KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI

COLLEGE OF SCIENCES

FACULTY OF BIOSCIENCES

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

EFFECT OF TRADITIONAL PROCESSING OF HAUSA KOKO (MILLET PORRIDGE) AND EKOEGBEME (COOKED DEHULLED MAIZE GRITS) ON AFLATOXINS.

BY

ALBERT ANKOMAH

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(MSc. Food Quality Management)

A thesis submitted to the **Department of Food Science and Technology**, Kwame Nkrumah University of Science and Technology, Kumasi in partial fulfilment of the requirements for the award of

MASTER OF SCIENCE IN FOOD QUALITY MANAGEMENT

JUNE, 2019

DECLARATION

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma at Kwame Nkrumah University of Science and Technology, Kumasi, or other educational institution, except where due acknowledgement is made in the thesis.

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DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

ABSTRACT

Aflatoxin is produced as secondary metabolites by fungi *Aspergillus flavus, A.parasiticus and A. nominusmoulds.* These fungi grow on various crop such as nuts, maize, millet and other grains. Chronic dietary ingestion of low dose of aflatoxin is a risk factor for liver cancer, however ingestion of high doses of aflatoxin contaminated food can result in aflatoxicosis with symptoms including vomiting, abdominal pains and jaundice. This study was aimed at investigating the effect of traditional processes of Hausa koko (millet porridge) and "ekoegbeme" (cooked dehulled maize grit) on aflatoxin. The level of aflatoxin G1, G2, B1, B2 and total aflatoxins were measured at the end of the various processing stages of each food product by HPLC method. The processing stage that produced significant reduction was identified by using ANOVA. Overall, processing of "ekoegbeme" resulted in more aflatoxin B1 and total aflatoxin reduction than the processes involved in preparing Hausa koko. Fermentation of millet during Hausa koko processing resulted in 13.97 % and 4.82 % reduction in aflatoxin B2 and B1 respectively with total aflatoxin reduction of 4.9% while cooking of the fermented filtrate to Hausa koko resulted in 0.70 % and 1.85 % reduction in aflatoxin B2 and B1 respectively as well as 1.49 % reduction in total aflatoxin. However the steeping of the millet caused an increase of 11.88% and 7.67% in the aflatoxin B1 and total aflatoxin respectively. The dehulling of the maize during preparation of ekoegbeme resulted in 100% reduction in aflatoxins while cooking of the maize resulted in 11.52 % reduction in total aflatoxin as well as 5.95 %, 3.97 %, 41.53 % reduction in aflatoxin B1, B2 and G1 respectively. There was no aflatoxin G2 detected in the maize and millet samples used for the investigations.

ACKNOWLEDGEMENTS

I am very much grateful to the Almighty God for granting me the strength, wisdom and favor to pursue this research, while keeping busy with other work schedules. I would like to acknowledge the efforts of my academic supervisor, Dr. Herman E. Lutterodt, who patiently guided me to choose this research topic, directing me to institutions where to get assistance and, finally writing up my findings. I would also like to say thank you to other distinguished members of the Department of Food Science and Technology, including Dr. Isaac W. Ofosu, Dr. Jacob K Dr. Agbenorhevi, Dr. Emmanuel de-Graft Johnson Owusu- Ansah. I would also like to extend my acknowledgement to Richard Bruce Lamptey, a librarian of College of Science as well as Williams Ofori Appaw and Redeemer K. Agbolegbe all of the Mycotoxin laboratory, of the Department of Food Science and Technology. I also thank my family for their support. May God richly bless you all.

ABBREVIATIONS

TLC	Thin layer Chromatographic
MPL	Maximum Permissible Limit
WHO	World Health Organization
FAO	Food and Agriculture Organization
AF-alb	Aflatoxin –albumin
UV	Ultraviolet
IARC	International Agency for Research on Cancer
EU	European Union
USA	United State of America
EDI	Estimated daily intake
AEZs	Agro-ecological zones
α -LA	Alpha –Lipoic Acid
HIV	Human Immunodeficiency Virus
D3G	Deoxynivalenol -3-glucose
DON	Deoxynivalenol
CSB	Chinese Steam Bread
HPLC	High Pressure Liquid Chromatography
PHRED	Photochemical Reactor for Enhanced Detection
AFT	Aflatoxin

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CHAPTER ONE

1.0. GENERAL INTRODUCTION

1.1. Background

Aflatoxin belongs to the group of secondary metabolites known as mycotoxins, which are produced by fungi. Like other mycotoxins, Aflatoxin is potentially harmful to the health of humans and animals when ingested in sufficient quantities over a period sof time. *Aspergillus flavus, A. parasiticus and A. nomiusmoulds* are the fungal species, known to produce aflatoxins (Verheecke *et al.*, 2016). It has been established that the consumption of aflatoxins by humans could lead to adverse health effects such as severe acute hepatitis, liver cancer, liver failure and ultimately, death (Whittle *et al.*, 2008). The International Agency for Research on Cancer (IARC) has classified aflatoxin as a class 1 human carcinogen, thus reflecting the severity of its impact on human health (Assessment, 2010, Li *et al*., 2014).

Livestock which are fed with food contaminated by aflatoxin, tend to suffer a reduced growth rate, and also transfer the mycotoxin to humans, through animal products such as dairy products, eggs, and meat, which serve as a food source to humans. As such, aflatoxin contaminates the entire food chain, and has raised global concern in the area of food safety (Thirumala-Devi *et al.*, 2002).

Also, the Food and Agriculture Organization (FAO) has estimated that 25% of the world's food supply is contaminated with mycotoxins (Kange *et al.*, 2015). Currently, it is estimated that 25% of the tropical regions have been contaminated by aflatoxins (Okaru *et al*., 2017). Maize –base foods in Ghana, Benin, Kenya and other African countries have been found to be contaminated with aflatoxin (Atongbiik *et al.*, 2017). Climate conditions of the tropics and sub- tropics such as warm temperature, humidity, moisture, pH of food in storage make these regions favorable for growth of fungi that produce aflatoxins. The destructive activities of birds and insects on food crops such as corn, also contributes to aflatoxin contamination by

creating a portal of entry for *Aspergillus* species (Odoemelam *et al.*, 2009). Based on their characteristics upon exposure to Ultraviolet Light and their movement on a thin-layer Chromatographic (TLC) plate, aflatoxins are classified into four main groups: B1, B2, G1 and G2 (Assessment, 2010). The wide prevalence of mycotoxin contamination of global food reserves, combined with the devastating effect on human health, has created the need to develop an effective and objective means of controlling and monitoring the concentration of mycotoxins found in food.

Currently this is achieved by determining the aflatoxin concentrations of food substances, and comparing them with pre-determined reference values, known as the Maximum Permissible Limit (MPLs). The World Health Organization (WHO) and the FAO has proposed 15 ug/kg as the maximum tolerable limit for aflatoxin in food. In the United State of America (USA), the MPLs for total aflatoxin in food is set at 20 µg/kg. The European Union has set the MPL for aflatoxin B1 in ready-to-eat cereals and oil seeds as 2 µg/kg, while 4 µg/kg was set as the MPL for total aflatoxin. According to the Ghana Standard GS211PT.1:2003, Ghana has set the MPLs for aflatoxin in maize at 15 µg/kg (Atter *et al.*, 2015) while Ghana Standard GS728:2003 set maximum permissible limit of aflatoxin in millet at 20 µg.

Levels of aflatoxin in humans can be determined by identifying the quantities of aflatoxin-albumin (AF-alb) adduct in human blood (Asiki *et al.*, 2014). Unfortunately, in most developing countries, food shortage, combined with the lack of regulatory enforcement and an inadequate understanding of the harmful effects of aflatoxins on human health and control strategies, contributes to a high degree of exposure to aflatoxin contamination of food.

There are a number of detoxification methods, used to effectively reduce the levels of aflatoxins present in food substances, to make them safer for human consumption. They can be broadly classified into chemical, biological and physical methods. An effective physical means of reducing the concentration of aflatoxin B1 in maize, involves the removal of all

external layers such as the hull, bran or pericarp, germ and tip cap, so as to obtain the endosperm, which is processed into various maize based foods (Iacomo *et al.*, 2006). Also, heating of food to extremely high temperatures above 150 ^{o}C, is reported to reduce aflatoxins to about 33%. However, it is very difficult to eliminate mycotoxins by this method since they are heat resistant, and so this is only achieved at temperatures of between 237-306 ^{o}C, a process that is most often impractical (Martins *et al.*, 2017).

Biological treatment of aflatoxin involves the use of microorganisms to reduce the levels of aflatoxin B1 in food. Lactic acid producing bacteria such as *Streptococcus sp., Lactobacillus sp. and Leuconostoc sp,* are used during the processing of carbohydrate food. This method of food processing, known as lactic acid fermentation, which is practiced by many African cultures, is known to reduce the levels of Aflatoxins in food (Afsharmanesh *et al.*, 2018) . In Ghana, lactic acid fermentation is employed in the preparation of food from cereals such as maize and millet (Owusu-Kwarteng *et al.*, 2010).

Reduction of mycotoxin levels in food can also be achieved by the use of chemical substances such as ozonated water, hydrogen peroxide, ozone gas, lactic acid and citric acid. Ammoniation, a chemical method is reported to be efficient in detoxifying aflatoxin in raw materials. It involves the treatment of food substances with ammonia or other ammonium compound (Pankaj *et al.*, 2018).

Many other methods of food processing techniques such as nixtamalization, flaking, frying, brewing, cooking, baking, milling, canning and cleaning are all methods of processing food that can help reduce the level of aflatoxin contamination in food (Bullerman & Bianchini, 2007).

1.2: Statement of Problem

There is an increase in the consumption of food products prepared with cereals like maize, millet, rice, peanut, spices and soya beans which may contain high levels of aflatoxin. Awareness of the potential danger posed by aflatoxins contamination of foodstuffs has been on the increase in recent times. Aflatoxins are generally considered a potential hazard to public health due to their toxicity, mutagenicity, teratogenicity and carcinogenicity. An outbreak of aflatoxin poisoning in Kenya (January – July 2004) resulted in 125 recognized deaths and hospitalization of over 300 others. (Odoemelam & Osu, 2009).

1.3. Hypothesis

The level of aflatoxin concentration in maize and millet can be reduced by traditional processing methods such as "ekoegbeme" and Hausa koko processing respectively.

1.4. Main Goal

To identify if aflatoxin concentration level in food can be controlled by traditional processing methods.

1.5: Specific Objective

To determine the effect of traditional processing on aflatoxin in maize and millet used to prepare Hausa kooko and cooked dehulled maize grates.

1.6: JUSTIFICATION

This current study is to identify the effect of traditional processing in controlling the levels of aflatoxin in millet and maize used in preparing Hausa koko and "ekoegbeme" which will reduce the negative health effect from consuming foods with high levels of aflatoxins.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1: Health effects of Mycotoxins

There are several kinds of mycotoxins which are produced by fungi as secondary metabolites, including aflatoxin, fumonisin, nivalenol, ochratoxin, moniliformin, zerealenone as well as deoxynivalenol also known as vomitoxin. As the name suggests, mycotoxins are harmful to human and animal health. The kind of disease or disorder that affect humans depends on the type of mycotoxin humans are exposed to, the rate of consumption and the quantities of mycotoxin consumed (Chala *et al*., 2014; Udomkun *et al*., 2017).

Aspergillus flavus, A. parasiticus and A. nomiusmoulds are fungi that produce aflatoxins. These fungi grow on various crops like nuts (peanuts, pistachio nuts, chest nuts), cotton seed, maize and many other grains (Flanders *et al*., 2011; Algül & Kara, 2014; Okaru *et al*., 2017). Chronic dietary ingestion of low doses of aflatoxins is a risk factor for liver cancer. Afltoxicosis, which is sever, acute hepatitis can result when highly contaminated food products are ingested. Symptoms associated with aflatoxicosis include abdominal pain, vomiting, and jaundice. This can lead to liver failure and death. When supportive care is provided, the case fatality rate of acute poisoning is between 25% to 40%. However, there is no specific treatment for acute aflatoxicosis (Flanders *et al*., 2011).

Food considered to be major African dietary food product are reported to be contaminated with aflatoxin levels above the maximum acceptable limits, examples are peanut and peanut butter used in preparing groundnut soap in Burkina Faso, Sudan and Egypt are contaminated with aflatoxin concentration of 170 µg/kg, 21 – 170 µg/kg and 24 µg/kg respectively (Kuhumba, Simonne, & Mugula, 2018).

Many people in Sub-Saharan Africa and some parts of Latin America as well as Asia are regularly eating foods contaminated with aflatoxin B1 which has the potential to cause high

death rate in the population as well as high morbidity. Eating food contaminated with aflatoxin B1concentration of 20 to 120 µg/kg body weight per day will cause acute aflatoxicosis with signs and symptoms such as abdominal pains and vomiting. This may result in death if it occurs in children. For people infected with hepatitis B, chronic intake of aflatoxin B1 leads to cancer of the liver (McMillan *et al.*, 2018).

Mycotoxin affect the food chain since crops contaminated by mycotoxin are also use as animal feed. Mycotoxin contaminated feed when fed to animals, reduces their growth rate and also it can be bio-transferred to livestock product such as milk, eggs, dairy products and meat since reports has showed that the digestive systems of animals are not able to catabolize mycotoxins (Udomkun *et al*., 2017).

2.2: Characteristics of Aflatoxin

There are 17 isomers of aflatoxin which includes B1, B2, G1, G2, M1 and M2. Aflatoxin B1, G1 and M1 are more carcinogenic as compared with aflatoxin B2, G2 and M2 respectively (Bullerman & Bianchini, 2007). Aflatoxin M1 and M2 are usually found in milk of mammals fed with feed contaminated with aflatoxin B1 and B2 (Udomkun *et al*., 2017). The main species of moulds that produce aflatoxin B1, B2, G1 and G2 are *Aspergillus flavus* and *Aspergillus parasiticus*, these fungi thrive in the tropics and sub-tropical regions where there is warm conditions (Taylor *et al*., 2009).

In the presence of Ultra Violet radiation (ca. 365nm), aflatoxin produce a strong fluorescence. At room temperature aflatoxin appears as colorless to pale-yellowish crystals (Tan *et al*., 2014).

Aflatoxins are classified into four main groups base on their characteristic under ultraviolet (UV) light and movement on the thin-layer chromatographic (TLC) plate. Under UV light aflatoxin may fluorescence a blue or green color, which is used to separate them into B1, B2,

G1 and G2. The extent of aflatoxin contamination in tropical regions is said to be 25% (Okaru *et al.*, 2017).

Aflatoxin B1, aflatoxin B2, aflatoxin G1 and aflatoxin G2 occur naturally. Aflatoxins in crops and animal source food is a global health issue in agriculture. The International Agency for Research on Cancer (IARC) has classified aflatoxin as a class 1 human carcinogen. The ability of aflatoxin to bind to proteins and deoxyribonucleic acid resulting in the formation of adduct like aflatoxin B1-lysine in albumin makes it toxic (Sirma, 2016; Asiki *et al.*, 2014)

The carcinogenic effect of aflatoxin B1 is as a result of the epoxidation of aflatoxin B1 by cytochrome P450. When aflatoxin B1 is epoxidated by cytochrome P450, it leads to the formation of exo-8, 9-epoxide. This highly reactive compound react with nitrogen 7 atom of the DNA, the depurination forms aflatoxin B1-N guanine adduct which is eliminated in urine (McMillan *et al.*, 2018).

During growth, harvest and storage of different kinds of food crops and animal feeds, *A. flavus and A. parasiticus* have been found. Metal ions, reduced coenzymes level and catabolic activities influence the production of aflatoxin which is classified as a secondary metabolites. Crops that can be affected include spices, peanuts, cereals, as well as cassava. To be exposed to aflatoxin, humans and animals must eat or consume contaminated food. Aflatoxins are mutagenic, tetratogenic and toxic hence it is a potential hazard to public health (Odoemelam & Osu, 2009).

There are several factors that promote the growth of *A. flavus*, hence the production of aflatoxin in foods, making the presence of aflatoxin in food unpreventable. These factors includes relative humidity, temperature, soil properties, injury on food, attack by rodents and insects, pH within the food, moisture content or water activity of the food as well as nutrient content of the food (Atongbiik *et al.*, 2017).

2.3: How foods are contaminated by Aflatoxin

The contamination of corn by *A. flavus* could be through various steps which include airborne or insect transmitted inoculum which contaminate the corn's silk and grows into the ear which is developing. Corn ear damaged by birds or insects are then infected with *A. flavus* which produce aflatoxin to contaminate the crop. The available *A. flavus* sporogules in the soil or residues of the crop can lead to contamination of the silks during cultivation of adjacent farm (Freitag & Ryder, 1973).

It has been estimated by the Food and Agriculture Organization (FAO) that 25% of the world's food crops are affected by mycotoxin. The World Health Organization (WHO) and the FAO has proposed 15 µg/kg as the maximum tolerable limit for aflatoxin in food (Kange *et al*., 2015). The Maximum Permissible Limit (MPLs) set for aflatoxin B1 by the European Union (EU) for aflatoxin in ready to eat cereals and oil seeds was 2 µg/kg while 4 µg/kg was set for total aflatoxin (Algül & Kara, 2014 ; Apen *et al*., 2016 ; Taylor *et al*., 2009). However in the United State of America (USA) the MPLs for total aflatoxin in food is set at 20µg/kg (Apen *et al*., 2016). According to the Ghana Standard GS211PT.1:2003, Ghana has set the MPLs for aflatoxin in maize at 15µg/kg (Atter *et al*., 2015) while Ghana Standard GS728:2003 set maximum permissible limit of aflatoxin in millet at 20 ppb.

2.4: Methods for Detoxifying Aflatoxins in foods

Detoxification methods for reducing the level of aflatoxin have been classified into chemical, biological and physical method. Ammoniation, a chemical method is reported to be efficient in detoxifying aflatoxin in raw materials as well as roasting reducing aflatoxin to about 40% in peanuts. Also extrusion method at high temperature (>150 ^{o}C) is reported to reduce aflatoxin to about 33%. The biological treatment of aflatoxin make use of microorganism to reduce aflatoxin B1. Apart from roasting, sorting and extrusion, other food processing methods such as nixtamalization, flaking, frying, brewing, cooking, baking, milling, canning

and cleaning are all methods of processing food that can help reduce the level of aflatoxin contamination in food. (Bullerman & Bianchini, 2007; Lutfullah & Hussain, 2012).

The physical process of removing the hull, bran or pericarp, germ and tip cap to bring out the endosperm of a maize during milling is one of the steps used in making maize – base food products. The endosperm obtained during milling is used in making different kinds of milling fractions like maize grits, maize rice, coarse maize and maize flour. The external part of the maize that is removed during milling i.e. the bran is usually used as animal feed. It is being reported in various studies that dry milling fractions such as maize grits, maize meal, maize flour resulting after milling which is used for human consumption has reduced levels of aflatoxin B1 while the hull, pericarp, tip cap and the germ used as animal feed has higher concentration of aflatoxin B1 (Lutfullah & Hussain, 2012).

One of the processes Africans use to prepare maize before they eat as food is through lactic acid fermentation. Lactic acid is produced through the fermentation of carbohydrates by a group of lactic acid bacteria such as *Streptococcus sp., Lactobacillus sp. and Leuconosto sp.*(Mokoena *et al*., 2006).

It is very difficult to control or reduce mycotoxin since they are heat resistance, hence the inability to destroy or eliminate them by conventional thermal processes. Temperatures used to decompose aflatoxin is between 237-306 ^{o}C. When aflatoxin is heated to temperatures that can decompose it, aflatoxin generate acrid smoke. Degradation of aflatoxin in animal feed has been done by the use of chemicals such as ozonated water, hydrogen peroxide, ozone gas, lactic acid and citric acid. Also methods involving the use of biological, ammoniation, adsorption and fermentation has been used to reduce aflatoxin in animal feed (Pankaj *et al*., 2018).

2.5: Traditional processed foods

Maize and millet are part of the staple foods consumed by many Ghanaians to obtain greater portion of their total calories in the diet. Maize is widely consumed by many people within the southern part of Ghana (Kpodo *et al*., 1996).

Fermentation is widely used as part of traditional food processing in Africa. It is used to process cassava, cereals and dairy foods. These fermented food products includes breads, beverages which includes alcoholic and non-alcoholic, porridges, fermented meat, fish, vegetables and condiments. Also there are examples of fermented foods such as ogi and mawe in Benin, koko and kenkey in Ethiopia and Ghana. There are different porridges in Ghana made from maize and millet which are all called koko. However porridges made by the use of pearl millet also called *Pennisetum glaucum* is known as Hausa koko. This type of koko from millet is widely eaten as breakfast in Ghana as it is mostly sold in a ready –to- eat food product (Akabanda *et al*., 2010, Owusu-Kwarteng *et al.*, 2010).

During food processing, pH, food matrix, moisture content, temperature for processing and concentration of the toxin are among the factors that affect mycotoxin in food. Heating, fermentation and enzymes can change mycotoxin or their concentration in food during food processing (Zhang & Wang, 2014 ; Khaneghah *et al*., 2018).

Bran obtained during milling of cereals which is used as animal feed contains mycotoxins in concentrated amount, this results in less mycotoxin concentration in the other milled fraction (flour). During wet milling of cereals the mycotoxin dissolve in the water used during wet milling. Therefore there is less mycotoxin in the starch or endosperm that is obtained after wet milling (Khaneghah *et al*., 2018).

Maize production in Ghana accounted for 3% of the Agricultural Gross Domestic Product which amounted to 2 billion metric tons in the year 2012. There are several traditional foods

that is prepared from maize in Ghana, these include koko (fermented maize porridge), banku (stiff porridge prepared with fermented whole maize dough mixed with fermented cassava dough), aboloo (steamem maize dumpling made from fermented dehulled maize), touzafi, kenkey (a sour stiff dumpling made with fermented maize dough) Ekoegbeme (porridge made with dehulled maize grits) and oblayo (porridge made with dehulled maize grains) (Oduro-Yeboah *et al*., 2016)

White maize forms majority portion-90% of all maize produced in Africa intended for human consumption. Maize in Africa can be contaminated with mycotoxin through pre and post-harvest contamination. Due to climate change the mycotoxin contamination in maize is expected to worse (Matumba *et al*., 2015).

If maize, millet, groundnut and other crops can be sold for better or higher price then it will provide more incentive for farmers to concentrate their efforts and resources in aflatoxin risk mitigation technologies and better agricultural practices. In developing countries, it is not common to find grains with low aflatoxin level sold for higher price however it is common to find quality grains for export or as international food aid programs. There is also differentiated market found when grains are used as raw materials to feed animals for the production of meat and milk. There are a lot of small scale farm operated outside the control of government which dominated the food systems in poor countries (Grace *et al*., 2015).

2.6: Measuring Aflatoxin

Levels of aflatoxin in humans can be determined by identifying the quantities of aflatoxin-albumin (AF-alb) adduct in human blood while levels of aflatoxin contamination in food stuffs can be measured directly. In most developed world level of aflatoxin contamination in animal feed as well as human food are measured for routine monitoring due to stringent regulatory measures being implemented, however in most of the developing countries, lack of proper regulatory enforcement and food shortage increase the level of exposure to aflatoxin

contamination. The understanding of the harmful effect of aflatoxin on human health, its exposure and means of prevention has not been strengthened in underdeveloped settings or countries (Asiki *et al*., 2014)

2.7: Investigations on Aflatoxin

A study in Accra, Ghana, on the aflatoxin contamination in 25 processed cereals – based food products (mixed grain, wheat-based, corn –based, and rice -based) for infants and young children indicated total aflatoxin concentration ranging from 0.18±0.01 – 44.42± 0.38 μgkg^{-1}. 72% of the total infant foods analyzed contained 0.18± 0.01 to 35.79±0.34 μgkg^{-1} aflatoxin B1 was above the 0.1 μgkg^{-1} permissible limits by the European Union. Individual estimated consumption rates of aflatoxin and aflatoxin concentration in food were used to assess the aflatoxin intake. Those who consumed mixed cereals had the highest intake of aflatoxin B1 contamination of 0.005 -0.852 $\mu gkg^{-1}bwb^{-1}$ for infants and 0.004 -0.657 $\mu gkg^{-1}bwb^{-1}$ for young children. Also infants and young children had mean estimated daily intake (EDI) of 0.23±0.16 $\mu gkg^{-1}bwb^{-1}$ and 0.153± 0.13 $\mu gkg^{-1}bwb^{-1}$ respectively. Comparing the tolerance limit for aflatoxin in Ghana to that of the test samples indicated that 32% of the samples tested had aflatoxin concentrations above the Ghanaian tolerance limit for foods made with cereals intended for human use (Blankson & Mill-Robertson, 2016).

Studies indicate that all samples of millet, wheat, guinea corn, groundnut and breadfruit analyzed in Nigeria specifically the Niger Delta region were contaminated with aflatoxin B1. There were significant differences in the concentrations of aflatoxin B1 obtained for the sample ($p<0.01$). The concentration of aflatoxin obtained for millet ranged from 34.00 - 40.30μgkg^{-1}, that of wheat ranked from 17.01 - 20.53μgkg^{-1}, The value for that of guinea corn 27.22 - 36.13 μgkg^{-1}, groundnut had a rank of 74.03 - 82.12 μgkg^{-1} and breadfruit had aflatoxin B1 concentration of 40.06 - 48.59 μgkg^{-1}. All the grains analyzed had aflatoxin

concentration above the 20 μgkg^{-1} tolerance level of aflatoxin in food (Odoemelam & Osu, 2009).

Sirma *et al*. (2016) reported on the aflatoxin concentration in sorghum, maize, and millet sampled from five Kenyan counties namely Bungoma, Kwale, Tharaka-Nithi, Isoilo and Kissi which represented the countries four (4) agro-ecological zones (AEZs). The studies were conducted in two phases between February and October 2014. Out of the total of 164 sorghum, 497 maize and 205 millet sampled, 60% of Sorghum, 76% of maize and 64% of millet were found to contain aflatoxin B1 respectively. Of these percentages, 11% of sorghum, 26% of maize and 10% of millet were found to have aflatoxin B1 concentration above the five (5) part per million of the Kenya Bureau of Standards limit for aflatoxin B1. The second phase of the studies indicated significant difference ($p<0.05$), in the mean levels of aflatoxin B1 found between the agro ecological zones. Millet and sorghum from the humid and semi-arid AEZs represented by Tharaka-Nithi and Isiolo had the highest mean of aflatoxin B1 while maize from the temperate agro ecological zone had the lowest mean of aflatoxin B1 contamination respectively.

Investigation of aflatoxin and fungi in millet, sesame and sorghum and their products carried out in Nigeria indicated that aflatoxin level was reduced by beer processing from sorghum grains to beer and *Aspergillus flavus* was the most common fungus found in the test samples (Apen *et al*., 2016).

A report by Kange *et al*.(2015), on conditions of sorghum grains and it effect on occurrence of mycotoxin producing fungi indicates that *Aspergillus, Fusarium and Penicillium* species were present in both grains of sorghum found on the fields in Kenya as well as those kept in storage (Kange *et al*., 2015). In their studies it was reported that *Aspergillus* species was more in sorghum from the stores of farmers. However Fusarium species was dominant in harvested grains which were fresh. It was observed that Aflatoxin B1 was found in 10.81% of the

sampled tested. Aflatoxin B2 was found in 5.41% of the samples, G1 was 18.92% of the samples and G2 was found in 32.42%.

A study which compared dehulling, flotation and sorting in removing deoxynivalenol, fumonisin, aflatoxin, nivalenol, and alternariol which occurs naturally in maize found that hand sorting has the greatest efficiency in removing mycotoxin in maize as compared to flotation and dehulling. It was observed that mycotoxin are dominant in non-matured, shriveled, discolored and broken grains which when picked out of the grains can reduce significantly, mycotoxins by 95% efficiency or even higher (Matumba *et al*., 2015).

In a study by Afsharmanesh *et al*. (2018) to investigate the use of microbes to control aflatoxin concentration in food revealed that *Baccillus subtilis* UTB1 had a negative effect on the growth of fungi there by reducing aflatoxin levels as well as degrading aflatoxin in food. It was observed that metabolite such as bacilysin is very important in the biological control action by *Baccilus subtilis* UTB1 against *Aspergillus flavus.* BacC oxidoreductase, a homologus enzyme of mycobacertum helps in the degradation of aflatoxin in pistachio fruits by bacteria.

Other studies have also found that in addition to *Bacillus subtilis* other microbes that can inhibit the growth of fugal and hence reduce production of aflatoxin by *Aspergillus flavus* are *Pseudomonas spp., Burkholderia spp., and Ralstonia spp*. (Bhatnagar-Mathur *et al*., 2015).

In a research review by Pankaj *et al*. (2018) on update on current research on reduction of aflatoxin by new technologies such as electrolyzed water, gamma and electron beam irradiation, ultraviolet and pulse light, microwave heating and cold plasma revealed that direct treatment of gamma radiations does not decontaminate food products with aflatoxin contamination since aflatoxins are resistance to the direct treatment with gamma radiations. However aflatoxin can indirectly be degraded by gamma radiation by reaction of free radicals

generated by radiolysis of water during treatment with gamma radiations at the terminal furan ring of aflatoxin. When artificially contaminated feed was heated by microwave at power level of 1.4 Kw for 10 min there was a degradation of aflatoxin to about 20 – 35%.

A study in Tanzania where farmers who adopted aflatoxin mitigation technologies on grains quality were monitored for two years, it was reported that factors such as storage conditions, post-harvest handling, crop variety and time for planting influence the susceptibility of the crops to aflatoxin contamination. Due to the poor post-harvest handling by actors in the supply chain as well as farmers, *Aspergillus flavus* infection which begun in the field increases during storage of the grains. When farmers are trained on the hazards of aflatoxin B1, and they adopted aflatoxin mitigation technologies on grain quality, the level of aflatoxin contamination was significantly lowered in their stored grains (Seetha *et al.*, 2017)

Martins *et al*. (2017) reported in a study which investigated the effect of initial aflatoxin concentration on aflatoxin degradation in raw peanut, aflatoxin degradation kinetics when roasting peanut as well as the result roasting time and temperature has on peanut color, that, initial aflatoxin contamination concentration has an influence on reduction of aflatoxin when roasted. Blanching, sorting by use of color and roasting are stages in peanut processing that can be used to reduce aflatoxin contamination however, roasting alone cannot reduce the aflatoxin concentration in peanut (Martins *et al*., 2017).

To break aflatoxin in artificially contaminated corn oil or in naturally contaminated peanut by dry heating, it has to be heated to a temperature range between 237 to 306 ^{o}C. At 250 ^{o}C aflatoxin begins to decomposes, indicating it's a very heat resistant chemical. However, there has been studies that indicated degradation of pure aflatoxin when heated in furnace at 150 ^{o}C for 1 hour, and there was 70% reduction in aflatoxin and when heated to 180 ^{o}C there was complete breakdown of aflatoxin B1. When aflatoxin in food is wet heated below or at 100 ^{o}C there were reduction of aflatoxin by 10 to 80%. There could be different mechanisms

involved in the degradation of aflatoxin since there is a difference between the degradation temperature during dry heating and wet heating which is ≥150 and ≥100 ^{o}C respectively (Shi, 2016).

Mokoena *et al*. (2006) researched on how lactic acid fermentation can help reduce aflatoxin concentration in fermented maize foods. It was reported after the experiment that lactic acid fermentation has the effect of reducing the aflatoxin concentration in maize significantly with p-value < 0.05. This was observed after some samples of maize was fermented by a mixture of *Streptococcus lactis* and *Lactobacillus delbrueckii* for 4 day and other maize samples were made to fermented spontaneously.

Kpodo *et al*. (1996) report in study that investigated the level of mycotoxins in fermented maize dough and Ga kenkey in Accra by studying the effect of spontaneous fermentation and cooking on the levels of aflatoxin and citrinin. It was reported that aflatoxin and citrinin were still present throughout the fermentation stages however cooking of the fermented maize dough for 3 hours as in the traditional kenkey production method, reduced aflatoxin B1 and G1 levels by 80% while there was 35% reduction in aflatoxin B2 and G2. However citirinin was reduced to a non-detectable level after the boiling of fermented maize dough.

Fandohan *et al*. (2005) report in a study that investigated effect of traditional maize food processing such as mawe, makume, ogi, akassa and owo in Bennin, West Africa, on aflatoxin and fumonisins reported that food processes that combined sorting, winnowing, washing, dehulling as well as fermentation such as mawe and makume had the significant reduction of the mycotoxins as compared to the food process that involved only fermentation and cooking such as ogi and akassa. The process of makume reduced aflatoxin to about 93% and also had 87% reduction in fumonisins. There was 92% reduction of aflatoxin as well as 50% reduction in fumonisins during the processing of akassa. The reduction in mycotoxins was more

significant in makume and akassa than owo which processing reduced aflatoxin to about 40% and fumonisins to about 48%.

To reduce post-harvest aflatoxin in groundnuts during storage the moisture content of the kennel must be reduced to less than 8%, infestation of insect must be prevented by the addition of preservative in order to reduce fungal contamination. Pods and kernels contaminated must be sorted out as well as re-drying of the kernels and pods. There must be combination of different options to reduce aflatoxin contamination during storage after harvesting of the grains or kernel which should also include appropriate storage conditions and educating or informing farmers on technologies available to reduce aflatoxin contaminations (Waliyar *et al.*, 2015).

Li *et al.* (2014) reported in a study on the effect of alpha- Lipoic acid (α-LA) against oxidative damage of the liver in broilers feed with aflatoxin B1 that, α-LA has the potential to reduce the oxidative damage induced in liver by aflatoxin B1 and may help in stopping oxidative stress. This was observed when 300 mg/kg α-LA was added to the feed of birds as supplementation and observed for 3 weeks. There was a significant change with p value less than 0.05 in plasma concentration of total protein, glutamic-pyruvic transaminase, glutamic-oxalacetic transaminase, alkaline phosphatase as well as albumin. The addition of 300 mg/kg α-LA also prevented the damage of the liver tissues as compared to that of birds which were not fed with feed with α-LA. It was also reported that, the α-LA may be as a result of the inhibition of excessive production of lipid peroxides and maintenance of intracellular antioxidant by the aflatoxin B1.

McMillan *et al.* (2018) reported that stunted children in Nigeria had higher concentration of aflatoxin B1-lysine as compared to children who are not stunted, also children suffering from severe acute malnutrition had more aflatoxin- lysine concentration than those who were not malnourished. Children suffering from kwashiorkor also had more aflatoxin-lysine

concentration as compared to those who were marasmic. These were observed in an investigation that measured aflatoxin –lysine in children suffering from severe acute malnutrition in Nigeria.

Consuming food contaminated with aflatoxin at an early age is harmful to children due to the immature detoxification ability of younger children, their rapid growth rate as well as the higher amount of exposure compared to their body size (Kuhumba *et al.*, 2018)

Matumba *et al*. (2015) reported in an experiment that investigated the possible mycotoxin reduction method in maize that hand sorting is the effective method of reducing aflatoxin with 95% efficiency. This was observed when hand sorting, dehulling and floatation of maize were compared. At the end of the experiment it was recommended that the last line of defense to reduce human exposure to aflatoxin in maize is hand sorting since mycotoxin are concentrated in discolored, broken or shriveled immature grains.

Grace *et al*. (2015) reported that maize milled commercially and those sold informally have been found to be contaminated with aflatoxin in Keyan. In the districts that were affected by aflatoxicosis outbreak, it was found that 55% of maize flour and kenel bought were having aflatoxin contamination above the allowable limit, out of these 35% of the samples having over 5 times the regulator limit and 7% of them having over 20 times the regulatory limits. The aflatoxin in maize purchased from the informal, open-air market compared to those bought form the formal retailers showed no significant difference.

In a study conducted by Asiki *et al*. (2014) to investigate the aflatoxin exposure in rural south-western Uganda, it was reported that all 100 adults involved in the study and 92 out of 96 children were found to have aflatoxin-albumine (AF-alb) adduct, the children included 5 babies who were exclusively breast fed. The AF-alb level in children who were mix-feeding were higher than those who were exclusively breast fed. The difference in AF-alb levels

between men and women were not significant as well as the difference between age or by HIV serostatus. About 25% of the test group were found to have AF-alb adduct above 15.1 pg/mg albumin. Those of the subjects who leave closer to commercial trading area had higher concentration of AF-alb adduct, indicating evidence of heterogeneity between villages with p-value of 0.003. There was also significant difference in the level of AF-alb adduct between adults who consumed more food made from maize (posho) and those who eat more food made with banana (matooke) with p-value of 0.02. The study also found that children who ate food made with soya not grown within their village had twice the level of AF-alb adduct as compared to that found in children who did not eat food from soya with p-value of 0.04.

Reports indicates that strains of microbes used as biocontrol can reduce aflatoxin –producing population only by four to five folds. The application of competitive non-toxic strains of *Aspergillus flavus* and A. parasiticus during pre-and post –harvest has to be successful biological control of aflatoxin. There has been a reduction of 70-90% in aflatoxin contamination in peanuts and cotton seed field using non-toxigenic Aspergillus strains. Afla-guard and AF36 are two product that have been approved by U.S. Environmental Protection Agency for prevention of aflatoxin in maize, cotton seed and peanut. Also strains of atoxigenic of A. flavus from African have been found to remove toxigenic fungi in peanut and maize fields, they can reduce aflatoxin to about 70- 99% (Bhatnagar-Mathur *et al*., 2015).

Algül & Kara, (2014) reported a relationship which was statistically stronger between heavy metals (lead, cadmium, arsenic, zinc, copper, chromium and mercury), and mycotoxins such as aflatoxin B1, total aflatoxin and ochratoxin in corn flour sampled in Turkey as well as in UK. Using PCA analysis and correlation, the experiment indicated a strong statistical relationship between chromium and total aflatoxin and aflatoxin B1, which suggest that the corn flour samples were contaminated with aflatoxin as well as heavy metal and that the

heavy metal chromium may have promoted the growth of aflatoxin. However, ochratoxin A had a stronger relationship with copper and zinc.

In a study conducted by Okaru *et al*.(2017) to evaluate the extent of aflatoxin contamination in unrecorded beer and wine and the risk it possess to consumers in Kenya, it was found out that, 10% of unrecorded beer sampled were contaminated with aflatoxin but none of the recorded beer were had aflatoxin contamination. The unrecorded beer samples (locally called bussa) had

3.5 µg /L as their mean aflatoxin concentration with a range between 1.8 – 6.8 µg/L. The mean aflatoxin concentration of 3.5 µg/L corresponded with an average consumption of 500 mL, (the volume for a standard drink) and a Margin of Exposure (MOE) of 36 with range of 15-58 which possess a risk to the consumers.

Kuhumba *et al*. (2018) reported in a study conducted in Tanzania to evaluate the level of aflatoxin contamination in peanut- enriched flour used mainly as weaning food that all 65 samples collected from 17 manufactures were contaminated with aflatoxin B1, B2, G1 and G2 with 71% of the samples containing total aflatoxin above the 10 µg/kg acceptable level. The study also revealed that the type of packaging material (low density poly ethylene [LDPE] or paper) used by manufactures to package the peanut-enrich flour did not have an effect on the mean values of aflatoxin B1, B2, G1 and G2.

In a study that investigated the level of contamination of aflatoxin in maize grains sampled from Ghana, Togo and Benin showed that, maize from Ghana were having aflatoxin contamination ranging from 0.4 to 490.6 ng/g, Togo with 0.7 to 108 ng/g and Benin with 24 to 117.5 ng/g. Also a report from Ejura-Sekyedumase, North-Kwahu and Nkransah all in the middle belt of Ghana revealed a range of 12 to 50 ppb as aflatoxin contamination. In another

study in six (6) districts in Upper West and Upper East of Ghana also indicated a range of aflatoxin contamination of 0.011 – 308 ppb in 240 maize samples (Atongbiik *et al*., 2017).

Bullerman & Bianchini, (2007) reported in a study that investigated the reduction of aflatoxin B1 in Korean and US wheat by home cooking methods such as washing, heating and steaming, that washing time was directly proportional to level of reduction in aflatoxin B1 in both wheat from Korea and US, however aflatoxin was reduced more by heating more than washing. It was reported that heating at 150 and 200 ^{o}C reduced aflatoxin B1 by 50% to 90% respectively. Aflatoxin was reduced more in wet wheat than in dry wheat. Again the report indicated that traditional Korean food processes like sujebi (soup with wheat flakes) and steaming of bread reduced aflatoxin contamination by 71% and 43% respectively.

In an experiment by Raksha *et al.* (2017) it was reported that *Bacillus licheniformis* CFR1 can reduce aflatoxin by 94.7%. This was observed in their experiment that isolated and characterized bacteria that can detoxify aflatoxin B1. During the study 7 strains of bacteria out of 56 bacteria that were isolated, were observed to reduce aflatoxin B1 to70% in liquid culture media, out of the 7 strains, B. licheniformis CFR1 was the one that was more efficient in reducing aflatoxin B1 with optimal temperature of 34 ^{o}C at pH of 7 for 24 hours.

It was reported in an experiment by Atter *et al*.(2015) to evaluate the safety of ice-kenkey (a fermented street vended maize drink in Ghana) sample from four markets in Accra that there is high level aflatoxin in all the ice-kenkey sampled. The ice-kenkey sampled from Madina, Ashiaman, Mallam Atta and Agbogloshie markets had mean concentration of aflatoxin as 17.04, 7.99, 11.99 and 22.17 µg/kg respectively with that of Madina and Agbogloshie markets exceeding the 15 µg/kg set as the total maximum limit by the Ghana Standard Authority.

A survey by Lutfullah & Hussain (2012) to investigate the contamination of aflatoxin in some cereals and beans it was observed that out of the 160 samples of cereals and bean bought from the retail shops and local market in different locations in Pakistan, 40% of maize, 25% of rice, 15% of broken rice, 20% of wheat, 20% of barley, 30% of sorghum, 20% of red kidney beans, 27% of split peas, 10% of chick pea, 20% of cow pea and 15% of soya beans were contaminated. It was also found that one wheat sample with 15.5 µg/kg, one maize sample with 13.0 µg/kg, on one barley sample with 12.6 µg/kg were the cereals with the highest aflatoxin contamination level. Comparing the EU permitted level of aflatoxin of 4 µg/kg, it was found that 10% of soybean, 10% of sorghum, 20% of barley, 20% of maize, 5% if broken rice, 10% or rice, 6% of split peas and 10% of red kidney beans were above the EU permitted level of aflatoxin.

An experiment by Taylor *et al*. (2009) to investigate the aflatoxin as well fumonisins proportions in milling fractions obtained after commercial dry-milling of maize that was contaminated reported that after the milling processes and analysis, it was observed that the maize sample that was having aflatoxin B1 contamination of 3.6 µg/kg and that with 91.1 µg/kg had aflatoxin reduction level of 8 and 57% respectively after the cleaning of the maize. The cleaning processes included winnowing, destoning and aspiration which eliminated broken kenels and dust from the maize. The 1st maize sample with fumonisins B1 levels of 5379 µg/kg had a reduction of 11% and the maize with 8841µg/kg fumonisins contamination had a reduction of 34%. There was more reduction in the aflatoxin and fumonisins levels after the bran and germ of the maize were removed. With the milled maize that was ment for human consumption (grit and flour), aflatoxin was uniformly distributed as compared to fumonisins B1 which was concentrated in the finer size fraction. There was also an observation that indicated that, in the cleaned maize kennels, aflatoxin B1 was found on the surfaces of the kernel and germ while fumonisins was more concentrated in the inner layers

of the kernels. The aflatoxin concentration found in the maize bran ad germ obtained after the de-germination process had more aflatoxin concentration than the clean maize, with the animal feed made from the waste of the maize and the bran had aflatoxin concentration similar to that of the raw maize.

Zhang & Wang (2014) reported in their study that investigated the effect of milling of wheat and processing of Chinese Steam bread on deoxynivalenol (DON) and deoxynivalenol-3-glucoside (D3G). The processing of the Chinese steamed bread involves mixing of two kinds of wheat flour (break and reduction flour). The milled flour samples were fermented by the addition of yeast solution at 38 ^{o}C for 60 minutes. After moulding the fermented dough and allowing it to rest for 15 minutes it was steamed for 100 ^{o}C. It was observed after the analysis that the wheat bran had higher DON and D3G with 1-2- 2.2 times and 2.9 -4.4 time respectively as compared to that in the grains. After milling the grains are collected in the grains. After milling the grains into wheat flour, DON reduced by 79-90% and D3G had a reduction of 23 -39% compared to wheat grains. It was observed that after milling DON had decreased however D3G had increased. This may be attributed to starch molecules binding to DON during milling. After the steaming of the fermented dough to obtain the Chinese Steamed Bread (CSB) the level of D3G in the flour and fermented dough did not change significantly i.e 50% lower than flour however the level of DON doubled. Chinese steam bread processing decreased the D3G during dough making stages but may have released more DON in wheat flour after steaming.

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1: Source of Materials

1 kg of maize was purchased randomly from nine retailers of cereals in the Takoradi market circle. All the different 1 kg maize samples purchased were mixed together and stored in brown paper bags and kept under refrigerating temperature (4 ^{o}C) until it was time for processing into dehulled maize grits. The millet was also sampled by purchasing 1 kg of millet each from nine retailers of millet. The individual millet purchased were mixed together and stored into brown paper bags and kept at 4 ^{o}C until it was time for processing into the Hausa koko (Matumba *et al*., 2015).

3.2. Preparation of Samples

3.2.1 Spiking of the Millet and Dehulled maize

A sample of 20 g of contaminated peanut with visible *Aspergillus niger* moulds were added to 500 g of the test samples (dehulled maize and millet) respectively. The contaminated peanuts were added to introduce *Aspergillus niger* moulds to the test samples to aid contamination of the dehulled maize and millet. A volume of 15 ml of distilled water was sprayed using a spray gun and kept at 38 ^{o}C and relative humidity of 97% for 6 days in an incubator. A volume of 15 ml distilled water was sprayed daily onto the test sample for 6 days. The added peanuts were carefully removed and the remaining test samples were tested for aflatoxin presence prior to use in the experiment. Natural contaminated groundnut with *Aspergillus flavus* species was added to the maize and millet samples in order to contaminate the maize samples with aflatoxin prior to the start of the experiment.

3.3: Hausa koko processing

In processing the millet into hausa koko, 1 kg of the millet was steeped in 4 L portable water for 72 hours after the water was decanted. The millet was wet milled with mixer grinder at its 4th speed level (Crompton Greaves consumer Electricals limited, Worli, Mumbai, India). After which 3 L of water was added to the milled dough and kneaded, it was then sieved in cheese cloth to remove all the chaff. The filtrate was allowed to settle and ferment for 72 hours. After the 72 hours fermentation, the supernatant were separated from the decanted paste. The supernatant was heated to boiling point (100 ^{o}C) after which the decanted paste were added and stirred in for 10 minutes at 80 ^{o}C to 90 ^{o}C to form porridge.

3.4: Processing of ekoegbeme (cooked dehulled maize grits)

A total of 9 kg of maize purchased from the market was divided into 4 kg and 5 kg groups. Each group of maize was tempered with 50 ml of water and milled (Jacobson Machine Works, Minneapolis, USA) to dehulle the maize. The remaining bran was separated from the endosperm by winnowing. The dehulled maize was milled into grits by mixer grinder (Crompton Greaves Consumer Electricals Limited, Worli, Mumbai, India) and kept in air tight plastic bags and stored under refrigerating temperature of 4 ^{o}C. About 800 ml of water was boiled to 100 ^{o}C after which 200 g of dehulled maize grit was poured into the boiling water while stirring. Stirring was continued while the maize grit was cooking at 95 ^{o}C for 45 minutes (Njapau *et al*. 1998).

3.5: Aflatoxin Analysis

3.5.1: Sample Extraction

Aflatoxin was extracted using methods as described by Tan *et al*.(2014) with modifications. Samples were milled and homogenized using a Preethi Mixer Grinder. A weight of 2 g of sample was weighed into a 15 ml centrifuge tube, 5 ml of distilled was added and the tube vortex for 1mins. The solution was allowed to stand for 5 minutes. A volume of 5ml 1% (v/v)

acetic acid in acetonitrile solution was added. The resultant mixture was vortexed using Genie Vortex machine for 3mins. A mass of 1.32 g of anhydrous $MgSO_4$ and 0.2 g of NaCl were added to the mixture and the vortexed for 1 min. The tube was centrifuged for 5minutes at 4000 rmp and the upper organic layer filtered through a 0.45 µm nylon syringe prior to injection. A volume of 50 µL of the filtered extract was injected into the HPLC.

3.5.2: HPLC Determination

HPLC Determination was done based on AOAC Official Method 2005.08 (AOAC, 2006) (Senyuva & Gilbert, 2006.). Substituting Photochemical Reactor for Enhanced Detection (PHRED) with Kobra Cell for post-column derivatization. A Cecil-Adept Binary Pump HPLC coupled with Shimadzu 10 AxL fluorescence detector (Ex: 360 nm, Em: 440 nm) with YMC C18 Column (150 x 4.60 mm, 5 µm). 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 350 µL of 4 M nitric acid (required for post column electrochemical derivatization with Kobra Cell, R-Biopharm Rhone). Aflatoxin Mix (G_1, G_2, B_1, B_2) standards (ng/g) were prepared from Romer Labs® aflatoxin standard of 5.02 ng/µL in acetonitrile. Aflatoxins in samples were detected by using the retentions of the standard solution run and quantification done using the calibration curves of each respective toxins. Limit of Detection and Limit of Quantification of total aflatoxin were established at 0.5 ng/g and 1 ng/g respectively.

3.5.3: Quality control

Recovery studies were conducted to check for precision and accuracy. Blank samples were spiked at 5 (five) replicated maize samples at 13 µg/kg, 26 µg/kg and 104 µg/kg with recoveries 91±1.75%, 98±1.33 and 102±1.87 respectively. Blanks that were run periodically contained no detectable amount of target analyte. Trueness was further validated using a certified reference material (TR-A1000) from Triology Labs, USA. The value obtained was

20.17±1.14 µg/kg from ten replicates was within the recommended range of the certified value of 21.0 ± 2.9 µg/kg. Coefficient of variation was less than 15 % for replicates.

3.6: Statistical Analysis

Statistical analysis was performed using IBM SPSS version 23. One way Analysis of variance (ANOVA) and Turkey's HSD test were used to compare the means concentration of the aflatoxin G1, G2, B1, B2 and total aflatoxin levels detected in the samples collected at the different processing stages.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 Mean aflatoxin G1, G2, B1, B2 and total aflatoxin concentrations at different processing stage of Hausa koko

There were some changes observed in the levels of aflatoxins after the processing of the Hausa koko. The measured levels of aflatoxin G1, G2, B1, B2 and total aflatoxin concentrations at different stages of processing of Hausa koko are shown in table 1. All values are average of two replicates, (mean ± standard deviation).

Table 1: Mean concentrations of total aflatoxin, aflatoxin G2, G1, B2 and B1 and their respective standard deviation at different processing stage of Hausa koko

Processing steps	**Aflatoxin G2** (µg/kg)	**Aflatoxin G1** (µg/kg)		**Aflatoxin B2** (µg/kg)		**Aflatoxin B1** (µg/kg)		**Total Aflatoxin** (µg/kg)	
Raw Millet	0.00	0.00	a	0.00	a	0.00	a	0.00	c
Raw Millet (Spiked by Natural Contamination)	0.00	2.7 ± 0.35	b	13.295 ± 0.97	b	72.255 ± 0.46	b	88.25 ± 0.86	b
Before Wet milling (After steeping for 72hrs)	0.00	1.575 ± 0.021	c	12.56 ± 0.255	bc	80.845 ± 2.284	c	94.98 ± 2.051	a
After Fermentation of filtrate for 72hrs	0.00	2.585 ± 0.021	b,d	10.8 ± 0.367	cd	76.93 ± 0.679	bc	90.315 ± 1.067	b
After cooking for less than 100oC	0.00	2.74 ± 0.297	b,e	10.71 ± 0.480	ce	75.515 ± 1.435	be	88.965 ± 0.658	b

Values in columns are means of two replicates with standard deviation

Means in the same column followed by the same letter are not significantly different ($p>0.05$) according to Turkey's HSD test.

There was no aflatoxin found in the millet samples purchased from the market, indicating that when farmers and other stakeholders along the food supply chain properly handle cereals and grains through proper storage conditions, there would be very little or no aflatoxin contamination in grains in the market as indicated by Verheecke, *et al*. (2016). The raw millet purchased for the study was spiked by naturally contaminating it with *Aspergillus niger*. There was no aflatoxin G2 detected after spiking the raw millet and after any of the processing steps in the preparation of Hausa koko. After spiking the raw millet, the levels of aflatoxin G1, B2, B1and total aflatoxin concentrations found were 2.7 ± 0.35 µg/kg, 13.295 ± 0.97 µg/kg, 72.255 ± 0.46 µg/kg and 88.25 ± 0.86 µg/kg respectively. The total aflatoxin concentration detected in the spiked millet samples was less as compared to contamination of test materials done by Njapau, *et al*. (1998) which resulted in 1000 µg/kg of aflatoxin. Both aflatoxin G1 and B2 levels reduced to 1.575 ± 0.021 µg/kg and 12.560 ± 0.255 µg/kg respectively after steeping the spiked millet for 72 hours, representing 41.11% and 5.35% reduction respectively as shown in figure 1.

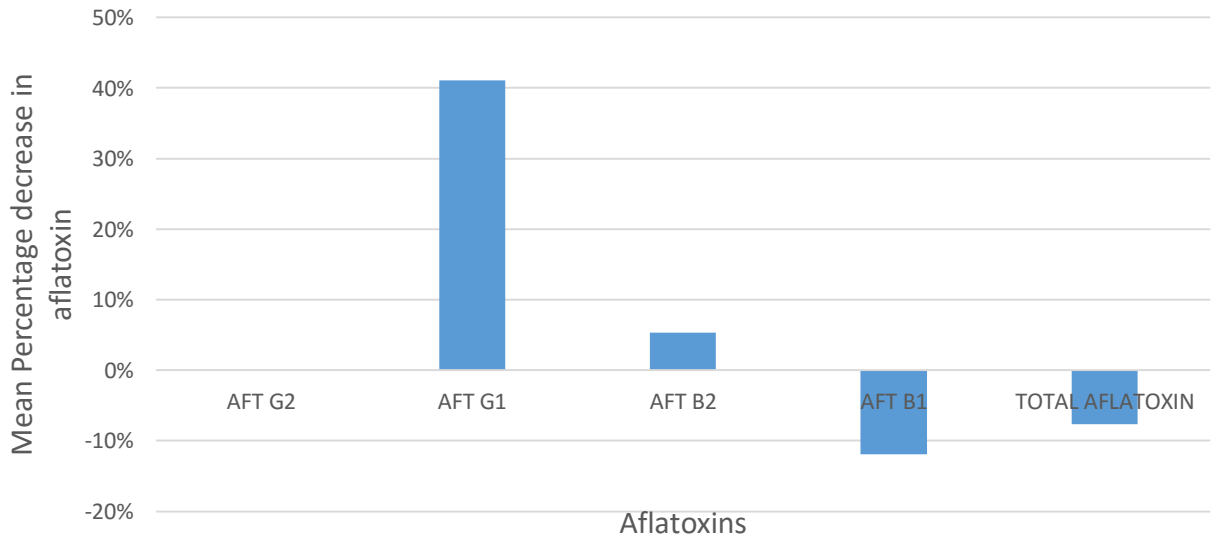

Figure 1: Mean percentage decrease in aflatoxin concentration from raw millet (spiked) to steeping for 72 hours.

The decrease in aflatoxin G1 concentration was significant ($p<0.05$). However, the decrease of aflatoxin B2 concentration after steeping the spiked millet was not significant ($p>0.05$). There was a significant increase in the aflatoxin B1 ($p \leq 0.05$) as well as total aflatoxin after steeping the raw millet contaminated naturally by spiking. The aflatoxin B1 and total aflatoxin concentrations after steeping of spiked millet for 72 hours increased to 80.845 ± 2.284 µg/kg and 94.98 ± 2.051 µg/kg representing 11.88% and 7.67% increase respectively. The increase in the aflatoxin B1 and total aflatoxin observed after steeping may be due to the fact the *Aspergillus niger* used during spiking were still active during the steeping period as the millet sample came in contact with the moisture. After the fermentation of the filtrate of the wet milled millet for 72 hours, the level of aflatoxin G1 increased to 2.585 ± 0.021 µg/kg, representing 64.13% increase. The level of B2, B1 and total aflatoxin concentrations decreased to 10.8 ± 0.367 µg/kg, 76.93 ± 0.679 µg/kg and 90.315 ± 1.067 µg/kg indicating 13.97 %, 4.82% and 4.9% respectively as shown in figure 2.

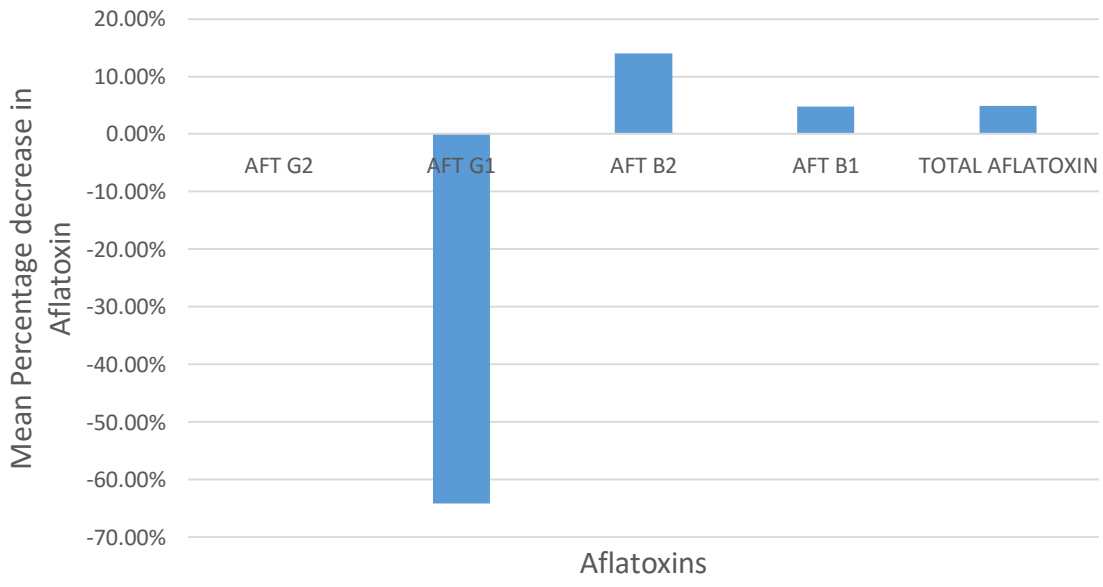

Figure 2: Mean percentage decrease in aflatoxin concentration from steeping millet to 72 hours fermentation of filtrate.

After fermentation of the filtrate of the wet milled millet, the increase in the aflatoxin G1 level was significant ($p<0.05$) from that detected after steeping of the spiked millet. However, the decrease in aflatoxin B2 and B1concentration were not significant ($p>0.05$) from that detected after steeping the spiked millet for 72 hours. The decrease in the total aflatoxin level detected after fermentation of the filtrate was statistically significant ($p<0.05$). Fandohan, *et al*. (2005) detected reduction of 80% in aflatoxin level during the production of ogi which involves steeping and milling of maize as well as further reduction in aflatoxin level (18%) when they fermented the Ogi, which is a maize meal. Their study did not report any significant effect on mycotoxin level in the maize product even with prolong fermentation time from 24 hours to 72 hours. They further indicated in their research that the persistence of aflatoxin during fermentation process is by the presence of aflatoxin precursors in maize grains, and that there is only rearrangement of toxin in acid condition but there is no reduction of aflatoxin. This condition is created by the pH levels when fermenting the maize product as well as the organic acids produced by the microorganisms available in the product being fermented.

In this present study the types of microorganisms responsible for the fermentation of the millet were not analyzed, however a research by Okeke *et al*. (2015) revealed 142 bacteria isolates during steeping of maize for the production of ogi. Identification of the 142 bacteria revealed that part of them were lactic acid bacteria belonging to *Lactobacillus* and *Pediococcus spp*. *Lactobacillus* strains can effectively eliminate aflatoxin B1 from cellular solution, the cell wall of lactic acid bacterial binds to mutagen non-covalently to remove toxins. During fermentation, aflatoxin B1's lactone ring is opened up thereby ending up in complete detoxification Mokoena, *et al*. (2006).

After cooking (80-90 ^{o}C) of the fermented filtrate from the wet milled millet into Hausa koko, the level of aflatoxin G1 increased to 2.74 ± 0.297 µg/kg, representing 6.05% increase.

This increase in aflatoxin G1 was not significant ($p>0.05$) from that detected after fermentation of the filtrate. The level of aflatoxin B2, B1 and total aflatoxin concentrations decreased to 10.71 ± 0.48 µg/kg, 75.515 ± 1.435 µg/kg and 88.965 ± 0.658 µg/kg, representing 0.7 %, 1.85 % and 1.49 % respectively as shown in figure 3.

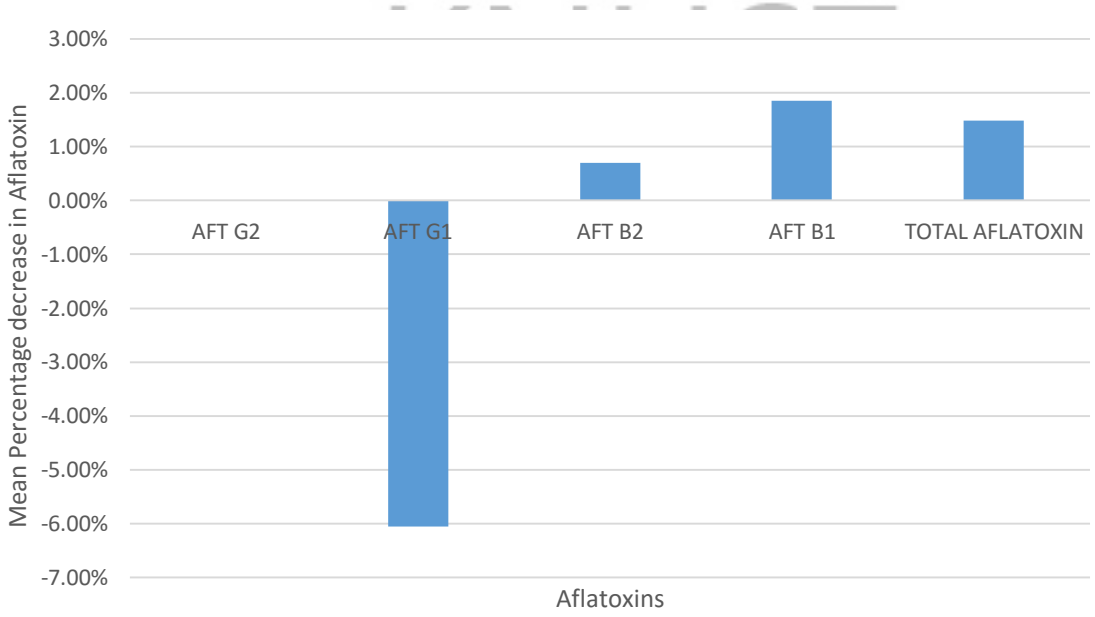

Figure 3: Mean percentage decrease in aflatoxin concentration after cooking of fermented filtrate to Hausa koko

However, these decreases were not significantly different ($p>0.05$) from that of the level of aflatoxin B2, B1 and total aflatoxin concentrations detected after 72 hours fermentation of the filtrate respectively. This results is similar to that observed by Fandohan *et al*. (2005) when 24 hours fermented ogi was cooked to akassa (a fermented maize meal in Benin). However, in their study there was a significant decrease in the aflatoxin levels when they cooked the ogi which was fermented for 72 hours.

The total aflatoxin concentration levels detected after cooking of the Huasa koko as compared to that measured after spiking the raw millet was not significantly different

($p>0.05$) since there was a significant increase in the level of aflatoxin B1 ($p<0.05$) during the 72 hours steeping of the naturally spiked millet.

4.1.2 Mean aflatoxin G1, G2, B1, B2 and total aflatoxin concentrations at various processing stages of Ekoegbeme (cooked dehulled maize grit).

At the end of the processing methods for preparing Ekoegbeme (cooked dehulled maize grit), there were changes in the levels of aflatoxins concentrations. Measured levels of aflatoxin G1, G2, B1, B2 and total aflatoxin concentrations at different processing stages in cooking dehulled maize grit (ekoegbeme) are shown in table 2. All values are average of two replicates, (mean ± standard deviation). There was no aflatoxin G2 detected in the raw maize purchased from the market for the research as well as at the different processing stages of cooking ekoegbeme. Also there was no aflatoxin B2 detected in the raw maize samples purchased from the market. The mean aflatoxin G1 and B1concentrations detected in the raw maize sample were 0.77 ± 0.17 µg/kg and 1.36 ± 0.0 µg/kg respectively. The mean total aflatoxin concentrations detected in the raw maize sample was 2.13 ± 0.17 µg/kg. The total aflatoxin level found in the raw maize was below the World Health Organization (WHO), FAO and Ghana Standard Authority's maximum tolerable limit for aflatoxin in food of 15 µg/kg. After the maize samples were dehulled to remove the bran and the germ, no aflatoxin G1, G2, B1, B2 were detected in the remaining dehulled maize hence total aflatoxin was also zero. This represents 100% reduction in aflatoxin G1, B1 and total aflatoxin concentrations respectively as shown in figure 4. This reduction to zero level of aflatoxin G1, B1, B2 and total aflatoxin concentrations found after dehulling of the maize was significant ($p<0.05$) as compared to that detected in the raw maize samples purchased from the market. This was similar as compared to what was observed in an experiment by Njapau, Muzungaile, & Changa (1998) in Zambia, after dehulling a maize sample for the preparation of a traditional maize meal, detected that there was 11% and 9 % aflatoxin B1 and G1 respectively

remaining in the endosperm of the maize. There was also a significant ($p<0.001$) reduction in the aflatoxin B2 and G2 concentrations in the endosperm. Even though in this present study the bran removed from the maize were not analyzed for aflatoxin, an experiment by Taylor, *et al* (2009) indicated that the maize bran, germ and waste obtained after the dehulling of 2 different maize samples had more aflatoxin contamination as close to that of the raw maize samples while the clean maize samples after degerming had very low levels of aflatoxins. This means during the dehulling process the aflatoxin found on the outer portion of the maize were eliminated as the bran and germ were removed. During the preparation of the ekoegbeme, the maize samples were not pre-cleaned by hand sorting and flotation as compared to the study carried out by Matumba *et al*., (2015) where the maize samples used in their experiment were cleaned by hand sorting, flotation before dehulling the maize.

After eliminating all the aflatoxin from the maize samples by dehulling the maize, the dehulled maize was spiked to naturally contaminate them. This was to allow for the detection of the effect of the cooking stage of the processes of ekoegbeme on the level of aflatoxins. After allowing the dehulled maize to be contaminated by *Aspergillus niger for* 6 days, there was no aflatoxin G2 detected in the contaminated dehulled maize. Aflatoxin G1 concentration level detected was 16.87 ± 0.28 µg/kg. Aflatoxin B2 and B1 concentrations detected after the spiking of the dehulled maize were 11.02 ± 0.55 µg/kg and 76.01 µg/kg respectively. However, the total aflatoxin level detected was 103.9 ± 1.64 µg/kg.

The process of preparing ekoegbeme was continued by cooking (95 ^{o}C) the spiked dehulled maize grit.

After cooking the spiked dehulled maize grit samples for 45 minutes at 95 ^{o}C, no aflatoxin G2 was detected in the cooked dehulled maize grit. Aflatoxin G1 concentration decrease to 9.86 ± 0.33 µg/kg, representing a decrease of 41.53% as shown in figure 5. This decrease in aflatoxin G1 concentration was significant ($p<0.05$) as compared to that found in the spiked

dehulled maize grit. After cooking the maize grit, the aflatoxin B2 and B1 concentration levels decreased to 10.565 ± 0.163 µg/kg and 71.48 ± 1.65 µg/kg, representing 3.97% and 5.95 % reduction respectively. The decrease in the aflatoxin B2 and B1 levels as compared to that found in the spiked dehulled maize grit before cooking, were not significant ($p>0.05$). The total aflatoxin concentration level decreased to 91.905 ± 2.143 µg/kg, which represented 11.52 % decrease, after cooking the spiked dehulled maize grit as shown in figure 5. This decrease in the total aflatoxin level was significant ($p<0.05$) as compared to that found in the dehulled maize grit before cooking. The percentage reduction in the total aflatoxin concentration after cooking of the spiked dehulled maize grit was less as compared to that obtained in an experiment by Kirui (2016) where boiling of aflatoxin contaminated maize in distilled water reduced total aflatoxin from 59.5 ppb to 44.1 ppb which is 25.9% reduction. Matumba *et al*. (2015) also reported that the cooking of milled whole maize reduced aflatoxin B1 and G1 concentrations by 13% and 12% respectively which is more than the percentage reduction observed in this current study. More moisture in food samples increase the destruction of the aflatoxins and also the duration of exposure of the aflatoxin to heat and moisture affect the level of destruction of the aflatoxins. There was 40 % and 47 % reduction in aflatoxin B1 concentration when wet soft red winter wheat from USA and wet Anbaekmil wheat from Korea were heated as compared to heating of dry wheat samples. To hydrolyze the lactone ring of aflatoxins during cooking, temperature should be from 85 to 95 ^{o}C for effective detoxification (Hwang & Lee, 2006). Boiling at high temperatures (100 ^{o}C) can lead to opening of the ring in the structure of aflatoxin which is followed by decarboxylation which result in the methoxy group being lost from the aromatic ring (Kirui, 2016).

Table 2: Mean aflatoxin G2, G1, B2, B1 and Total aflatoxin concentration levels at different processing stages of cooking dehulled maize grit –Ekoegbeme.

Processing steps	Aflatoxin G2 (µg/kg)	Aflatoxin G1 (µg/kg)		Aflatoxin B2 (µg/kg)		Aflatoxin B1 (µg/kg)		Total Aflatoxin (µg/kg)	
Raw Maize	0	0.77+/-0.17	a	0	a	1.36+/- 0	a	2.13+/- 0.17	c
Dehulled maize	0	0	b	0	b	0	b	0	c
Dehulled Maize (spiked by natural contamination	0	16.87+/- 0.28	c	11.02 +/- 0.55	b	76.01 +/- 0.81	c	103.9 +/- 1.64	a
After cooking of dehulled maize grit	0	9.86 +/-0.33	b	10.565 +/- 0.163	cb	71.48 +/- 1.65	bc	91.905 +/-2.143	b

Values in columns are means of two replicates and standard deviation

Means in the same column followed by the letter are not significantly different (p>0.05) according to Turkey's HSD test

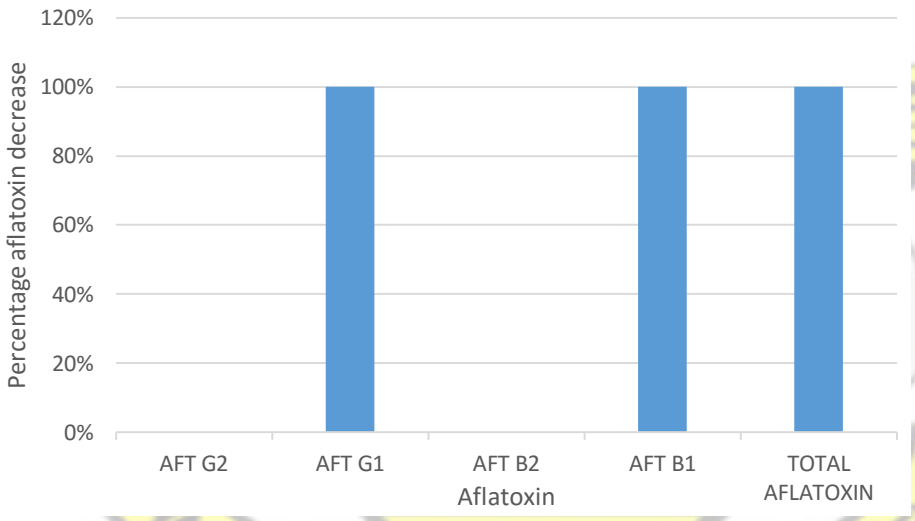

Figure 4: Mean percentage decrease in aflatoxins concentrations after dehulling the maize.

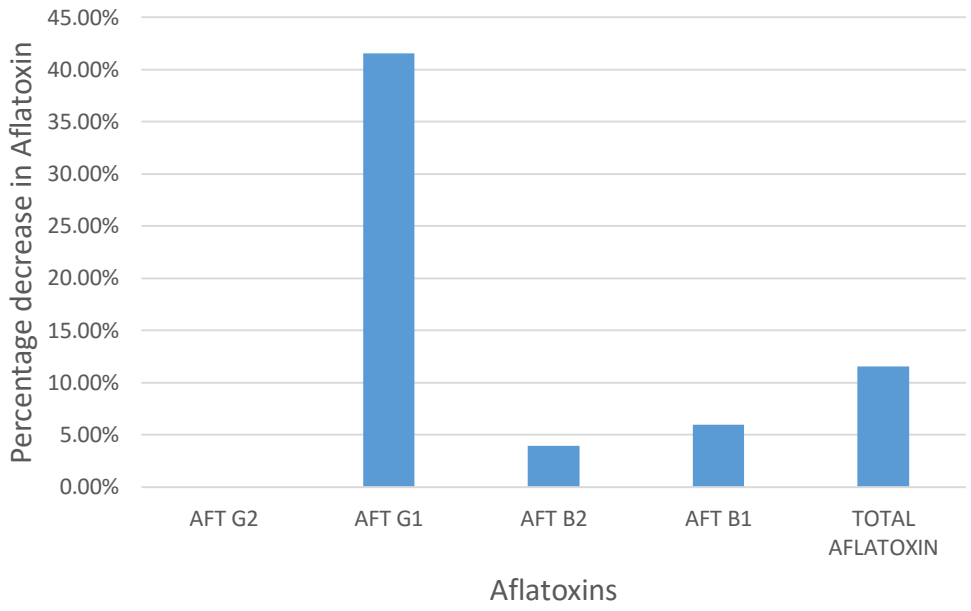

Figure 5: Mean percentage reduction in aflatoxins concentration after cooking the maize grits.

In this current study, the cooking process of the ekoegbeme (dehulled maize grit) resulted in greater percentage reduction of aflatoxins concentration than the cooking process involved in the preparation of the Hausa koko. Cooking of the 72 hours fermented filtrated into Hausa koko did not have significant effect on the aflatoxin concentrations in the millet meal since there was less than 0.7%, 1.85% and 1.49% reduction in the aflatoxin B2, B1 and total aflatoxins concentrations respectively.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1: Conclusion

In this current study it was found that the aflatoxin contamination in the maize grains is mostly found on the surfaces and germ of the maize. If the aflatoxin contamination in maize is very little it is possible to achieve 100% decontamination through the dehulling process involved in the preparation of ekoegbeme. After steeping the millet for 72 hours in this study there was an increase in the aflatoxin B1 and total aflatoxin concentrations hence there was no significant reduction in the initial total aflatoxin concentration in the spiked raw millet at the end of the processing methods used to prepare the Hausa koko.

5.2: Recommendations

In subsequent studies the moisture content and pH of the millet steeped must be considered to study how they could affect the levels of the toxins.

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7.0 APPENDIX

Appendix 1:

Results of aflatoxin G1, G2, B1, and B2 concentrations at various cooking stage of Hausa koko and ekoegbeme

		G2	G1	B2	B1
Hausa Kooko	Raw Millet	0.00	0.00	0.00	0.00
	Raw Millet (Spiked by Natural Contamination)	0.00	2.45	12.61	72.58
	Before Wet Milling (After steeping for 72hrs)	0.00	1.59	12.38	82.46
	After fermentation of filtrate for 72hrs	0.00	2.60	11.06	77.41
	After cooking of porridge	0.00	2.53	10.37	76.53
	Raw Millet	0.00	0.00	0.00	0.00
	Raw Millet (Spiked by Natural Contamination)	0.00	2.95	13.98	71.93
	Before Wet Milling (After steeping for 72hrs)	0.00	1.56	12.74	79.23
	After fermentation of filtrate for 72hrs	0.00	2.57	10.54	76.45
	After cooking of porridge	0.00	2.95	11.05	74.50
		G2	**G1**	**B2**	**B1**
Maize Grit	Raw Maize	0.00	0.89	0.00	1.36
	Degemed Maize	0.00	0.00	0.00	0.00
	Degemed Maize (Skipped by Natural Contamination)	0.00	17.07	11.41	76.58
	After Cooking	0.00	9.63	10.45	70.31
	Raw Maize	0.00	0.65	0.00	1.36
	Degemed Maize	0.00	0.00	0.00	0.00
	Degemed Maize (Skipped by Natural Contamination)	0.00	16.67	10.63	75.44
	After Cooking	0.00	10.09	10.68	72.65

Appendix 2:

Mean percentage reduction of aflatoxins G1, G2, B1, B2 and total aflatoxin concentrations at various processing stages for Hausa koko

Between Raw millet (spiked by natural contamination and steeping for 72 hrs	AFT G2	AFT G1	AFT B2	AFT B1	TOTAL AFLATOXIN
Replicate 1	0%	35.10%	1.82%	-13.61%	-10.03%
Replicate 2	0%	47.12%	8.87%	-10.15%	-5.26%
Mean	0%	41.11%	5.35%	-11.88%	-7.65%
Standard deviation	0	0.084994235	0.049851028	0.024465895	0.033728993
P-value					
Between after steeping for 72 hrs and fermentation of filtrate for 72 hrs	AFT G2	AFT G1	AFT B2	AFT B1	TOTAL AFLATOXIN
Replicate 1	0.00%	-63.52%	10.66%	6.12%	5.56%
Replicate 2	0.00%	-64.74%	17.27%	3.51%	4.24%
Mean	0.00%	-64.13%	13.97%	4.82%	4.90%
Standard deviation	0	0.008626703	0.046739758	0.018455487	0.00933381
P-value					
Between Fermentation for 72hrs and cooking for less than 100^{o}C	AFT G2	AFT G1	AFT B2	AFT B1	TOTAL AFLATOXIN
Replicate 1	0.00%	2.69%	6.24%	1.14%	1.80%
Replicate 2	0.00%	-14.79%	-4.84%	2.55%	1.18%
Mean	0.00%	-6.05%	0.70%	1.85%	1.49%
Standard deviation	0	0.123602265	0.078347431	0.009970206	0.004384062

Appedix 3:

Results of ANOVA analysis of aflatoxin concentration at different processing stage

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
G2	Between Groups	0.000	4	0.000		
	Within Groups	0.000	5	0.000		
	Total	0.000	9			
G1	Between Groups	11.057	4	2.764	64.555	.000
	Within Groups	.214	5	.043		
	Total	11.271	9			
B2	Between Groups	234.332	4	58.583	213.861	.000
	Within Groups	1.370	5	.274		
	Total	235.702	9			
B1	Between Groups	9411.780	4	2352.945	1480.035	.000
	Within Groups	7.949	5	1.590		
	Total	9419.728	9			

Multiple Comparisons							
Tukey HSD							
Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
G1	Raw Millet	Raw Millet (Spiked by Natural Contaminatio n)	-2.70000*	.20693	.000	-3.5301	-1.8699
		Before wet milling (after steeping for 72hrs)	-1.57500*	.20693	.003	-2.4051	-.7449
		After fermentation of filtrate for 72hrs	-2.58500*	.20693	.000	-3.4151	-1.7549
		After cooking of porridge	-2.74000*	.20693	.000	-3.5701	-1.9099
	Raw Millet (Spiked by Natural Contamination)	Raw Millet	2.70000*	.20693	.000	1.8699	3.5301
		Before wet milling (after steeping for 72hrs)	1.12500*	.20693	.015	.2949	1.9551
		After fermentation of filtrate for 72hrs	.11500	.20693	.976	-.7151	.9451
		After cooking of porridge	-.04000	.20693	1.000	-.8701	.7901
	Before wet milling (after steeping for 72hrs)	Raw Millet	1.57500*	.20693	.003	.7449	2.4051
		Raw Millet (Spiked by Natural Contaminatio n)	-1.12500*	.20693	.015	-1.9551	-.2949
		After fermentation of filtrate for 72hrs	-1.01000*	.20693	.023	-1.8401	-.1799
		After cooking of porridge	-1.16500*	.20693	.013	-1.9951	-.3349

	After fermentation of filtrate for 72hrs	Raw Millet	2.58500*	.20693	.000	1.7549	3.4151
		Raw Millet (Spiked by Natural Contaminatio n)	-.11500	.20693	.976	-.9451	.7151
		Before wet milling (after steeping for 72hrs)	1.01000*	.20693	.023	.1799	1.8401
		After cooking of porridge	-.15500	.20693	.935	-.9851	.6751
	After cooking of porridge	Raw Millet	2.74000*	.20693	.000	1.9099	3.5701
		Raw Millet (Spiked by Natural Contaminatio n)	.04000	.20693	1.000	-.7901	.8701
		Before wet milling (after steeping for 72hrs)	1.16500*	.20693	.013	.3349	1.9951
		After fermentation of filtrate for 72hrs	.15500	.20693	.935	-.6751	.9851
B2	Raw Millet	Raw Millet (Spiked by Natural Contaminatio n)	-13.29500*	.52338	.000	-15.3946	-11.1954
		Before wet milling (after steeping for 72hrs)	-12.56000*	.52338	.000	-14.6596	-10.4604
		After fermentation of filtrate for 72hrs	-10.80000*	.52338	.000	-12.8996	-8.7004
		After cooking of porridge	-10.71000*	.52338	.000	-12.8096	-8.6104
	Raw Millet (Spiked by	Raw Millet	13.29500*	.52338	.000	11.1954	15.3946

	Natural Contamination)	Before wet milling (after steeping for 72hrs)	.73500	.52338	.651	-1.3646	2.8346
		After fermentation of filtrate for 72hrs	2.49500*	.52338	.026	.3954	4.5946
		After cooking of porridge	2.58500*	.52338	.022	.4854	4.6846
	Before wet milling (after steeping for 72hrs)	Raw Millet	12.56000*	.52338	.000	10.4604	14.6596
		Raw Millet (Spiked by Natural Contamination)	-.73500	.52338	.651	-2.8346	1.3646
		After fermentation of filtrate for 72hrs	1.76000	.52338	.094	-.3396	3.8596
		After cooking of porridge	1.85000	.52338	.079	-.2496	3.9496
	After fermentation of filtrate for 72hrs	Raw Millet	10.80000*	.52338	.000	8.7004	12.8996
		Raw Millet (Spiked by Natural Contamination)	-2.49500*	.52338	.026	-4.5946	-.3954
		Before wet milling (after steeping for 72hrs)	-1.76000	.52338	.094	-3.8596	.3396
		After cooking of porridge	.09000	.52338	1.000	-2.0096	2.1896
	After cooking of porridge	Raw Millet	10.71000*	.52338	.000	8.6104	12.8096
		Raw Millet (Spiked by Natural Contamination)	-2.58500*	.52338	.022	-4.6846	-.4854

		Before wet milling (after steeping for 72hrs)	-1.85000	.52338	.079	-3.9496	.2496
		After fermentation of filtrate for 72hrs	-.09000	.52338	1.000	-2.1896	2.0096
B1	Raw Millet	Raw Millet (Spiked by Natural Contaminatio n)	-72.25500*	1.2608 7	.000	-77.3130	-67.197 0
		Before wet milling (after steeping for 72hrs)	-80.84500*	1.2608 7	.000	-85.9030	-75.787 0
		After fermentation of filtrate for 72hrs	-76.93000*	1.2608 7	.000	-81.9880	-71.872 0
		After cooking of porridge	-75.51500*	1.2608 7	.000	-80.5730	-70.457 0
	Raw Millet (Spiked by Natural Contamination)	Raw Millet	72.25500*	1.2608 7	.000	67.1970	77.313 0
		Before wet milling (after steeping for 72hrs)	-8.59000*	1.2608 7	.006	-13.6480	-3.5320
		After fermentation of filtrate for 72hrs	-4.67500	1.2608 7	.067	-9.7330	.3830
		After cooking of porridge	-3.26000	1.2608 7	.208	-8.3180	1.7980
	Before wet milling (after steeping for 72hrs)	Raw Millet	80.84500*	1.2608 7	.000	75.7870	85.903 0
		Raw Millet (Spiked by Natural Contaminatio n)	8.59000*	1.2608 7	.006	3.5320	13.648 0
		After fermentation of filtrate for 72hrs	3.91500	1.2608 7	.122	-1.1430	8.9730

		After cooking of porridge	5.33000*	1.26087	.041	.2720	10.3880
	After fermentation of filtrate for 72hrs	Raw Millet	76.93000*	1.26087	.000	71.8720	81.9880
		Raw Millet (Spiked by Natural Contamination)	4.67500	1.26087	.067	-.3830	9.7330
		Before wet milling (after steeping for 72hrs)	-3.91500	1.26087	.122	-8.9730	1.1430
		After cooking of porridge	1.41500	1.26087	.791	-3.6430	6.4730
	After cooking of porridge	Raw Millet	75.51500*	1.26087	.000	70.4570	80.5730
		Raw Millet (Spiked by Natural Contamination)	3.26000	1.26087	.208	-1.7980	8.3180
		Before wet milling (after steeping for 72hrs)	-5.33000*	1.26087	.041	-10.3880	-.2720
		After fermentation of filtrate for 72hrs	-1.41500	1.26087	.791	-6.4730	3.6430
*. The mean difference is significant at the 0.05 level.							

Appendix 4:

ANOVA analysis for Total aflatoxin concentration in Hausa koko processing stages

ANOVA analysis for Total aflatoxin in Hausa koko processing stages

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```

One-way ANOVA: Raw millet, Raw millet s, After steepi, After fermen, ...

```
Source  DF        SS       MS        F      P
Factor   4  13196.27  3299.07  2529.30  0.000
Error    5      6.52     1.30
Total    9  13202.79

S = 1.142   R-Sq = 99.95%   R-Sq(adj) = 99.91%



Level                     N    Mean  StDev
Raw millet                2   0.000  0.000
Raw millet spiked to con  2  88.250  0.863
After steeping fro 72hr   2  94.980  2.051
After fermentation for 7  2  90.315  1.068
After cooking             2  88.965  0.658

                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level                     -+---------+---------+---------+--------
Raw millet                (*)
Raw millet spiked to con                                (*)
After steeping fro 72hr                                    (*)
After fermentation for 7                                 (*)
After cooking                                            (*
                          -+---------+---------+---------+--------
                           0        25        50        75

Pooled StDev = 1.142


Grouping Information Using Tukey Method

                                N   Mean  Grouping
After steeping fro 72hr         2  94.98  A
After fermentation for 72hr     2  90.31    B
After cooking                   2  88.97    B
Raw millet spiked to contaminat 2  88.25    B
Raw millet                      2   0.00      C

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 98.98%


Raw millet subtracted from:
```

```
                          Lower  Center  Upper
Raw millet spiked to con  83.67   88.25  92.83
After steeping fro 72hr   90.40   94.98  99.56
After fermentation for 7  85.74   90.31  94.89
After cooking             84.39   88.97  93.54

                          ----+---------+---------+---------+-----
Raw millet spiked to con                                   (*-)
After steeping fro 72hr                                      (-*)
After fermentation for 7                                   (*-)
After cooking                                              (-*)
                          ----+---------+---------+---------+-----
                              0        30        60        90


Raw millet spiked to contaminat subtracted from:

                          Lower  Center  Upper
After steeping fro 72hr    2.15    6.73  11.31
After fermentation for 7  -2.51    2.06   6.64
After cooking             -3.86    0.72   5.29

                          ----+---------+---------+---------+-----
After steeping fro 72hr        (*-)
After fermentation for 7     (-*)
After cooking                (*-)
                          ----+---------+---------+---------+-----
                              0        30        60        90


After steeping fro 72hr subtracted from:

                           Lower  Center  Upper
After fermentation for 7   -9.24   -4.67  -0.09
After cooking             -10.59   -6.02  -1.44

                          ----+---------+---------+---------+-----
After fermentation for 7    (*-)
After cooking             (-*-)
                          ----+---------+---------+---------+-----
                              0        30        60        90


After fermentation for 72hr subtracted from:

               Lower  Center  Upper  ----+---------+---------+---------+-----
After cooking  -5.93   -1.35   3.23     (-*)
                                     ----+---------+---------+---------+-----
                                       0        30        60        90
```

Appendix 5:

Mean percentage reduction in aflatoxin G2, G1, B2, B1 and total aflatoxin concentrations in Ekoegbeme processing

Percentage reduction of aflatoxin G2, G1, B2, B1 and total aflatoxin

Processing steps	AFT G2	AFT G1	AFT B2	AFT B1	TOTAL AFLATOXIN
Between Raw maize and Dehulled maize grit	0.0%	100%	100%	100%	100%
	0.0%	100%	100%	100%	100%
P-Value					
	AFT G2	AFT G1	AFT B2	AFT B1	TOTAL AFLATOXIN
Between Dehulled maize (spiked by Natural contamination) to cooking of maize grit)	0.00%	43.59%	8.41%	8.19%	13.96%
	0.00%	39.47%	-0.47%	3.70%	9.07%
Mean Percentage decrease (%)	**0.00%**	**41.53%**	**3.97%**	**5.95%**	**11.52%**
Standard Deviation	0.00%	2.91%	6.28%	3.17%	3.46%
P-Value					

Means Percentage decrease of AFLATOXIN G2, G1, B2, B1 AND TOTAL AFLATOXIN

Processing steps	**AFT G2**	**AFT G1**	**AFT B2**	**AFT B1**	**TOTAL AFLATOXIN**
Between Dehulled maize (spiked by natural contamination and cooking of maize grit	**0.00%**	**41.53%**	**3.97%**	**5.95%**	**11.52%**

Appendix 6:

ANOVA results for the Processing steps involved in ekoegbeme preparation.

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```
Welcome to Minitab, press F1 for help.
Retrieving project from file: 'C:\USERS\ALBERT\DOCUMENTS\2ND SEM IDL-KNUST
MATERIALS\STATISTICAL METHOD\MINITAB WORK IN CLASS.MPJ'
```

One-way ANOVA: Raw maize, Dehulled maize, Dehulled maize s, After cooking ma

```
Source  DF        SS       MS        F      P
Factor   3  18903.42  6301.14  3447.74  0.000
Error    4      7.31     1.83
Total    7  18910.73

S = 1.352   R-Sq = 99.96%   R-Sq(adj) = 99.93%



Level                     N    Mean  StDev
Raw maize                 2    2.13   0.17
Dehulled maize            2    0.00   0.00
Dehulled maize spiked     2  103.90   1.64
After cooking maize grit  2   91.91   2.14

                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level                     -+---------+---------+---------+--------
Raw maize                  (*)
Dehulled maize            (*)
Dehulled maize spiked                                      (*)
After cooking maize grit                                 (*)
                          -+---------+---------+---------+--------
                           0        30        60        90

Pooled StDev = 1.35


Grouping Information Using Tukey Method

                          N    Mean  Grouping
Dehulled maize spiked     2  103.90  A
After cooking maize grit  2   91.91    B
Raw maize                 2    2.13      C
Dehulled maize            2    0.00      C

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 98.48%


Raw maize subtracted from:

                          Lower  Center   Upper
Dehulled maize           -7.64   -2.13    3.38
Dehulled maize spiked    96.26  101.77  107.28
After cooking maize grit 84.27   89.78   95.28

                          --------+---------+---------+---------+-
```

```
Dehulled maize                          (*)
Dehulled maize spiked                                   (*)
After cooking maize grit                              (*)
                          --------+---------+---------+---------+-
                                -60         0        60       120


Dehulled maize subtracted from:

                          Lower  Center   Upper
Dehulled maize spiked     98.39  103.90  109.41
After cooking maize grit  86.40   91.91   97.41

                          --------+---------+---------+---------+-
Dehulled maize spiked                                       (*)
After cooking maize grit                              (*)
                          --------+---------+---------+---------+-
                                -60         0        60       120

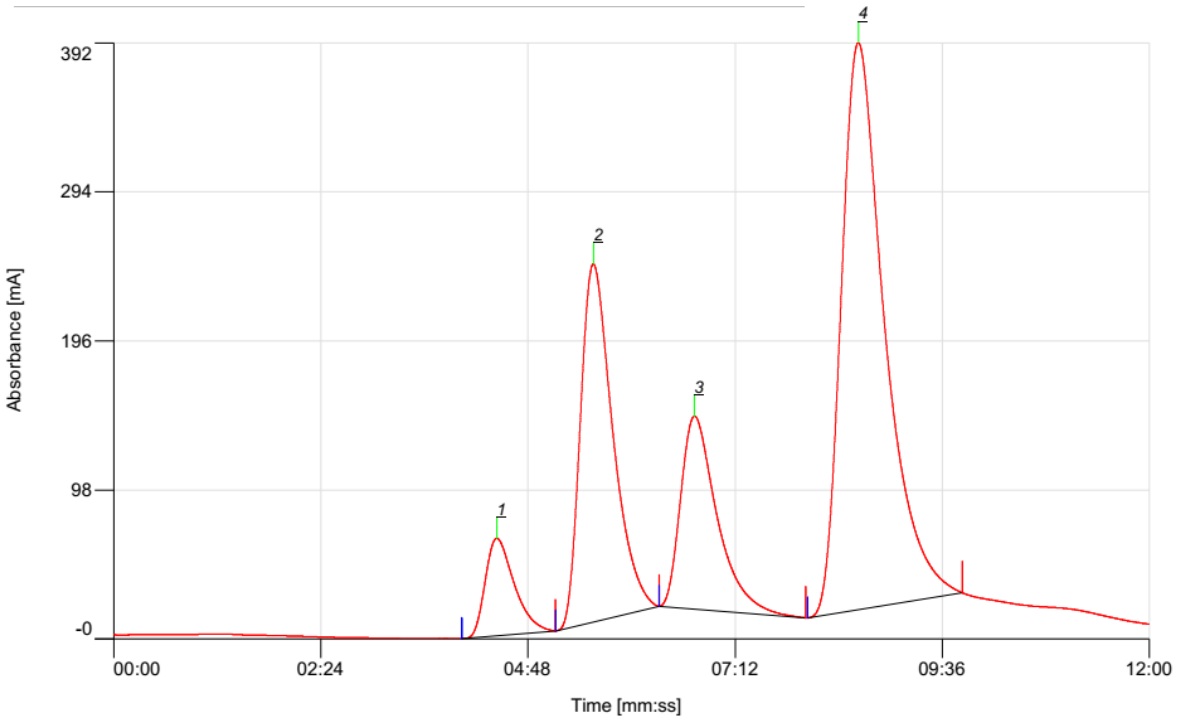

Dehulled maize spiked subtracted from:

                           Lower  Center  Upper
After cooking maize grit  -17.50  -12.00  -6.49

                          --------+---------+---------+---------+-
After cooking maize grit                (*)
                          --------+---------+---------+---------+-
                                -60         0        60       120
```

Appendix 7:

Chromatogram for aflatoxin determination



Appendix 8:

Retention time/retention time window for different aflatoxins

Peak	Aflatoxin	Retention Time (mm:ss.ms)	% Window
1	G2	04:26.4	10.15
2	G1	05:33.6	10.08
3	B2	06:43.9	10.76
4	B1	08:37.8	13.54

Appendix 9: Aflatoxin 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 (µL),

I = volume eluate injected into LC (µL), W = mass (g) of commodity represented by final extract.