KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI

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FACULTY OF BIOSCIENCES

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

EXPOSURE AND RISK ASSESSMENT OF AFLATOXIN INGESTED CEREALS AND GRAINS BY UNIVERSITY STUDENTS

MASTER OF FOOD QUALITY MANAGEMENT

BY

TETTEH LUKE PAA MENSAH

(BSc. CHEMISTRY)

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UNIVERSITY STUDENTS

A THESIS SUBMITTED TO THE DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF

MASTER OF FOOD QUALITY MANAGEMENT IN FOOD SCIENCE AND TECHNOLOGY (MSC. FOOD QUALITY MANAGEMENT)

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DECLARATION

I declare that this project report is an authentic record of my own work carried out under the supervision of Dr. Isaac W. Ofosu and that except portions where references have been duly cited, this project is the outcome of my research.

TETTEH LUKE PAA MENSAH …… ……..

PG1050117 Signature Date

Supervisor.

Dr. ISAAC W. OFOSU …… …….

Signature Date

Dr. (MRS.) FAUSTINA DUFIE WIREKO-MANU …… ……..

Certified by Head of Department Signature Date

DEDICATION

This work is dedicated to God almighty whose abundant grace has seen me through my one-year degree program safely. Also, to my parents Mr. and Mrs. Simon Kofi Ohene, my siblings and finally my grandmother Nana Adowa Duku for their prayers, guidance and sacrifices they made for me towards my education.

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Thank you all and I wish the blessings and grace of God fall on you abundantly.

ABSTRACT

The objective of this study was to assess the exposure and health risks associated with the consumption of kenkey by students of KNUST. Students completed a food dietary questionnaire to gather their exposure frequency and amount of kenkey eaten. Kenkey samples were collected from various students' food joints and homogenized. Sirhan method of extraction and purification was used to extract the aflatoxin from the kenkey. HPLC was used to determine the concentrations of each group of aflatoxin. The probabilistic approach was used to model the chronic exposures using the Monte Carlo simulation of the Palisade @Risk software. The mean concentration of aflatoxin was 4.78 ng/g. Probabilistic approach was used to estimate the chronic daily exposure. The chronic exposures ranged between 0.024-1.09 ng/g bw/day. The chronic exposure and reference dose of aflatoxin were used to estimate the hazard quotient. The hazard quotient was below the tolerable limit (1). The chronic exposure for both carcinogen and noncarcinogen groups of aflatoxins were also estimated of exposure. There was a significant health concern as the margin

of exposure (792.06) for the carcinogen group was below the threshold level (10,000). Aflatoxin contamination must be given a serious attention and effective measures must be in place to curb the contamination of aflatoxin.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Aflatoxins are mycotoxins primarily produced by *Aspergillus nomius, Aspergillus flavus* and *Aspergillus parasiticus*. They are known to be acutely toxic, mutagenic, carcinogenic, teratogenic and immunosuppressive (Wild and Gong, 2009) and classified as G1, G2, B1 and B2 based on their chemical structures. Aflatoxins are found in most staple foods in their raw forms such as maize (Murphy *et al.*, 2006). Previous reports in several studies have reported the occurrence of aflatoxins in foods from the Ghanaian market (Khlangwiset *et al.*, 2011). Out of the eighteen known aflatoxins (AF), just AFG1 AFG2, AFB1 and AFB2 are reported in agricultural food crops (Riba *et al.*, 2010). Aflatoxin B1 is the only metabolite among the four known groups that is very carcinogenic (Assunção *et al.*, 2018). The International Agency for Research on Cancer (IRAC) reported Aflatoxin B1 is a hepato carcinogen when hazard is chronically exposed and causes acute hepatitis and can end up causing cancer of the liver (IARC, 2002 and IARC, 2012). According to Assunção *et al.* (2018) aflatoxin B1 have been grouped as class 1 potential carcinogen to humans.

Kenkey, which is a cereal-based food, prepared from maize, is consumed by most Ghanaians and several studies have reported the occurrence of aflatoxin in kenkey (Shephard, 2003). According to Assunção *et al.* (2018), cereal-based foods is one of the commonest means to human exposure to aflatoxin and other mycotoxins. Previous studies have shown that approximately 25% of cereals produced globally are contaminated by mycotoxins (FAO/WHO, 2002). Aflatoxin in maize is mostly produced as a result of poor handling of maize in the supply chain including storage. If the moisture exceeds 12% and humidity is not right, stored maize is prone to be contaminated with aflatoxin.

To be able to estimate the exposure and risk of aflatoxin in kenkey, several methodologies such as dietary recall, and dietary history or food-frequency methods are used. Assuming that the consumption of kenkey is considered as chronic daily intake, dietary recall should be appropriate as it could be expedient to recollect the exact food intake measurements (Cano-Sancho *et al.*, 2013). In order to make an accurate judgment on the risk that aflatoxin in kenkey poses, it is imperative to take into consideration all aspects risk indices. Risk characterization estimates the severity of an identified hazard and the probable adverse health effects of the hazard (CanoSancho *et al.*, 2013). At the present time, the contamination cereal-based foods by aflatoxin needs a close attention, specifically because of the adverse health effects as a result of mycotoxins exposure could lead to diverse toxicity and carcinogenicity (Speijers *et al*., 2004). Previous studies only focused on the occurrence of aflatoxin and not much on exposure and risk assessment especially in Ghana (Assunção *et al*., 2015). The chemical compositions and knowledge of the toxicities of aflatoxin has been used to envisage the toxicity and risk it presents. For example, in order to quantify the risk associated with aflatoxin exposure, hazard quotient is used, and it is expressed as the ratio of the chronic human exposures to the reference dose of aflatoxin. On the other hand, margin of exposure (MOE), which is defined as the ratio of bench mark dose lower limit ($BMDL_{10}$) to estimated exposure of a hazard. According to Syberg *et al*. (2008) these procedures of risk assessment are based on the theories of Independent Action (IA) and Concentration Addition (CA). According to Assunção *et al.* (2016), MOE is aimed at both carcinogenic and genotoxic risk assessment and is mostly used for the cumulative risk assessment. Lifetime risk is another way to estimate risk of a hazard. It is multiplication of the potency factor and chronic daily intake that are exposed to the population. The slope factor (also known as the potency factor) which is the risk produced by a lifetime average dose of 1 mg/kgday is mostly originated from certified studies. Very few studies have been conducted on the exposure and risk assessment exist on cereal-based

products such as kenkey Murphy *et al*., 2006). The European Food Safety Authority (EFSA) instituted a minimum limit of 5 µg/kg for total aflatoxin in cereals (van Egmond *et al.*, 2007). The objective of this study was to estimate the exposure and risk associated with aflatoxin exposure through the consumption of kenkey.

1.2 Problem Statement and Justification

As a result of the high standard of living on university campuses in Ghana, many students resort to consuming cheaper food alternatives such as cereal-based (kenkey). However, there have been documented evidence of poor handing of cereals on the market especially if the grains are not sourced from certified maize grits producers (Shephard, 2003). Thus, it can be assumed that consumers of these cereal-based foods are at risk of adverse health effects that result from aflatoxin known to be present in the poorly handled grains as a result of the growth of *Aspergillus flavus.* Therefore, there is the need to quantify the exposure and determine if the concentrations of aflatoxins in kenkey consumed by many students on KNUST campus are enough to pose a significant health concern or risks such as cancers or any related toxicities. Since reports from the KNUST Hospital indicates increasing number of students who are treated of Hepatitis B.

1.3 Main Objective

To estimate the dietary exposure and health risks associated with the consumption of kenkey.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Aflatoxin

Aflatoxins are mycotoxins, which can be carcinogenic and are produced by some *Aspergillus* species in a huge variety of agricultural commodities, typically *Aspergillus flavus* in maize and peanuts. Aflatoxin B_1 became initial known within the UK in 1960 in an exceeding payload of peanuts from Brazil. Turner *et al.* (2007) discovered that aflatoxin B1 caused the outbreaks of acute liver disease in animals and humans leading to carcinoma and the associated diseases. According to Wagacha *et al*. (2008), the risks of foodstuffs contaminated with aflatoxin at levels higher than tolerable limit were once more demonstrated in 2004 in the Republic of Kenya where a hundred and twenty-five individuals died following the consummation of locally-cultivated maize containing high levels of aflatoxin.

2.2 Types of aflatoxin

Hundreds of fungal species produce over 300 known mycotoxins which might be a threat to the health of humans. Aflatoxin is naturally present mycotoxins made by three fungus species: *Aspergillus flavus, Aspergillus paraciticus* and *Aspergillus nominus.* The most commonly found is *Aspergillus flavus*. They primarily found in soil decaying vegetation. The main reported groups of aflatoxin are B_1, B_2, G_1 and G_2, and two sub-major ones, M_1 and M_2 normally found in milk products. Aflatoxin B1 is the commonest member of this family of mycotoxins and has very high carcinogen potency. It is possible that cereals contaminated by aflatoxin may not include aflatoxin B_2, G_1 and G2 but aflatoxin B_1 is always present (Creppy, 2002).

Goto *et al*. (1996) revealed the probability of more fungi species included to the existing fungi as a result of their growth. New strains of *Aspergillus* sp. such as *Aspergillus ochraceoroseus* and *Aspergillus tamarii* extracted from the Tai rainforest and sclerotium growth respectively, has been stated to produce aflatoxins (Goto *et al.*, 1996). However, there have not been any documentations on the aflatoxin produced by other *Penicillia, Aspergilli* and other species of *Rhizopus*. Just as amongst the aflatoxin producer's species, *Aspergillus flavus* simply yields the group B, whereas *Aspergillus parasiticus* and *Aspergillus nomius* from G group produces a more complex toxic forms of aflatoxins with an oxidative ring. Generally, aflatoxin B1 is considered the commonest with both chronic and acute chronic toxicity which is mostly toxic.

Even though aflatoxins, are produced from a little fraction of mold species of *Aspergillus flavus* and *Aspergillus parasiticus*, they are extremely prevalent within subtropics as well as tropics where is marked consumption of staple food commodities (Liu and Wu, 2010).

With favorable conditions such as surfaces of plants like maize and peanuts, of *Aspergillus flavus* and *Aspergillus parasiticus* spores is capable of growing. The mycelium is capable of setting up an endotrophic connection that does not cause any harm to the vigorous plant. During the time that the plant is stressed, of which the normal stress period is drought, then an appreciable level of aflatoxin is likely be developed or produced in the tissue of the plant for the growth period on the farm. With these situations, there could be contamination of farm produce before harvest (Hill *et al*., 1985). Even though intensities are by no means higher as compared those developed in farm produce that are stored, by which from economical point of view is very significant.

2.3 Aflatoxin contamination and food quality

Agricultural produce is often contaminated by aflatoxin throughout production, storage, process and transit once the humidity and temperature states are appropriate. Mold growth and aflatoxin development are favoured by poor storage conditions and factors such as temperature and humidity (Hell *et al.*, 2000). Aflatoxin content in ng/g of a product can show a sign of product quality decline. As a result, the market value is affected.

2.4 Impact on nutrition, health and environment

Shephard (2003) revealed that aflatoxin is amongst the most food contaminants with severe adverse health effects, food and nutritional security and economic losses. Cereals and grains contaminated with aflatoxin could lead to lethal aflatoxicosis and chronic mutagenic and carcinogenic effects with long dormancy periods. Aflatoxin is related to exacerbation of the protein deficiency disease syndrome kwashiorkor in children. Assessing the human health effects of aflatoxin in terms of primary cancer of the liver needs information on human exposure to aflatoxins. In developing countries, people do not seem to be solely malnourished but are also endangered inveterately to high levels of aflatoxin in the diet of individuals (Cardwell, 2004). The health risk decreases labour productivity, whereas increasing health cost and overall income losses because of the chance cost coupled to lost days of work (Asenso-Okyere *et al*, 2011). Signs of acute aflatoxicosis involve hemorrhagic necrosis of the liver, epithelial duct proliferation, oedema, and lethargy (Yaman *et al.,* 2016). According to Gong *et al*. (2004), aflatoxins are evidenced carcinogens, immunotoxins and causes retardation of growth and implicated within the stunning of children.

2.5 Childhood aflatoxin exposure patterns in West Africa

Aflatoxin exposure has been tough to characterize (Piekkola *et al.*, 2012). The inability to accurately measure individual exposure has successively held back efforts to better understand

aflatoxin health effects. Overcoming the difficulties inherent to those forms of assessment has been the thrust for the development of aflatoxin exposure biomarkers (Kensler e*t al.*, 1999; Wild and Turner, 2002). Bio-activation is needed in order for aflatoxin to be reactive. Due to that 8, 9epoxide exert their carcinogenic harmful effects as the metabolic activation enzymes is found in humans (Mannaa *et al*., 2014). Once activated to the epoxide, aflatoxin can bind to and cause damage to cellular targets like DNA and proteins. According to Wild and Turner (2002), aflatoxin binds covalently to albumin and this Aflatoxin-albumin (AF-alb) adduct are often measured in the peripheral blood as a helpful biomarker of exposure over the 30-60 days before sampling. However, this means of aflatoxin exposure assessment is limited in most African countries.

The exposure pattern among infant is quite dynamic as a result of the fact that children at the start are breastfed and gradually introduced to cereal-based porridge. The period of introducing cereal-based porridge increases the level of aflatoxin exposure. According to Gong *et al*. (2004), the general pattern as substitution foods slowly replace breast milk increases aflatoxin exposure markedly. El–Serag and Rudolph (2007) found out that aflatoxins have long been investigated on the aetiology of Hepatocellular Carcinoma (HCC), and very little attention paid to the potential adverse health effects due to unavailability of substantial exposure and risk information.

2.6 Risk analysis

Improvements in mycotoxins detection and also the implication for health, trade and food convenience led to the development of risk analysis methodology that needs additional elaborated data on mycotoxin action and interaction. Risk analysis strengthens the flexibility of tradition food safety system by providing a guideline to practically manage, assess and communicate risks operational with numerous beneficiaries permitting effective decisionmaking. This approach will increase the flexibility to identify

hazards food by safety regulators, characterize them, assess exposure, and estimate the probability of the resulting risk to the potential impact on human health.

2.7.1 Risk assessment

Food risk assessment includes the method of detecting, studying and characterizing a foodrelated health risk (Spink *et al.*, 2011). Risk assessment estimates the probability and severity of an adverse health effect occurring from exposure to a hazard. It is used to study contaminants or any other substance that are deliberately added to food or naturally-occurring toxins in foods such as pathogenic microorganisms, food preservative and aflatoxins (Moss, 2002). According to WHO (2015), risk assessment is made of three main processes namely hazard identification, hazard characterization and risk assessment.

Assessing food risk is very important as the outcomes of the assessment play a vital role in policy making for the food consumers and also for food safety and security reasons. A representative exposure observation must be achieved in order to achieve an accurate risk assessment (FAO/WHO, 2015).

2.6.1 Hazard identification

Hazard identification mostly concentrates on hazards likely to be found in a certain food and to cause foodborne illness. The causal relationship between the hazard, sickness and a food as one vector of a particular sickness are identified during hazard identification (Hedberg *et al.*, 2006).

Wild and Turner (202) established that the people in West Africa are exposed to aflatoxin at the early stage of their lives. El–Serag *et al.* (2007) found out that aflatoxins have long been investigated on the aetiology of Hepatocellular Carcinoma (HCC), and very little attention paid to

the potential adverse health effects due to unavailability of substantial exposure and risk information.

2.6.2 Exposure assessment

Food dietary exposure assessment is the method of quantifying how much of hazard or food chemical that has been consumed by a population or a selected group of people for certain or definite time period. It is expressed as mass per time unit (mg/kgBw-day). Food exposure assessment must be the actual food intake pattern of the selected populace (Marin *et al*., 2013).

Dietary exposure to food hazards is estimated by the combination food consumption data with food hazard concentration data. According to Biro *et al.* (2002) food consumption is an estimate of the daily average per capita amount of a food or group of foods consumed by a given population and is employed for estimating long term-hazards. Different population groups depict variable food consumption patterns therefore completely different consumption data are needed for assessing dietary exposure to food chemicals.

The prospective methods (dietary records and dietary recall techniques) which record data at the time of eating and the retrospective methods (diet history and food frequency questionnaire) which use data about the food eaten over a specified duration of time are the two main methods to estimate and assess for food consumption (Merten *et al.*, 2011).

According to Dougherty *et al.* (2000), identification of potential pathways, identification of potentially exposed populace and estimation of exposed populace, estimation of the severity, duration and time-pattern and frequency must be considered during dietary exposure assessment. African countries that are exposed to aflatoxin could be determined by a single-point using the mean levels because both food consumption and contamination levels are a function of it (Shephard, 2008). Excessive exposures are as

a result of excessive consumption of contaminated foods or excessive contamination levels of foods that are consumed in moderate quantities. Most people in developing countries consume the staple foods in excessive quantities that are highly contaminated at levels beyond the tolerable level (Shephard, 2008). Aflatoxins exposure and risk assessment data set for most developing countries information is not available (Wilkinson *et al.,* 2000). Evidently, it is imperative that one must have a full data on consumption of staple foods in communities in order to fully estimate the risk associated with a staple food. Studies have shown that most human populace is opened to dietary consumption of aflatoxin that differs significantly from one fraction of the world to another and among societal classes (Doll *et al.,* 1981).

Most staple foods such as maize consumed by the people in the developing countries are mostly foods that have been cultivated by indigenous farmers or families, put in storage, sold and cooked with little or no concern for the threats of aflatoxin. For economic benefits, the minimum contaminated produce is exported (Otsuki *et al.,* 2001). This undoubtedly increases the level of exposure of aflatoxin to the people as the highly contaminated cereals and grains are left in the country for the consumption by the populace. Though aflatoxin detection of in most foods suggests the probability of exposure, it is difficult to measure the dietary consumption as no farreaching data set is available to estimate the level and severity of exposure and risk of individuals in low-income countries of which Ghana is part (Chen *et al.*, 2012; Wu *et al.,* 2004).

2.6.3 Acute exposure to aflatoxin

According to Bankole *et al*. (2006) mortality percentage of 25% of acute poisonings occurs due to high exposure levels. Scientific journals in the mid-1990s reported of this nature, however succeeding reports are sometimes found mainly trendy by the electronic media. Death information and severe illness are most at times recorded in the advancing countries inside the risk zone.

Reported situations of acute poisoning don't seem to be massive relative to the population in danger, most likely as a result of individuals sometimes avoid foods that are evidently moldy, and individuals are at times tolerant to aflatoxin (Kimanya *et al.*, 2014). Conversely, during times of insufficiency of food, or in circumstances of poverty individuals normally don't have any choice but to use cheaper, inferior-quality food, which ordinarily is unclean (Bankole *et al*., 2006).

2.6.4 Chronic exposure to aflatoxin

Chronic exposure of aflatoxin in humans is estimated in two key methods (Wagacha *et al.*, 2008). The first approach involves food samples. Samples of food are gathered either from ready foods and food ingredients or in markets is the utmost frequently accessed information. The highly dependable source of sample for exposure estimation is by way of analysis of cooked dishes. Then again, samples from the market offers data by exposure risk as of varied meals, notably when native food manufacturers carry out set-ups like milling with no Standard Operation Procedures any quality assurance or control. The next method comprises the use of biological markers of exposure. In this method, urine, blood, or milk samples are gotten from individuals and probed for any aflatoxin derivatives that may be present, every of that holds incorporates a distinctive half-life within the body (Landrigan *et al.*, 2005; Osei-Tutu and Anto, 2016). The technology is comparatively modern, and findings centered on it are physically limited to a little low portion of advancing countries (Jemal *et al*., 2010).

2.7 Risk Characterization

Risk characterization is the quantitative or qualitative estimation of the adverse effects associated with the hazard that may be present or found in food (Hedberg *et al.*, 2006). A dose-response assessment is conducted for the hazard to characterize the hazard (Choy *et al*., 1993). As a result of the combined effects of aflatoxins and hepatitis B virus in inducing Hepatocellular carcinoma

(HCC), the evaluation is individually conducted for inhabitants with and without chronic hepatitis B virus infection. Many epidemiologic studies ascertain aflatoxin as a carcinogen (Groopman *et al*., 1985; Qian *et al*., 1994).

The final risk characterization step involves comparison of the TMDI or EDI with the healthbased toxicological value i.e. or Cancer Benchmark Concentration (CBC), ADI or ARfD in the case of chronic exposure, acute exposure or cancer risk respectively. Hazard Index (HI) concept of quantifying the ratio of the exposure to the health-based toxicological value (otherwise known as hazard quotient) has repeatedly been used in other research works for the characterization of risk (Chun and Kang, 2003; Peduzzi *et al.,* 2009; Akoto *et al.*,2015).

The tolerable levels are established for both total aflatoxin and AFB1 as low as fairly moderately possible (EFSA, 2013). Sorting or different physical treatments enable the reduction of the aflatoxin of cereals and grains such as maize. To minimize the consequences on trade, higher aflatoxin contents are allowed for the products that do not seem to be consumed by humans or that are constituent in foods. However, most low-come countries do not adhere to this protocol as they consume higher aflatoxin cereals and grain and as a result, exceed the maximum exposure limit (Williams *et al.,* 2004).

2.8 Challenges affecting estimation of aflatoxin exposure

Since mycotoxins are natural contaminant that is notably tough for risk assessors, as they sometimes are considered inevitable and thus should be regulated otherwise than artificial chemicals that are considered avertable (Van Egmond *et al.,* 2002). In developing countries, most local food industries are not regulated by quality-control measures due to the limited sources of food and the and under equipped laboratory food institutions (Chen *et al.,* 2012). As a result of this, the tolerable limit of aflatoxin permissible in foods by *Codex Alimentarius* is of no

significance to most developing countries. Though aflatoxin detection of in most foods suggests the probability of exposure, it is difficult to measure the dietary consumption as no data set is available to estimate the level and severity of exposure and risk of individuals in low-income countries such as Ghana (Wu *et al.,* 2004). For the developed countries, due to their strong food quality-control measures, data set for exposure and risk to aflatoxin exist just for most countries. Few challenges exist in getting chronological estimations of exposure and risk assessment of aflatoxin (Wu *et al.,* 2004). Evidently, it is imperative that one must have a full data on consumption of staple foods in communities in order to fully estimate the risk associated with a staple food. Studies have shown that most people are exposed to dietary consumption of aflatoxin that differs significantly (Doll *et al.,* 1981). Despite, having an extensive and precise estimates of aflatoxin exposure, risk assessment is however, a problem (Wu, 2004). With these gaps being the motivation for this study, this study seeks to estimate the exposure and risk assessment of aflatoxin through the consumption of kenkey. Observations from this study could be a landmark for food regulators in Ghana in for monitoring and regulating aflatoxin in foods for the general public.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Study area

The study area was Kwame Nkrumah University of Science and Technology (KNUST), in the Kumasi metropolis, Ashanti Region. It is surrounded by communities such as Bomso, Kotei, Ayigya, Deduako and Ayeduase.

3.1.2 Survey

Skilled field assistants were employed and trained to help in the collection of the data and the administration of the questionnaires. These include how to serve the respondent with the questionnaire and the human relationship. English language was used throughout as means of directive during the administration of the questionnaire since the KNUST students were the target respondents. The 300 respondents were randomly selected with an absolute willingness to provide the information needed to complete the survey.

3.1.3 Questionnaire and food consumption data framework

A food dietary questionnaire (Appendix 1) was used to collect dietary history from the students. The structure of questionnaire was made to provide biodata of respondents; weight, age and religion, weight of kenkey eaten at a sitting: the quantity of kenkey consumed at a sitting, the number of times that they consume kenkey in a week and the number of years that they have been consuming kenkey. The amount of kenkey bought was also included in the questionnaire.

The data from the completed questionnaires were captured on Microsoft excel spreadsheet 2016 version and processed.

3.2 Methods

3.2.1 Sampling of foods

Food samples from the KNUST hall of residents, Bomso, Kotei, Ayigya, Deduako and Ayeduase in the Kumasi Metropolis were bought from the food joints for the analysis. A total of 100 food samples were bought. The food samples were bought during the time of day where the food was ready to be sold to students.

3.2.2 Food groups sampling and sample preparation

The most consumed foods were sampled randomly. Quantified amount of water was homogenized quantitatively with the sampled foods in a Crompton blender (cq Sierra 500, India) and Ziploc bags used to bag them and stored further analysis at -2°C.

3.2.3 Extraction and clean-up

Aflatoxin extraction from the kenkey was done using the method described by Sirhan *et al.* (2014). Samples were milled and homogenized in Preethi mixer grinder (mg 139, India). In the extraction process, an amount of 2 g of homogenized kenkey was weighed was into a 15 mL centrifuge tube, 5 mL of distilled was added and the tube vortex for 1 min. The solution was allowed to stand for 5 min. A volume of 5 mL 1% (v/v) acetic acid in acetronitrile solution was added. The resultant mixture was vortexed using Genie vortex (si-p238, USA) for 3 min. 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 5 min 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.

3.2.3 HPLC analysis

HPLC determination was done based on AOAC Official Method 2005.08 (AOAC, 2005) A Cecil-Adept Binary Pump HPLC (ce 4100, UK) coupled with Shimadzu 10AxL 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 1 mL/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 acetronitrile. Aflatoxins in samples were detected by using the retentions of the standard solution run and quantification done using the calibration curves of each respective toxin. Limit of Detection and Limit of Quantification of total aflatoxin were established on 0.5 ng/g and 1 ng/g respectively.

3.2.4 Aflatoxin calculation

$$Aflatoxin\ \left(\frac{ng}{g}\right) = A\left(\frac{t}{l}\right)x\left(\frac{1}{W}\right) \qquad (1)$$

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.

3.2.5 Analysis of data and Risk characterization

The datasets for the variables in Equation 2; contact rate (CR), weight (BW), exposure frequency (EF) and exposure duration (ED), were entered into Microsoft excel. They were initially fitted to their distributions based on the Akaike information criterion (AIC). The variables were then

integrated in Equation 2 to calculate the chronic daily intake (CDI) using an averaging time (AT) of 70 years since AFB1 is a carcinogen, and iterating at 100,000.

$$CDI = \frac{C_L \times C_R \times EF \times ED}{B_W \times AT}. \quad (2)$$

The margin of exposure (MOE), which is the ratio of the bench mark dose lower limit ($BMDL_{10}$) to the estimated exposure of aflatoxin was similarly estimated using Equation 3. A benchmark dose lower limit ($BMDL_{10}$) of 870 ng/kg bw/day reported by Cano-Sancho *et al.* (2013) was used for aflatoxins B1, B2, G1, G2..

$$MOE = \frac{BMDL_{10}}{CDI}. \quad (3)$$

The hazard quotient (HQ) which is expressed as the ratio of the chronic human exposures to the reference dose of aflatoxin. The HQ was (non-carcinogens, AFG1, AFG2 and AFB2) estimated similarly based on Equation 4 using a reference dose (R_fD) of 5 ng/g (Kimanya *et al.*, 2014).

$$HQ = \frac{CDI}{RfD} \quad (4)$$

The lifetime risk of cancer (R) (determined for AFB1) which is the product of the potency factor (PF) and human exposures of hazards (CDI) was estimated using Equation 5 based on a potency factor of 0.013 $(ng/g\ day)^{-1}$ recommended by JECFA (1998).

$$R = CDI \times PF. \quad (5)$$

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Occurrence, consumption and exposure assessment

All the foods analyzed were found to contain aflatoxin (Table 1). Similar results reported by Andrade and Caldas (2015) who recorded 54.2% of the maize samples analyzed as contaminated with aflatoxin. The variation could be due to poor post-harvest handling of raw materials sourced to prepare to kenkey in this study.

Table 1: Statistical distributions of aflatoxin exposure in students

Hazard (ng/g)	Statistical distribution	Central tendencies metrics					Percentiles	
		Min	Max	Mean	Mode	Median	5th	95th
G2	Expon (0.32595, -0.0032595)	0.00	9.96	0.32	0.00	0.22	0.02	0.97
G1	Expon (1.7283, (-0.017283)	0.00	27.86	1.73	0.00	1.18	0.07	5.16
B2	Expon (2.4690, (-0.024690)	0.00	14.21	2.47	0.00	1.69	0.10	7.37
B1	InvGauss (14.848,0.62200, -0.18480)	0.00	124.08	14.66	0.02	3.02	0.00	56.81
Mass of food (g)	NegBin (40,0.10060)	240	510	358	410	330	265	461
EF (days)	IntUniform (30,356)	30	356	218	300	230	46	340
ED (years)	Binomial (6,0.38611)	1	2	2.32	2	2	1	4
BW (kg)	NegBin (43,0.37221)	45	116	72.53	56	71	54	90

4.3 Risk Characterization

4.3.1 Margin of Exposure

The observations from this study show that the mean (729), mode, median and 5th percentile MOE values for the carcinogen group was below the threshold limit (10,000) established by the European Food Safety Authority (Table 2) which presents a health concern. However, the 95th percentile value was above the threshold limit. It was again observed that the mean (53,694), median (56,538)

and 95th percentile (141,294) MOE values for the non-carcinogen group also recorded values greater than the threshold limit. This implies a safe health concern. On the other hand the mode (1638) and 5th percentile (908) values were below the threshold limit indicating a health concern for the 5th percentile consumers. Similar results (carcinogen: mean (850) and 95th percentile (90,000) non-carcinogen: mean 11,000 and 95th percentile (150,000) trend were reported by Assunção *et al.* (2018). The carcinogen MOE values obtain from this study was less than results reported by Assunção *et al.* (2018) and threshold limit. The CDI value could be attributed to the difference though same benchmark dose limit ($BMDL_{10}$) of 870 ng/kg bw/day was used in both studies. The mean MOE values for the non-carcinogen ranged between 7629 and 1, 711,540 (Table 2). The carcinogen group AFB1 recorded the least MOE value which ascertains AFB1 as the most potent carcinogen among the group.

Table 2: Margin of Exposure for both Carcinogen and Non-carcinogen group

Hazard	Central tendencies			Percentiles	
	Mean	Mode	Median	5th	95th
		Non-carcinogen			
AFG1	7,629	2,050	10,789	1,281	196,376
AFG2	1,711,540	8,796	56,538	6,893	1,053,111
AFB2	53,694	1,638	7,476	908	141,295
		Carcinogen			
AFB1	792	21.18	7,766.15	0	264,625

Lower MOE value shows a higher probability for aflatoxin concentration to get to or exceed toxicity levels. Observations from this study reveals that students generally presented higher risk of cancer and could be as result of high chronic exposure. Similar studies by Assunção *et al.*

(2018) reported the likely health trepidation may possibly result from the consumption of cereals, especially for high consumers with AFB1 as the focal contributor (87.3%) for the risk (MoE < 10,000).

According to Wild and Turner (2002), in developing or low-income countries, people living in the rural communities mostly consume or have greater levels of aflatoxin exposure compared to the urban settlers since the urban settlers have many options of consuming more varied foods than the rural inhabitants who have quite a limited option and may perhaps have diet that is well regulated for contaminants. Tajkarimi *et al*. (2007) added that a robust cyclical variation exists in the exposure of aflatoxin which relates with the availability of food. But observations from this study show that the respondents are urban dwellers with high cost of living are exposed just as those in the rural areas though the degree may vary (Kilonzo *et al.*, 2014).

4.3.2 Hazard Quotient

Again, observations from the study indicated that the mean, mode, median 5th and 95th percentiles HQ values for the non-carcinogen groups of aflatoxin were way less than the tolerable limit (1) as established by the European Food Safety Authority. (Table 3), which on the contrary demonstrating no health concern.

Table 3. Hazard Quotient for Non-carcinogen group

Hazard	Central tendencies			Percentiles	
	Mean	Mode	Median	5th	95th
		Non-carcinogen			
AFG1	0.03	0.00	0.01	0.00	0.12
AFG2	0.01	0.00	0.03	0.00	0.02
AFB2	0.04	0.00	0.02	0.00	0.12

Comparing this observation to the study by Assunção *et al.* (2018) similar results (0.01-0.04) trend was obtained because a reference dose of 5 ng/g (established by European Food Safety Authority) was used in both studies.

4.3.3 Lifetime Risk

The lifetime human cancer risk observed in this study as a result of dietary exposure of AFB1 (tumorigenic) was 0.10 which is greater than estimated by EFSA estimated ranges of 0.004– 0.007 and 0.002–0.009 cancer/year per 100,000 habitants. The concentration of hazard, exposure frequency, exposure duration, body weight, mass of food, and averaging time are the key components or elements needed to estimate a hazard. Also risk values from this study were greater than computed estimates in Japan (Sugita-Konishi *et al.*, 2010). The disparity could be due to the concentration level of aflatoxin detected in the food sampled. The rate of implication is proportional to the risk value. Also, it was observed that cancer risk is directly proportional to exposure frequency, exposure duration and aflatoxin concentration (topmost contributor). Mass of food and body weight had a minimal impact on cancer risk (Kleter and Marvin, 2009). From Table 2, aflatoxin concentration and exposure frequency are the highest contributors on the cancer risk of the students' means that students have a greater chance of developing cancer if they are habitually exposed to aflatoxin through food.

Table 4: Life time risk for carcinogen group

Hazard	**Central tendencies**			**Percentiles**	
	Mean	Mode	Median	5th	95th
		Carcinogen			
AFB1	0.10	0.10	0.00	0	0.371

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

Aflatoxin was detected in all the kenkey that students consume. The students' chronic dietary exposures to aflatoxin were greater than the tolerable limit (5ng/kg) which consequently put them at risk to cancer and other related health issues that aflatoxin presents. The Hazard Quotient for non-carcinogen group of aflatoxin were below the tolerable limit (1), implying no health concern. The mean, median and 95th percentile consumers margin of exposure were above the threshold (10000) for non-carcinogen group of aflatoxins. There was a health concern of students as the MOE for the carcinogen group was lower than the threshold limit. Students also are at risk of cancer as the risk value was above the estimated range of values.

Government agencies and food manufacturers need to make policies that will help decrease aflatoxin contamination. Thus, programs and actions targeted at lessening in severity of the aflatoxin exposure should be seriously put in place in order to save the lives of students on KNUST campus that are at risk.

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APPENDIX

APPENDIX 1: FOOD DIETARY QUESTIONNAIRES

DIETARY QUESTIONNAIRE

QUESTIONS ON CEREAL PRODUCTS YOU USUALLY EAT

INSTRUCTIONS:

This questionnaire is about your cereal products eating habits over the past 2 months. Give only 1 answer for each question.

BIODATA								
Gender		**Occupation**		**Age** (Yrs.)		**Weight** (Kg)		
M	◯	Student	◯	0	0	0	0	0
F	◯			1	1	1	1	1
				2	2	2	2	2
				3	3	3	3	3
				4	4	4	4	4
				5	5	5	5	5
				6	6	6	6	6
				7	7	7	7	7
				9	9	8	8	8
						9	9	9

A. BREAKFAST

1. What type of food do you usually eat for breakfast?

   ○ a) I don't eat breakfast

   ○ b) Tom brown and bread

   ○ c) Porridge and bread/ koose

   ○ d) Banku and fish/meat

   ○ e) Kenkey and fish

2. How much do you usually eat (GH¢)?

   ○ a) 1.00

   ○ b) 1.50

   ○ c) 2.00

   ○ d) 2.50

   ○ e) 3.00

   ○ f) 3.50

   ○ g) 4.00

   ○ h) 4.50

   ○ i) 5.00

3. How many times do you consume this breakfast in a week?

- ○ a) Once
- ○ b) Twice
- ○ c) Thrice
- ○ d) Four times
- ○ e) Everyday

4. For how long (years) have you been eating this food?

○	○	a. 0	0						
○	○	b. 1	1						
○	○	c. 2	2						
○	○	d. 3	3						
○	○	e. 4	4						
○	○	f. 5	5						
○	○	g. 6	6						
○	○	h. 7	7	l.	8	8	j.	9	9
○	○								
○	○								
○	○								

B. LUNCH

1. What type of food do you usually eat for lunch?

- ○ a) I don't eat lunch
- ○ b) Rice and fish/meat
- ○ c) Kenkey and fish
- ○ d) Abitie and fish/meat
- ○ e) Banku and fish/meat
- ○ f) Fufu and fish/meat
- ○ g) Pito

2.0 How much do you usually eat (GH¢)?

- ○ a) 1.00
- ○ b) 1.50
- ○ c) 2.00
- ○ d) 2.50
- ○ e) 3.00
- ○ f) 3.50
- ○ g) 4.00
- ○ h) 4.50
- ○ i) 5.00

3. How many times do you consume this lunch in a week?

○ f) Once

○ g) Twice

○ h) Thrice

○ i) Four times

j) ○ Everyday

4. For how long (years) have you been eating this food?

○ 0 ○ a) 0

○ 1 ○ b) 1

○ 2 ○ c) 2

○ 3 ○ d) 3

○ 4 ○ e) 4

○ 5 ○ f) 5

○ 6 ○ g) 6

○ 7 ○ h) 7

○ 8 ○ i) 8

○ ○ j) 9 9

C. SUPPER

1. What type of food do you usually eat for supper?

○ h) I don't eat lunch

○ i) Rice and fish/meat

○ j) Kenkey and fish

○ k) Abitie and fish/meat

○ l) Banku and fish/meat

○ m) Fufu and fish/meat

○

n) Pito

2. How much do you usually eat (GH¢)?

- ○ 1. 1.00
- ○ 2. 1.50
- ○ 3. 2.00
- ○ 4. 2.50
- ○ 5. 3.00
- ○ 6. 3.50
- ○ 7. 4.00
- ○ 8. 4.50
- ○ 9. 5.00

3. How many times do you consume this supper in a week?

- ○ k) Once
- ○ l) Twice
- ○ m) Thrice
- ○ n) Four times
- ○ o) Everyday

4. For how long (years) have you been eating this food?

2. 0
3. 1
4. 2
5. 3

○ 0	○	6.	4
○ 1	○	7.	5
○ 2	○	8.	6
○ 3	○	9.	7
○ 4	○	10.	8
○ 5	○	11.	9 9
○ 6	○		
○ 7	○		
○ 8	○		
○	○