SULEIMAN ABDULRAHMAN HARUNA
INCIDENCE OF AFLATOXINS IN "*Garri*" AND "*Egusi*" MELON SEEDS CONSUMED IN TURKISH REPUBLIC OF NORTHERN CYPRUS
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A THESIS SUBMITTED TO THE GRADUATE SCHOOL OF APPLIED SCIENCES OF NEAR EAST UNIVERSITY

By
SULEIMAN ABDULRAHMAN HARUNA

In Partial Fulfillment of the Requirements for the Degree of Master of Science in Food Engineering

NICOSIA, 2017

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I hereby declare that, all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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To my uncle Alhaji Ubale Haruna…

ABSTRACT

Tremendous amounts of food are destroyed each year due to harmful organisms or fungal metabolic products of *Aspergillus flavus* and *Aspergillus parasiticus*. This study is aimed at investigating the incidence of aflatoxins in "*garri*" and "*egusi*" melon seeds consumed in Turkish Republic of Northern Cyprus (TRNC). Fifteen (15) samples (both "*garri*" and "*egusi*") collected from the cities of Nicosia, Famagusta and Kyrenia were analyzed after immunoaffinity column clean-up using HPLC coupled fluorescence detection and post-column derivatization system.

Two "*egusi*" samples out of the fifteen (15) samples from Nicosia markets were containing AFB1 (0.707 µg/kg and 0.743 µg/kg, respectively) at a concentration below the maximum tolerable limit of 2 µg/kg set by EU. Aflatoxins recoveries were seen in the range of 70 – 120%. For "*egusi*", the overall recovery, SD and RSD values were at 94%, 4.8% and 5.1%. While, for "*garri*" the overall recovery, SD and RSD values were seen at 89%, 4.3% and 4.8%, respectively. Overall recovery, individual recoveries and RSD were within the identified acceptance guideline $70\% \leq Q \leq 120\%$ and $RSD \leq 20\%$).

Overall results indicated the suitability of the consumption of "*garri*" and "*egusi*" in TRNC. However, regular control checks should be carried out on the other mycotoxins like ochratoxin and fumonisins which may be present.

Keywords: Aflatoxin; "*egusi*"; "*garri*"; HPLC; TRNC

ÖZET

Her yıl önemli miktarda ürün zararlı mikroorganizmalar veya *Aspergillus flavus* ve *Aspergillus parasiticus* gibi küflerin metabolik ürünleri nedeniyle kayba uğramaktadır. Bu çalışmada Kuzey Kıbrıs Türk Cumhuriyeti (KKTC)'nde tüketilen bazı tipik Afrika gıdalarından "*garri*" ve "*egusi*" deki aflatoksin varlığı araştırılmıştır. Bu amaçla Lefkoşa, Girne ve Magusa'daki marketlerden toplam 15 adet "*garri*" ve "*egusi*" numunesi toplanmış; immunoaffinite kolon ve HPLC kullanılarak aflatoksin içerikleri analiz edilmiştir.

15 örnek içinde sadece Lefkoşa'ya ait örneklerin iki tanesinde 0.707 µg/kg ve 0.743 µg/kg düzeyinde aflatoksin B1 bulunmuştur, ancak bu değerler Avrupa Birliğinin maksimum izin verilen sınırı olan 2 µg/kg'dan düşüktür.

"*Garri*" ve "*egusi*" için aflatoksin geri kazanımlarının % 70-120 arasında olduğu saptanmıştır. Egusi için geri kazanım ortalaması (Q), standart sapma (SD) ve bağıl standart sapma (RSD) değerleri sırasıyla % 94, 4.8 ve % 5.1; garri için ise % 89, 4.3 and % 4.8 olmuştur. Her iki ürün için de geri kazanım ortalaması (Q) ve bağıl standart sapma (RSD) değerleri kabul edilebilir sınırlar (% 70 ≤ Q ≤ % 120 ve RSD ≤ % 20) içindedir.

Araştırma sonuçları, Kuzey Kıbrıs Türk Cumhuriyeti (KKTC)'nde tüketilen "*garri*" ve "*egusi*" nin tüketime uygun olduğunu göstermektedir. Bununla beraber bu gıdalardaki aflatoksin ve okratoksin, fumonisin gibi diğer mikotoksin içeriklerinin de düzenli olarak kontrol edilmesi önerilmektedir.

Anahtar Kelimeler: Aflatoksin; "*egusi*"; "*garri*"; HPLC; KKTC

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LIST OF ABBREVIATIONS AND SYMBOLS

AFs:	Aflatoxins
ALARA:	As-Low-As-Reasonably-Achievable
AOAC:	Association of Official Analytical Chemists'
ASE:	Accelerated solvent extraction
CAST:	Council for Agricultural Science and Technology
CE:	Capillary electrophoresis
CIT:	Citrinin
CNP:	Cyanogenic potential
EC:	European Commission
EFSA:	European Food Safety Authority
ELISA:	Enzyme-linked immunosorbent assay
EU:	European Union
FAO:	Food and Agriculture Organization
FAOSTAT:	Food and Agriculture Organization Corporate Statistical Database
FDA:	Food and Drug Administration
HCN:	Hydrogen cyanide
HNO_3:	Nitric acid
HPLC:	High-performance liquid chromatography
IAC:	Immunoaffinity column
IARC:	International agency for research on cancer
ISO:	International standard organization
KCl:	Potassium bromide

LC:	Liquid chromatography
LFIA:	Lateral-flow immunoassay
LLE:	Liquid-liquid extraction
LOD:	Limit of Detection
LOQ:	Limit of Quantitation
LSE:	Liquid-solid extraction
MFC:	Multifunctional column
MSPD:	Matrix solid-phase dispersion
OTA:	Ochratoxin A
PBPB:	Pyridinium Hydrobromide Perbromide
PBS:	Phosphate-buffered solution
PCD:	Post-Column Derivatization
PPB:	Parts per billion
QuEChERS:	Quick, easy, cheap, effective, rugged and safe
RASFF:	Rapid Alert System for Food and Feed
RPM:	Revolutions per minute
RSD:	Relative Standard Deviation
SD:	Standard Deviation
SFE:	Supercritical fluid extraction
SPE:	Solid-phase extraction
SPME:	Solid-phase micro extraction
TLC:	Thin-layer chromatography
TRNC:	Turkish Republic of Northern Cyprus

USA:	United State of America
USDA:	United State Department of Agriculture
UV:	Ultraviolet
WHO:	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background

Tremendous amounts of food are wasted each year since they are attacked by harmful organisms or defiled by fungal metabolic products. Such waste happens most conspicuously in more sweltering nations where nourishment deficiencies may as of now be an issue (Leslie et al., 2008).

Mycotoxins have afflicted humanity for a large number of years bringing on death, pipedream and hopelessness. Since the 1960s with the distinguishing proof of aflatoxin the toll extracted by these mixes from human populaces in developed nations has consistently diminished with progressively powerful government directions and routine checking of the food supply decreasing perceived issues by requests of magnitude. The truth in less created nations couldn't be in starker difference. Food uncertainty, few if any implemented controls, and harsh situations that support fungal development and toxin evolution join to make perpetual mycotoxin defilement and its related medical issues another cruel fact certainty in an effectively troublesome life. The absence of valuation for these issues among strategy makers and the general people restrains the general population interest for a logical answer, and for the most part as a non-tariff exchange hindrance (Leslie et al., 2008).

Council for Agricultural Science and Technology (2003) defined mycotoxins "as toxic secondary metabolites that for the most part have a place with one of three genera – *Aspergillus*, *Penicillium* or *Fusarium*. Mycotoxins might be adverse to the strength of people and/or and may be produced on an extensive variety of agricultural products under a different scope of conditions. A portion of mycotoxins, for example, aflatoxins are among the stronger mutagenic and cancer-causing substances known." Mycotoxins are connected with numerous interminable well-being dangers, including the induction of cancer, immune suppression, and stomach related, blood and nerve absconds (CAST, 2003; Shephard, 2006).

Due to high demand and consumption of "*egusi*" and "*garri*" by the people especially Africans, the incessant epidemics of toxins-causing cancer and liver related diseases to mention but a few. The increasing number of people (Africans to be precised) trooping into Turkish Republic of Northern Cyprus either for studies or as tourists coupled with TRNC as one of the tourists nation. There is need to examine the occurrence of aflatoxins level in "*egusi*" and "*garri*" consumed in TRNC.

The likely dangers of aflatoxins to the wellbeing of human has received monitoring programs for toxin existing in different commodities and thereby leading to the imposition of regulatory measures by almost all countries. Frequent report have been made on aflatoxins infestation in foods above the described amount as contained in the regulatory safety levels; the foods in question are commodities believed to be from tropical regions. Most of the producers of "*egusi*" and "*garri*" are countries of the tropics and fungal development specifically *Aspergillus species* is extremely rampant in areas of the tropics (Bankole et al., 2006; Junior and Campos, 2004).

The aim of this research is to examine the occurrence (if any) and quantify the level of aflatoxins in "*egusi*" and "*garri*" consumed in TRNC, with the following specific objectives:

i. To detect the presence of aflatoxins in the samples of "*egusi*" and "*garri*" consumed in TRNC.
ii. To determine the quantity of aflatoxins in "*egusi*" and "*garri*"
iii. To ascertain the possible hazards of aflatoxins (based on levels approved by safety agencies) in "*egusi*" and "*garri*".

The study is restricted to Turkish Republic of Northern Cyprus (TRNC). Areas of sample collection are: Nicosia (Lefkoşa), Kyrenia (Girne) and Famagusta (Mağusa).

1.2 Aflatoxins

Aflatoxins are cancer-causing mycotoxins generated by some species of *Aspergillus* in a variety of agricultural products, basically by *Aspergillus flavus* and *Aspergillus parasiticus* in peanuts and maize. The emergence of aflatoxin B1 was first observed in 1960 in peanut consignment from Brazil to United Kingdom. Thereafter, there is epidemics of dreadful hepatitis in humans and animals, causing cancer of liver in both animals and humans

specifically in conjunction with virus responsible for hepatitis B in Southeast Asia and sub-Saharan Africa are all associated to aflatoxin B1 (Turner et al., 2002). Aflatoxin B1 has been described as carcinogen Group 1 in humans by International Agency for Research on Cancer (IARC, 1993). In almost most countries worldwide, there is regulation of aflatoxins levels tolerated maximally and is within the range of 4-20 ng/g (Food and Agricultural Organization, 2004).

Aflatoxins are a class of closely member of heterocyclic compounds generated specifically by two types of filamentous fungi, *A. flavus* and *A. parasiticus*. Studies conducted recently, revealed that strains of *A. tamarii* and *A. nominus* are capable of causing aflatoxins, because the phenotype arrangement of *A. nominus* is almost the same to that of *A. flavus* (Kurtzman et al., 1987; Goto et al., 1996). Another strain, *A. pseudotamarii* was isolated and ascertained to produce aflatoxin (Ito et al., 2001). They belong to the family *Aspergillaceae*, class Hyphomycetes and subdivision Deuteromycotina (Beuchat, 1987). They infest agricultural products and a number of foods. Under normal and different environmental conditions, species of *Aspergillus* have the capability to grow on diversified substrates. The process of production, processing, transporting and storing is where food become susceptible to fungi-causing aflatoxins (Palmgren and Hayes, 1987).

1.2.1 Classification of aflatoxins

Aflatoxins are naturally prevailing mycotoxin developed by species of fungi: *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nominus*, with *A. flavus* regarded as most common among them and with soil and rotting vegetable as their principal place of occurrence. Four major groups of aflatoxins are in existence (B1, B2, G1 and G2), with minor groups as an addition (M1 and M2 normally related with milk). Considering the extreme carcinogenic potency, aflatoxin B1 is the most recurrent class of the family of mycotoxins. Aflatoxin limits for B1 and the summation of B1, B2, G1, and G2 in foods and feeds have been regulated in 2003 by all nations with mycotoxin regulations (FAO, 2004). Therefore, it's very rare that products will be contaminated with aflatoxins B2, G1 and G2 and not aflatoxin B1 (Yabe and Nakajima, 2004), aflatoxins B2, G1 and G2 summation is drastically less than aflatoxin B1. Pitt (2000) reported that 'B' and 'G' indicates blue and green fluorescent colours

exhibited under ultraviolet (UV) light by these toxins during plate visualisation of thin layer chromatography. Numbers 1 and 2 are subscript numbers which show major and minor compounds.

1.2.2 Occurrence and biosynthesis

Aflatoxins are regarded as the most vital mycotoxins due to their existence, toxicological impacts and effect on human prosperity and trade exchange (Gnonlonfin et al., 2013). A variety of soil occupying *Aspergillus* strains are aflatoxin producers. These incorporate *A. flavus, A. parasiticus, A. nomius* (Wilson et al., 2002), *A. pseudotamarii* (Ito et al., 2001), and *A. bombycis* (Peterson et al., 2001).

Aflatoxins found in food are categorised as B1, B2, G1, and G2. “B” and “G” allude to the blue and green fluorescent colours delivered by these toxins under UV light during the thin layer chromatography plate perception; the subscript numbers 1 and 2 show major and minor mixes, individually. At the point when aflatoxins B1 and B2 are ingested by lactating dairy animals, an extent (around 1.5%) is hydroxylated and discharged in the milk as aflatoxins M1 and M2. In view of their high harmful effects, low levels of aflatoxins have been set and controlled in foods and feeds by numerous nations. Aflatoxin M1 has been recognized in human breast milk from Victoria, Australia and Thailand and also in crude milk from dairy animals and water wild oxen in Iran at large amounts (Rahimi et al., 2010; Lanyasunya et al., 2005; Pitt, 2000).

Two *Aspergillus* species, *A. flavus* and *A. pseudotamarii*, result in B aflatoxins. They can't synthesize G aflatoxins because of cancellation (0.8-to 1.5-kb) in the aflatoxin biosynthesis 28-gene group (Ehrlich et al., 2004). The other aflatoxigenic species including *A. nomius*, *A. parasiticus* and *A. bombycis* deliver each of the four aflatoxins (El-Nezami et al., 1995).

Since the disclosure of aflatoxins, *A. flavus* has turned into the most generally announced food-borne microorganism. This mirrors its significance in human health services and economy (Pitt, 2000). *A. flavus* is a ubiquitous and morphologically complex species including two classes in light of its sclerotia measure: L strains (Group I) with sclerotia >400 mm in width and S strains (Group II) with sclerotia <400 mm in breadth (Cotty, 1989). Both *A. flavus* strains produce aflatoxins B1 and B2, however *A. flavus* S strains can also produce aflatoxins G1 and G2. S strains are geologically distributed worldwide however uncommon in the United

States (Tran-Dinh, et al., 1999). The sexual phase of *A. flavus* has been distinguished as Petromyces where ascospores are found to evolve inside sclerotia (Horn, et al., 2009a). *A. parasiticus* is an essential plant pathogen and yields B and G aflatoxins. Despite the fact that its sexual stage also being a member of *Petromyces* sp., its host specificity is for the most part restricted to ground crops though *A. flavus* taints an extensive variety of plant hosts (Horn, et al., 2009b). Few varieties having a place with segment Ochraceorosei including *A. ochraceoroseus* and *A. rambellii* have been described to develop aflatoxin (Cary et al., 2005 and 2009). Also, a few other Aspergilli deliver aflatoxin precursors, for example, sterigmatocystin and o-methyl sterigmatocystin, which have comparative natural properties to aflatoxin (Brown et al., 1996).

Aflatoxins are thermo-stable. So they may pollute the dairy items and fermented food in spite of purification and sanitization. It has been ascertained not long ago that aflatoxin M1 (hydroxylated metabolites of aflatoxin B1) infestation in milk is a potential hazard for animal and human wellbeing (Prandini et al., 2009). The prevalence of aflatoxin M1 in crude milk relies on upon the climatic conditions. Milk tainting by this mycotoxin was remarkably influenced in dry periods. However, Picinin et al. (2013) in their study find that fresh milk tests contained aflatoxin M1 since the food fed to cattle was most likely debased with the toxin. This pollution happened especially in the dry time frame with <8.0 mm precipitation and low temperatures. Under these climatic conditions, as the cows are normally kept in internment, there is a requirement for supplementary feedstuff. It gives the idea that the extra encourage was contaminated. Be that as it may, in the windy period, when the animals are typically allowed to graze on vegetation, the danger of this contamination is reduced.

A survey conducted recently recommends that specific food and food constituents in African nations are exceedingly tractable to pollution by a few mycotoxins. Among these, maize is the primary wellspring of fumonisin, deoxynivalenol and zearalenone while groundnuts are the fundamental root of contamination by aflatoxin and ochratoxin A. Aflatoxin (principally aflatoxin B1) applies harmful impacts on people, represent the significant danger as a potential hazard element for some human sicknesses in Cameroon. This danger is probably going to be more significant if aflatoxins exist together with different mycotoxins (Abia et al., 2013).

Aflatoxin biosynthetic pathway begins with the location of the toxins formations. On the other hand, at a sub-atomic level, the fundamental biochemical strides and the resulting hereditary

constituents of aflatoxin biosynthesis have been lit up only in the most recent decade (Bhatnagar et al., 2002; Yu, 2003). In a nutshell, the aflatoxin biosynthesis genes of *A. parasiticus* and *A. flavus* are remarkably homologous and the formation of the genes inside the bunch being the same (Yu et al., 1995). Minto and Townsend (1997) established that aflatoxins are integrated in two stages from malonyl CoA, previous with the arrangement of hexanoyl CoA, and formerly with the development of a decaketide anthraquinone. For exchange of acetyl coenzyme A (acetyl CoA) to its definitive items, that is, AFB1, AFB2, AFG1, and AFG2, no less than 18 enzymes processes are needed (Yabe & Nakajima, 2004; Roze et al., 2007). Various genes encoding the catalysts and the interpretation variables have been cloned and described.

1.2.3 Factors affecting biosynthesis

The contamination of food with *Aspergillus*, and its resultant aflatoxins are connected with warm and dry atmospheres (Hell et al., 2003). A few numerical models on climatic danger of *Aspergillus* species developing plus there *in situ* generation of aflatoxins have been distributed (Chauhan et al., 2008; Pitt, 1993). In any case, it has additionally been accounted for that aflatoxin pollution may significantly vary year by year at a similar area because of variable ecological conditions in various growing seasons and also irregular management exercises (Hell et al., 2003). For examples, growing yields sequentially in a similar field builds the danger of contamination by toxin year by year (Hennigen et al., 2000).

The impact of temperature on the development of *A. flavus* and *A. parasiticus*, and their aflatoxin generation has been examined in various commodities utilizing artificial media. In one review, the ideal temperature for aflatoxin production by *A. flavus* was characterized at 25ºC on ground nuts, while the ideal temperature for *A. parasiticus* generation of aflatoxin was 25-30 ºC. Likewise, this review demonstrated an adjustment in the extents of aflatoxin B1 and G1 produced by *A. parasiticus*, with a reduction in aflatoxin G1 as temperatures expanded (Diener and Davis, 1967). Molina and Giannuzzi (2002) with utilizing research facility media and numerical modelling found that ideal temperatures for aflatoxin generation by *A. parasiticus* were 27.8ºC and 27.3ºC at pH 5.9 and 5.5, individually. The ideal temperature for aflatoxin production by *A. bombycis* and *A. nomius* was 25ºC (Peterson et al., 2001).

The ideal water activity (a_w) for development of *A. flavus* is demonstrated as 0.996, with a low supporting growth at 0.80-0.82. At higher water exercises (0.98-0.99), aflatoxins are produced in more quantity but toxin generation stops at or close a_W 0.85 (Gqaleni et al., 1997; Northolt et al., 1977). It is additionally announced that over 70% of high moisture grains (>18%) are contaminated with *A. flavus* with a positive relationship between the rate of contamination and aflatoxin improvement. toxin infestation is specifically corresponded with the moisture constituent of yields (Mora and Lacey, 1997). In line with the research conducted on restorative plants, no aflatoxin was distinguished with water movement underneath 0.81 and temperatures of 25 ± 2ºC, 30 ± 2ºC and 40 ± 2ºC. Comparative perception was made when the water action was more than 0.81 and temperature lower than 10 ± 2ºC (Kulshrestha et al., 2008).

Ideal states of temperature and water activity are critical for mycotoxigenic fungi. Moreover, the nations with cool or mild atmospheres may turn out to be more at risk to aflatoxins when the temperature increases. An illustration is Italy in the recent years (FAO, 2000). Moreover, tropical nations may turn out to be excessively unfriendly for fungal growth and mycotoxin generation. Countries which stand to control the storage environment might have the capacity to maintain a strategic distance from postharvest pollution however at high extra cost. The absence of concern about the connection between food safety and environmental change could be more at risk of aflatoxin infestation in Africa (Gnonlonfin et al., 2013).

The production of aflatoxin is widely regarded as an aerobic process. Production of aflatoxin by *A. flavus* cultures emerged on a groundnuts medium in oxygen-drained atmosphere is lower than under typical conditions (Diener and Davis, 1967; Dobson and Sweeney, 1998). An estimate yield of 212 mg of aflatoxin for every litre of liquid culture was delivered at an air circulation (aeration) rate of 9 L/min while a significant decrease in aflatoxin seen at lower air circulation rates (Heathcote and Hibber, 1978). Diener and Davis (1967) researched the impacts of various levels of the ordinary atmospheric gasses, carbon dioxide, oxygen and nitrogen on aflatoxin production under states of shifting temperature and humidity. It was noted that aflatoxin generation in sound develop peanut pith diminished with higher amounts of carbon dioxide from 0.03% to 100%. Decreasing the oxygen amount also reduced aflatoxin production. Other examination announced declared that a considerable reduction in mycotoxin

development came about when the oxygen was decreased from 5% to 1% irrespective of the carbon dioxide number, so that storage under low oxygen or in an improved atmosphere could be seen as one of the reason for the reduction of aflatoxin biosynthesis (Magan and Aldred, 2007).

Nutrients availability plays an important role in mould growth and determines the amount of mycotoxins to be produced. Selection of adversity of toxin infestation strains of certain species is determined by substrate. For example, cottonseed and peanuts isolates are found to contain high content of toxin-inducing strains of *A. flavus* other than from sorghum or rice (Rachaputi et al., 2002).

1.2.4 Control of aflatoxins

The crops contamination to aflatoxin is still a serious problem among countries of the world posing considerable health issues in high number of *A. flavus*-induced *Aspergillosis*. Therefore, the danger of aflatoxin infestation can be limited by embracing different preventive measures in early hour at farm level. Suitable agronomic exercises and early harvesting have significant impact on aflatoxins pollution in product at farm level (Avantaggio et al., 2002; Rachaputi et al., 2002). Quick drying protects the grains for longer timeframe from insects and moulds by limiting the water movement which is essential for their improvement (Hamilton, 2000). Other drying methods like microwave and sonic drying are much efficient and fast, but could not be applicable in some developing countries. Likewise, by the use of enhanced storage structures and improved yields (Lozovaya, 1998; Lanyasunya et al., 2005; Turner et al., 2005; Zain, 2010) lead toward security measure against aflatoxins defilement in farm crops.

The formation of mycotoxin and growth of mycotoxigenic mould are known to be retarded by certain chemical and physical agents. Extremely toxic gas like phosphine, which is applied to halt the invasion of mould and insects in cereals, has been found to be productive (range of 1000 to 2000 ppm) in obstructing the metabolism of mycotoxin and in suffocating the growth of *Aspergillus flavus* and *Aspergillus parasiticus* (Fernanda et al., 1996; Antonacci et al., 1999).

1.3 Cassava

Cassava (*Manihot esculenta Crantz*): Belongs to the class of root and tuber crops in tropical regions and considered second in utilization to cereals (Lebot, 2009; Owusu-Darko et al., 2014; Villardon et al., 2014). Cassava is a good source of food and nutrition to the most malnourished and poorest population of Africa and Asia (Scott, 2000). However, taking into consideration of production volume annually, it is among the top 10 food crops in developing countries of the world over other root and tuber crops in the tropics (Srinivas, 2009; Tavva and Nedunchezhiyan, 2012).

1.3.1 History and cultivation

Cassava was initially tamed in neo tropical marsh South America 8,000– 10,000 years back and turned into the staple yield of the Amerindians**.** From South America, cassava was transferred to Africa in the sixteenth century and has since turned into essential food as well. Despite the fact that Africa owes the instigation of cassava to the Portuguese, Asia owes it to the Spanish, who built up the main plantations in the Philippines and from where it was introduced all through tropical Asia toward the start of the nineteenth century. The cultivation of cassava was expanded by colonial administrators who similarly saw cassava as a starvation hold and trade commodity (Piperno, 2011).

The products mostly consumed from cassava by the people from Asia, South America and Africa is normally products of fermentation. Africa has a deep rooted history of producing conventional fermented foods and is maybe the regions with the richest variety of lactic acid-fermented foods. These foods have significantly influenced the level of nutrition, health and socio-economy to the lives of the continent, who are often hit by drought, famine, disease and war (Franz et al., 2014).

The germination of cassava involves a distinct range of cultivation situations (Edison et al., 2006). Some countries notably Thailand, Southern Brazil and some parts of India cultivate cassava as monocrop and also as high commercial crop in a number of countries (Edison et al., 2000; Howeler, 2000). Whereas, in some parts of the world like Asia which include India, Vietnam, Thailand and Indonesia (Edison, 2000; Amanullah et al., 2006; Howeler et al.,

2013), and most African nations (Adekunle et al., 2014), as a semi-commercial and backyard crop, intercropping and mixed cropping is mainly the cultivation systems (Moreno, 1992; Osundare, 2007).

1.3.2 Nutritional composition

The protein content of cassava is as 0.7% to 1.3% low as fresh weight (Ngiki et al., 2014). There is an approximately low protein content in cassava peels 5.5%, leaves 21% and flour 3.6% (Iyayi and Losel, 2001). Nassar and Sousa (2007) reported that the total lysine and amino acid contents of cassava are approximately 0.010 g per 100 g and 0.254 g per 100 g respectively. Cassava protein content is rich in arginine and low in threonine, cysteine, phenylalanine, isoleucine, proline and methionine (Onwueme, 1978).

The lipid content of cassava is relatively low and has been found to be only 0.1% as compared with maize which has an approximate content of 6% (Gomes et al., 2005). Cassava roots flour has an average 2.5% lipid and only half of it can be extracted using traditional solvent systems and also contains primarily saturated fatty acids (Hundson and Ogunsua, 1974). Cassava is high in vitamin C and low levels of vitamins A, B_1, B_2 and niacin have been reported (Onwueme, 1978). Cassava starch is compared with maize starch and found cassava 17% amylose and 83% amylopectin, while that of maize 28% amylose and 72% amylopectin (Gomes et al., 2005; Promthong et al., 2005).

1.3.3 Public health issues

The hazardous problem linked with cassava are two members of cyanogenic glycoside compound, linamarin and lotaustralin (methyl-linamarin) mostly present in tubers and the leaves and are regarded as edible parts. Cyanogenic potential (CNP) is present in the estimated 5000 varieties of cassava with fresh pulp content in the range of 10 to above 500 mg HCN/kg (Wilson and Dufour, 2002). On the basis of CNP in the fresh pulp, cultivars of cassava are categorised into three; Non-toxic cultivars with a concentration below 50 mg HCN/kg, moderately toxic cultivars 50–100 mg HCN/kg and above 100 mg HCN/kg as high toxic cultivars (AttahDaniel et al., 2013). One extremely toxic variety is available in Nigeria and is termed as “eat and die” (AttahDaniel et al., 2013). Cassava varieties on the basis of organoleptic attributes exhibited by cyanogenic compounds are grouped as “sweet” or “bitter”

with high non-toxic level by the sweet cultivars. This classification is ascertained by individuals with rare ability to quantify the constituents of cyanogenic compounds, but can as well feel the taste of the cassava (Wilson and Dufour, 2002).

"Sweet" cassava is certainly described and appraised fit for the consumption of human and only need essential processing (e.g., peeling and cooking). While, the cyanogens in "bitter" cassava need to be remove or decrease to physiological amount (10 mg HCN equivalent/kg dry weight) that can be tolerated (as set by FAO/WHO) and therefore requires further processing in order to achieve above and also to make it wholesome prior to consumption (Codex Alimentarius Commission, 2013).

1.3.4 "*Garri*"

"*Garri*" is a coarse product derived from peeled, washed and shredded cassava roots (Figure 1.1). The pulverised roots are kept in a container usually bag and allow a fermentation of 3-4 days or more while slowly pressed to remove the substance as juice. Fermentation plays a vital role in the detoxification and also gives taste of acidity to the final product. Sieving is done on fermented pulp in order to obtain fine squash, followed by roasting and frying in palm oil. White "*garri*" is obtained as end product after roasting of the fermented pulp (Figure 1.2), while, the yellow "*garri*" is as a result of frying (Figure 1.3). Yellow "*garri*" is 10-30% more nutritious than white "*garri*" and is due to the presence of vitamin A in palm oil (Hahn et al., 1988).

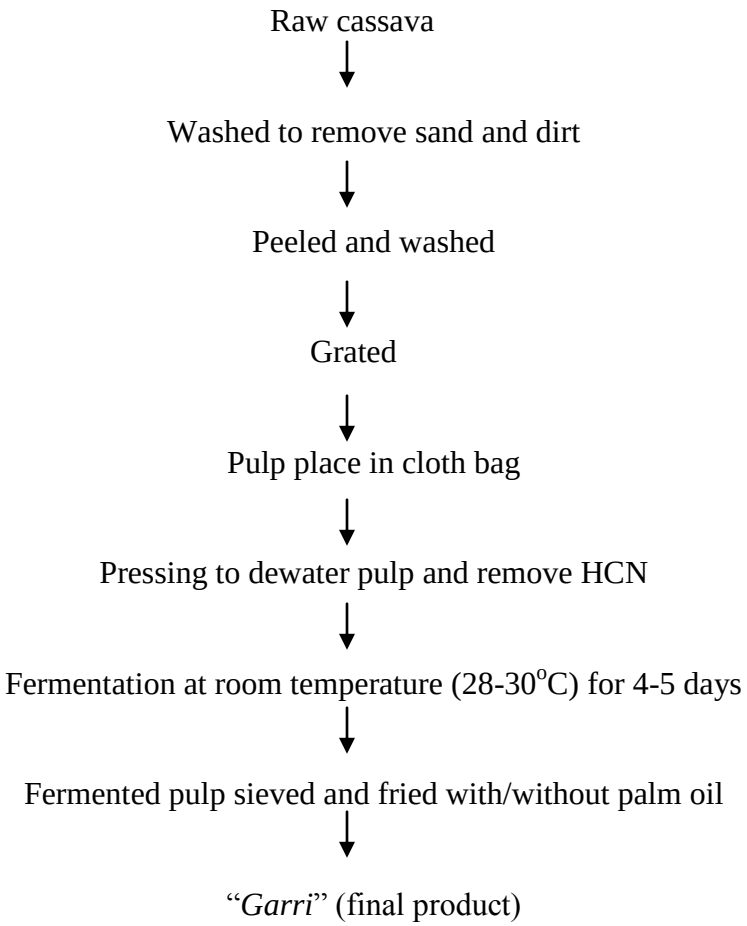

Figure 1.1: Flow chart for "*garri*" production (Uzogara et al., 1990)

The processing of "*garri*" is a traditional one usually by women though can be processed industrially, medium and small scale (Ene, 1992; Lahai, 1991). The average moisture content (mc) is 12-14% and 8-10% for traditional and industrial processing respectively (Vlavonou, 1988). Because of its organoleptic attributes, the traditional techniques of processing is widely the consumers preference coupled with the limitations in low yield, unstable utilisation conditions, inadequate efforts toward decreasing losses as well as poor hygiene (Chinsman and Fiagan, 1987). The rate at which crude cassava roots are transformed into gari (garification) is 15–20% (Hahn et al., 1988). Ejiofor and Okafor (1981) reported that the sour flavour of "*garri*" is induced by lactic acid bacteria in the bagging process.

Figure 1.2: White "*garri*"
Source: http://microbiotics.com.ng/protein-fortified-garri-improving-nutritional-quality-of-a-staple-food/

Figure 1.3: Yellow "*garri*"
Source: http://www.foodstantly.com/item/1006/Red_Garri_Bag#.WVVjZnIzrIU

1.4 "*Egusi*" Melon

"*Egusi*" melon (*Colocynthis citrullus lanatus*) is broadly cultivated crop in West Africa and some parts of Asia, a member of the family 'Curcurbitaceae' and ordinarily called *'egusi'* in Nigeria. It looks like watermelon in appearance but bears hard, small fruits with the pulp bitter in taste different from that of watermelon (Ajuru and Okoli, 2013) (Figure 1.4 and Figure 1.5). Farmers use it to stop the growth of unwanted grasses where mixed cropping system is practiced, leaves are also used to provide the soil with cover and this is because of the prowling nature and ability, and therefore plays a key role in the development of the farming system and progress of village people in West Africa (Achigan-Darko et al., 2008).

Kushwaha et al (2005) reported that the removal of pod, fermentation, washing, drying, cleaning and shelling as the major steps involves in melon processing. Pods are normally allowed to remain on the field for 3-4 days thereby making it to rot, prior to washing stage, there are rotten and soft pods, and therefore, the process of fermentation and depodding is a simultaneous one. The reason for the fermentation is that it allows easy removal of seeds from the pod (Figure 1.6).

Figure 1.4: 'Curcurbitaceae**'** melon

Figure 1.5: Shelled "*egusi*" melon

However, there is possibility of snake bites and scorpion stings which cached in the rotten pods during washing and fermentation process and renders it irritating, hard and vicious. The

level of fermentation can be attributed by the emergence of mucilage and tones of ammonia generated due to amino acids meltdown during fermentation (Omafuvbe, 1998).

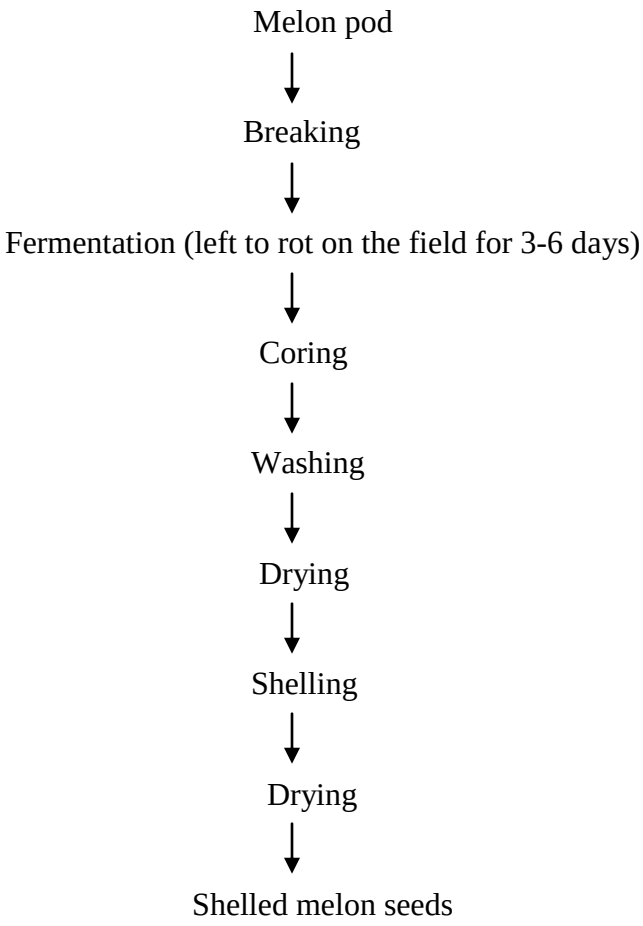

Figure 1.6: Unit operations in melon seed processing (Jackson et al., 2013)

1.4.1 Agronomy and nutritional significance

In terms of production of melon seed, Nigeria is rated high in the world, with 530 kilotonnes in 2004, resulting to 75% melon seed generation globally (FAOSTAT, 2015, as cited in Somorin et al., 2016). Northern parts of Nigeria is regarded as a thriving place for the production of the crop and is due to the availability of land for the cultivation thereby making it possible for mixed and sole cropping practices (Yusuf et al., 2008).

"*Egusi*" melon seed contains a high significant amount of some major proteins, oil and nutrients. The essential amino acids content of the proteins in the flour attributed to its goodness as a source of vegetable protein. Conventional West African diets and other source

of protein from plant are supplemented with egusi flour there by leading to bumper development in growth (Sanchez et al., 1972).

The protein quality of "*egusi*" in relation to the indices biologically was found to be below that of soybean (Oyenuga and Fetuga, 1975). Umoh and Oke (1974) found that the ratio of protein efficiency, net utilization of the protein and value existing biologically in "*egusi*" seed to be higher in comparison with majority of oilseeds. There is potential of the melon seed oil to be utilised feedstock for the production of biodiesel (Giwa et al., 2010).

1.4.2 Complications and regulations

The demand for "*egusi*" is increasingly becoming high at both local (Nigeria) and international level (Bankole, 1993). The output of the production of egusi is low, in spite of its acclaimed value and socio-economic advantage. Fragmentation of land resulting to inadequate land, exorbitant prices of inputs, application of conventional techniques and in appropriation in resources are the factors contributing to this throw out (Debertin, 1986).

In storage conditions of West Africa, fungi evolvement specifically *Aspergillus* species poses stern challenge peeved by promoting the growth of fungi in humid tropical climate (Bankole et al., 2006). These *Aspergillus* species have a tendency to multiply in different type of food and in appropriate storage conditions can emit their secondary toxic metabolites referred to as "mycotoxins" with which aflatoxins widely known as an example and most fatal. Aflatoxin B1 is regarded as most common and highly destructive amongst all the four paramount classes of aflatoxins (Aflatoxins B1, B2, G2 and G2) (International Agency for Research on Cancer, 1993).

Products of melon seed from Nigeria have been checked to be contaminated by aflatoxins and therefore, based on the fact above, border rejection principle is imposed to melon seed shipments from Nigeria to United Kingdom, Ireland and other EU countries as accentuated in the Rapid Alert System for Food and Feed (RASFF, 2012) notifications 2013.BQC; 2012.BNB; 2012.BLV; 2012.CPF; 2012.AQM; 2011.AGG among the rest, containing aflatoxin B1 level of more than 147 µg/kg.

Research conducted by Adebajo et al. (1994) revealed a significantly high amount of aflatoxins contamination in samples of melon seed believed to be from markets and farmers

stows in Nigerian. Oilseeds and products derived from it have been outlined as the major promoters of aflatoxins exposure in human and also ascertained that the sources of aflatoxin exposure should be measurable and rationally low (European Food Safety Authority, 2007). In line of the claim above, the European Commission in the year 2010, enacted a law for the limitation of aflatoxins in oilseeds which before human utilisation requires other physical treatment and sorting as 8 µg/kg for AFB1 (15 µg/kg for total aflatoxins) and for oilseeds designed for straight human consumption as 2 µg/kg for AFB1 (4 µg/kg for total aflatoxins). The enactment of codification further mandated that 50% of the total consignment of "*egusi*" and its products should be thoroughly examined before being permitted into the EU from Nigeria (European Commission, 2014).

CHAPTER 2

THEORETICAL FRAMEWORK

2.1 Toxicological Effects of Aflatoxins and Risk Assessment

Acute and chronic toxicity of aflatoxins were evidently recorded. Apart from their ability to cause cancer and acute liver problem, recent investigations revealed other strong effects, such as impaired growth in children, intestinal abnormalities, neurotoxicity, compromised immunity, nephrotoxicity, chronic fatigue syndrome, protein synthesis interference and various micronutrient that are dangerous to health. As shown by analysis in rats, the nature of small intestine to quickly absorb aflatoxin has been described as toxic potential (Ramos and Hernandez, 1996). Speijers and Speijers (2004) reported the synergistic toxicological effects of aflatoxins.

An estimate of 4.5 billion people, for the most part in developing nations, are at danger of chronically exposed to aflatoxins from infested food produces, and this brings about the need of an additional knowledge of aflatoxins health consequences (Aguilar et al., 1993). Most of the researches conducted on aflatoxins concentrated on the levels of contamination in different foods. However, in order to precisely ascertain the exposure and its contrast among number of groups (diet habits, age, geographical distribution etc), dietary analysis are therefore very essential because of the risk of aflatoxin exposure to consumers through normal diet.

In setting up maximum tolerable level of aflatoxins in foodstuffs, the As-Low-As-Reasonably-Achievable (ALARA) approach is implemented. The ALARA, simply expresses an amount of a substance which may not necessarily be obliterated from a food without attempting to repudiate that food altogether (FAO, 2004).

2.2 Regulations and Maximum Levels

General principles and requirements of food law were drawn up by European Commission in 2002, setting guidelines in areas of food safety leading to European Food Safety Authority

(EFSA). It could be seen as a steppingstone with which other significant food safety measures can be established (European Commission, 2002).

Several institutions gradually developed regulations for food and feed in relation to aflatoxins. Aflatoxin B1 maximum limits and entire aflatoxin amounts (AFB1, AFB2, AFG1 and AFG2) has been described as the criterion commonly implemented to restrict aflatoxin presence in certain number of foods (Hernandez-Hierro et al., 2008). Bennett and Klich (2003) reported that food content of aflatoxins are 5 and 10 μg/kg for AFB1 and total aflatoxins, respectively, in over 75 countries. In its establishment for aflatoxins levels in food, the European Union (EU), described 2 μg/kg and 4 μg/kg for AFB1 and total aflatoxins in cereal and their subsidiaries, and in cereal-based processed food and in food for young children and infants, the AFB1 is 0.1 μg/kg (European Commission, 2006a; Quinto et al., 2009). Espinosa-Calderon et al (2011) revealed that the U.S. Food and Drug Administration (FDA) and the United State Department of Agriculture (USDA) have entrenched aflatoxin total amounts in animal feed products to be 15-20 μg/kg. For assurance of milk safety, the aflatoxins residue limits for milk (AFM1) have been established by the EU and the FDA. The EU established 0.05 μg/kg as level in milk (EC, 2001; EC, 1998), and in food for the baby as 0.025 μg/kg (EC, 2004). According to the U.S. FDA (1996) the level admissible in milk (AFM1) is 500 ng/L. The legislations established by national and international organizations have encouraged high number of countries to set their own regulation. For example, in Brazil, 20 μg/kg is set aside as aflatoxin maximum level safe in peanuts and corn (Abbas, 2005). In Korea, for all food, the AFB1 maximum level is 10 μg/kg (Abbas, 2005).

The levels of AFB1 in dried fruits and nuts are 20 μg/kg, 5 μg/kg and 1 μg/kg for USA, Netherlands and Switzerland, respectively (Bacaloni et al., 2008; Creppy, 2002). Moreover, in Switzerland and Austria, the AFM1 maximum limits acceptable are 0.02 μg/kg, 0.25 μg/kg and 0.05 μg/kg for butter, cheese and milk, respectively (Manetta et al., 2005). In China, according to GB 2761-2011 (2011), the safe limit for AFB1 in fermented food, beans and other grains is 5 μg/kg, while for peanut kernel, peanut oil and corn is 20 μg/kg. The European Union (EU) legislations tend to have impact in some countries like Turkey, and Bosnia and Herzegovina.

2.3 Benefits of Mycotoxins Determinations

Fungi pathogenic plants are ubiquitous in nature and are described as the major agents responsible for the spoilage of certain foods and feedstuffs. Various fungi are responsible for plants infection amounting to decrease in crop quality with high significant economic waste; they also contaminate agricultural products with toxic fungi known as mycotoxins (Bhat and Vasanthi, 2003). The result of the attack by fungi has led to immense amounts of loss of foods each year. Such wastes happen most conspicuously in nations where shortages of food may have been a problem (Leslie et al., 2008). According to CAST (2003) about 25 % of the food crops in the world are affected by mycotoxins which include staple foodstuffs, animal feed, crops like coffee with huge economic gain. Research conducted through several decades indicated the potential pernicious impacts to the wellbeing of man and animals a few mycotoxins may bring about if devoured in amounts adequate to inspire harm. The risks might be huge in economically downward areas of the globe where the way to identify and isolate the infected products, and contrasting options utilising food that could be degraded, might be low (Njapau, 2006).

Analytical identification and evaluation of contaminants at the limits approved has to be conducted using dependable techniques, and this is because of the potential toxic effects. In order for an effective control of the possible infestation of food and feedstuffs, the results must be accurate and precise. In line of the above and for the official control of mycotoxins particularly aflatoxins, performance criteria for the techniques to be employed in the analysis has been set by EC (European Commission, 2006b).

The synergy existing in food security and food safety is critical while tending to the issue of aflatoxins (Leslie et al., 2008). Therefore, keeping up food quality and food safety must be adjusted by the food security and adequacy of supply which can only be attained through the analysis and application of reliable techniques and also compliance of the limit set by regulatory agencies (Abbas, 2005).

2.4 Aflatoxin Analysis

The multiplicity of agricultural products infested with aflatoxins, make the procedure for identification complex and broad. In view of the inherent nature of agricultural product, certain assay approaches distinct for commodities like corn, green coffee, mixed feeds, cottonseed and peanuts have been developed (Ellis et al., 1991).

2.4.1 Sampling and sample pre-treatment

For analysis to be done, representative sample are selected from population or sample lot. Therefore, in aflatoxin assay, sampling has crucial importance. Aflatoxins are not distributed uniformly in a commodity. Invasion sites of toxigenic mould are where aflatoxin normally found in high proportions and small percentage of the commodity may be formed by the sites (Smith and Moss, 1985). For instance, report has it that, separate peanut kernels tainted with aflatoxin B1 at 1.1 mg/g, and just single out of such kernel would be enough to infect about 10,000 viable kernels at high aflatoxin amounts (Cucullu et al., 1966). Thus, for sub-sample and homogeneity, the sample is efficiently mixed (Association of Official Analytical Chemists, 1984).

Stoloff (1972) proposed that, for a representative sample to be taken for analysis, large amount of sub-samples which are finely grounded should be well mixed and randomly taken. Complete mixing is needed for liquids, pastes and free-flowing powders (Association of Official Analytical Chemists, 1984). Smith and Moss (1985) reported that the heterogeneous characteristic of toxin division in infected raw products is the major stumbling block in sampling for aflatoxins, and so, the larger the separate particle of food, the greater the problem for sampling adequacy.

In order to decrease matrix effects before analysis for certain analytical techniques, the aflatoxins should be appropriately extracted from the sample tested and purified subsequently. For improvement in selectivity and in other performance characteristics and also to simplify protocols, the immunoaffinity column (IAC) (for both extraction and purification), liquid-solid extraction (LSE) with various alternative solvent mixtures, liquid-liquid extraction (LLE), modern sample clean-up methods like solid-phase extraction (SPE), solid-phase micro extraction (SPME) and ultrasound extraction are the techniques commonly used (Bacaloni at

al., 2008; Bakirdere et al., 2012). Other techniques in used for extraction are accelerated solvent extraction (ASE), and quick, easy, cheap, effective, rugged and safe (QuEChERS), supercritical fluid extraction (SFE), and other clean-up techniques like multifunctional column (MFC) (Akiyama et al., 2001; Khayoon et al., 2010; Krska et al., 2008), and matrix solid-phase dispersion (MSPD) (Dors et al., 2011; Krska et al., 2008; Shephard, 2009).

2.4.2 Extraction of aflatoxins from matrices

Turner et al. (2009) suggested that use of organic solvents such as chloroform, acetonitrile and methanol is the major factor considered in the extraction of aflatoxins from many food matrices. In spite of the low water solubility of numerous toxins, blends of a few solvents with water are extremely viable because the penetration of hydrophilic tissues is better with aqueous solvents than organic solvents alone (Sheibani and Ghaziaskar, 2009). Acetonitrile /water and methanol/water are regularly applied for aflatoxins extraction (Bankole et al., 2010; Dors et al., 2011). For standard procedures, methanol/water process is used for aflatoxins recovery from original samples (Stroka and Anklam, 2000). Though, in the application of Thin-layer chromatography technique, certain researchers, still find chloroform useful in the extraction of aflatoxins (Shouman et al., 2012; Tripathi and Mishra, 2011; Var et al., 2007). However, because it's ecologically hazard, the use of chloroform solvent in the extraction has been drastically diminished (Shephard, 2009; Turner et al., 2009).

2.4.3 Purification of aflatoxin extract

If the identification selectivity is less, the analysis sensitivity relies firmly on the clean-up of sample. In order to efficiently remove the food or feed matrix and to concentrate the specific analytes, certain variety of protocols for sample clean-up have been set aside (Zöllner and Mayer-Helm, 2006). Many clean-up procedures have been employed to certain matrices of food before aflatoxins analysis using High-Performance Liquid Chromatography (HPLC). For cereals and their derivatives and prior to IAC and SPE clean-up pace, methanol/water is normally used in the extraction and purification (Huang et al., 2010; Reiter et al., 2010).

Clarifying agents like lead acetate are used in order to achieve precipitation of substances (such as fatty acids, lipids and pigments) interfering. For quantitation process, solution of

extracted aflatoxin is also collected and concentrated (Groopman and Donahue, 1987). According to Bullerman (1987), for effective concentration, any of the following methods can be used:

1. Steam bath evaporation
2. Use of an enclosed hot plate to evaporate solvent
3. Rotary evaporation under reduced pressure

**To avoid aflatoxin loss through evaporation, Applications 1 and 2 are performed under nitrogen stream.

2.4.4 Separation

The adherence of regulatory levels of aflatoxins in food relies heavily on exact and solid analytical approaches for their analysis. Various methods are applied, including non-destructive optical techniques like imaging technology and spectroscopy, immunological techniques such as lateral-flow immunoassay (LFIA), immunosensors and Enzyme-linked immunosorbent assay (ELISA), capillary electrophoresis (CE) and chromatographic techniques such as TLC and HPLC together with various detectors (Xie et al., 2015). However, the most frequently used techniques are TLC and HPLC.

2.4.4.1 Thin-layer chromatography (TLC)

For the analysis of aflatoxins in pistachio samples, the TLC technique has been approved and officially recommended by the AOAC (AOAC, 1988; Sheibani and Ghaziaskar, 2009). It is the earliest and developed technique for the measurement of aflatoxins (Marutoiu et al., 2004). Because of the technical advancement in HPLC and later in fluorometric methods and ELISA, the use of TLC technique was substituted in the early 1980s by developed countries. However, due to its practicability and simplicity, it is still useful in developing nations (Stroka and Anklam, 2002).

In TLC, silica is used in glass plate coating and by a baseline application of aflatoxin concentrated sample. The occurrence of separation can be seen by the migration of solvent and follows, is the resultant spots drying and characterization. Silica gels are utilised for plates cladding and to achieve effective resolution, high-cleaned silica gels are needed (Bullerman,

1987). Drying is activated after glass plate was spread by the adsorbent of thin layer. Temperature and time controls the property of activation. The coated and activated home-made plates or ready-to-use TLC plates are spotted with microlitre amounts of the concentrated extract and put in an upright position with the near spot submerged in solvent. Capillary action helps in the migration of solvent through sorbent layer effecting division into separate spots straight to the baseline. Solvent evaporation is achieved by drying after desired level of migration prior to removal of the plate. For developing spots, detection techniques are then employed (Pomeranz and Meloan, 1987).

Recent invention for analysis of aflatoxin by TLC is the application of two-dimensional process. TLC method is highly suitable for specimen with extreme portion of co-extracted substances. As a clean-up phase, development is normally in the first direction, while the actual quantitation is in the second direction (Smith and Moss, 1985).

2.4.4.2 High-Performance Liquid Chromatography (HPLC)

The use of HPLC for the analysis of aflatoxins and other toxins is progressively high and it's due to reliability and enhanced sensitivity when compared with the TLC technique. It is a method of separating sample constituents, their identification and subsequent quantitation. Between a mobile liquid phase and a static liquid or solid phase reinforced in column (around 25 cm by 4 mm internal diameter), there is sample competitive distribution which is attained through separation. Effectiveness of the column parameters, specifically the size of the particle is the dependent factor upon which the strength of the separation could be actualized. The movement of the mobile phase is controlled by pressure with the help of pump and moves through column of the concentrate and afterward streams to UV absorption fluorescence detector. Under stable conditions, aflatoxin has a steady retention time. A correlation of retention times and that of the standards empowers the outcomes to be looked on the basis of quantitation as the territory under each peak the chromatograph comparable to the aggregation of the individual class of aflatoxin. HPLC is normally attached to responsive and modern equipment for data recovery thereby allowing the detection of the tremendously low amounts of aflatoxin (Smith and Moss, 1985). HPLC is highly effective for the analysis of aflatoxin in various foods, for example, corn, figs and peanut products (Pons and Franz, 1977). And for

cottonseeds, milk and its derivatives for aflatoxin M1, and in mammals blood (AOAC, 1984; Smith and Moss, 1985; Thiel, 1986).

2.5 Validation of Analytical Methods for Aflatoxin Determination

The entire analytical techniques ranging from sampling, preparation of sample, purification and final verification employed by laboratories for the control of regulatory amounts must be reliable to a confirmation strategy to demonstrate that the technique produces comparable results and is in line to the set guidelines (Josephs et al., 2004; Krska et al., 2008). Accuracy, precision (reproducibility and repeatability), linearity, limit of detection (LOD), limit of quantitation (LOQ), working range, selectivity (based on the components of matrix and other compounds interference) and ruggedness, are the typical quantitative criteria for evaluating the method performance characteristics. With regard to method validation, various guidelines and procedures have been developed (Ataş et al., 2010; Ataş et al., 2011; Ataş et al., 2012). Massive number of analytical techniques accessible are recognized and authorised by official authorities, such as AOAC international, international standard organization (ISO) and the European Committee for Standardization (Gilbert and Anklam, 2002). Every laboratory should execute quality control checks, for example, regular check of the accuracy and linearity of their techniques (Josephs et al., 2004).

2.6 Safety Considerations and Precautions

Aflatoxins are toxic compounds, mutagenic, teratogenic and carcinogenic causing wide variety of health problems (Bakirdere et al., 2012). For this reason, preparation and management of concentrates, standards and working solutions must be carried out with mask, gloves, research centre coat and precisely in a fume hood. It is always ideal to soak the glassware used for preparing samples or standards in aqueous sodium hypochlorite (5%) to enable the destruction of the remaining aflatoxins prior to scrubbing and subsequent use (Desmarchelier et al., 2010). Because of the nature of aflatoxins in undergoing light degradation, amber bottles should therefore be used to keep the standard solutions (Bacaloni et al., 2008; Nakai et al., 2008).

CHAPTER 3

RELATED RESEARCH

This chapter summarizes the previously conducted studies on cassava and its derivatives, some cereal crops and various forms of melons in relation to the prevalence of aflatoxins in certain areas of the world.

Aflatoxins are members of toxic fungal secondary metabolites called mycotoxins. Fungi are ubiquitous in nature and are the major agents to the spoilage of foods and feedstuffs (Bhat and Vasanthi, 2003). Aflatoxins was first discovered in England in 1960 (widely known as Turkey "X" disease), and since then several research have been carried out to investigate its exposure and detrimental health problems (Groopman et al., 1988 and Blount, 1961). Aflatoxin exposure differs greatly and it's due to the difference in diets consumed between countries (Farombi, 2006; Binder et al., 2007).

Analysis from various countries demonstrated that AFB1 is a contaminant of pellet wheat, corn silage, cotton seed, soybean, peanut shells and barley (Decastelli et al., 2007; Sassahara et al., 2005).

In Sweden, AFB1 level was found to be above 2 µg/kg in 9.5% of pistachio nut sample (Thuvander et al., 2001). In 17 of 29 samples of pistachio nut, in the Netherland, AFB1 was found to be in the range of 0.8 to 165 µg/kg (Scholten and Spanjer, 1996). In Spain, according to Arino et al (2009), AFB1 contaminated about 50% of bulk pistachio nuts analysed. In similar percentage (50.5%) in Turkey, the contamination levels of AFB1 in 95 unpacked pistachio nuts samples was found to be in the range of 0.007 to 7.72 µg/kg (Set and Erkmen, 2010).

Samples of melon seed and tigernut (*Cyperus esculentus*) were detected with aflatoxins. Thirty – two per cent (32%) of melon seed samples from markets in Nigeria representing forest and savannah zones were detected with aflatoxin B1 with a means of 14 and 11 µg/kg,

respectively (Bankole et al., 2004). A reported 35% of tigernut samples acquired from various areas in Nigeria were contaminated at amount in the range of 10 – 120 µg/kg (Bankole and Eseigbe, 1996). In western parts of Nigeria, Aflatoxin B1 have been reported to contaminate 54% of the dried yam chips for sale at levels of 4 – 190 µg/kg with a mean of 23 µg/kg, aflatoxin B2 contaminated 32% at a mean of 2 – 55 µg/kg, aflatoxin G1 was detected in 5% of the samples at 4 – 18 µg/kg and aflatoxin G2 in two of the samples (Bankole and Adebanjo, 2003).

Aflatoxins were detected in foodstuffs obtained from various markets in Benin City of Nigeria and they are; "ogili – ugba" (made from castor bean, *Riccinus communis*), "*garri*" (cassava farinha), "ogbono" (*Irvingia gabunesis*), and "*egusi*" meal (*Cucumeropsis edulis*) (Alozie et al., 1980).

Obtained from households and retailers in Ireland and the United Kingdom (UK), the natural co-occurrence of aflatoxins (AFs), ochratoxins A (OTA) and citrinin (CIT) in melon seed samples was evaluated. AFB1 was found in 100% of the samples with mean of 9.7 µg/kg and a range of 0.2 – 66.5 µg/kg. Total aflatoxins mean was 12.0 µg/kg with a range of 0.3 – 82 µg/kg. Samples from retailers were significantly higher ($p < 0.05$) than the samples from household. Both AFs and OTA were analysed by HPLC coupled with fluorescence detection, while HPLC-MS/MS was used in CIT. The study demonstrated that, 68% of the melon seed samples were contaminated with AFB1 in oilseeds and was found to be above the limit of 2 µg/kg set by European Union (EU). The need for the strategies to reduce contamination of aflatoxins in "egusi" for the consumption of human was highlighted (Somorin et al., 2016).
In a study to determine and quantify the level of contamination of aflatoxin in cassava flour from three (3) major markets in Zaria, Nigeria. An overall of 36 samples were collected from Sabon Gari, Samaru and Zaria City markets and the analysis was carried out using enzyme linked immunosorbent assay (ELISA). The total aflatoxins were detected in 22 of the 36 samples at a range of 2.0 – 7.5 µg/kg. The result indicated that, the contamination of aflatoxin in cassava flour is low and therefore safe for human consumption (Saleh et al., 2016).

Study conducted in Nigeria, farmers in the Northern guinea savannah and rain forest zones usually make use of bags made of woven polypropylene to keep melon seeds in their stores, or

in rare cases in a sacks made of jute. For re-drying and sorting, they often brought the seeds out after the emergence of moulds as the major obstacle in storage. The detection showed that the samples were contaminated with AFB1 at levels above 5 µg/kg in 27.4 % of savannah samples (mean; 12.1 µg/kg) and 35.6 % of the forest samples which stood at 13.7 µg/kg mean. Samples stored for more than two months are at risk of increased aflatoxins incidence (Bankole et al., 2004).

In Southern part of Nigeria, analysis for proximate composition, fungi detection and amount of aflatoxins B1, B2, G1 and G2 contamination were conducted on eight samples of both fresh and stored "garri ijebu" mainly consumed in Ijebu land and in some people from Yoruba. The result showed that stored samples (18 month's period of storage) were detected with aflatoxin B2 at concentration level of 0.0085 µg/kg and the detection of total aflatoxin representation at about 8.8% (Jonathan et al., 2013).

In a study to investigate the role of palm oil in relation to the fermentation period on cyanide and aflatoxin levels of processed cassava tubers (Garri) from three different places; Durungwu, Mkporo – Oji and Akunna in Njaba Local Government Area, Imo State of Nigeria. Tubers of mature cassava (*Manihot esculenta crantz*) were harvested and processed into "*garri*". For aflatoxin determination, samples of "gari" were stored for a period of 30 consecutive days prior to spectrophotometric analysis. The result indicated no significant difference ($p > 0.05$) in amounts of aflatoxin which was ranged between 0.26 ± 0.07 and 0.55 ± 0.04 µg/kg in various samples of "*garri*" (Chikezie and Ojiako, 2013).

Intended for human consumption in Nigeria in the year 2005 and 2006, derivable from "egusi" melon seeds, three of the products; "robo" (melon ball snacks), "*egusi*" soup and "*ogiri*" (fermented melon seed condiment) were analysed for aflatoxin B1 content using thin layer chromatography (TLC) with fluorescent detection. The percentage AFB1 detected in samples was 19.5%, 31.8%, and 25% for "*egusi*" soup, "*ogiri*" and "*robo*", respectively. In all samples, the range of aflatoxin B1 was from 2.3 – 15.4 ppb. To mean levels of AFB1 were 7.2 ppb, 9.7 ppb and 8.9 ppb for "*egusi*" soup, "robo" and "ogiri", respectively. Aflatoxin B1 was found to contaminate all the melon seeds derived products at levels lower than the permissible amount

of 20 ppb recommended in Nigeria. Overall outcome indicated less risk by human consumption (Bankole et al., 2010).

In a coordinated survey earlier conducted on feedstuffs, feeds ingredients and commodities revealed that in samples from the Northern Europe, the maximal AFB1 levels to be 60 µg/kg, in samples from the Mediterranean region and the Southern Europe to be 656 µg/kg, and in samples from the Central Europe to be 311 µg/kg (Binder et al., 2007). Kos et al (2013) postulated that in southeast part of Europe, research undertaken in 2002 in Serbia, revealed AFB1 contamination in maize and attributed that changes in weather might be the possible causes.

A report has that, 70% or more of the grains with extreme high moisture (> 18 %) are contaminated with *Aspergillus flavus* and the correlation between the level of contamination and aflatoxin development could be seen as positive. There is a direct correlation between the moisture level of crop and the toxin occurrence (Mora and Lacey, 1997).

CHAPTER 4

MATERIALS AND METHOD

4.1 Study Area

Cyprus, an island in the Mediterranean sea of the Eastern basin and considered the third largest island in the Mediterranean (after Sardinia and Sicily of the Italian islands) and by area, it's the world's 81st largest island. It is 100 km (60 miles) to the west of Lebanon and Syria and 60 km (40 miles) to the south of Turkey.

The city of Nicosia is the capital and largest. It's close to Northern Africa and Southern part of Europe, and has had long times of predominantly Greek and intermittent Anatolia, Turkish, Byzantine, Levantine, and influence of Eastern European (Grove and Rackham, 2003; Kademi, 2016).

In the Turkish Republic of Northern Cyprus (TRNC), the consumption of "*garri*" and "*egusi*" is increasing mainly by Africans and is prone to contamination by aflatoxins due to high humidity and warm temperatures during winter and summer period respectively. Therefore, it is highly beneficial to ascertain the degree of safety by determining level of aflatoxins in these food products.

4.2 Sampling

"*Garri*" and "*egusi*" samples were taken from the major markets of Nicosia (Lefkoşa), Kyrenia (Girne) and Famagusta (Mağusa) in August and February of 2016 and 2017 respectively according to TS EN ISO 13690 standard (1999) (Figure 4.1).

Figure 4.1: Picture from sampling of "*garri*" and "*egusi*"

Overall, fifteen (15) samples of both white/yellow "*garri*" and "*egusi*", (4 packaged) and (11 open) containing 500 g of "*garri*" and "*egusi*", were purchased from the markets of the above regions as seen in Figure 4.2 and Table 4.1 below:

Figure 4.2: Map of TRNC (KKTC) depicting the study locations

Table 4.1: Regions where samples were taken in TRNC

Region	Sample	Type of sample	Number of samples
Nicosia (Lefkoşa),	Yellow "*garri*"	Packaged	1
	Yellow "*garri*"	Packaged	1
	"*Egusi*"	Packaged	1
	"*Egusi*"	Packaged	1
	Yellow "*garri*"	Open	1
	White "*garri*"	Open	1
	"*Egusi*"	Open	1
Famagusta (Mağusa)	"*Egusi*"	Open	1
	Yellow "*garri*"	Open	1
	"*Egusi*"	Open	1
	Yellow "*garri*"	Open	1
Kyrenia (Girne)	"*Egusi*"	Open	1
	White "*garri*"	Open	1
	"*Egusi*"	Open	1
	White "*garri*"	Open	1
Total			15

The samples (both from open and packaged market sources) were packed and sealed in polythene bags and labelled accordingly, and were then transferred to Ankara Food Control Laboratory in Turkey to be analyzed.

4.3 Principle

Test portion was extracted with MeOH and hexane. The extract was filtered and diluted with H_2O to a concentration of specified solvent, and was applied to an affinity column with antibodies of aflatoxins B1, B2, G1, and G2 specifics. Aflatoxins were extracted from the affinity column with methanol and quantified by reversed-phased liquid chromatography (LC) with post-column derivatization (PCD) involving the process of bromination. The PCD was achieved by electrochemically generated bromine (Kobra cell) and was determine by fluorescence detection.

4.4 Performance Standards for Immunoaffinity Column (IAC)

The capacity of the column was maintained at less than 100 ng and for aflatoxins B1, B2, and G1, the recovery was not less than 80% and 60% for aflatoxin G2 after the application of standard solution in aqueous form of 10% methanol which contained each toxin (5 ng).

4.5 Calibration

Prior to chromatographic analysis of the samples, 9 points calibration standards were used for the calibration of HPLC instruments. As recorded, 0.1µg/kg was the lowest level of calibration for B1; others were 0.15 µg/kg, 0.2 µg/kg, 0.25 µg/kg, 0.5 µg/kg, 1 µg/kg, 2 µg/kg, 2.5 µg/kg and 5 µg/kg.

Figure 4.3: HPLC machine

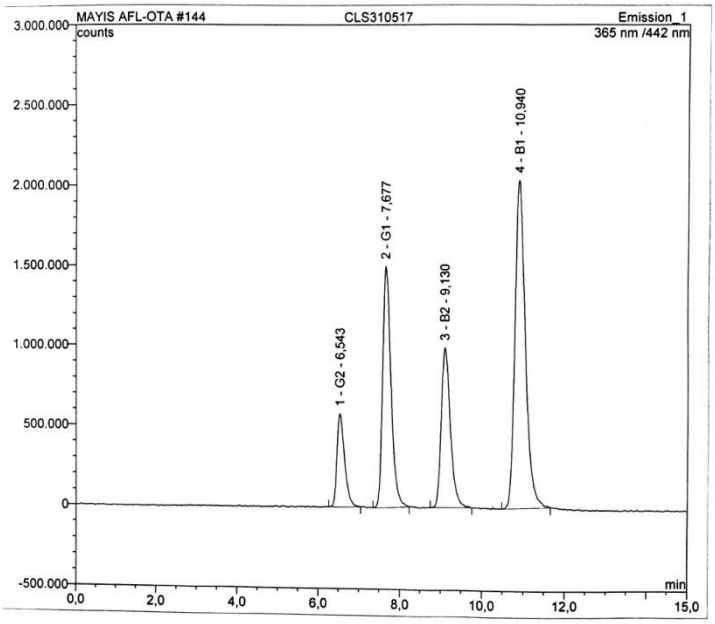

Figure 4.4: Sample chromatogram of calibration standard of 1 µg/kg

4.6 Reagents and Chemicals

Phosphate buffered saline solution	pH 7.4, 0.2 g KCl
Extraction solvent	Methanol - water solution (8+2, v/v)
Methanol	LC grade
Methanol	Technical grade, distilled
Pyridinium hydrobromide perbromide (PBPB)	CAS 39416-48-3
Water	LC grade, ISO 3696 grade 1
Toluene acetonitrile	98+2 (v/v)
Nitric acid	26.1 mL 70% HNO_3
Post-column reagent (B)	25 mg PBPB in 500 mL H_2O
Acetonitrile	LC grade

LC mobile phase solvent (A)	Water, acetonitrile, methanol LC grade solution (6+2+3, v/v/v)
LC mobile phase solvent (B)	Electrochemically generated bromide
Hexane	
Potassium bromide	
Sodium chloride	
LC aflatoxin standard solutions	

4.7 Apparatus

Laboratory balance:	Readability 0.1 g
Analytical balance:	Readability 0.1 mg
Pipettes:	10 mL
LC pump:	1.000 ± 0.005 mL/min
Reservoir:	75 mL/Luer tip connector for affinity column
Fluorescence detector:	Wavelengths 360 nm excitation filter × 420 nm cut-off emission filter
Reversed-phase LC column:	4.6 mm × 25 cm
Glass microfiber filter paper:	5 cm diameter, retention: 1.6 µm
Blender:	Explosion proof (8000 rpm)
Hand pump:	20 mL syringe
Vertical shaker:	Adjustable for max. solid-liquid agitation
Injection system:	200 µL valve loop
Calibrated microlitre syringe:	25 and 500 µL

Disposable filter unit:	Cellulose, 0.45 µm
Volumetric glassware:	2, 3, 10, and 20 mL (0.5% accuracy)
Filter paper:	24 cm diameter, pre-folded, and 30 µm retention
Affinity columns:	Vicam (313 Pleasant St, Watertown, MA 02472, USA.
Post-column derivatization system:	Electrochemically generated bromine – Kobra cell; Rhone Diagnostic Technologies Ltd., Lyon, France.
Erlenmeyer flask:	500 mL, screw top

4.8 Preparation of Extracts

i. "*Egusi*" melon: 50 ± 0.1 g of "*egusi*" test portion were mixed (8:20 v/v), followed by addition of 100 mL hexane, 5 g NaCl, and 300 mL methanol-water extraction solvent. They were shaken for 3 min using high speed shaker. After filtration, 10.0 mL clear filtrate was pipetted and placed on conditioned immunoaffinity column in a reservoir of 60 mL PBS solution. Plastic spatula was used in mixing and later the residues were rinsed in 1 mL PBS and then transferred to column.

ii. "*Garri*": 50 ± 0.1 g of "*garri*" test portion were mixed into 500 mL screw top Erlenmeyer flask (11:9 v/v). Sodium chloride (5 g) and methanol-water solvent (300 mL) were added. They were intensively shaken for 30 m by a shaker. Pre-folded filter paper was used in extract filtration. A 10.0 mL clear filtrate was pipetted poured into PBS (60 mL) solution of the reservoir and was placed on conditioned immunoaffinity column. They were mixed with spatula of plastic material and residues were rinsed using 1 mL PBS and later the solution was applied to the column.

Figure 4.5: Flask containing 50 g of test portions

Figure 4.6: Extraction by shaking

Figure 4.7: Filtration of extracts

4.9 Immunoaffinity Column Clean-up

Under gravity, filtrate was passed through the column at a flow rate of ca 3 mL/min. The column was washed with water (15 mL), and was dried by an application of small vacuum at 5-10 s.

Figure 4.8: Immunoaffinity column clean-up

Aflatoxins were eluted by addition of methanol on column at 0.5 mL/min and were passed through gravity. Calibrated volumetric flask (3.0 mL) was used in the collection of the eluate.

Prior to application of the second portion of methanol (0.75 mL), one minute gap was observed. Applied elution solvent was collected after an air was pressed through, and was marked and mixed after dilution. The solution was clear and was immediately used for LC analysis.

4.10 LC Determination with Fluorescence Detection and Post-column Derivatization

In order to achieve post-column derivatization, the electrochemically generated bromide was used and operated with the flow rate for the mobile phase set as 1 mL/min and the current fixed at 100 µA.

200 µL working standard solution was injected into the injector with concentration of aflatoxin B1 within the range 1-4 ng/g. The injector loop was completely filled per the manufacturer's instruction. Aflatoxins G2 eluted first with the retention time of 6 minutes, followed by G1, B2 and B1 with 8, 9 and 11 minutes retention times respectively with the baseline taken into consideration.

Again, 200 µL extract was injected into the injector; the peak of each aflatoxin in the chromatogram was identified by comparing their retention times with those of a reference standards. The quantity of aflatoxin contained in the injected eluate was determined from the standard curve.

4.11 Calculations

The level of concentration of aflatoxin in the test sample was calculated as follows:

A graph of aflatoxins B1, B2, G1 and G2 (ng/mL; y-axis) concentration was plotted against peak area (units; x-axis). In order to calculate the amount of aflatoxins in the measured solution, the resulting function ($y = ax + b$) was used.

From the measured solutions (ng/mL), the contamination (ng/g) was calculated as:

$$C_{smp}\ (ng/mL) = ax\ Signal_{smp}\ (units) + b$$

$$\text{Contamination} = \frac{C_{smp} \times \text{solvent} \times \text{elution}}{Wt \times \text{aliquot}} \left[\frac{ng \times mL \times mL}{mL \times g \times mL}\right]$$

Where:

Wt (g) = test portion taken for analysis

Solvent (mL) = solvent taken for extraction

Aliquot (mL) = aliquot taken for IAC cleanup

Elution (mL) = final volume collected after elution from IAC

C_{smp} (ng/mL) = conc. of aflatoxin calculated from linear regression

Contamination (ng/g) = contamination of test sample with aflatoxin

$Signal_{smp}$ (units) = area of aflatoxin peak obtained from the measured solution

4.12 Recovery Check: Acceptability of Analytical Performance for Routine Recoveries

The performance of the analytical method should be checked, both during its development and during its subsequent use.

The main purposes of performance verification are:

- Monitor the performance of the method under the actual conditions prevailing during its use;
- Take into account the effect of inevitable variations caused by, for instance, the composition of samples, performance of instruments, quality of chemicals, varying performance of analysts and laboratory environmental conditions;
- Demonstrate that the performance characteristics of the method are broadly similar to those established at method validation, showing that the method is under "statistical control", and the accuracy and uncertainty of the results are comparable to those

expected of the method. For this purpose, data obtained during method validation may be updated with data collected from performance verification during the regular use of the method.

The calculation of mean recovery is normally from one group of commodity. To achieve the set acceptable limits within a single recovery, the range of the result is expected to normally be within the mean recovery +/- 2 x RSD, and which by the use of routine ongoing recovery data (laboratory reproducibility) can be adjusted. However, in routine analysis involving multi aflatoxins, a generalised 60-140% range may be used, and therefore, re-analysis of the batch is required in recoveries outside the range mentioned above. In the event, where the individual recovery is high and unacceptable, and no aflatoxins detected, then re-analysis of the samples is not necessary for aflatoxins absence that needs to be proven. This necessitates the investigation of consistent high recovery. However, the causes must be checked, where certain change occurs in recovery or that the results attained are not accepted potentially (RSD beyond ± 20%) (SANCO, 2009).

For the recovery check, control "*egusi*" and "*garri*" samples were fortified with the mixture of aflatoxins as three replicates at MRL and half MRL levels. MS Excel was used in calculating the Relative Standard Deviation (RSD), Standard Deviation (SD) of recoveries and Average recovery by the following formula:

$$\% \, \boldsymbol{RSD} = \left(\frac{\boldsymbol{SD}}{\textbf{Mean Rec.}}\right) * \mathbf{100} \qquad (4.1)$$

To give a good estimate on the content of aflatoxins in the sample and also to certify commodity compliance, RSD value is used below:

$$\boldsymbol{R_{max-min} = R_i \pm (k * RSD * R_i)} \qquad (4.2)$$

$R_{max-min}$: Maximum and minimum aflatoxin amount (µg/kg)

R_i: Aflatoxin amount measured in the sample (µg/kg)

k: Expansion factor which is 2 by International Union of Pure and Applied Chemistry (IUPAC) at the level of 95% confidence

RSD: Repeatability

RSD * Ri: Combined standard uncertainty of the analysis

k* RSD* Ri: Expanded uncertainty of the analysis

Aflatoxins recoveries were seen in the range of 70 – 120%. For "*egusi*", the overall recovery, SD and RSD values (n=24) were at 94%, 4.8% and 5.1%. While, for "*garri*" the overall recovery, SD and RSD values (n=24) were seen at 89%, 4.3% and 4.8%, respectively. Overall recovery, individual recoveries and RSD were within the identified acceptance guideline 70% ≤ Q ≤ 120% and RSD ≤ 20%), therefore, the method was considered valid for both "*egusi*" and "*garri*" (Table 4.2).

Table 4.2: Recovery check results

Sample	**Recovery of the Method (%)**						**Accuracy[a] (%)**	**SD**	**RSD (%)**
"*Garri*"		MRL			MRL/2				
G2	94.5	95.6	89.2	94.9	88.5	96.5	**89**	**4.3**	**4.8**
G1	86.5	88.7	82.4	90.9	86.2	91.9			
B2	87.5	93.2	86	89.8	86.1	92.5			
B1	82.6	87.1	80.5	88.1	85.1	88.6			
"*Egusi*"									
G2	96.6	93.3	93.3	104	96.7	99..2	**94**	**4.8**	**5.1**
G1	90.9	87.9	88.1	100.2	92.1	94.9			
B2	94.9	91.8	89.9	100.7	94.4	96.8			
B1	90.8	85.5	86	98.6	89	92.6			

[a]As percent average recovery (n=24)

CHAPTER 5

RESULTS AND DISCUSSION

The Tables (Table 5.1 and Table 5.2) below represent the results and concentration of aflatoxins after the analysis was carried out.

Table 5.1: Concentrations of aflatoxins from "*garri*" samples

Region	**Sample Name**	**Concentration of Aflatoxins (µg/kg)**				
		B1	**B2**	**G1**	**G2**	**Total**
Nicosia (Lefkoşa)	Yellow "*garri*"	n.d	n.d	0.006	0.015	0.021
	Yellow "*garri*"	0.021	n.d	0.015	0.029	0.065
	Yellow "*garri*"	n.d	n.d	n.d	0.021	0.021
	Yellow "*garri*"	n.d	n.d	n.d	n.d	n.d
Famagusta (Mağusa)	Yellow "*garri*"	n.d	n.d	n.d	n.d	n.d
	Yellow "*garri*"	n.d	n.d	n.d	0.007	0.007
Kyrenia (Girne)	White "*garri*"	0.034	n.d	n.d	n.d	0.034
	White "*garri*"	0.041	0.006	n.d	n.d	0.047

*n.d = not detected

Table 5.2: Concentrations of aflatoxins from "*egusi*" samples

Region	Sample Name	Concentration of Aflatoxins (µg/kg)				
		B1	**B2**	**G1**	**G2**	**Total**
Nicosia (Lefkoşa)	"*Egusi*"	**0.707**	0.150	n.d	0.027	0.884
	"*Egusi*"	**0.743**	0.121	n.d	0.007	0.871
	"*Egusi*"	0.114	0.011	n.d	n.d	0.125
Famagusta (Mağusa)	"*Egusi*"	0.099	0.011	0.006	n.d	0.116
	"*Egusi*"	0.130	n.d	n.d	n.d	0.130
Kyrenia (Girne)	"*Egusi*"	0.116	0.020	n.d	n.d	0.136
	"*Egusi*"	0.095	0.014	n.d	n.d	0.109

*n.d = not detected

Out of the fifteen (15) samples collected, only two "*egusi*" samples were found to contain aflatoxin B1 at a concentration of 0.707 µg/kg and 0.743 µg/kg (Figure 5.1 and Figure 5.2). Others were below the detectable limit (as seen in the tables above) and therefore considered compliant. Several institutions gradually developed regulations for food and feed in relation to aflatoxins. Aflatoxins B1 maximum limits and the entire aflatoxin amounts (AFB1, AFB2, AFG1 and AFG2) has been described as the criterion commonly implemented to restrict the presence of aflatoxins in certain number of foods (Hernandez-Hierro et al., 20008).
The concentration of aflatoxin B1 found is below the maximum tolerable limit of 2 µg/kg set by European Union for oilseeds.

The result is contrary to the findings of Somorin et al. (2016) which reported the amount of aflatoxin B1 as 9.7 µg/kg and a range of 0.2 – 66.5 µg/kg in "*egusi*" melons obtained from households and retailers in Ireland and the United Kingdom (UK). The study demonstrated that, 68 % of the melon seed samples were contaminated with AFB1 in oilseeds and was found to be above the limit of 2 µg/kg set by European Union (EU). HPLC coupled with fluorescence was used in the analysis. Total aflatoxins mean was 12.0 µg/kg with a range of

0.3 – 82 µg/kg, and therefore concluded that there is need for strategies to reduce contamination of aflatoxins in "*egusi*" for human consumption. However, it is still in line with our finding, since 32 % of the "*egusi*" samples were contaminated with AFB1 below the maximum tolerable limit (2 µg/kg) of the European Union.

In another similar study conducted in Nigeria, farmers in the Northern guinea savannah and rain forest zones usually make use of bags made of woven polypropylene to keep melon seeds in their stores, or in rare cases in a sacks made of jute. The detection showed that the samples were contaminated with AFB1 at levels above 5 µg/kg in 27.4 % of savannah samples (mean; 12.1 µg/kg) and 35.6 % of the forest samples which stood at 13.7 µg/kg mean. The study also revealed the amount of AFB1 as above the maximum tolerable limit (2 µg/kg) set by EU (Bankole et al., 2004).

Depending on the food products, certain factors are believed to be the driven force in the variation of the concentration of aflatoxins especially food content and water activity of the samples during processing and storage affect aflatoxin amount. However, prolonged storage of the "*egusi*" products plays a role in increasing the risk of aflatoxins incidence (Bankole et al., 2004).

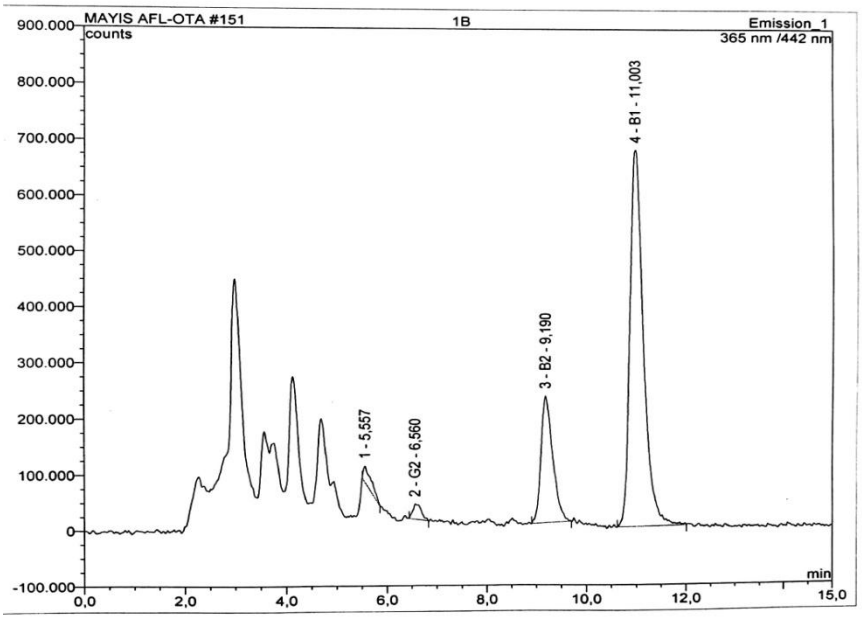

Figure 5.1: Chromatogram of "*egusi*" sample (1B)

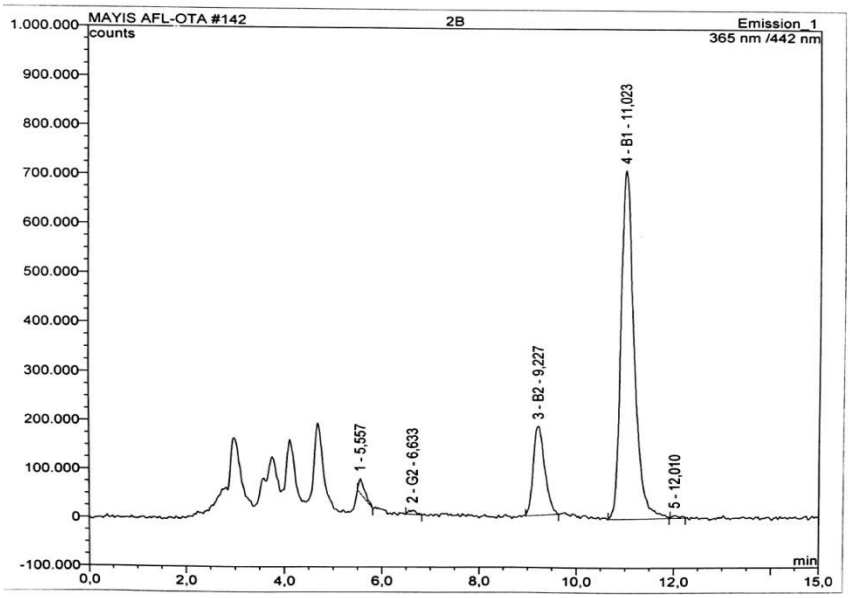

Figure 5.2: Chromatogram of "*egusi*" sample (2B)

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

On the basis and outcome of this study, two "*egusi*" samples out of the fifteen (15) from Nicosia (Lefkoşa) markets were containing AFB1 at the level 0.707 µg/kg and 0.743 µg/kg below maximum tolerable limit (2 µg/kg) set by EU. Therefore, "*egusi*" and "*garri*" consumed in TRNC are safe with regard to aflatoxins.

Considering the high number of mycotoxins present in foods, it is of paramount importance to control the incidence of aflatoxins and also ochratoxins regularly.

REFERENCES

Abbas, H.K. (Ed.). (2005). *Aflatoxin and food safety*. CRC Press. Taylor and Francis Group. 600 Broken Sound Parkaway NW, Suite 300, USA.

Abia, W. A., Warth, B., Sulyok, M., Krska, R., Tchana, A. N., Njobeh, P. B., Dutton, M. F., & Moundipa, P. F. (2013). Determination of multi-mycotoxin occurrence in cereals, nuts and their products in Cameroon by liquid chromatography tandem mass spectrometry (LC-MS/MS). *Food Control, 31*, 438-453.

Achigan-Dako, G. E., Vodouhe, S. R., & Sangare, A. (2008). Caracterisation morphologique des cultivars locaux de Lagenaria siceraria (Cucurbitaceae) collectes au Benin et au Togo. *Belgian Journal of Botany, 141*, 21-38.

Adebajo, L. O., Bamgbelu, O. A., & Olowu, R. A. (1994). Mould contamination and the influence of water activity and temperature on mycotoxin production by two *Aspergilli* in melon seed. Nahrung, *38*, 209–217.

Adekunle, Y. A., Olowe, V. I., Olasantan, F. O., Okeleye, K. A., Adetiloye, P. O., & Odedina, J. N. (2014). Mixture productivity of cassava- based cropping system and food security under humid tropical conditions. *Food and Energy Security, 3*(1), 46-60.

Aguilar, F., Hussain, S. P., & Cerutti, P. (1993). Aflatoxin B1 induces the transversion of G→T in codon 249 of the p53 tumor suppressor gene in human hepatocytes. *Proceedings of the National Academy of Science,* USA. *90*(18), 8586–90.

Ajuru, M. G., & Okoli, B. E. (2013). The morphological characterization of the melon species in the family cucurbitaceae juss., and their utilization in Nigeria. *International Journal of Modern Botany, 3*(2), 15-19.

Akiyama, H., Goda, Y., Tanaka, T., & Toyoda, M. (2001). Determination of aflatoxins B1, B2, G1 and G2 in spices using a multifunctional column clean-up. *Journal of Chromatography A., 932*, 153-157.

Alozie, T. C., Rotimi, C. N., & Oyibo, B. B. (1980). Production of aflatoxin by *Aspergillus flavus* (UBMI) in some Nigerian indigenous beverages and foodstuffs. *Mycopathologia, 70*, 125-128.

Amanullah, M. M., Alagesan, A., Vaiyapuri, K., Pazhanivelan, S., & Sathyamoorthi, K. (2006). Intercropping and organic manures on the growth and yield of cassava (Manihot esculenta Crantz.). *Research Journal of Agriculture and Biological Sciences, 2*(5), 183-189.

Antonacci, L., Salvat, A. E., Faifer, C., & Godoy, H. M. (1999). Suppression of spore germination and aflatoxin biosynthesis in Aspergillus parasiticus during and after exposure to high levels of phosphine. *Mycopathologia*, *147*, 83–87.

Ariño, A., Herrera, M., Estopañán, G., Rota, M. C., Carramiñana, J. J., Juan, T., & Herrera, A. (2009). Aflatoxins in bulk and pre-packed pistachios sold in Spain and effect of roasting. *Food Control, 20*, 811-814.

Asi, M. R., Iqbal, S. Z., Ariño, A., & Hussain, A. (2012). Effect of seasonal variations and lactation times on aflatoxin M 1 contamination in milk of different species from Punjab, Pakistan. *Food Control*, *25*(1), 34-38.

Association of Official Analytical Chemists International (1984). Official Methods of Analysis, 14th ed., AOAC, Washington, D.C., 1984.

Association of Official Analytical Chemists International. (1988). Official Method No. 969.22. Official methods of Analysis of the AOAC International.

Ataş, M., Temizel, A., & Yardimci, Y. (2010). Classification of aflatoxin contaminated chili pepper using hyperspectral imaging and artificial neural networks. *In: Signal Processing and Communications Applications Conference* (SIU), 2010 IEEE 18th, IEEE. 9-12.

Ataş, M., Yardimci, Y., & Temizel, A. (2011). Aflatoxin contaminated chili pepper detection by hyperspectral imaging and machine learning. Proc. of SPIE 8027:8027F.

Ataş, M., Yardimci, Y., & Temizel, A. (2012). A new approach to aflatoxin detection in chili pepper by machine vision. *Computers and Electronics in Agriculture, 87,* 129-141.

Attah Daniel, B. E., Ebisike, K., Adeeyinwo, C. E., Ojumu, T. V., Olusunle, S. O. O., & Adewoye, O. O. (2013). Towards arresting the harmful effect of cyanogenic potential of cassava to man in the environment. *The International Journal of Engineering and Science*, *2*(9), 100-104.

Avantaggio, G., Quaranta, F., Desidero, E., & Visconti, A. (2002). Fumonisin contamination of maize hybrids visibly damaged by Sesamia. *Journal of the Science of Food and Agriculture, 83,* 13–18.

Bacaloni, A., Cavaliere, C., Cucci, F., Foglia, P., Samperi, R., & Laganà, A. (2008). Determination of aflatoxins in hazelnuts by various sample preparation methods and liquid chromatography–tandem mass spectrometry. *Journal of Chromatograph A., 1179,* 182-189.

Bakirdere, S., Bora, S., Bakirdere, E. G., Aydin, F., Arslan, Y., Komesli, O. T., Aydin, I., & Yildirim, E. (2012). Aflatoxin species: their health effects and determination methods in different foodstuffs. *Central European Journal of Chemistry*, *10,* 675-685.

Bankole, S. A. (1993). Moisture content, mould invasion and seed germinability of stored melon. *Mycopathologia*, *122*(2), 123-126.

Bankole, S. A., & Adebanjo, A. (2003). Aflatoxin contamination of dried yam chips marketed in Nigeria. *Tropical Science*, *43,* 201-203.

Bankole, S. A., & Eseigbe, D. A. (1996). Occurrence of mycoflora and aflatoxins in marketed tigernut. *Crop Research, 11,* 219-223.

Bankole, S. A., Adenusi, A. A., Lawal, O. S., & Adesanya, O. O. (2010). Occurrence of aflatoxin B1 in food products derivable from "*egusi"* melon seeds consumed in Southwestern Nigeria. *Food control*, *21*(7), 974-976.

Bankole, S. A., Lawal, O. A., & Adebanjo, A. (2004). Storage practices and aflatoxin B1 contamination of "*egusi"* melon seeds in Nigeria. *Tropical Science*, *44*(3), 150-153.

Bankole, S. A., Ogunsanwo, B. M., & Mabekoje, O. O. (2004). Natural occurrence of moulds and aflatoxins in melon seeds from markets in Nigeria. *Food and Chemical Toxicology*, *44*, 1209-1214.

Bankole, S. A., Ogunsanwo, B. M., Osho, A., & Adewuyi, G. O. (2006). Fungal contamination and aflatoxin B1 of 'egusi' melon seeds in Nigeria. *Food Control*, *17*(10), 814-818.

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

Beuchat, L. R. (1987). Food and Beverage Mycology, 2nd ed., Van Nostand Reinhold, New York, USA.

Bhat, R. V., & S. Vasanthi, (2003). *Food Safety in Food Security and Food Trade. Mycotoxin Food Safety Risk in Developing Countries*. International Food Policy Research Institute.

Bhatnagar, D., Yu, J., & Ehrlich, K. C. (2002). Toxins of filamentous fungi. In: Fungal Allergy and Pathogenicity. *Chemical Immunology and Allergy*, Vol. *81*, 167–206, Breitenbach, M., Crameri, R., and Lehrer, S. Eds., Basel, Karger.

Binder, E. M., Tan, L. M., Chin, L. J., Handl, J., & Richard, J. (2007). Worldwide occurrence of mycotoxins in commodities, feeds and feed ingredients. *Animal feed science and technology*, *137*(3), 265-282.

Blount, W. P. (1961). Turkey "X" disease. *Turkeys 9*(2), 55-58.

Brown, D. W., Adams, T. H., & Keller, N. P. (1996). *Aspergillus* has distinct fatty acid synthases for primary and secondary metabolism. *Proceedings of the National Academy of Science*, USA, *93*, 14873–77.

Bullerman, L. B. (1987). Methods of detecting mycotoxins in foods and beverages, in Food and Beverage Mycology, 2nd ed., Beuchat, L. R., Ed., AVI, New York, 571.

Cary, J. W., Ehrlich, K. C., Beltz, S. B., Harris-Coward, P., & Klich, M. A. (2009). Characterization of the Aspergillus ochraceoroseus aflatoxin /sterigmatocystin biosynthetic gene cluster. *Mycologia*., *101*, 352–62.

Cary, J. W., Klich, M. A., & Beltz, S. B. (2005). Characterization of aflatoxin- producing fungi outside of *Aspergillus* section Flavi. *Mycologia*., *97*, 425– 32.

Chauhan, Y. S., Wright, G. C., & Rachaputi, N. C. (2008). Modelling climatic risks of aflatoxin contamination in maize. *Australian Journal of Experimental Agriculture, 48*, 358– 366.

Chikezie, P. C., & Ojiako, O. A. (2013). Cyanide and aflatoxin loads of processed cassava (*Manihot esculenta*) tubers (Garri) in Njaba, Imo state, Nigeria. *Toxicology international*, *20*(3), 261.

Chinsman, B., & Fiagan, Y. S. (1987). Postharvest technologies of root and tuber crops in Africa: evaluation and recommended improvements. In *Tropical root crops: root crops and the African food crisis: proceedings of the Third Triennial Symposium of the International Society for Tropical Root Crops--Africa Branch held in Owerri, Nigeria, 17-23 August 1986*. Ottawa, Ont., Canada: International Development Research Centre.

Codex Alimentarius Commission, (2013). Proposed draft maximum levels for hydrocyanic acid in cassava and cassava products, Joint FAO/WHO Food Standard Programme, Codex Committee on Contaminants in Food. Accessed on 2nd February, 2017 From ftp://ftp.fao.org/codex/meetings/cccf/cccf7/cf07_10e.pdf.

Cotty, P. J. (1989). Virulence and cultural characteristic of two *Aspergillus flavus* strains pathogenic on cotton. *Phytopathol*ogy, *79*, 808–14.

Council for Agricultural Science and Technology. (2003). Mycotoxins: Risks in Plant and Animal Systems. Task Force Rep No 38. Council for Agricultural Science and Technology (CAST), Ames, Iowa, USA.

Creppy, E. E. (2002). Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicology letters, 127*, 19-28.

Cucullu, A. F., Lee, L. S., Mayne, R. Y., & Goldblatt, L. A. (1966). Determination of aflatoxin in individual peanuts and peanut sections. *Journal of the American Oil Chemists' Society, 43*, 89.

Debertin, D. L. (1986). Agricultural Production Economics. New York: Macmillan Publishing Company.

Decastelli, L., Lai, J., Gramaglia, M., Monaco, A., Nachtmann, C., Oldano, F., Ruffer, M., Sezian, A., & Bandirola, C. (2007). Aflatoxins occurrence in milk and feed in Northern Italy during 2004–2005. *Food Control, 18*, 1263– 1266.

Desmarchelier, A., Oberson, J. M., Tella, P., Gremaud, E., Seefelder, W., & Mottier, P. (2010). Development and comparison of two multiresidue methods for the analysis of 17 mycotoxins in cereals by liquid chromatography electrospray ionization tandem mass spectrometry. *Journal of Agricultural and Food Chemistry, 58*, 7510-7519.

Dobson, D. W., & Sweeney, M. J. (1998). Mycotoxin production by Aspergillus, Fusarium and Penicillium species. *International Journal of Food Microbiology, 43*, 141–158.

Dors, G. C., Caldas, S. S., Feddern, V., Bemvenuti, R. H., dos Santos Hackbart, H. C., de Souza, M. M., dos Santos Oliveira, M., Garda-Buffon, J., Primel, E. G., & Badiale-Furlong, E. (2011). Aflatoxins: Contamination, Analysis and Control. In: *Aflatoxins-Biochemistry and Molecular Biology*, 415-438. Guevara-Gonzalez, R. G., Eds., In Tech, Rijeka.

Edison, S. (2000). Present situation and future potential of cassava in India. In *Cassava's Potential in Asia in the 21st Century: Present Situation and Future Research and Development Needs. Proc. 6th Regional Workshop, held in Ho Chi Minh city, Vietnam* 61-70.

Edison, S., Anantharaman, M., & Srinivas, T. (2006). Status of cassava in India: An overall view, Central Tuber Crops Research Institute (Indian Council of Agricultural Research). *Technical Bulletin Series*, *46*.

Ehrlich, K. C., Chang, P. K., Yu, J., & Cotty, P. J. (2004). Aflatoxins biosynthesis cluster gene cyp A is requiered for G aflatoxin formation. *Applied Environmental Microbiology, 70*, 6518–6524.

Ejiofor, M. A. N., & Okafor, N. (1981). Comparison of pressed and unpressed cassava pulp for gari making. In *1. Triennial Root Crops Symposium of the International Society for Tropical Root Crops, Ibadan (Nigeria), 8-12 Sep 1980*. IDRC.

Ellis, W. O., Smith, J. P., Simpson, B. K., Oldham, J. H., & Scott, P. M. (1991). Aflatoxins in food: occurrence, biosynthesis, effects on organisms, detection, and methods of control. *Critical Reviews in Food Science & Nutrition, 30*(4), 403-439.

El-Nezami, H. S., Nicoletti, G., Neal, G. E., Donohue, D. C., & Ahokas, J. T. (1995). Aflatoxin M1 in human breast milk samples from Victoria, Australia and Thailand. *Food and Chemical Toxicology*, *33*, 173–179.

Ene, L. S. O. (1992). Prospects for processing and utilization of root and tuber crops in Africa. In *Proc. 4th Symposium, ISTRC–AB*. 7-15.

Espinosa-Calderón, A., Contreras-Medina, L. M., Muñoz-Huerta, R. F., Millán-Almaraz, J. R., González, R. G. G., & Torres-Pacheco, I. (2011). Methods for Detection and Quantification of Aflatoxins. In: *Detection, Measurement and Control.* 109-128. Torres-Pacheco, I., Eds., In Tech, Rijeka.

European Commission. (1998). Commission Regulation (EC) No 1525/98 of 16 July 1998, amending Regulation (EC) No 194/97 of 31 January 1997 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Communities L 201*, 43-46.

European Commission. (2001). Commission Regulation (EC) No 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Communities L., 77*, 1-13.

European Commission. (2002). Commission Regulation EC No 178/2002 – Laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. https://ec.europa.eu/food/sites/food/files/safety/docs/gfl_fitc_survey_ev_stakeholders en.pdf

European Commission. (2004). Commission Regulation (EC) No 683/2004 of 13 April 2004 amending Regulation (EC) No 466/2001 as regards aflatoxins and ochratoxin A in foods for infants and young children. *Official Journal of the European Union L., 106*, 3-5.

European Commission. (2006a). Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union L., 364*, 5-24.

European Commission (2006b). Commission Regulation (EC) No. 401/2006. *Official Journal of the European Communities, L., 70*, 12-34.

European Commission. (2010). Commission regulation (EU) No 165/2010 of 26 February 2010 amending regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. *Official Journal of the European Commission*. *L., 50*, 8–12.

European Commission. (2014). Commission regulation (EC) No 884/2014 of 13 August 2014 imposing special conditions governing the import of certain feed and food from certain third countries due to contamination risk by aflatoxins and repealing regulation (EC) No 1152/2009. *Official Journal of the European Commission. L., 242*, 4–19.

European Food Safety Authority (2007). Opinion of the scientific panel on contaminants in the food chain [CONTAM] related to the potential increase of consumer health risk by a

possible increase of the existing maximum levels for aflatoxins in almonds, hazelnuts and pistachios and derived products. *Journal of European Food Safety Authority*, *446*, 1–127.

Farombi, E. O. (2006). Aflatoxin contamination of foods in developing countries: implications for hepatocellular carcinoma and chemopreventive strategies. *African Journal of Biotechnology, 5*, 1–14.

FAO/WHO (1990). FAO/WHO Standards Programme. Codex Alimentarius Commission, Alinorm 91/29.

FAO/WHO (1992). FAO/WHO Standards Programme. Codex Alimentarius Commission, Alinorm 93/12.

Fernanda, M., De Castro, P. P. M., Pachelo, I. A., Soares, M. V., Furlant, R. P. Z., De Paula, D. C., & Bolonhezi, S. (1996). Warehouse control of *Aspergillus flavus* link and *Aspergillus parasiticus* speare on peanuts (*Arachis hypogaea*) by phosphine fumigation and its effect on aflatoxin production. *Journal of Food Protection*, *59*, 407–411.

Food and Agriculture Organization (2000). *Drought in Kenya: Food situation in deteriorating rapidly first starvation-related deaths*. Press release 00/40. http://www.fao.org/WAICENT/OIS/PRESS_NE/PRESSENG/2000/pren0040.htm. Accessed on February 4, 2017.

Food and Agriculture Organization (2004). *Worldwide Regulations for Mycotoxins in Foods and Feeds in 2003*. FAO Food and Nutrition Paper 81. Rome, Italy.

Franz, C. M., Huch, M., Mathara, J. M., Abriouel, H., Benomar, N., Reid, G., & Holzapfel, W. H. (2014). African fermented foods and probiotics. *International journal of food microbiology*, *190*, 84-96.

GB 2761-2011 (2011). National food safety standard of maximum levels of mycotoxins in foods. Beijing, China.

Ghali, R., Belouaer, I., Hdiri, S., Ghorbel, H., Maaroufi, K., & Hedilli, A. (2009). Simultaneous HPLC determination of aflatoxins B1, B2, G1 and G2 in Tunisian sorghum and pistachios. *Journal of Food Composition and Analysis, 22*(7), 751-755.

Gilbert, J., & Anklam, E. (2002). Validation of analytical methods for determining mycotoxins in foodstuffs. *TrAC Trends in Analytical Chemistry, 21*, 468-486.

Giwa, S., Abdullah, L. C., & Adam, N. M. (2010). Investigating "*egusi*" (*Citrullus colocynthis L.*) seed oil as potential biodiesel feedstock. *Energy., 3*, 607–618.

Gnonlonfin, G. J. B., Hell, K., Adjovi, Y., Fandohan, P., Koudande, D. O., Mensah, G. A., Sanni, A., & Brimer, L. (2013). A Review on Aflatoxin Contamination and Its Implications in the Developing World: A Sub- Saharan African Perspective. *Critical Reviews in Food Science and Nutrition, 53*, 349-365.

Gomes, E., Souza, S. R. D., Grandi, R. P., & Silva, R. D. (2005). Production of thermostable glucoamylase by newly isolated *Aspergillus flavus* A 1.1 and *Thermomyces lanuginosus* A 13.37. *Brazilian Journal of Microbiology, 36*(1), 75-82.

Goto, T., Wicklaw D. T., & Ito Y. (1996). Aflatoxin and cyclopiazonic acid production by a sclerotium producing starin of *Aspergillus tamarii. Applied Environmental Microbiology, 62*, 4036–4038.

Gqaleni, N., Smith, J. E., Lacey, J., & Gettinby, G. (1997). Effects of temperature, water activity and incubation time on production of aflatoxins and cyclopiazonic acid by an isolate of *Aspergillus flavus* in surface agar culture. *Appleid Environmental Microbiology, 63*, 1048–1053.

Groopman, J. D., & Donahue, K. F. (1988). Aflatoxin, A human carcinogen: determination in foods and biological samples by monoclonal antibody affinity chromatography. *Journal of Association of Official Analytical Chemists', 71*, 861.

Groopman, J. D., Cain, L. G., Kensler, T. W., & Harris, C. C. (1988). Aflatoxin exposure in human populations: measurements and relationship to cancer. *CRC Critical reviews in Toxicology*, *19*(2), 113-145.

Grove, A. T., & Rackham, O. (2003). The nature of Mediterranean Europe: an ecological history. Yale University Press.

Hahn, S. K., Reynolds, L., & Egbunike, G. N. (1988). Cassava as livestock feed in Africa. In *Proceedings of the IITA/ILCA/University of Ibadan. Workshop on the Potential Utilisation of Cassava as Livestock Feed in Africa. International Institute of Tropical Agriculture. International Livestock Centre for Africa*.

Hamilton, D. (2000). Toxic fungus threatens health of consumers: Mycotoxins in food in West Africa: current situation and possibility of controlling it. *African Journal of Biotechnology, 2*(9), 254-263.

Heathcote, J. G., & Hibbert, J. R. (1978). Aflatoxins: *Chemical and Biological Aspects*. Elsevier, New York, USA. 212.

Hell, K., Cardwell, F. K., & Poehling, H. M. (2003). Relationship between management practices, fungal infection and aflatoxin for stored maize in Benin. *Journal of Phytopathology, 151*, 690–698.

Hennigen, M. R., Valente Soares, L. M., Sanchez, S., Di Benedetto, N. M., Longhi, A., Eyherabide, G., & Zanelli, M. (2000). Fumonisin in corn hybrids grown in Argentina for two consecutive seasons. In *Proceeding of the Xth international IUPAC symposium on mycotoxins and phycotoxins*. (21-25).

Hernández-Hierro, J. M., García-Villanova, R. J., & González-Martín, I. (2008). Potential of near infrared spectroscopy for the analysis of mycotoxins applied to naturally contaminated red paprika found in the Spanish market. *Analytica Chimica Acta, 622*, 189-194.

Horn, B. W., Moore, G. G., & Carbone, I. (2009a). Sexual reproduction in *Aspergillus flavus*. *Mycologia, 101*, 423–29.

Horn, B. W., Ramirez-Prado, J. H., & Carbone, I. (2009b). The sexual state of *Aspergillus parasiticus*. *Mycologia, 101*, 275–80.

Howeler, R. H. (2000). Cassava agronomy research in Asia: Has it benefited cassava farmers. In *Cassava's Potential in Asia in the 21st Century: Present Situation and Future Research and Development Needs. Proc. 6th Regional Workshop, held in Ho Chi Minh city, Vietnam* (345-382).

Howeler, R. L., Thomas, N., Arrieta Bolaños, S., Ochoa Henríquez, J. M., Odio Echeverría, F., Moreno, V., & Boerger, J. (2013). *Save and grow: cassava. A guide to sustainable production intensificationProduire plus avec moins Ahorrar para crecer* (No. FAO 633.6828 S266). FAO, Roma (Italia).

Huang, B., Han, Z., Cai, Z., Wu, Y., & Ren, Y. (2010). Simultaneous determination of aflatoxins B1, B2, G1, G2, M1 and M2 in peanuts and their derivative products by ultra-high-performance liquid chromatography–tandem mass spectrometry. *Analytica Chimica Acta*, *662*, 62-68.

Hudson, B. J. F., & Ogunsua, A. O. (1974). Lipids of cassava tubers (*Manihot esculenta Crantz*). *Journal of the Science of Food and Agriculture*, *25*(12), 1503-1508.

International Agency for Research on Cancer (1993). Aflatoxins. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: *Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins*, 56, 245-395.

ISO 13690 (1999). International Standard. Cereals, pulses and milled products – sampling of static batches.

Ito, Y., Peterson, S. W., Wicklow, D. T., & Goto, T. (2001). *Aspergillus pseudotamarii*, a new aflatoxin producing species in *Aspergillus* section *Flavi*. *Mycological Research*, *105*, 233–239.

Iyayi, E. A., & Losel, D. M. (2001). Protein enrichment of cassava by-products through solid state fermentation by fungi. *The Journal of Food Technology in Africa*, *6*(4), 116-118.

Jackson, B. A., Adamade, C. A., Azogu, I. I., & Oni, K. C. (2013). Melon pod fermentation and its effects on physio-chemical characteristics of melon seeds. *Scientific Research and Essays*, *8*(17), 664-669.

Jonathan, S. G., Abdul-Lateef, M. B., & Ayansina, A. D. (2013). Fungal and aflatoxin detection in fresh and stored "*garri"* Ijebu (locally processed food). *Report and Opinion*, *5*, 13-9.

Josephs, R. D., Derbyshire, M., Stroka, J., Emons, H., & Anklam, E. (2004). Trichothecenes: reference materials and method validation. *Toxicology Letters*, *153,* 123-132.

Junior, C. B., & Campos, L. (2004). Identification and characterization of a novel cassava (Manihot esculenta Crantz) clone with high free sugar content and novel starch. *Plant Molecular Biology*, *56*(4), 643-659.

Kademi, H. I. (2016). Investigating the occurrence of *Vibrio parahaemolyticus* in various seafood consumed in Turkish Republic of Northern Cyprus. Master's thesis, Near East University.

Khayoon, W. S., Saad, B., Yan, C. B., Hashim, N. H., Ali, A. S. M., Salleh, M. I., & Salleh, B. (2010). Determination of aflatoxins in animal feeds by HPLC with multifunctional column clean-up. *Food Chemistry, 118*, 882-886.

Kos, J., Mastilović, J., Hajnal, E. J., & Šarić, B. (2013). Natural occurrence of aflatoxins in maize harvested in Serbia during 2009–2012. *Food Control*, *34*(1), 31-34.

Krska, R., Schubert-Ullrich, P., Molinelli, A., Sulyok, M., MacDonald, S., & Crews, C. (2008). Mycotoxin analysis: An update. *Food Additives and Contaminants A, 25*, 152-163.

Kulshrestha, R., Gupta, C. P., Shukla, G., Kundu, M. C., Bhatnagar, S. P., & Katiyar, C. K. (2008). The effect of water activity and storage temperature on the growth of *Aspergillus flavus* in Medicinal herbs. *Planta Medica*, *74*, 1308-1315.

Kurtzman, C. P., Horn, B. W., & Hesseltine, C. (1987). *A. nominus*, a new aflatoxin producing species related to *A. flavus* and *A. tamarii*. Antonie van Leeuwenhoek *53*, 147–158.

Kushwaha, H. L., Srivastava, A. P., & Singh, H. (2005). Development and performance evaluation of an okra seed extractor.

Lahai, B. N. (1991). An evaluation of the level of female participation in cassava and sweet potato on-farm trials and demonstrations in Sierra Leone. In *Symposium on Tropical Root Crops in a Developing Economy 380* (55-61).

Lanyasunya, T. P., Wamae, L. W., Musa, H. H., Olowofeso, O., & Lokwaleput, I. K. (2005). The risk of mycotoxins contamination of dairy feed and milk on small holder dairy farms in Kenya. *Pakistan Journal of Nutrition, 4*, 162–169.

Lebot, V. (2009). *Tropical root and tuber crops: cassava, sweet potato, yams and aroids* (No. 17). CABI.

Leslie, J. F., Bandyopadhyay, R., & Visconti, A. (Eds.). (2008). *Mycotoxins: detection methods, management, public health and agricultural trade*. CABI.

Lozovaya, V. V. (1998). β-1,3-Glucanase and resistance to *Aspergillus flavus* infection in maize. *Crop Science, 38*, 1255–1260.

Magan, N., & Aldred, D. (2007). Post-harvest control strategies: Minimizing mycotoxins in the food chain. *International Journal of Food Microbiology, 119*, 131–139.

Măruţoiu, C., Puiu, S., Moise, M. I. Soran, L., Măruţoiu, O. F., & Boboş, L. (2004). Optimization of the separation of some aflatoxins by thin-layer chromatography. *JPC-Journal of Planar Chromatography, 17*, 372-374.

Minto, R. E., & Townsend, C. A. (1997). Enzymology and molecular biology of aflatoxin biosynthesis. *Chemical Reviews, 97*, 2537–2555.

Molina, M., & Giannuzzi, L. (2002). Modelling of aflatoxin production by *Aspergillus parasiticus* in a solid medium at different temperatures, pH and propionic acid concentrations. *Food Research International, 35*, 585–594.

Mora, M., & Lacey, J. (1997). Handling and aflatoxin contamination of white maize in Costa Rica. *Mycopathologia, 138*, 77–89.

Moreno, R. A. (1992). Recent developments in cassava agronomy. *Roots, tubers, plantains and bananas in animal feeding. FAO Animal Production and Health Paper*, (95).

Nakai, V. K., de Oliveira Rocha, L., Gonçalez, E., Fonseca, H., Ortega, E. M. M., & Corrêa, B. (2008). Distribution of fungi and aflatoxins in a stored peanut variety. *Food Chemistry, 106*, 285-290.

Nassar, N. M., & Sousa, M. V. (2007). Amino acid profile in cassava and its interspecific hybrid. *Genetics and Molecular Research, 6*(2), 192-197.

Ngiki, Y. U., Igwebuike, J. U., & Moruppa, S. M. (2014). Utilisation of cassava products for poultry feeding: A review. *The International Journal of Science and Technology, 2*(6), 48.

Njapau, H. (Ed.). (2006). *Mycotoxins and Phycotoxins: Advances in Determination, Toxicology, and Exposure Management: Proceedings of the XIth International IUPAC Symposium on Mycotoxins and Phycotoxins*, May 17-21, 2004, Bethesda, Maryland, USA. Wageningen Academic Pub.

Northolt, M. D., van Egmond, H. P., & Paulsch, W. E. (1977). Differences in *Aspergillus flavus* strains in growth and aflatoxin B1 production in relation to water activity and temperature. *Journal of Food Protection, 40*, 778–781.

Omafuvbe, B. O. (1998). Evacuation of the microbiological and biochemical properties of two cowpea (*Vigna unguiculata*) varieties. *Plant Foods for Human Nutrition, 53*, 321-332.

Onwueme, I. C. (1978). *The tropical tuber crops: yams, cassava, sweet potato, and cocoyams*. John Wiley and Sons.

Osundare, B. (2007). Effects of different interplanted legumes with cassava on major soil nutrients, weed biomass, and performance of cassava (*Manihot esculenta Crantz*) in the Southwestern Nigeria. *ASSET: An International Journal (Series A), 7*(1), 216-227.

Otsuki, T., Wilson, J. S., & Sewadeh, M. (2001). Saving two in a billion: Quantifying the trade effect of European food safety standards on African export. *Food Policy, 26*, 495–514.

Owusu-Darko, P. G., Paterson, A., & Omenyo, E. L. (2014). Cocoyam (corms and cormels)—An underexploited food and feed resource. *Journal of Agricultural Chemistry and Environment*, *3*(01), 22.

Oyenuga, V. A., & Fetuga. B. L. (1975). Some aspects of the bio- chemistry and nutritive value of the watermelon seed (*Citrullus vulgaris*, Schrad). *Journal of the Science of Food and Agriculture, 26,* 843.

Palmgren, M. S., & Hayes A. W. (1987). Aflatoxins in food. In Mycotoxins in Food, Palle Kroghs, Ed., Academic Press, London, 65-96.

Peterson, S. W., Ito, Y., Horn, B. W., & Goto, T. (2001). *Aspergillus bombycis*, a new aflatoxigenic species and genetic variation in its sibling species, *Aspergillus nomius*. *Mycologia*, *93*, 689–703.

Picinin, L. C. A., Cerqueira, M. M. O. P., Vargas, E. A., Lana, A. M. Q., Toaldo, I. M., & Bordignon-Luiz, M. T. (2013). Influence of climate conditions on aflatoxin M1 contamination in raw milk from Minas Gerais State, Brazil. *Food Control*, *31*, 419-424.

Piperno, D. R. (2011). The origins of plant cultivation and domestication in the New World tropics: patterns, process, and new developments. *Current anthropology*, *52*(4), 453-470.

Pitt, J. I. (2000). Toxigenic fungi and mycotoxins. *British Medical Bulletin, 56,* 184-192.

Pitt, R. E. (1993). A descriptive model of mold growth and aflatoxin formation as affected by environmental conditions. *Journal of Food Protection, 56,* 139–146.

Pomeranz, Y., & Meloan, C. E. (1987). *Food Analysis Theory and practice*, An Avi Book. London, England.

Pons, W. A., & Franz, A. O. (1977). High performance liquid chromatography of aflatoxins in cotton products, *Jornal of Association of Official Analytical Chemists', 60*, 89.

Prandini, A., Tansini, G., Sigolo, S., Filippi, L., Laporta, M., & Piva, G. (2009). On the occurrence of aflatoxin M1 in milk and dairy products. *Food and Chemical Toxicology, 47,* 984-991.

Promthong, S., Kanto, U., Tirawattanawanich, C., Tongyai, S., Isariyodom, S., Markvichitr, K., & Engkagul, A. (2005). Comparison of nutrient compositions and carbohydrate fractions of corn, cassava chip and cassava pellet ingredients: Animals. In *Proceedings of 43rd Kasetsart University Annual Conference,* Thailand.

Quinto, M., Spadaccino, G., Palermo, C., & Centonze, D. (2009). Determination of aflatoxins in cereal flours by solid-phase micro extraction coupled with liquid chromatography and post-column photochemical derivatization-fluorescence detection. *Journal of Chromatography A, 1216,* 8636-8641.

Rachaputi, N. R., Wright G. C., & Kroschi, S. (2002). Management practices to minimise pre-harvest aflatoxin contamination in Australian groundnuts. *Australian Journal of Experimental Agriculture, 42*, 595-605.

Rahimi, E., Bonyadian, M., Rafei, M., & Kazemeini, H. R. (2010). Occurrence of aflatoxin M1 in raw milk of five dairy species in Ahvaz, Iran. *Food and Chemical Toxicology, 48,* 129–131.

Ramos, A. J., & Hernández, E. (1996). In-situ absorption of aflatoxins in rat small intestine. *Mycopathologia, 134*(1), 27-30.

Rapid Alert System for Food and Feed (2012). Notification details. Rapid alert system for food and feed. European Commission, Health and Food Safety. Annual Report. Retrieved on 5th February, 2017. Available at. https://ec.europa.eu/food/sites/food/files/safety/docs/rasff_annual_report_2012_en.pdf

Reiter, E. V., Vouk, F., Böhm, J., & Razzazi-Fazeli, E. (2010). Aflatoxins in rice - A limited survey of products marketed in Austria. *Food Control, 21*, 988-991.

Roze, L. V., Arthur, A. E., Hong, S. J., Chanda, A., & Linz, J. E. (2007). The initiation and pattern of spread of histone H4 acetylation parallel the order of transcriptional activation of genes in the aflatoxin cluster. *Molecular Microbiology, 66*, 713–726.

Saleh, A., Abdullahi, I. O., & Olonitola, O. S. (2016). Determination of aflatoxin contamination in cassava flour sold in selected markets in Zaria, Kaduna state; Nigeria. *Bayero Journal of Pure and Applied Sciences*, *9*(2), 145-147.

Sanchez, A., Fuller, A. B., Yahiku, P. Y., & Baldsin, M. V. (1972). Supplementary value of blackeyed peas, peanuts and egusi seed on the typical West African diet of plant origin. *Nutritional Reports International, 6*, 171.

SANCO. (2009). Method validation and quality control procedures for pesticide residue analysis in food and feed. Document No. SANCO/10684/2009, Implemented by 01/01/2010.

Sassahara, M., Netto, D. P., & Yanaka, E. K. (2005). Aflatoxin occurrence in foodstuff supplied to dairy cattle and aflatoxin M1 in raw milk in the North of Parana state. *Food and Chemical Toxicology, 43*, 981-984.

Scholten, J. M., & Spanjer, M. C. (1996). Determination of aflatoxin B1 in pistachio kernels and shells. *Journal of Association of Official Analytical Chemists', 79*, 1360-1364.

Scott, G. J. (2000). Roots and tubers in the global food system: *a vision statement to the year 2020*. Report to the Technical Advisory Committee (TAC) of the Consultative Group on International Agricultural Research (CGIAR). IITA.

Set, E., & Erkmen, O. (2010). The aflatoxin contamination of ground red pepper and pistachio nuts sold in Turkey. *Food and chemical Toxicology, 48*, 2532-2537.

Sheibani, A., & Ghaziaskar, H. S. (2009). Pressurized fluid extraction for quantitative recovery of aflatoxins B1 and B2 from pistachio. *Food Control, 20*, 124-128.

Shephard, G. S. (2006). Mycotoxins in the context of food risks and nutrition issues. Barug, D., Bhatnagar, D. Van Egmond, HP, Van Der Kamp, JW, Van Osenbruggen, WA und

A. Visconti (Editors): *The Mycotoxin Factbook: Food and Feed Topics.* Wageningen Academic Publishers, 21-36.

Shephard, G. S. (2009). Aflatoxin analysis at the beginning of the twenty-first century. *Analytical and Bioanalytical Chemistry, 395,* 1215-1224.

Shouman, B. O., El-Morsi, D., Shabaan, S., Abdel-Hamid, A. H., & Mehrim, A. (2012). Aflatoxin B1 Level in Relation to Child's Feeding and Growth. *Indian Journal of Pediatrics, 79,* 56-61.

Smith, J. E., & Moss, M. O. (1985). *Mycotoxins. Formation, analysis and significance*. John Wiley & Sons Ltd.

Somorin, Y., Akinyemi, A., Bertuzzi, T., & Pietri, A. (2016). Co-occurrence of aflatoxins, ochratoxin A and citrinin in "*egusi*" melon (*Colocynthis citrullus L.*) seeds consumed in Ireland and the United Kingdom. *Food Additives & Contaminants: Part B*, *9*(3), 230-235.

Speijers, G. J. A., & Speijers, M. H. M. (2004). Combined toxic effects of mycotoxins. *Toxicology Letters, 153,* 91–98.

Srinivas, T. (2009). Economics of sweet potato production and marketing. In *The Sweet potato* (235-267). Springer Netherlands.

Stoloff, L. (1972). Analytical methods for mycotoxins, *Clinical Toxicology, 5,* 465.

Stroka, J., & Anklam, E. (2000). Development of a simplified densitometer for the determination of aflatoxins by thin-layer chromatography. *Jouranl of Chromatography A, 904,* 263-268.

Tavva, S., & Nedunchezhiyan, M. (2012). Global status of sweet potato cultivation. *Fruit, Vegetable and Cereal Science and Biotechnology, 6*(1), 143-147.

Thiel, P. G. (1986). HPLC determination of aflatoxin and mammalian aflatoxin metabolites, in Mycotoxins and Phycotoxins, Steyn, P. S. and Vleggaar, R., Eds., Elsevier, New York, 329.

Thuvander, A., Moller, T., Barbieri, H. E., Jansson, A., Salomonsson, A. C., & Olsen, M. (2001). Dietary intake of some important mycotoxins by the Swedish population. *Food Additives and Contaminants, 18,* 696-706.

Tran-Dinh, N., Pitt, J. I., & Carter, D. A. (1999). Molecular genotype analysis of natural toxigenic and nontoxigenic isolates of *A. flavus and A. parasiticus. Mycological Research, 103*, 1485–90.

Tripathi, S., & Mishra, H. N. (2011). Modeling and optimization of enzymatic degradation of aflatoxin B (AFB) in red chili powder using response surface methodology. *Food and Bioprocess Technology, 4*, 770-780.

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

Turner, P. C., Sylla, A., Diallo, M. S., Castegnaro, J. J., Hall, A. J., & Wild, C. P. (2002). The role of aflatoxins and hepatis viruses in the etiopathogenesis of hepatocellular carcinoma: A basis for primary prevention in Guinea-Conakry, West Africa. *Journal of Gastroenterology and Hepatology, 17*, 5441-5448.

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

U.S. Food and Drug Administration. (1996). Sec. 527. 400 Whole milk, low fat milk, skim milk- aflatoxin M1 (CPG 7106.210). FDA compliance policy guides. FDA, Washington, DC, 219.

Umoh, I. B., & Oke, O. L. (1974). Nutritive value of some lesser known oilseeds in rats. *Nutrition Reports International, 9,* 453.

Uzogara, S. G., Agu, L. N., & Uzogara, E. O. (1990). A review of traditional fermented foods, condiments and beverages in Nigeria: Their benefits and possible problems. *Ecology of food and nutrition, 24*(4), 267-288

Var, I., Kabak, B., & Gök, F. (2007). Survey of aflatoxin B1 in helva, a traditional Turkish food, by TLC. *Food Control*, *18*, 59-62.

Villordon, A. Q., Ginzberg, I., & Firon, N. (2014). Root architecture and root and tuber crop productivity. *Trends in plant science*, *19*(7), 419-425.

Vlavonou, B. M. (1988). Cassava Processing Technologies in Africa. *In Praise of Cassava, Proceedings of the Interregional Experts' Group Meeting on the Exchange of Technologies for Cassava Processing Equipment and Food Products*, IITA, Ibadan, Nigeria. Apr 13–19, 1988; Hahn, N.D., Ed.; UNICEF House: New York, 19–25.

Wilson, D. M., Mubatanhema, W., & Jurjevic, Z. (2002). Biology and ecology of mycotoxigenic *Aspergillus* species as related to economic and health concerns. *Advances in Experimental Medicine and Biology*, *504*, 3–17.

Wilson, W. M., & Dufour, D. L. (2002). Why "Bitter" Cassava? Productivity of "Bitter" and "Sweet" Cassava in a Tukanoan Indian Settlement in the Northwest Amazon 1. *Economic Botany*, *56*(1), 49-57.

Xie, L., Chen, M., & Ying, Y. (2016). Development of Methods for Determination of Aflatoxins. *Critical reviews in food science and nutrition*, *56*(16), 2642-2664.

Yabe, K., & Nakajima, H., (2004). Enzyme reactions and genes in aflatoxin biosynthesis. *Applied Microbiology and Biotechnology*, *64*, 745–755.

Yu, J. (2003). Genetics and biochemistry of mycotoxins synthesis. In: Handbook of fungal biotechnology (2nd Edition). Arora, D.K. Ed., New York, Marcel Dekker, In press.

Yu, J., Chang, P. K., Cary, J. W., Wright, M., Bhatnagar, D., Cleveland, T. E., Payne, G. A., & Linz, J. (1995). Comparative mapping of aflatoxin pathway gene clusters in *Aspergillus parasiticus* and *Aspergillus flavus. Applied Environmental Microbiology*, *61*, 2365–2371.

Yusuf, O., Sanni, A., Ojuekaiye, E. O., & Ugbabe, O. O. (2008). Profitability of "*egusi*" Melon (*Citrullus lanatus Thunb*. Mansf). Production under sole and mixed cropping

Systems in Kogi State, Nigeria. *ARPN Journal of Agricultural and Biological Sciences, 3,* 14-18.

Zain, M. E. (2010). Impact of mycotoxins on humans and animals. *Journal of Saudi Chemical Society, 15*, 1–16.

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

APPENDICES

APPENDIX 1

AFLATOXINS MAXIMUM TOLERABLE LIMITS BY EU

Table 1.1: The current European Union legislative limits (FAO/WHO, 1990:1992; Otsuki et al., 2001).

	Aflatoxins µg/kg		
Product type	**B1**	**Total aflatoxins (B1+B2+G1+G2)**	**M1**
Groundnuts, nuts, dried fruit, and processed products thereof for direct human consumption or as a food ingredient	2	4	
Groundnuts to be subjected to sorting or other physical treatment, before human consumption or as a food ingredient	8	15	
Nuts and dried fruit to be subjected to sorting or other physical treatment, before human consumption or as a food ingredient	5	10	
Cereals and processed products thereof for direct human consumption or as a food ingredient	2	4	
Chilies, chili powder, cayenne pepper, paprika, white and black pepper, nutmeg, ginger, and turmeric	5	10	
Milk (raw milk, milk for the manufacture of milk-based products and heat-treated milk)			0.05

APPENDIX 2

FAO/WHO JOINT EXPERT COMMITTEE LIMITS ON AFLATOXINS

Table 2.1: Aflatoxin legislations set by the Joint FAO/WHO Expert Committee (FAO/WHO, 1990:1992; Otsuki et al., 2001).

Aflatoxin	Tolerance level (µg/kg)	Food/feed
B1	0.5	Feed for dairy cattle
M1	0.05	Milk
B1+G1+B2+G2	15	Raw peanut for human consumption

APPENDIX 3

CHEMICAL AND PHYSICAL PROPERTIES OF AFLATOXINS

Table 3.1: Chemical and physical properties of aflatoxins (Asi et al., 2012).

AF	Formula	Molecular weight	Melting point (º C)	Optical rotation $[\alpha]_D$ ($CHCl_3$)
B1	$C_{17}H_{12}O_6$	312	268-269	-558º
G1	$C_{17}H_{12}O_7$	328	247-250	-556º
B2	$C_{17}H_{14}O_6$	314	287-289	430º
G2	$C_{17}H_{14}O_7$	330	230	454º
M1	$C_{17}H_{12}O_7$	328	299	280º