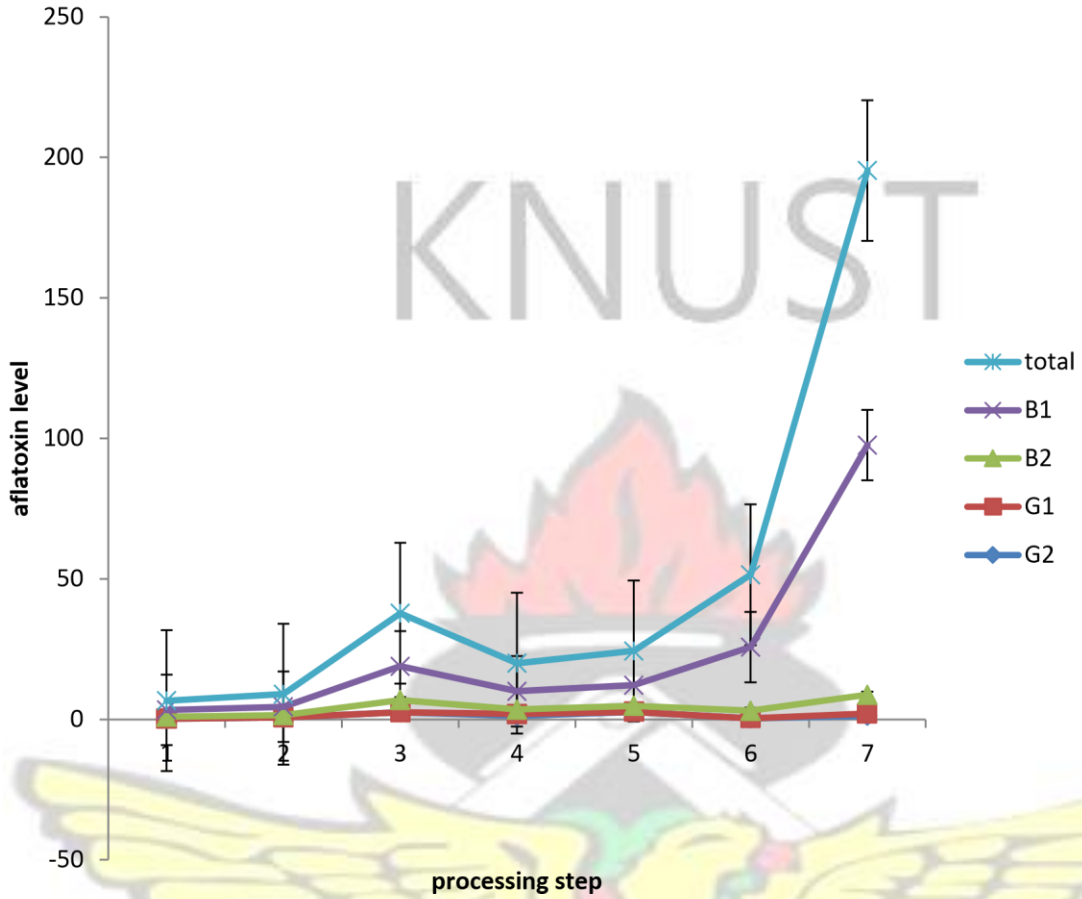


Levels of various aflatoxins in the maize at each processing step

Fig 4.5: Levels of G2, G1, B2, B1 and total aflatoxin in the maize at each processing stage. Results are means ± SD. Step 1- Sifting; Step 2- Washing; Step 3- Washing; Step 4- Soaking in water; Step 5- Washing; Step 6- Milling/fermentation for 72 hours; Step 7- Fermentation to 96 hours; Step 8- Cooking for 40 min; Step 9-Mixing with uncooked dough; Step 10- cooking of kenkey.

4.1.6 Levels of G2, G1, B2, B1 and total aflatoxin in the water used in processing the maize

The lowest level of the various aflatoxins was detected in water sample Nafw01. The level of the various aflatoxins increased from the sample 1 (Nafw01) reaching the highest level from the last water sample (Nafw07).



Levels of various aflatoxins in the water used to wash the maize after each step.

Fig 4.6: Total aflatoxin level in water in processing steps. Results are means ± SD. Nafw01- step 2 washing; Nafw02- step 3 washing; Nafw03- step 4 soaking in water; Nafw04- step 5 washing; Nafw05- step 5 second washing ; Nafw06- step 10 stock from cooked kenkey

4.2 DISCUSSION

The relevance of aflatoxin contamination of farm produce especially cereals including maize cannot be underrated due to its health implications. The study was conducted to determine the effect of various processes in the preparation of maize into kenkey on aflatoxin levels.

A mass of 10 kg of maize was first inoculated with the mycotoxin and found to be highly contaminated with aflatoxin after which the contaminated maize was sifted to remove all debris and other physical contaminants and the following processing techniques followed: sorting; washing; soaking in water; milling; fermentation and cooking.

Washing and eventually soaking of the maize in water was most useful in reducing the levels of aflatoxins G2, B2 and B1 significantly ($p<0.001$) in the maize. These steps were significant in eliminating moulds. Washing may have been efficient in removing much of the aflatoxins because these moulds are on the surface of the maize and thereby vigorous rubbing of the grains together could have helped in removing the moulds on the surface. This reflected in the level of aflatoxin in the water used in washing the maize grains. Mycotoxins that are soluble in water can easily be washed from the surface of grains and leached into the water which was in the case of soaking in water. Considerable amount of aflatoxins was found to be removed by simply washing the maize grains and immersing them in water (Shetty and Bhat, 1999). The role of maize washing in the cleaning and decontamination process cannot be underestimated.

The aflatoxin that gets removed from the maize eventually ends up in the washing water. For this, processes that recorded a reduced aflatoxin level in the maize could be matched with a significant hike in water. The washing and soaking steps that resulted in a significant reduction in aflatoxin levels in the maize also resulted in a rise in aflatoxin levels in the water and this further emphasizes the role of washing in the elimination of mycotoxins and for that matter aflatoxins.

Milling of the maize did not necessarily reduce aflatoxin levels but rather redistributed it (Thammawong *et al*., 2011; Tibola *et al*., 2015). Physical processes that are carried out before milling such as sorting and cleaning are effective ways of reducing mycotoxin content before milling. If these processes are enhanced and performed with much more emphasis, it will help reduce contaminations in our food products.

Fermentation also led to a decrease in the aflatoxin levels in the maize. The reduction could have been attributed to the fermentation process. Fermentation (also known as zymology) among other definitions is the process of transforming carbohydrates to alcohol or organic acids using microorganisms yeast and bacteria under anaerobic conditions. In food processing, fermentation may lead to the production of ethanol by converting sugars or producing lactic acid for the preservation of foods as a result of carbon dioxide produced by yeast activity (Oyeyiola, 1990). These processes are enhanced by the reduction in pH and oxygen and the increase in moisture during the fermentation process which could have created an unsuitable condition for mould growth and for that matter aflatoxin production (Oluwafemi and Taiwo, 2003). The lactic acid produced in the course of the fermentation process is known to reduce mould growth and aflatoxin production because of competition for nutrients between bacterial cells and mould/fungi. Other studies have shown that lactic acid efficiently removed aflatoxin B1. It has also been suggested that, removal of toxins is through noncovalent binding of mutagens by divisions of the cell wall skeleton of lactic acid bacteria (Assohoun, 2013). The lactic acid seems to ultimately bind to aflatoxins in grains, legumes and nuts. This corroborates with a similar finding where fermentation reduced aflatoxin B1 by 50% after fermentation (Oluwafemi and Ikeowa, 2005). Also, fermentation was demonstrated to result in a reduction of pH from 5.2 to 3.7, this change helped detoxify aflatoxin B (Oluwafemi and Taiwo, 2003). The lower pH of the media could also have contributed to the removal of the toxins from the media as other studies have indicated that treatment of LAB pellets with HCL significantly enhanced the binding ability of the bacteria (Assohoun, 2013).

Also AFB1 was degraded during fermentation to AFB2a, a less toxic and hydrated derivative of AFB1 and other compounds during beer production. AFB1 carry over was still present but was reduced in the case of wine fermentation because of hydration (Inoue *et al*., 2013).

Just after the fermentation processes, aflatoxin levels rose again. In beer fermentation, it was noticed that, although the AFB1 level decreased of its initial concentration on the first day, it increased again on the second day. This finding suggested that, AFB1was taken up by the foam produced during the early phase of fermentation and that, after the foam disappeared, AFB1was released again into the wort. The adsorption ratio for the yeast crop was also analyzed after the fermentation process was complete. In contrast with the results for bottom fermentation, AFB1 was not adsorbed on the yeast surface (Inoue *et al*., 2013). This could have resulted in the increase in aflatoxin after the fermentation processes

The process of cooking kenkey saw a reduction in aflatoxin levels which can be attributed to the presence of the high temperature at which kenkey was prepared. Aflatoxins are generally reported to be heat-stable and are not easily destroyed by ordinary cooking except at high temperatures (Bolger *et al*., 2001). They can be partially removed by cooking, particularly when this occurs under pressure in moist conditions (Sinha, 1998).

Total aflatoxin levels in the contaminated maize were reduced in a similar trend and the following processes: sorting; cleaning; soaking in water; milling; fermentation and cooking were proven to help in mitigating mycotoxins contamination in the maize.

There was a significant rise in the aflatoxin G1, G2, B1 and B2 levels in the water residue from the cooked kenkey. There were no signs of aflatoxin G1 in the maize but rather in the water residue and this signal a health threat since it’s often a delicacy in most communities. This is matched with the reduced level of the aflatoxin in the kenkey meaning the process of kenkey preparation significantly reduced aflatoxin levels.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The processing steps in the preparation of kenkey; washing, soaking in water, milling, fermentation and cooking were useful in reducing the aflatoxin levels in kenkey. This also means that in the case of low levels of contamination, these processes if enhanced can drastically remove a lot if not all of the aflatoxins from our foods.

The consumption of the stock from cooked kenkey should be avoided as most of the toxins and contaminants leach into it.

Maize is also mostly used in almost all weaning foods and at such tender ages, children might be vulnerable and therefore proper education and precautions must be taken to eliminate such toxins from our foods.

5.2. Recommendation

Based on the results obtained, it will be recommended that further work be done on other foods that use these aflatoxin susceptible grains and produce.

REFERENCES

Abbas A, Vales H., Dobson A.D.W. (2009). Analysis of the effect of nutritional factors on OTA and OTB biosynthesis and polychetide syntase gene expression in Aspergillus ochraceus. Int. J. Food Microbiol, 135:22-27

Agag, B. (2004). Mycotoxins in foods and feeds: 1-aflatoxins. *Ass. Univ. Bull. Environ. Res.* **7**:176-206

Adjetey, J. A. (1994). Maize physiology and growth requirements. Crops Research Institute (CRI) Management Guide 19. Kumasi. p22.

Addo S., Birkinshaw L.A., Hodges R.J. (2002). Ten years after the arrival in Ghana of larger grain borer: farmers' responses and adoption of IPM strategies Int. J. Pest Manag., 48 (2002), pp. 315-325

Akrobortu, D.E. (2008). Aflatoxin contamination of maize from different storage locations in Ghana. M.Sc. Thesis submitted to the Department of Agricultural Engineering, Kwame Nkrumah University of Science and Technology, Ghana. 27-32

Assohoun Marina C. N., Djeni Théodore N., Koussémon-Camara M., Brou K. (2013). Effect of Fermentation Process on Nutritional Composition and Aflatoxins Concentration of Doklu, a Fermented Maize Based Food

Awuah R. and Kpodo K, (1996). High incidence of Aspergillus flavus and aflatoxins in stored groundnut in Ghana and the use of a microbial assay to assess the inhibitory effects of plant extracts on aflatoxin synthesis. *Mycopathologia*.**134**:109-14.

Aycicek, H., Aksoy, A. and Saygi, S. (2005). Determination of aflatoxin levels in some dairy and food products which consumed in Ankara, Turkey. *Food Control* **16**:263-266.

Bakirdere, S., Bora, S., Bakirdere, E. G., Aydin, F., Arslan, Y., Komesli, O. T., Aydin, I., and Yildirim, E. (2012). Aflatoxin species: their health effects and determination methods in different foodstuffs. *Cent. Eur. J. Chem.* **10**:675-685.

Bankole, S. A., & Adebanjo, A. (2003). Review of mycotoxins in food in West Africa: current situation and possibilities of controlling it. *African Journal of Biotechnology*, **2**(9), 254-263.

Baydar, T., Engin, A.B., Girgin, G., Aydin, S. and Sahin, G. (2005). Aflatoxin and ochratoxin in various types of commonly consumed retail ground samples in Ankara, Turkey. Annals of Agriculturaland Environmental Medicine, 12: 193-197.

Bett, C. and Nguyo, R. (2007). Post-harvest Storage Practices and Techniques used by Farmers in Semi-arid Eastern and Central Kenya. *African Crop Science Proceedings* 8, pp.1023-1227 **Bhat, R., Rai, R. V., and Karim, A. A. (2010).** Mycotoxins in Food and Feed: Present Status and Future Concerns. *Compr. Rev. Food Sci. F.* **9**:57-81.

Becer, U. K. and Filazi, A. (2010). Aflatoxins, Nitrates and Nitrites Analysis in The

Commercial Cat and Dog Foods. *Fresenius Environmental Bulletin*, **18**(11), 2523-2527.

Bennett, J. W., and Klich, M. (2003). Mycotoxins. *Clin Microbiol Rev* **16**:497-516

Bolger, M., Coker, R.D., DiNovi, M., Gaylor, D., Gelderblom, W.,Olsen, M., Paster, N., Riley, R.T., Shephard, G., Speijers, G.J.A. (2001). Fumonisins. In: Safety Evaluation of Certain Mycotoxins in Food, WHO Food Additives Series 47, FAO Food and Nutrition Paper 74, Prepared by the 56th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). WHO, Geneva, pp. 103–279

Carrillo, L. (2003). Microbiología agrícola. En: Carrillo, L. Los hongos de los alimentos y forrajes. Universidad Nacional de Salta. Cap. 6, p.1-7.

CAST (2003). Mycotoxins: Risks in plant, animal, and human systems. Council for Agricultural Science and Technology (CAST), Ames, Iowa, USA

Calderwood, D.L., & H.W. Schroeder (1968). Aflatoxin development and grade of undried rough rice following prolonged storage in aerated bins USDA, ARS 52-26, 32p, illus

Carlson M. A., Bargeron C. B., Benson R. C., Fraser A. B., Phillips T. E., Velky J. T., Groopman J. D., Strickland P. T. & Ko H. W. (2000). An automated, handheld biosensor for aflatoxin. Biosensor and Bioelectronics, 14, 10-11, pp. 841-848

Castells, M., Marin, S., Sanchis, V., Ramos, A.J. (2005). Fate of mycotoxins in cereals during extrusion cooking: A review. Food Additives and Contaminants: Part A 22, 150-157.

Chao, T. C., Maxwell, S. M., and Wong, S. Y. (1991). An outbreak of aflatoxicosis and boric acid poisoning in Malaysia: Aclinic pathological study. *Journal of Pathology*, 164, 225-333.

Collier, L., A. Balows., Sussman M. (1998). Topley & Wilson's Microbiology and Microbial Infections, 9th Ed, vol. 4. Arnold, London, Sydney, Auckland, New York

Coulibaly O., Hell K., Bandyopadhyay R., Hounkponou S., and Leslie J. F. (2008). Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade, CAB International,

Coulter J, Asante E.O, Ofei Aboagye E. (1993). Financing of Agricultural trade. Report on a seminar organized by the Ministry of Food and Agriculture, Accra, Ghana, 4 May 1993. p. 24.

Cotty, P. J. (1994). Influence of field application of an atoxigenic strain of Aspergillus flavus on the population of A. flavus infecting cotton bolls and on the aflatoxin content of cottonseed. Phytopathology 84: 11, 1270-1277.

Delmulle B. S., De Saeger S. M. D. G., Sibanda L., Barna-Vetro I. & Van Peteghem C. H. (2005). Development of an immunoassay-based lateral flow dipstick for the rapid detection of aflatoxin B1 in pig feed. Journal of Agricultural and Food Chemistry, 53, 9, pp. 3364-3368

DARSA Department of Agriculture-Republic of South Africa (2003). Maize production. http://www.nda.agric.zaipublications. Date accessed: 3-08-2016. 09:24 pm.

Diener, U.L., Cole, R.J., Sanders, T.H., Payne, G.A., Lee, L.S., Klich, M.A. (1987). Epidemiology of Aflatoxin, in formation by Aspergillus flavus. Annual Review of Phytopathology 25, 240-270.

Espinosa-Calderón, A., Contreras-Medina, L. M., Muñoz-Huerta, R. F., Millán-Almaraz, J. R., González,R. G. G., and Torres-Pacheco, I. (2011). Methods for Detection and Quantification of Aflatoxins.

Fackler, P.L., Livingston, M.J. (2002). Optimal Storage by Crop Producers. *American Journal of Agricultural Economics*, **84(3)**, pp.645-659

Fallah, A. A. (2010). Aflatoxin M1 contamination in dairy products marketed in Iran during winter and summer. *Food Control* **21**:1478-1481

F.A.O (1983). Post-harvest losses in quality of food grains. Food and Agriculture

Organisation (Food and Nutrition Paper 29, 103

Fandohan P, Zoumenou D, Hounhouigan DJ, Marasas WF, Wingfield MJ, Hell K. (2005). Fate of aflatoxins and fumon- isins during the processing of maize into food products in Benin. International Journal of Food Microbiology 98(3):249–259.

Farombi E. O. (2006). “Aflatoxin contamination of foods in developingcountries: implications for hepatocellular carcinoma andchemopreventive strategies,” *African Journal of Biotechnology*, **5** :(1)1–14.

Fening J. O., Ewusi- Mensah, N. and Safo, E.Y. (2011). Short-term effects of cattle manure compost and NPK application on maize grain yield and soil chemical and physical properties. *Agricultural Science Research Journal 1 (3): 69- 83*

Fung F., and Clark R (2004). Health effectss of mycotoxins: a toxicological overview. *Journal of toxicologyClinical toxicology*. **98**(3); 249-259

Geetanjali K. (2015). Effect of Processing on Mycotoxin Content in Grains, Critical Reviews in Food Science and Nutrition, **55**:12, 1672-1683

Gong, Y. Y., Hounsal, A., Egal, S., Turner, P. C., Sutcliffe, A. E., Hall, A. J., Cardwell, K. F., & Wild, C. P. (2004). Postweaning exposure to aflatoxin results in impaired child growth. *A longitudinal study in Benin, West Africa.Environmental Health Perspective*, 112, 1334-1338.

Hazel, C. M. and Patel, S. (2004). Influence of processing on trichothecene levels. *Toxicol. Lett.* **153**:51–59

Hussain, I., Anwar, J., Munawar, M. A., and Asi, M. R. (2008). Variation of levels of aflatoxin M1 in raw milk from different localities in the central areas of Punjab, Pakistan. *Food Control* **19**:1126- 1129.

Hell, K., Cardwell, K. F., Setamou, M. and Schulthess, F. (2000b). Influence of insect infestation on aflatoxin contamination of stored maize in four agroecological regions in Benin. Afr. Entomol. **6**: 169-177.

IITA International Institute of Tropical Agriculture (2007). Annual report of the International Institute of Tropical Agriculture

Inoue, T., Nagatomi, Y., Uyama, A., & Mochizuki, N. (2013). Degradation of Aflatoxin B$_1$ during the Fermentation of Alcoholic Beverages. *Toxins*, *5*(7), 1219–1229. http://doi.org/10.3390/toxins5071219

Kaaya, N. A. and Warren H. L. (2005). A Review of Past and Present Research on Aflatoxin in Uganda: African J. of Food Agriculture Nutrition and Development (AJFAND), 5(1):1-18.

Kaushik G., Satya S., Naik S.N. (2009). Food processing a tool to pesticide residue dissipation—A review. Food Res Int 42:26–40. doi:10.1016/j.foodres.2008.09.009

Kawasugi, S., Kawasima, K., Siriacha, P. and Tanboon-Ek, P. (1988). Experimental studies on field dried Thai maize inoculated with Aspergillus flavus. Proc. Japanese Assoc. Mycotoxicol 28, 17-22.

Kimenju, S.C., Hugo, de G. (2010). Economic Analysis of Alternative Maize Storage Technologies in Kenya. Contributed Paper Presented at the Joint 3rd African Association of Agricultural Economists (AAAE) and 48th Agricultural Economists Association of South Africa (AEASA) Conference, Cape Town, South Africa, September, 19-23, 2010

Komen, J.J., Mutoko C.M., Wanyama J.M., Rono S.C. and Mose L.O. (2006). Economics of Post-Harvest Maize Grain Losses in TransNzoia and Uasin Gishu Districts of NorthWest Kenya. Kenya Agricultural Institute, Kitale

Kumar, P. L., Reddy, S. V., Waliyar, F. (2000). Management of aflatoxins in maize. International Research for the Semi-Arid Tropics. (ICRISAT). www.aflatoxin.info

Kumi, J. (2011). Measurement of aflatoxin levels in homemade Weanimix from the EjuraSekyeredumase District in the Ashanti Region of Ghana (Dissertation submitted in partial fulfillment for the award of Bachelor of Science in Medical Laboratory Sciences). Accra, Ghana: University of Ghana

Lopez, C., et al. (2002). Aflatoxin B1 in human serum: Aflatoxin B1 content in patients with hepatic diseases. *Medicina (Buenos Aires)*, 313-316.

Manetta, A. C. (2011). Aflatoxins: Their Measure and Analysis, Aflatoxins -Detection, Measurement and Control, Dr Irineo Torres-Pacheco (Ed.), ISBN: 978-953-307-711-6

McMillian, W.W., Widstrom, N.W., Wilson, D.M. (1987). Impact of husk type and species of infesting insects on aflatoxin contamination in pre-harvest corn at Tifton, Georgia. Journal of Entomological Science 22, 307-310

Mills, G. (2002). Annual Report to Congress. General Mills' Maize Processing Project, U. S. African Development Foundation, Kumasi, Ghana.

Méndez-Albores A & Moreno-Martínez E. (2009). Las Micotoxinas: Contaminantes naturales de los alimentos". Revista Ciencia, julio-septiembre 2009:1-7. Mycotoxin analysis: An update. *Food Addit. Contam. A* **25**:152-163.

MOFA (2005). Ministry of Food and Agriculture, Monitoring and Evaluation report for 2005, Ashanti Region.

MOFA (2006). Ministry of Food and Agriculture, Sector Development Policy FASDEP II.

MOFA (2013). Ministry of Food and Agriculture, Agriculture in Ghana: Facts and figures

Morris, M.L., Tripp,R. and Dankyi, A.A. (1999). Adoption and Impacts of Improved Maize Production Technology. A Case Study of the Ghana Grains Development Project, Economics Program Paper 99-01. Mexico, D.F., CIMMYT. Available online http://www.cimmyt.org/Research/economics/map/research_results/program_papers/pdf/EPP%2099_01.pdf

Nakai, V. K., de Oliveira Rocha, L., Gonçalez, E., Fonseca, H., Ortega, E. M. M., and Corrêa, B. (2008). Distribution of fungi and aflatoxins in a stored peanut variety. *Food Chem.*

106:285-290.

Nawaz S., Coker R. D. & Haswell S. J. (1992). Development and evaluation of analytical methodology for the determination of aflatoxins in palm kernels. Analyst, 117, pp. 67-74

Nasir M. S. & Jolley M. E. (2002). Development of a fluorescence polarization assay for the determination of aflatoxins in grains. Journal of Agricultural and Food Chemistry, 50, pp. 3116-3121

Nielsen, N. L. (2003). Maize: New Uses for an Old Crop. Far New Zealand Maize Conference Proceedings, 12-13 Feb. 2003, Hamilton, New Zealand.

Oluwafemi F. and Ikeowa M.C. (2005). Fate of aflatoxin B1during fermentation of maize into "Ogi". Niger. Food J. 23: 243-247.

Oluwafemi, F., and Taiwo, V. O. (2003) Reversal of toxigenic effects of aflatoxin B1 on cockerels by alcoholic extract of African nutmeg *Monodora myristica. J. Sci. Food Agric* 84: 333340.

Oyeyiola G.P. (1990) World J Microbiol Biotechnol 6:17 http://doi.org/10.1007/BF01200938

Paniel N., Radoi A. & Marty J. L. (2010). Development of an electrochemical biosensor for the detection of aflatoxin M1 in milk. Sensors, 10, pp. 9439-9448, ISSN 1424-8220

Pardez-Lopez, O., Gonzales-Casteneda, J., & Carabenz-Trejo, A. J. (1991). Influence of solid substrate fermentation on the chemical composition. Journal of Fermentation and Bioengineering, 71, 58–62

Park D.L. (2002). Effect of processing on aflatoxin. Advances in Experimental Medicine and Biology, 504:173–179.

Pelczar., Jr. M. J., Chan. E. C. S., Krieg, N. R., Pelczar, M. F., and Edwards, D. D. (1993). Microbiology: Concepts and Applications, McGraw-Hill, Inc. p 279-286.

Peraica, M., Radić B., Lucić A. and Pavlović M. (1999). Toxic effects of mycotoxins in humans. *Bulletin of the World Health Organization.*, http://whqlibdoc.who.int/bulletin/1999/9 bulletin_ 1999_77%289%29_754-766.pdf, 754-766.

Piermarini S., Volpe G., Micheli L., Moscone D. & Palleschi G. (2009). An ELIME-array for detection of aflatoxin B1 in corn samples. Food Control, 20, pp. 371-375

Probst, C., Schulthess, H., & Cotty, P. J. (2010). Impact of Aspergillus sect. Flavi community structure on the development of lethal levels of aflatoxins in Kenyan maize (Zea mays). *Journal of Applied Microbiology*, 108, 600-610.

Reddy, S. V., & Waliyar, F. (2012). Properties of aflatoxin and its producing fungi. *Aflatoxins*, http://www.icrisat.org/aflatoxin/aflatoxin.asp, (Accessed on 8th June 2012).

Rembold, F., Hodges, R., Bernard, M.H. Knipschild., Léo, O. (2011). The African Postharvest Losses Information System (APHLIS). European Union, Luxembourg.

Robens, J. K., & Richard, J. L. (1992). Aflatoxins in animal and human health. *Rev Environmental Contamination and Toxicology*, 127, 69-94.

Sallah, P.Y.K. (1986). Maize improvement research in Ghana with special reference to the Guinea Savannah Zone. *Proceedings from the 1st National Workshop on Improving Farming Systems in Savanna Zone of Ghana, Nyankpala, Ghana, 8-10 April. Pp 1- 12*

Sallah, P.Y.K. (1998). Studies on performance of some open pollinated maize cultivars in the guinea savanna III. Genetic contribution of four cultivars under varying population and nitrogen regimes. *African Crop Science Journal* 10: 35- 42

SARI (1996). Savanna Agricultural Research Institute. Annual Report. 1996**.**

Schoental, R. (1967). Aflatoxins. *Annu. Rev. Pharmacol.* **7**:343-356.

Scott, P. M., Kanhere, S. R., Lau, P. Y., Dexter, J. E. and Greenhalgh, R. (1983). Effects of Experimental flour milling and breadbaking on retention of deoxynivalenol (vomitoxin) in hard red spring wheat. *Cereal Chem.* **60**:421–424

Shetty P.H., Bhat R.V. (1999). A physical method for segregation of fumonisin-contaminated maize. Food Chem 66:371–374

Sinha, K. K., Sinha, A. K. (1992). Impact of stored grain pests on seed deterioration and aflatoxin contamination in maize. Journal of Stored Products Research 28, 211-219.

Sinha, K.K. (1998). Detoxification of mycotoxins and food safety. In: Sinha, K.K., Bhatnagar, D. (Eds.), Mycotoxins in Agriculture and Food Safety, Eastern Hemisphere Distribution. Marcel Dekker, New York, pp. 381–405.

Siriacha P, Kawashima K, Saito M, Tonboon ek P. and Buangsuwon D. (1990). Prevention of Thai maize from the infection by Aspergillus flavus and aflatoxin contamination in various packages. Source Proceedings of the Japanese Association of Mycotoxicology. 32:41-46.

Shephard, G.S. (2003). Aflatoxin and food safety: recent African perspectives. Journal of Toxicology, **22**(2&3):267–286.

Shephard, G. S. (2009). Aflatoxin analysis at the beginning of the twenty-first century. *Anal. Bioanal.Chem.* **395**:1215-1224.

Siontorou C. G., Nikolelis D. P., Miernik A. & Krull U. J. (1998). Rapid methods for detection of Aflatoxin M1 based on electrochemical transduction by self-assembled metal-supported bilayer lipid membranes (s-BLMs) and on interferences with transduction of DNA hybridization. Electrochimica Acta, 43, pp. 3611-3617

Strosnider, H., Azziz-Baumgartner, E., Banziger, M., Bhat, R. V., Breiman, R. and Brune, M. N. (2006). Workgroup report: Public Health strategies for reducing aflatoxin exposure in developing countries. *Environmental Health Perspective*, 114, 1898-1903.

Tannahill, R. (1995). Food in History. Three Rivers Press. p. 75.

Tanboon-ek, P. (1989). Control of aflatoxin in maize. In: Semple, R.L.,Frio, A.S., Hicks PA and Lozare, J.V. (eds.) Afycotoxin Prevention and Control in Food Grains. FAO, Rome, Italy, pp.p.166-172

Thammawong, M., Okadome, H., Shiina, T., Nakagawa, H., Nagashima, H.,Nakajima, T., Kushiro M. (2011). Distinct distribution of deoxynivalenol, nivalenol, and ergosterol in Fusarium-infected Japanese soft red winter wheat milling fractions.Mycopathologia, 172, 323 330

Tibola, C. S., Fernandes, J. M. C., Guarienti, E. M., Nicolau, M. (2015). Distribution of Fusarium mycotoxins in wheatmilling process. Food Control, 53:91□95.

Tombelli S., Mascini M., Scherm B., Battacone G. & Migheli Q. (2009). DNA biosensors for the detection of aflatoxin producing Aspergillus flavus and A. parasiticus. Monatshefte für Chemie / Chemical Monthly, 140, pp. 901-907.

Trucksess M. W., Stoloff L., Pons W. A., Cucullu A. F., Lee L. S. and Franz A. O. (1977).

Thin layer chromatographic determination of aflatoxin B1 in eggs. Journal of the Association of Official Analytical Chemists, 60, 4, pp. 795-798

Turner, P. C., Sylla, A., Gong, Y. Y., Diallo, M. S., Sutcliffe, A., E., Hall, A. J., and Wild, C. P. (2005). Reduction in exposure to carcinogenic aflatoxins by post-harvest intervention measures in West Africa: a common-based intervention study. *The Lancet*, 365, 1950-1956.

Turner, N. W., Subrahmanyam, S. and Piletsky, S. A. (2009). Analytical methods for determination of mycotoxins: A review. *Anal. Chim. Acta* **632**:168-180

Tweneboah, C. K. (2000). Modern Agriculture in the Tropics. Pp 37-40

Van Egmond, H.P., Jonker, M.A., (2004). Worldwide Regulations on Aflatoxins—The Situation in 2002. J. Toxicol. Toxin Rev., 23(2&3):273-293.

Variety release (2007). Variety release document submitted to the variety release committee. Ministry of Food and Agriculture, Ghana.

Variety release (2010). Variety release document submitted to the variety release committee. Ministry of Food and Agriculture, Ghana.

Walderhaug M. (1992). Ciguatera. Foodborne Pathogenic Microorganisms and Natural Toxins Handbook. Food and Drug Administration, U.S.

Widstrom N.W., Butron A., Guo B.Z., Wilson D.M., Snook M.E., Cleveland T.E., Lynch R.E. (2003). Control of preharvest aflatoxin contamination in maize by pyramiding QTL involved in resistance to ear-feeding insects and invasion by Aspergillus spp. Eur. J. Agron.19, 563–572.

Williams J.H., Phillips T.D., Jolly P.E., Stiles J.K., Jolly C.M., and Aggarwal D. (2004) Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. American Journal of Clin Nutr, **80**(5):1106–1122.

William, J., Philips, T. D., Jolly, P. E., Stiles, J. K., Jolly, C. M., & Aggarwal, D. (2003). Human aflatoxicoses in developing countries: a review of toxicology exposure, potential health consequences and interventions. *American Journal of Clinical Nutrition*, 80, 1106-1122.

Wild C.P. and Turner P.C. (2002). The toxicology of aflatoxins as a basis for public health decisions. Mutagenesis, 17(6):471-81.

Yin Y, Yan L, Jiang J. (2008). Biological Control of Aflatoxin Contamination of Crops. Journal of Zhejiang University Science B. 9(10):787-792.

Zöllner, P., and Mayer-Helm, B. (2006). Trace mycotoxin analysis in complex biological and food matrices by liquid chromatography–atmospheric pressure ionisation mass spectrometry. *J.Chromatogr. A* **1136**:123-169.

APPENDIX

G2 level in each processing step

Pairwise analysis: post test using the Tukey test

The Tukey Multiple comparison test was used to test the difference in the G2 levels between the processing steps as given below.

Tukey's Multiple Comparison Test	Significant? P < 0.05?	95% CI of diff
Step 1 vs Step 2	Yes	40.82 to 46.27
Step 1 vs Step 3	Yes	41.92 to 47.36
Step 1 vs Step 4	Yes	64.66 to 70.10
Step 1 vs Step 5	Yes	50.72 to 56.17
Step 1 vs Step 6	Yes	67.15 to 72.59
Step 1 vs Step 7	Yes	66.32 to 71.76
Step 1 vs Step 8	Yes	62.73 to 68.17
Step 1 vs Step 9	Yes	66.61 to 72.05
Step 1 vs Step 10	Yes	66.49 to 71.94
Step 2 vs Step 3	No	-1.623 to 3.821
Step 2 vs Step 4	Yes	21.12 to 26.56
Step 2 vs Step 5	Yes	7.179 to 12.62

Step 2 vs Step 6	Yes	23.61 to 29.05
Step 2 vs Step 7	Yes	22.78 to 28.22
Step 2 vs Step 8	Yes	19.19 to 24.63
Step 2 vs Step 9	Yes	23.07 to 28.51
Step 2 vs Step 10	Yes	22.95 to 28.39
Step 3 vs Step 4	Yes	20.02 to 25.46
Step 3 vs Step 5	Yes	6.080 to 11.52
Step 3 vs Step 6	Yes	22.51 to 27.95
Step 3 vs Step 7	Yes	21.68 to 27.12
Step 3 vs Step 8	Yes	18.09 to 23.53
Step 3 vs Step 9	Yes	21.97 to 27.41
Step 3 vs Step 10	Yes	21.85 to 27.29
Step 4 vs Step 5	Yes	-16.66 to -11.22
Step 4 vs step 6	No	-0.2318 to 5.212
Step 4 vs Step 7	No	-1.062 to 4.382
Step 4 vs Step 8	No	-4.654 to 0.7898
Step 4 vs Step 9	No	-0.7718 to 4.672
Step 4 vs Step 10	No	-0.8888 to 4.555
Step 4 vs Step 11	No	-1.801 to 3.643
Step 5 vs Step 6	Yes	13.71 to 19.15
Step 5 vs Step 7	Yes	12.88 to 18.32
Step 5 vs Step 8	Yes	9.284 to 14.73
Step 5 vs Step 9	Yes	13.17 to 18.61
Step 5 vs Step 10	Yes	13.05 to 18.49
Step 6 vs Step 7	No	-3.552 to 1.892
Step 6 vs Step 8	Yes	-7.144 to -1.700
Step 6 vs Step 9	No	-3.262 to 2.182
Step 6 vs Step 10	No	-3.379 to 2.065
Step 7 vs Step 8	Yes	-6.314 to -0.8702
Step 7 vs Step 9	No	-2.432 to 3.012
Step 7 vs Step 10	No	-2.549 to 2.895
Step 8 vs Step 9	Yes	1.160 to 6.604
Step 8 vs Step 10	Yes	1.043 to 6.487
Step 9 vs Step 10	No	-2.839 to 2.605

B2 level in each processing step

Pairwise analysis: post test using the Tukey test

The results of a pairwise post test analysis are given below;

Tukey's Multiple Comparison Test	Significant? P < 0.05?	95% CI of diff
step 1 vs step 2	Yes	36.37 to 43.51
step 1 vs step 3	Yes	43.54 to 50.68
step 1 vs step 4	Yes	55.27 to 62.40
step 1 vs step 5	Yes	49.49 to 56.62
step 1 vs step 6	Yes	51.55 to 58.69
step 1 vs step 7	Yes	55.21 to 62.34
step 1 vs step 8	Yes	51.39 to 58.52
step 1 vs step 9	Yes	56.80 to 63.93
step 1 vs step 10	Yes	56.57 to 63.70
step 2 vs step 3	Yes	3.603 to 10.74
step 2 vs step 4	Yes	15.33 to 22.46
step 2 vs step 5	Yes	9.547 to 16.68
step 2 vs step 6	Yes	11.61 to 18.75
step 2 vs step 7	Yes	15.27 to 22.41
step 2 vs step 8	Yes	11.45 to 18.58
step 2 vs step 9	Yes	16.86 to 23.99
step 2 vs step 10	Yes	16.63 to 23.77
step 3 vs step 4	Yes	8.157 to 15.29
step 3 vs step 5	Yes	2.376 to 9.512
step 3 vs step 6	Yes	4.443 to 11.58
step 3 vs step 7	Yes	8.098 to 15.23
step 3 vs step 8	Yes	4.276 to 11.41
step 3 vs step 9	Yes	9.686 to 16.82
step 3 vs step 10	Yes	9.458 to 16.59
step 4 vs step 5	Yes	-9.349 to -2.213
step 4 vs step 6	Yes	-7.282 to -0.1459
step 4 vs step 7	No	-3.627 to 3.509
step 4 vs step 8	Yes	-7.449 to -0.3129
step 4 vs step 9	No	-2.039 to 5.097
step 4 vs step 10	No	-2.267 to 4.869
step 5 vs step 6	No	-1.501 to 5.635
step 5 vs step 7	Yes	2.154 to 9.290
step 5 vs step 8	No	-1.668 to 5.468
step 5 vs step 9	Yes	3.742 to 10.88
step 5 vs step 10	Yes	3.514 to 10.65
step 6 vs step 7	Yes	0.08695 to 7.223

step 6 vs step 8	No	-3.735 to 3.401
step 6 vs step 9	Yes	1.675 to 8.811
step 6 vs step 10	Yes	1.447 to 8.583
step 7 vs step 8	Yes	-7.390 to -0.2539
step 7 vs step 9	No	-1.980 to 5.156
step 7 vs step 10	No	-2.208 to 4.928
step 8 vs step 9	Yes	1.842 to 8.978
step 8 vs step 10	Yes	1.614 to 8.750
step 9 vs step 10	No	-3.796 to 3.340

B1 level at each processing step

Pairwise analysis: post test using the Tukey test

A pairwise analysis Oof the mean B1 level is as listed below;

Tukey's Multiple Comparison Test	Significant? P < 0.05?	95% CI of diff
step 1 vs step 2	Yes	107.5 to 112.8
step 1 vs step 3	Yes	130.8 to 136.2
step 1 vs step 4	Yes	163.8 to 169.2
step 1 vs step 5	Yes	139.5 to 144.8
step 1 vs step 6	Yes	145.6 to 150.9
step 1 vs step 7	Yes	118.7 to 124.0
step 1 vs step 8	Yes	82.79 to 88.13
step 1 vs step 9	Yes	140.4 to 145.7
step 1 vs step 10	Yes	140.5 to 145.8
step 2 vs step 3	Yes	20.68 to 26.02
step 2 vs step 4	Yes	53.68 to 59.01
step 2 vs step 5	Yes	29.33 to 34.66
step 2 vs step 6	Yes	35.47 to 40.80
step 2 vs step 7	Yes	8.535 to 13.87
step 2 vs step 8	Yes	-27.35 to -22.02
step 2 vs step 9	Yes	30.24 to 35.57
step 2 vs step 10	Yes	30.33 to 35.66
step 3 vs step 4	Yes	30.33 to 35.66
step 3 vs step 5	Yes	5.978 to 11.31
step 3 vs step 6	Yes	12.12 to 17.45
step 3 vs step 7	Yes	-14.81 to -9.480

step 3 vs step 8	Yes	-50.70 to -45.37
step 3 vs step 9	Yes	6.887 to 12.22
step 3 vs step 10	Yes	6.977 to 12.31
step 4 vs step 5	Yes	-27.02 to -21.69
step 4 vs step 6	Yes	-20.88 to -15.54
step 4 vs step 7	Yes	-47.81 to -42.48
step 4 vs step 8	Yes	-83.70 to -78.36
step 4 vs step 9	Yes	-26.11 to -20.78
step 4 vs step 10	Yes	-26.02 to -20.69
step 5 vs step 6	Yes	3.474 to 8.808
step 5 vs step 7	Yes	-23.46 to -18.13
step 5 vs step 8	Yes	-59.34 to -54.01
step 5 vs step 9	No	-1.758 to 3.576
step 5 vs step 10	No	-1.668 to 3.666
step 6 vs step 7	Yes	-29.60 to -24.27
step 6 vs step 8	Yes	-65.49 to -60.15
step 6 vs step 9	Yes	-7.899 to -2.565
step 6 vs step 10	Yes	-7.809 to -2.475
step 7 vs step 8	Yes	-38.55 to -33.22
step 7 vs step 9	Yes	19.03 to 24.37
step 7 vs step 10	Yes	19.12 to 24.46
step 8 vs step 9	Yes	54.92 to 60.25
step 8 vs step 10	Yes	55.01 to 60.34
step 9 vs step 10	No	-2.577 to 2.757

Total Aflatoxin level in each processing step

Pairwise analysis

A pairwise analyses of the total aflatoxin level among the 11 processing steps using the Tukey's test. The result is given below for all the processing steps.

Tukey's Multiple Comparison Test	Significant? P < 0.05?	95% CI of diff
step 1 vs step 2	Yes	187.1 to 200.2
step 1 vs step 3	Yes	218.7 to 231.8
step 1 vs step 4	Yes	286.2 to 299.3
step 1 vs step 5	Yes	242.1 to 255.2
step 1 vs step 6	Yes	266.7 to 279.8
step 1 vs step 7	Yes	242.6 to 255.7
step 1 vs step 8	Yes	199.3 to 212.4
step 1 vs step 9	Yes	266.2 to 279.3

step 1 vs step 10	Yes	265.9 to 279.0
step 2 vs step 3	Yes	25.06 to 38.17
step 2 vs step 4	Yes	92.53 to 105.6
step 2 vs step 5	Yes	48.45 to 61.57
step 2 vs step 6	Yes	73.09 to 86.20
step 2 vs step 7	Yes	48.98 to 62.09
step 2 vs step 8	Yes	5.683 to 18.79
step 2 vs step 9	Yes	72.56 to 85.67
step 2 vs step 10	Yes	72.31 to 85.42
step 3 vs step 4	Yes	60.91 to 74.02
step 3 vs step 5	Yes	16.84 to 29.95
step 3 vs step 6	Yes	41.47 to 54.58
step 3 vs step 7	Yes	17.36 to 30.47
step 3 vs step 8	Yes	-25.94 to -12.83
step 3 vs step 9	Yes	40.94 to 54.05
step 3 vs step 10	Yes	40.69 to 53.80
step 4 vs step 5	Yes	-50.63 to -37.52
step 4 vs step 6	Yes	-25.99 to -12.88
step 4 vs step 7	Yes	-50.10 to -36.99
step 4 vs step 8	Yes	-93.40 to -80.29
step 4 vs step 9	Yes	-26.52 to -13.41
step 4 vs step 10	Yes	-26.77 to -13.66
step 5 vs step 6	Yes	18.08 to 31.19
step 5 vs step 7	No	-6.027 to 7.083
step 5 vs step 8	Yes	-49.33 to -36.22
step 5 vs step 9	Yes	17.55 to 30.66
step 5 vs step 10	Yes	17.30 to 30.41
step 6 vs step 7	Yes	-30.66 to -17.55
step 6 vs step 8	Yes	-73.96 to -60.85
step 6 vs step 9	No	-7.084 to 6.026
step 6 vs step 10	No	-7.339 to 5.771
step 7 vs step 8	Yes	-49.86 to -36.74
step 7 vs step 9	Yes	17.02 to 30.13
step 7 vs step 10	Yes	16.77 to 29.88
step 8 vs step 9	Yes	60.32 to 73.43
step 8 vs step 10	Yes	60.07 to 73.18

step 9 vs step 10	No	-6.810 to 6.300

G2 in water

Pairwise analysis

A multiple comparison test using the Tukey’s Multiple Comparison Test is as presented below.

Tukey's Multiple Comparison Test	Significant? P < 0.05?	95% CI of diff
Step 1 vs Step 2	No	-2.312 to 1.328
Step 1 vs Step 3	Yes	-4.154 to -0.5140
Step 1 vs Step 4	No	-2.867 to 0.7730
Step 1 vs Step 5	Yes	-4.273 to -0.6330
Step 1 vs Step 6	No	-2.014 to 1.626
Step 2 vs Step 3	Yes	-3.662 to -0.02200
Step 2 vs Step 4	No	-2.375 to 1.265
Step 2 vs Step 5	Yes	-3.781 to -0.1410
Step 2 vs Step 6	No	-1.522 to 2.118
Step 3 vs Step 4	No	-0.5330 to 3.107
Step 3 vs Step 5	No	-1.939 to 1.701
Step 3 vs Step 6	Yes	0.3199 to 3.960
Step 4 vs Step 5	No	-3.226 to 0.4140
Step 4 vs Step 6	No	-0.9671 to 2.673
Step 5 vs Step 6	Yes	0.4389 to 4.079

B2 in water

Pairwise analysis

A post test using the Tukey’s Multiple comparison Test to determine where difference from the one-way analysis come from is given below;

Tukey's Multiple Comparison Test	Significant? P < 0.05?	95% CI of diff
Nafw01 vs Nafw02	No	-1.340 to 1.160
Nafw01 vs Nafw03	Yes	-4.856 to -2.356
Nafw01 vs Nafw04	No	-2.139 to 0.3614
Nafw01 vs Nafw05	Yes	-2.684 to -0.1836
Nafw01 vs Nafw06	Yes	-3.194 to -0.6936
Nafw02 vs Nafw03	Yes	-4.766 to -2.266
Nafw02 vs Nafw04	No	-2.049 to 0.4514
Nafw02 vs Nafw05	Yes	-2.594 to -0.09362
Nafw02 vs Nafw06	Yes	-3.104 to -0.6036

Nafw03 vs Nafw04	Yes	1.467 to 3.967
Nafw03 vs Nafw05	Yes	0.9216 to 3.422
Nafw03 vs Nafw06	Yes	0.4116 to 2.912
Nafw04 vs Nafw05	No	-1.795 to 0.7054
Nafw04 vs Nafw06	No	-2.305 to 0.1954
Nafw05 vs Nafw06	No	-1.760 to 0.7404

B1 IN WATER

Pairwise analysis

With the p-value from the one-way analysis of variance less than 0.05, a post test was done with the Tukey’s Multiple Comparison test given the results as follows;

Tukey's Multiple Comparison Test	Significant? P < 0.05?	95% CI of diff
Nafw01 vs Nafw02	No	-6.127 to 4.937
Nafw01 vs Nafw03	Yes	-15.16 to -4.096
Nafw01 vs Nafw04	No	-9.599 to 1.465
Nafw01 vs Nafw05	No	-10.49 to 0.5704
Nafw01 vs Nafw06	Yes	-25.80 to -14.74
Nafw02 vs Nafw03	Yes	-14.57 to -3.501
Nafw02 vs Nafw04	No	-9.004 to 2.060
Nafw02 vs Nafw05	No	-9.899 to 1.165
Nafw02 vs Nafw06	Yes	-25.21 to -14.14
Nafw03 vs Nafw04	Yes	0.02855 to 11.09
Nafw03 vs Nafw05	No	-0.8664 to 10.20
Nafw03 vs Nafw06	Yes	-16.17 to -5.110
Nafw04 vs Nafw05	No	-6.427 to 4.637
Nafw04 vs Nafw06	Yes	-21.74 to -10.67
Nafw05 vs Nafw06	Yes	-20.84 to -9.776

Total aflatoxin level in water

Pairwise analysis

Following from the one-way analysis of variance with a p-value<0.05, a post test analysis was carried out using the Tukey’s Multiple Comparison Test. The result from the post test analysis is given as follows;

Tukey's Multiple Comparison Test	Significant? P < 0.05?	95% CI of diff
Nafw01 vs Nafw02	No	-7.354 to 5.000

Nafw01 vs Nafw03	Yes	-21.74 to -9.391
Nafw01 vs Nafw04	Yes	-12.85 to -0.4920
Nafw01 vs Nafw05	Yes	-15.03 to -2.672
Nafw01 vs Nafw06	Yes	-28.59 to -16.23
Nafw02 vs Nafw03	Yes	-20.57 to -8.214
Nafw02 vs Nafw04	No	-11.67 to 0.6850
Nafw02 vs Nafw05	Yes	-13.85 to -1.495
Nafw02 vs Nafw06	Yes	-27.41 to -15.05
Nafw03 vs Nafw04	Yes	2.722 to 15.08
Nafw03 vs Nafw05	Yes	0.5420 to 12.90
Nafw03 vs Nafw06	Yes	-13.02 to -0.6631
Nafw04 vs Nafw05	No	-8.357 to 3.997
Nafw04 vs Nafw06	Yes	-21.92 to -9.562
Nafw05 vs Nafw06	Yes	-19.74 to -7.382