UNIVERSITY FOR DEVELOPMENT STUDIES

FACULTY OF BIOSCIENCES

DEPARTMENT OF BIOTECHNOLOGY

THE POTENTIAL OF NATURAL ALKALINE SOURCES IN THE DETOXIFICATION OF AFLATOXIN-CONTAMINATED GROUNDNUT AND MAIZE

BY

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THESIS SUBMITTED TO THE DEPARTMENT OF BIOTECHNOLOGY, FACULTY OF BIOSCIENCES, UNIVERSITY FOR DEVELOPMENT STUDIES, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN BIOTECHNOLOGY AND MOLECULAR BIOLOGY

AUGUST, 2021

DECLARATION

Candidate's declaration

I hereby declare that this thesis is the result of my original work and that no part of it has been presented for another degree in the university or elsewhere. Research works that were consulted have been duly cited

Agbayiza Ben …….. ……...
(Student) Sign Date

Supervisors Certification

We certify that this study was carried out under our supervision in accordance with the guidelines on supervision of thesis laid down by the University for Development Studies**.**

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ABSTRACT

Groundnut and maize are part of staple crops consumed mostly in Ghana but gets contaminated easily by aflatoxin. Consumption of aflatoxin contaminated food causes several adverse health effects including liver cancer. Several detoxification methods and techniques have been employed to reduce aflatoxin levels in groundnut but these have not been fully effective. The study was carried out to assess the potential of saltpetre, whitewash and wood ash in detoxifying aflatoxin-contaminated groundnut and maize. Two separate experiments were carried out in this study. The first experiment was conducted in a completely randomized design with the concentrations of 0%, 1%, 5%, and 10% (w/v) and soaking time of 12 h, 18 h, and 24 h. The second experiment was factorial in a completely randomized design with the concentrations of 0%, 1%, 5%, and 10% (w/v), cooking times of 5, 10, 15 minute, and steeping time of 0 h, 6 h, 12 h. All experiments were replicated three times. Total aflatoxin level was determined using Rapid Test Kit for a Quantitative Test with Mobile Diagnostic Reader (Mobile Assay Inc., Boulder, CO). Alkaline concentration and soaking time significantly (p = 0.002) affected aflatoxin levels in both maize and groundnut. The result revealed that 5% saltpetre solution could reduce aflatoxin level by 89% in groundnut and 90% in maize while wood ash solution resulted in 28% total aflatoxin reduction in groundnut. In maize however, whitewash and wood ash solutions were able to cause a total aflatoxin reduction of 94% and 91% respectively. Consumer sensory analysis carried on the final product resulted in the overall acceptability of texture, colour, taste and aroma by the consumers. Saltpetre (5%, 10% (w/v)) was able to detoxify aflatoxin and maintained the proximate and sensorial quality in groundnut suggesting its potential to be used as aflatoxin decontamination agent in groundnuts. It is recommended that further assessment of the effects of the natural lime on nutrient bioavailability and toxicity in humans should be carried out.

DEDICATION

I dedicate this work to the almighty God, my parents, siblings, supervisors, researchers and scientists working hard to overcome the issue of aflatoxin.

ACKNOWLEDGEMENT

I thank God for his grace, protection and guidance over my life throughout the study which has made this dream possible.

My profound gratitude and appreciations go to my supervisors, Dr. Nelson Opoku and Dr. James Owusu-Kwateng. I also want to thank USAID for providing me with test kits for my analysis. I am grateful to Dr. Joseph X. Kugbe for the support he offered me.

Getting this far will not have been possible without the financial and spiritual support of my father, the late Mr. Kwame Agbayiza and my mother Mary Agbayiza.

I am also highly indebted to Selina Apana, Musah Sualah Haris, Aminu Shaibu and all staff of the Faculty of Agriculture, particularly the department of biotechnology for their support. Your assistance was key to the successful completion of this research.

May God bless you all.

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LIST OF ACRONYMS

%	Percentage
AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AOAC	Association of Official Analytical Collaboration
Ca(OH)2	Calcium hydroxide
CaO2	Calcium Oxide
EU	European Union
G	Gram
HCL	Hydrochloric Acid
Nejayote	Wastewater of alkaline cooking process
Nixtamalization	Alkaline cooking
^{O} C	Degree Celsius
Ppb	Part per billion
SP	Saltpetre
UDS	University for development Studies
USAID	United States Agency for International Development
W/V	Weight by Volume
WHO	World Health Organization

WW	Whitewash
Ml	Microlitres

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Mycotoxins are a structurally varied group of compounds having small molecular weight, which are primarily produced by fungi or moulds under suitable conditions. They are produced on a wide range of foodstuff which is hazardous to humans and animals. Fungi are highly adaptable organisms that can metabolize different types of substrates over a wide range of environmental conditions and produce mycotoxins under aerobic conditions (Daou, 2021; Stanaszek-Tomal, 2020). Mycotoxins are commonly found in arid, humid, and temperate regions. They are known to contaminate food and feed causing diseases in both humans and animals (Bennett, 2003; Haque et al., 2020). A wide range of food commodity especially cereals and legumes can be contaminated with mycotoxins at post-harvest stages (Council for Agricultural Science, USA, 2003; Patriarca & Pinto, 2017). Aflatoxin is a notable mycotoxin that contaminates most crops and when consumed by humans could cause serious health problems (Tinham, 2000; Winter & Pereg, 2019).

The issue of aflatoxin is a serious problem that has become more important due to its implication in crop production, food quality, and human and animal health. Since the recognition of aflatoxin as a significant worldwide problem in 1960 (Strosnider *et al*., 2006; Asao *et al*., 1965), researchers have studied a number of ways to fight it, but the battle lingers on because humans are unable to manipulate effectively, essential factors that influence its production and contamination of agricultural produce and products (Strosnider *et al*., 2006). Aflatoxins are most commonly ingested, but the most toxic form, aflatoxin, B_1, can permeate the skin (Boonen *et al*., 2012). Aflatoxins affect almost everything that is eaten be it cereals, legumes (Atongbiik *et al*., 2017), cassava,

nuts, dry fruits, (Paster, 2008), spices (Mekalar *et al*., 2011), wines, milk (Products & Mohammadi, 2001), and chocolates (Copetti *et al*., 2012).

According to the FAO (2011), 25% of the world's food crops production is lost as a result of aflatoxin contamination (Proctor *et al*., 2004). High levels of aflatoxin-contaminated foods consumption have been linked with the incidence of certain cancers that are very deadly in humans (Dhanasekaran & Shanmugapriya, 2011) and are considered first-class carcinogens in humans (International Agency for Research on Cancer, 2002).

In Ghana, the most common staple food consumed include groundnut and maize and these are the crops mostly affected by aflatoxin contamination. Several studies have focused on looking at ways to reduce pre and or post-harvest aflatoxin contamination in groundnut and maize. Controlling aflatoxin in groundnuts is very difficult as compared to maize due to the nature of the groundnut. Groundnut seed has high oil contents coupled with fat making it difficult for treatment when infected with aflatoxin. Alkaline cooking has been reported to cause a significant reduction of aflatoxin in maize up to about 90% (Moureen, 2020). This is prominent and if successfully employed in groundnuts could help reduce aflatoxin contamination in groundnuts. Alkaline cooking which is mostly referred to as nixtamalization is a traditional process of cooking and steeping cereals, particularly maize (Owusu-kwarteng & Akabanda, 2013) with alkaline solution for consumption and other purposes. Nixtamalization is an alkaline cooking process original employed by the people of Mexico which is applied in corn tortillas and has been effective in the destruction of aflatoxin in corn. It consists of the cooking of the grain in abundant water and lime (2–3 L of water/kg of maize processed, with 1–3% $CaOH_2$) at boiling temperatures for 20–70 min, with a steeping period of 8–16 h. After the steeping, the lime cooking solution (nejayote) is decanted,

and the grain is thoroughly washed to leave the grain ready for making further products (Méndez-Albores *et al*., 2004). It has been shown that traditional nixtamalization is capable of destroying 85% of the aflatoxin present in maize (Razzaghi-Abyaneh, 2013). During the nixtamalization process, the diffusion of water and calcium into the corn kernel is one of the most important processes and produce important physicochemical changes in the pericarp, endosperm, and germen (Bressani *et al*., 1990). Although nixtamalization is popular practice for processing maize in Mexico and other southern American countries, the process is less known to Ghanaians (Sefa-Dedeh *et al*., 2004). Considering its demonstrated ability to reduce aflatoxins in maize (Razzaghi-Abyaneh, 2013), the process of nixtamalization may find useful application in reducing aflatoxins in staple crops such as maize and groundnuts in Ghana and other tropical countries.

1.2 Problem statement and justification

Aflatoxin exposure is particularly problematic in low-income populations in the tropics that consume relatively large quantities of staples, particularly maize and groundnuts. The best-documented health impact of chronic exposure to aflatoxins is liver cancer. It has been reported that 4.5 billion of the world population are exposed to aflatoxin (Williams *et al*., 2004; Alshannaq *et al*, 2018). It is also estimated that 26,000 Africans living south of the Sahara die annually of liver cancer associated with aflatoxin exposure (Wild, 2009). Broader health effects such as immune suppression with higher rates of illness and child stunting have also been associated with aflatoxin exposure (Owaga, Muga, Mumbo, & Aila, 2011). Khlangwiset *et al*., (2011) summarized an epidemiological study that showed an association between child growth impairment and aflatoxin exposure. Studies have indicated that aflatoxin M1 in mothers' breast

milk was associated with reduced length and weight of infants at birth (Khlangwiset *et al*., 2011).

The two main food commodity that are easily susceptible to aflatoxins contamination are groundnuts and maize. Consequently, the increased populations and food insecurity in most African countries makes people more exposed to the health problems associated with aflatoxin contamination of food. The issue gets worsen because there is lack of dietary diversity so many people heavily rely on the staples maize and groundnuts which makes them more likely to aflatoxins exposure. One of the largest and most recent occurrences of severe aflatoxin poisoning was in Kenya where 125 people died whiles 317 were ill as a results of consuming extremely high aflatoxin contaminated maize (Fellow, 2011).

It is recommended that aflatoxin-contaminated food such as maize and groundnut and their products has to be discarded by burning or burying to avoid human or animal exposure (WHO, 2018; Tinham, 2000). However, this is not possible especially in Sub Saharan African countries such as Ghana where food security is at stake. In Ghana for example, there is evidence that farmers after sorting of their groundnuts sell the good nuts and consume the bad nuts which have high possibility of aflatoxin contamination (SPRING, 2017). The relatively bad nuts are also sold at very low prices and are mixed with good ones for the preparation of groundnut-based food products such as groundnut paste and ‘‘kulikuli’’ among others (De *et al*., 2013). In some cases, heavily contaminated aflatoxin groundnuts are used to prepare locally Ghanaian food additive known as ‘‘Dawa Dawa’’ and this food additive is used by most Ghanaians especially in the Northern region to prepare food for consumption (Abdul-Majeed, n.d). However, due to the heat stable characteristics of aflatoxin, cooking aflatoxin contaminated maize and groundnut with or without food additive have been reported to have little effect on

aflatoxin especially in groundnut. After the preparation of aflatoxin contaminated food product, aflatoxin still remain in the final product for human consumption. Notwithstanding these menaces, reducing aflatoxin concentration is paramount to help curb the issue of aflatoxin exposure.

1.3 Objective

1.3.1 Main Objective

The main objective of this study was to assess the detoxification potential of natural alkaline sources in reducing aflatoxin levels in groundnut and maize.

1.3.2 Specific Objectives

1. To assess the detoxification potential of different alkaline (saltpetre, whitewash, and wood ash) treatments in aflatoxin contaminated groundnuts and maize.
2. To determine the effect of alkaline treatment on the proximate composition of groundnuts.
3. To assess consumer's acceptability of the alkaline treated (nixtamalized) groundnuts.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview

Aflatoxin is a serious disease coursing toxic secondary metabolite produced mainly by *Aspergillus parasiticus* and *Aspergillus flavus* in food commodities such as maize grain, groundnut, cowpea, millet etc. and even animal feed (Jalili, 2015). Due to its contamination and infestation of numerous food items, there has been a significant effect on crop losses resulting in an economic recession in most countries (Kabak, 2006; Jalili, 2015). Mycotoxin can cause a lot of toxic effects including chronic diseases in humans and animals, heart disease, and even abortion in some farm animals (Zain, 2011). For all the known mycotoxins, aflatoxins B_1, B_2, G_1, and G_2 are the ones that frequently occur in food and feed (Tinham, 2000). These mycotoxins are highly dangerous to animal and human health due to their toxic, carcinogenic, teratogenic, hepatogenic and mutagenic nature in food and feed (Pal, *et al*., 2015). The issue of aflatoxin gets worsen when it was found out that even hepatitis B and C carriers can develop liver cancer when exposed to aflatoxin (Chu *et al*., 2018). The complexity encountered when dealing with aflatoxin has ever remained a challenge as it is colourless, odourless (Lalah, *et al* 2019), and tasteless, which can be consumed when found in food items without knowing. A lot of studies have focused on the methods of reducing aflatoxin and its exposure for years (Guzmán-Ortiz, & Ramírez-Wong, 2001; Hwang & Lee, 2006; Moreno-Pedraza *et al*., 2015; Torres & Kirui, 2016) . Some of these methods of detoxification include physical, chemical and biological methods (Kumar, 2018). Among the chemical methods, nixtamalization (alkaline cooking) has been reported to be effective in reducing aflatoxin concentration drastically to about 90% in maize (Temba *et al*., 2016; Torres *et al*., 2001). The case is different for

groundnut in the sense that, nixtamalization technology has not been reported in reducing aflatoxin levels in groundnut. A study conducted by Takaba (1994) saw no aflatoxin degradation when acidic potassium nitrate was used to detoxify raw aflatoxin. A study conducted by Temba *et al.* (2016) shows that reformation of aflatoxin could occur in an acidic medium which might have contributed to the failure of the detoxification process exhibited in the findings of (Tabata *et al*.,1994).

Nixtamalization is a technology that describes the olden food processing methods developed by indigenous Mesoamericans (Titcomb *et al*., 2020) which is used recently in reducing aflatoxin in most cereals that mostly involves alkaline cooking of maize kernels. Traditionally, lime can be used to cook the maize which is followed by steeping at room temperature overnight. During this process, the absorption of calcium and pH enhances the softening of the endosperm and the release of the pericarp (Argun *et al.*, 2016; Schaarschmidt & Fauhl-Hassek, 2019) exposing possible mycotoxin that might be present. During nixtamalization, aflatoxin can be impacted in different ways including physical removal during the steeping and washing, degradation, modification or released by high pH (Schaarschmidt & Fauhl-Hassek, 2019). The gap in the research is the fact that the nixtamalization process and other methods used in reducing aflatoxin concentration in legumes and cereals have not been fully harnessed especially in groundnut.

2.2 History and taxonomy of mycotoxin producing fungi *Aspergillus*

Aspergillus was formally described in 1729 where its name was given as a result of its resembling asexual spore-forming structure to the *aspergillum* (Scazzocchio, 2019). *Aspergillus* species are morphologically identical and difficult to differentiate (Silva *et al*., 2011). Their genomic variation is in parity with the phylum Vertebrata (Rokas,

2013), and can be present in a wide range of different environmental conditions because their survival depends on the availability of water or moisture (Seyedmousavi *et al*., 2015; Paulussen *et al*., 2017; Richardson & Rautemaa-Richardson, 2019). Due to these characteristics, they can infect most food commodities with the available moisture contents and has resulted in difficulties in controlling them.

2.3 Morphological features of *Aspergillus* genus

There are different varieties of the genus *Aspergillus* which include *Aspergillus A. niger, A. parasiticus, A. flavus* etc. (Hedayati*et al*., 2007; Amaike & Keller, 2011) which all share similar morphological characteristics. Some are filamentous fungi that form filaments called hyphae (García-Reyes *et al*., 2017). They consist of colourless and smooth spores, mycelia and conidiophores (Scazzocchio, 2019). The colourless nature of *Aspergillus* makes them difficult to even recognize when they are found in food commodities. Other genera are saprophytic found in soil that mostly infects and contaminates agricultural produce (Silva *et al*., 2011). Other morphological feature includes the formation of stipe with grey around the apex**,** *conidia*, *vesicle***,** *metal***,** and *phialide* (García-Reyes *et al*., 2017).

2.4 Factors promoting the growth of mycotoxin producing fungi

The growth of fungi species has become a great issue of concern because of the wide range of commodities they can grow on. These factors according to Opoku *et al*., (2018), can be extrinsic or intrinsic which indicate that there are both internal and external factors that work together to contribute to the growth and development of fungi. Some of these factors include minimum and maximum daily temperature, the genotype of the crop planted, the soil type, the dairy net evaporation, and the climate

of the region (Strosnider *et al*., 2006; Negash, 2018). In addition, the contamination of aflatoxin is also promoted by improper drying of the crop before storage, the heavy rains during and after harvest, insect activity, poor timing of harvest, and stress or damage to the crop (Lizárraga-paulín, *et al*., 2011).

The favourable environmental or weather conditions found in subtropical and tropical countries makes it very easy for crops grown in these areas to be more prone to aflatoxin contamination than those in the temperate zones (Dhanasekaran *et al*., 2011). Cereals and legumes have a high risk of aflatoxin contamination (Atongbiik *et al*., 2017). Groundnuts and groundnut meal are by far the two agricultural commodities that seem to have the highest risk of aflatoxin contamination. The combination of fungi growth, the host and the environment contribute to the manifestation of the aflatoxin. Also, other factors that enhance the growth of aflatoxin includes high-temperature stress, poor fertility, weed competition, high crop densities and water stress of the host plant (Dhanasekaran *et al*., 2011).

2.4.1 Climate condition

Soil moisture and temperature plays a very crucial role in the case of influencing the activity of the microorganisms in the soil (Moretti *et al.*, 2018). Environmental factors such as changes in pH, temperature, drought conditions or periods of waterlogging may contribute to the production of aflatoxin during the pre and post-harvest stage (Medina *et al*., 2015).

To be able to ascertain the effect of climate on aflatoxin contamination of food commodities is a very complex and integrated approach (Cotty & Jaime-garcia, 2007). Climate change partially affects the growth of aflatoxin producing fungi in the sense that as there is a change in the climate, the complex community of toxin-producing

fungi also gets affected which involves a change in the number of aflatoxins producing fungi (Shekhar *et al*., 2018). The unstable climate also contributes to the manifestation of insects that create wounds which predisposes the host plant to aflatoxin producing fungi (Cotty & Jaime-garcia, 2007). It has been argued that aflatoxin contamination in temperate areas could be high during drought (Fouché, 2020). This might have contributed to the reason why higher levels of aflatoxins are recorded in crops cultivated in most developing countries including Ghana. Although small scale field tests have been carried out by researchers to determine the influence of climate on the overall total crop contamination but such conclusions may even be wrong. This is because other agronomic practices such as insect control, and irrigation might even eliminate contamination (Cotty & Jaime-garcia, 2007).

2.4.2 Temperature

The effect of temperature on aflatoxin production is certain. Aflatoxin is very stable at high temperatures. According to Shi (2016), a temperature of 150 ^{0}C of dry heating is required to initiate the decomposition of aflatoxin in corn grain. The study further indicated that during wet heating for 1 hour at a temperature of 80 ^{0}C, 73% of aflatoxin B_1 was decomposed. The decomposition of the aflatoxin B_1 was possible through the hydrolysis of furofuran moiety and the lactone ring along with further decarboxylation (Shi, 2016). However, concerning normal or conventional cooking or heating where there is no addition of additives or dry heating, aflatoxin B_1 could reach up to a melting temperature of 260 ^{0}C with a decomposition temperature of 269 ^{0}C (Samarajeewa *et al*., 1990). Furthermore, temperatures ranging from 237 to 306°C have been studied to require the decomposition of aflatoxin (Pankaj *et al*., 2017; Kabak, 2009). The deactivation of aflatoxins by temperature is affected by its immediate environment or

what it finds itself. This makes it very difficult to easily detoxify food commodities contaminated with aflatoxin by conventional heating because the temperature requires for decomposition will render most food products to lose their nutrient components. Also, most apparatus used for cooking in our various homes cannot withstand these high temperatures and even if they withstand, by the time the food item is cooked up to that temperature most of them might have bent into ashes.

2.4.3 Type of soil

The type of soil in which a particular crop is cultivated is crucial when it comes to the contamination of agricultural produce. Crops cultivated in different soil types have a different levels of aflatoxin contamination and occurrence (Opoku *et al*., 2018a). Under dry condition, there is an enhanced fungi growth in light sandy soil as compared to heavier soil (Codex, 2004). Thus, there is less contamination as a result of increased water retention capacity. Zhang*et al*. (2017), estimated that the distribution and growth of aflatoxin producing fungi in soil worldwide are influenced by soil water retention, geographical regions and soil type. The soil type in Ghana was found to be sandy, clay, and loamy soil (Braimoh & Vlek, 2004). These soil types can be found in different geographical locations (Fearon, 2000). For instance, the soil types found in the Eastern part of Ghana where most cereal and legumes are grown are fairly different from those in the western part. Most soils where cereals and legumes are grown in Ghana mostly varies from clayey loamy to loamy soils which have been reported to support the occurrence and growth of mycotoxin producing fungi (Winter & Pereg, 2019). This could add up to the reason why there is a high level of aflatoxin contamination in most crops cultivated in these soil types. This was true as it was observed by Opoku *et al*.,

(2018) that most cereal-legume blends commodities were found to be contaminated with aflatoxin beyond the acceptable limit in Ghana.

Soil moisture also contributes significantly to the growth and contamination of mycotoxin specifically aflatoxin. Soil moisture content during the pod development stage of most legumes determines aflatoxin content in it at harvest (Chalwe *et al*., 2019). During drought season there is excessive force impose on the pod and seed coat which can be weakened by high soil moisture to predispose the host plant for aflatoxin infestation. (Opoku *et al*., 2018a).

2.5 Pathogenic and mycotoxin producing *Aspergillus* species

Table 2.1: Some selected *Aspergillus* and the type of mycotoxin they produce

***Aspergillus* species**	**Mycotoxin**
Aspergillus flavus, Aspergillus parasiticus	*Aflatoxin*
Aspergillus flavus	*Aflatrem*
Aspergillus ustus	*Austdiol*
Aspergillus ustus	*Brevianamide*
Aspergillus terreus	*Citreoviridin*
Aspergillus clavatus	*Cytochalasin E*
Aspergillus versicolor	*Cyclopiazonic acid*
Aspergillus ochraceus	*Destruxin B*
Aspergillus ochraceus, penicillium viridictum	*Ochratoxin*
Aspergillus niger	*Oxalic acid*

Aspergillus ochraceus	*Penicillic acid*
Aspergillus flavus	*Sterigmatocystin*
Aspergillus fumigates	*Viriditoxin*
Aspergillus ochraceus	*Destruxin B*
Aspergillus fumigates	*Fumagilin*

Source: (Blumenthal, 2004; Azziz *et al*., 2005; Hedayati *et al*., 2007;)

2.6 Molecular Structure of the various aflatoxin types

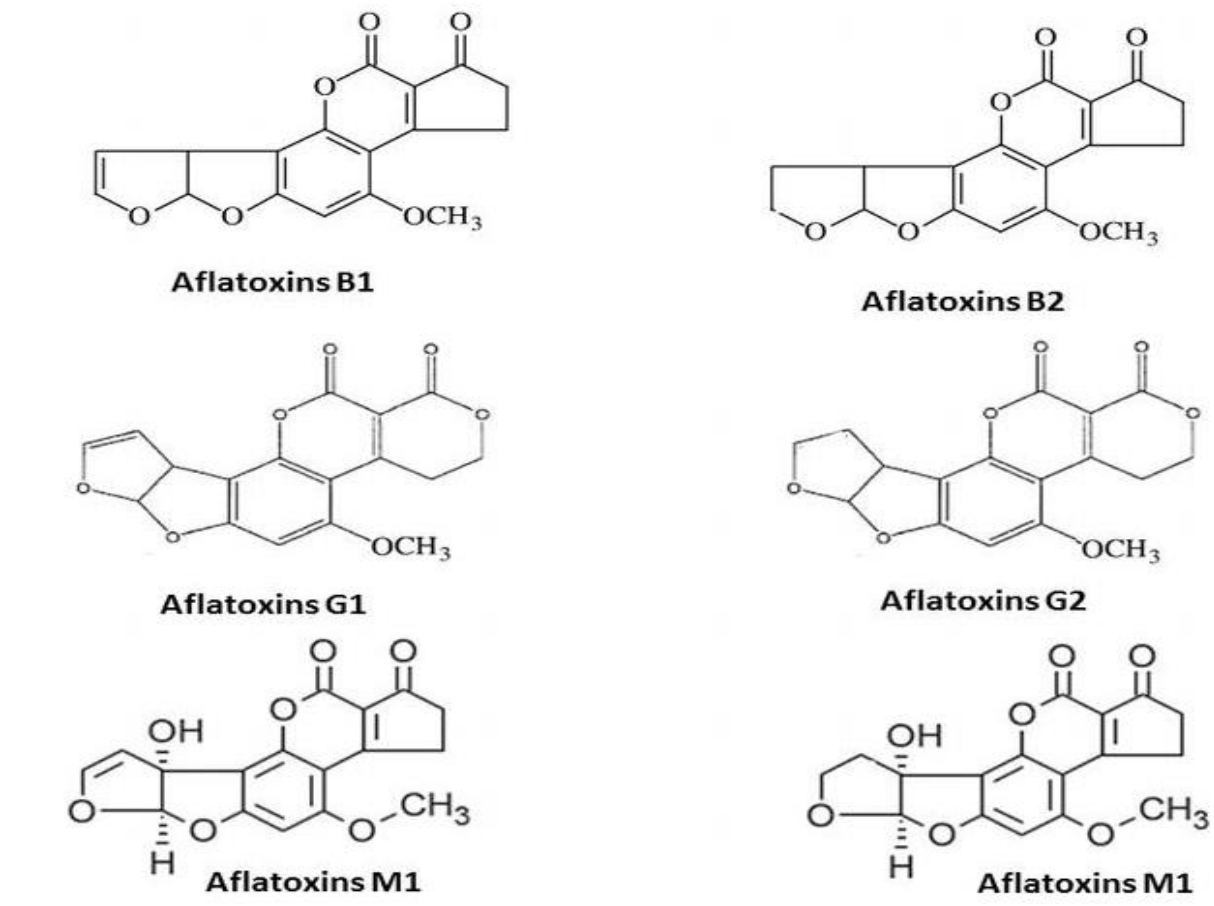

Figure 2. 1: Molecular Structure of the various aflatoxin types

Source: (Dhanasekaran *et al*., 2011; Saleem *et al*., 2017)

2.7 Aflatoxin contamination in maize

Maize (*Zea mays* L) is one of the most cultivated and consumed crops in Africa and particularly Ghana. It nonetheless also happens to be susceptible to aflatoxin contamination (Atongbiik *et al*., 2017; Hoffmann, 2018). In Ghana, Maize production is not limited to particular geographical locations since the commodity does well in almost all the agro-ecological regions (Agyare *et al*., 2014). Maize is consumed in nearly every part of the continent. In the years 2016 and 2017 alone, more than 1000 million tons of maize commodity was produced (Ramírez-jiménez *et al*., 2018). It is therefore not surprising that more than half of the total volume of food consumed in Mexico is maize (Carmen, 2015). One of the most common sources of aflatoxins is contaminated maize and its by-products (Negash, 2018a). According to Carroll (2018), aflatoxin contamination in maize results in the yellow-green or grey mold colouration of the grain, husk or its stem. It is worth mentioning that the presence of mold in food substances including maize reduces the taste of the food by altering its flavour and physical composition (Negash, 2018a). Crops such as maize are usually infected with aflatoxins even before their harvest (Peles *et al*., 2019). Aflatoxin contamination in grains which was initially assumed to be largely a storage problem was recently found not to be so always. Some findings have revealed that aflatoxins were found present in corn on the field at various levels of its growth, precisely at the late milk stage until its harvest (Anderson, 2010). Maize can be infested simultaneously with both *Aflatoxin flavus* and *Fusaria* which produces fumonisin. But this is not usually the case with groundnuts (ShepHard *et al*., 2013).

2.8 Aflatoxin contamination in groundnut

Groundnut also called peanut, earthnut or monkey-nut (*Arachis hypogea* L) is a very important source of oilseed crop on the globe. It is the third oilseed crop mostly

cultivated globally and has played a vital role in the economic development of some African countries (KepHe *et al*., 2020). In Ghana, more than 90% of the groundnuts are produced in the Northern part of the country (Agbetiameh *et al*., 2018). It is also estimated that about 80% of Ghanaians consume groundnuts in some form (either roasted, boiled, processed paste etc.) at least once a week (Hoffmann, 2018). The Groundnut kernel contains about 40-50% fat, 20-50% protein and 10-20% carbohydrates (Guchi, 2015b). With groundnuts, it is quite easy to identify the highly contaminated ones. They are mostly characterized by their small size, shriveled nature, moldiness and colour alteration from the regular ones (Jalili, 2015). The synchronic occurrence of extremely high soil temperature coupled with stress from the late seasonal drought has been identified as two conditions that easily causes the contamination of groundnut seeds with aflatoxin even whiles on the field (Mamadou, 2013). Also, insect and mechanical damage to the groundnut pod can as well expose it to *Aspergillus* invasion, and then subsequent contamination by aflatoxin (Guchi, 2015a). Many countries have now established legal limits for aflatoxin levels allowed in foods, specifically groundnuts intended for human consumption, to safeguard the lives of their people (Guchi, 2015a). However, some study shows that about 60 to 85% of consumers from undeveloped parts of the world are not adequately protected and informed about commercial food safety regulations (Article & Sugri, 2020). According to Chen *et al*. (2013), higher aflatoxins levels which are above the regulatory limit are more likely to be identified in the processed groundnut than in its unprocessed form. Furthermore, a research finding has shown that the reason for the higher aflatoxin concentration in processed groundnuts compared to its unprocessed form is likely to be due to the milling process involved (Clifford, 2020). This is because the increased surface area created by the milling process easily exposes the groundnuts to oxygen and

molds (Clifford, 2020). Aflatoxins B1, B2, G1, and G2 is mostly associated with groundnuts, pulses, and other agricultural products (Yeboah & Jk, 2020). Aflatoxin-producing fungi A. *flavus* are found across the groundnut-growing regions, and they can manufacture aflatoxin in groundnuts whenever conditions are appropriate for fungal growth (Sserumaga *et al*., 2020a). Insects and mites in the soil may encourage infection and subsequent aflatoxin production before groundnuts are dug during periods of drought. The growth of A. *flavus* in groundnuts is aided by prolonged periods of hot, rainy weather, insufficient drying after harvest, and inadequate protection from moisture during temporary storage and transportation (Jordan *et al*., 2018). Moisture condensation on roofs and sidewalls could be a source of A. *flavus* growth (Codex Alimentarius Commission, 2006). Treating aflatoxin-contaminated groundnut with alkaline medium containing calcium has been proven to be difficult. The groundnut grains contain some nutritional and antinutritional factors which prevents the penetration of calcium in to the grain to allow for aflatoxin detoxification. Phytate however is believed to have the ability to bind to calcium and prevent or interfere with its absorption (Titcomb *et al*., 2020). The nature of the groundnut grains also contributes to the interference of the detoxification process as compared to maize.

2.9 The incidence of aflatoxin in humans, animals and the economy

The occurrence of aflatoxin in food and crop is alarming in many parts of the world declining public health and development. It is a highly cancer-causing fungal metabolite known to cause immune system suppression, growth and mental retardation in children (Countries *et al*., 2007; ShepHard, 2008), and even low birth weight (Lombard, 2014), depending on the level of the exposure. It has been estimated that over 4.5 billion people in developing countries are at high risk of aflatoxin exposure

(Williams, 2004) which may result in liver cancer. This could be as a result of many people getting in contact with aflatoxin-contaminated foods. The persistence and the stable nature of aflatoxin make it very difficult to detoxify in food or feed. It can bioaccumulate in the tissue and cell of the human and animals for a longer period while incorporating its toxicity in the body and block the action of some enzymes (Feddern *et al*., 2013; Peles *et al*., 2019). Aflatoxin contamination poses a dangerous risk to the agricultural industry with moderate to high levels of aflatoxin causing morbidity for both humans and livestock (Negash, 2018b). The issue of aflatoxin ranges from pre-harvest to finished or the end product, accompanied by environmental conditions (Torres *et al*., 2014). About 25% of the world's crops are affected by mycotoxins of which aflatoxin are the major cause of these losses (Tinham, 2000; Eskola *et al*., 2019). Due to this, there have been adverse economic effects which include lower yields for food and fibre crops in most countries across the globe (Zahra *et al*., 2014). Aflatoxin is the most dominant problem regarding the quality of groundnut worldwide (N'dede *et al*., 2012), causing serious production losses, loss of export markets and rejection of produce at import ports (Njoroge, 2018; Wu, 2015). The cost and the losses incurred as a result of aflatoxin exposure could result in a serious economic recession contributing to a high rate of hunger as reported by many researchers, especially in developing countries (Unnevehr et al., n.d.).

Aflatoxin contributes to a lot of health concern issues to both humans and animals due to its wide spread in food commodities. This happens as the majority of the toxin are found in food staff. Aflatoxin b1 and to a lesser extent G1 are responsible for the biological potency of aflatoxin-contaminated foods (Lizárraga-paulín *et al*., 2011). Aflatoxin B_2 and G_2 are biologically inactive unless they are metabolized and oxidized into B1 and G1 in vivo (Verma, 2004). Aflatoxin impairs growth and is

immunosuppressive in farm animals (Dhama & Singh, 2014). Hepatocellular carcinoma and an increase in nutritional deficiencies are all caused by Aflatoxin exposure (Wambui *et al*., 2017). Aflatoxin can easily be injected into the gastrointestinal tract due to their small molecular weight (Yunus *et al*, 2011). The effect of aflatoxins in humans and animals have been grouped into two which include acute aflatoxicosis and chronic aflatoxicosis (Bbosa *et al*., 2013). Acute aflatoxicosis is produced when moderate to high levels of aflatoxins are consumed while chronic aflatoxicosis occurs as a result of ingestion of low to moderate levels of aflatoxins (Bbosa *et al*., 2013).

2.10 Aflatoxin impact across the groundnut value chain

The fungi that produce aflatoxin could right away contaminate crops during the pre-harvest stage due to bad agronomic practices (Sserumaga *et al*, 2020b). In some cases, during farming season preharvest activities including weeding, spraying etc. could result in the plants getting wounded making it easy for fungi infection (Muqit *et al*, 2016). This when not handled or dealt with but coupled with poor post-harvest activities such as poor storage conditions, could result in aflatoxin contamination (Pretari, & Tian, 2019). This could be transported into the groundnut food chain where they are used to feed animals and processing factories. Product made from these animals and factories gets contaminated by aflatoxin which eventually goes back to the final consumers. In Ghana, due to the high levels of aflatoxin in some groundnut, there has always been a high rate of export rejection (Agbetiameh *et al*., 2018) where those aflatoxin-contaminated groundnut find their way back into the local market for consumers to buy. This may pose a lot of people at risk in terms of aflatoxin exposure which could result in a global burden (Figure 2.2). It has been reported that about 5

billion people are at risk of aflatoxin exposures (Pandey *et al*., 2019). And out of these about 25% of liver cases have been linked to aflatoxin exposure (Pandey *et al*., 2019).

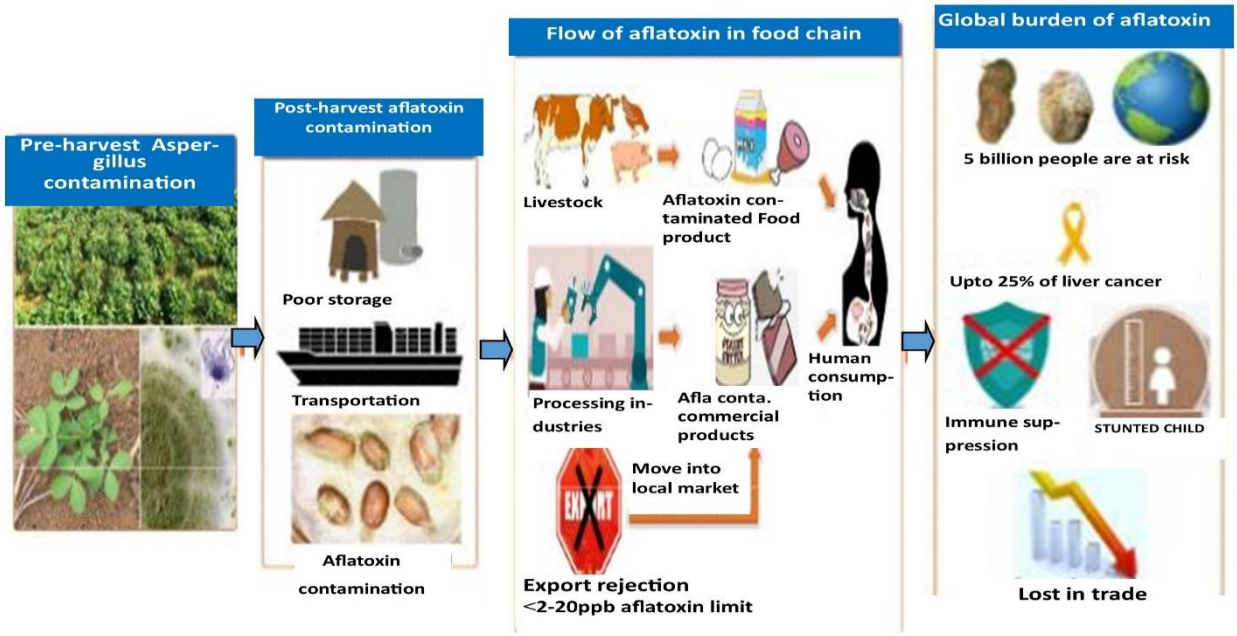

Figure 2. 2: Aflatoxin and impact justification across the groundnut value chain

Source: (Pandey *et al*., 2019)

2.11 Physicochemical property of aflatoxin

Aflatoxins have been indicated to dissolve in a polar solvent including dimethyl sulfoxide chloroform, methanol, and also partially dissolve in water (Feddern *et al*., 2013; Wacoo *et al*., 2014). They fluoresce under UV radiation (Karki *et al*., 2011). The lactone ring opens during the process of alkaline hydrolysis contributing to the detoxification of its molecular structure from food commodities (Agag, 2003). They are tasteless, colourless, and odourless, and have low molecular weight.

2.12 Regulatory limits for aflatoxin in human and animal foods

Owing to the higher prevalence of aflatoxin in food and feed staff, many countries have set a regulatory limit for consumable food and feeds (Table 2.2).

Table 2.2: Regulatory limit for aflatoxin

Country	**Food Category**	**Aflatoxin Limit (ppb)**
Indonesia	Nuts, Maize, Spices	15
Malaysia	Groundnut	15
PHilippines	Shelled corn (Feed grade) Nut for processing	50 15
Singapore	All foods except food for infants or young children Food for infants and young children	5 0.1
Vietnam	Groundnuts and oil seeds used for raw materials	8
EU	Human food	4
USA	Human food	20
Ghana	Human Food Shelled nut	10 15
Argentina	Infant food	20
Australia	Nuts and nuts product	15
Bahamas	All foods and grains	20
Canada	Nut and nut products Animal feedstuffs	15 20
Colombia	Cereals Oil seeds and groundnut	30 10
Cote D'Ivoire	Complete feed stuff for pigs and poultry	38
Egypt	Groundnut, oilseed, cereals and their product	10
Kenya	Groundnut and its products and vegetable oil	20

Malawi	Groundnut for export	5

Source: (Mazumder & Sasmal, 2001; Van Egmond & Jonker, 2004; GSA, 2021)

2.13 Mycotoxin exposure and detection

Humans and animals can be exposed to mycotoxin through contamination of other seeds with untraced fungi cereal grains. There are five broad groups of mycotoxins including, fumonisin, aflatoxin, ochratoxin A, vomitoxin, and zearalenone (Chhonker *et al*., 2018). The frequent occurrence of aflatoxin contamination has been observed in groundnuts, maize, fruits, and the rest (Afsah-Hejri *et al*., 2013; Monyo *et al*., 2012; Mutegi *et al*., 2009). The occurrence of ochratoxin contamination has been studied in cereals wine and coffee, whiles traces of fumonisin are reported in maize and maize made products (Chhonker *et al*., 2018). To determine the concentration of mycotoxins, there are a lot of chromatographic methods that have been used notable being HPLC to detect mycotoxins concentration in plasma (Chhonker *et al*., 2018). Aflatoxins are metabolized by hepatic enzymes and through the process generate reactive epoxide species which can form a covalent bond with guanine (Wild, 2002). Recently, several detection methods have been developed including the use of rapid test kits for total aflatoxin for food and feeds.

2.14 Methods of reducing aflatoxin in legumes and cereals

Legumes and cereals are the crops been affected by aflatoxin mostly especially maize and groundnut. Several methods have been proposed to have been effective in reducing aflatoxin in food commodities such as cereals and legumes. These methods can be broadly categorised into three which include a chemical method, physical method, and biological methods (Wu *et al*., 2009; Samarajeewa *et al*., 1990). All these methods focus on destroying, modifying or absorbing the toxin.

2.14.1 Biological method

The biological method of controlling aflatoxin has been studied in numerous agricultural products such as groundnut, cotton and corn (Yin *et al*., 2008). This method included the use of biological agents such as bacteria, yeast, enzyme, and fungi, as competitors for the containment of aflatoxin producing fungi growth and toxin production (Yin *et al*., 2008). A lot of bacterial species such as *Bacillus subtilis*, *Lactobacilli spp*., and *Ralstonia spp*. have indicated the ability to prevent fungal growth and production of aflatoxins by *Aspergillus* spp. in laboratory experiments (Yin *et al*., 2008). Saprophytic yeast species *Candida krusei* and *Pichia anomala* have proved potent as an agent to control aflatoxin producing fungi *A. flavus* (Niknejad *et al*., 2012). A. flavus, consists of toxigenic strains that produce a lot of aflatoxins and also atoxigenic strains which cannot produce aflatoxin (Probst *et al*., 2011). During the extrusion competitive process, the atogenic strains are introduced to out-compete and eliminate toxigenic from colonizing grains where by reducing the level of aflatoxin production in contaminated grains (Udomkun *et al*., 2017).

However, the mechanism involving the interference or the competition of non-aflatoxigenic strain with aflatoxin buildup of toxigenic strains has not been conclusively clarified (Ehrlich *et al*., 2015). The biological method sometimes involves the use of enzymes (Mishra & Das, 2003). These enzymes may react with other nutrients or components in the food to produce toxic or compound not suitable for human or animal consumption. Also, with the application of the biological method, microorganisms are used. These microbes can produce metabolites when found in food and feed which can bring health concern issues (Roager & Dragsted, 2019). The application of some of these enzymes and microbes to reduce aflatoxin requires an

expert to achieve the desired results. The cost involves in buying enzymes or extracting microbes are sometimes unbearable and many people cannot afford it.

2.14.2 Physical method

Aflatoxins are very stable under several conditions encountered during food storage, handling, and processing (Tian & Chun, 2017) However, detoxification of aflatoxin is required for food already contaminated with aflatoxin. Methods including extrusion cooking, magnetic carbon, and other absorbents have been studied to have proven to reduce the level of aflatoxin especially in cereals (Peng *et al*., 2018) especially in maize but have been difficult in groundnut. Although these various methods have been described for the detoxification of aflatoxins in foods, they usually result in a high cost and complex processes, and many also result in nutrient loss and food safety issues (Tian & Chun, 2017). Post-harvest drying, adequate storage, shelling, dehulling, product sorting, early harvest, cleaning, and insect control have also been found to reduced aflatoxin concentration (Aidoo, 2016; Matumba *et al*., 2015; Hell *et al*., 2008). However, before the crop will even finish maturing and store under good storage conditions, fungi infestation can occur at preharvest resulting in aflatoxin production which could eventually cause a significant production loss (Waliyar *et al*., 2008).

2.14.3 Chemical method

The chemical method has also been proved to reduce aflatoxin in food. Insecticides and fungicides were the first chemicals used in controlling aflatoxin producing fungi and insects. This was however followed by the use of food additives, chemical reagents such as citric acid and lactic acid (Martı, 2008), hydrogen peroxide, (Elias-Orozco *et al*., 2002) and ozone gas (Nantua *et al*., 2013). Often time, the use of insecticides to spray especially crops has gained little attention due to the high toxic remains they

generate (WHO, 2008). Chemicals applied to control aflatoxin often leaves carcinogenic and mutagenic residues (Li *et al*., 2020, Sarma *et al*., 2017) and are also expensive due to the cost of some of these chemicals which hiders their application and usage. It also does not completely deactivate or detoxify the toxin been produced by mycotoxin producing fungi (Li *et al*., 2020). Moreover, some of these chemicals may not be safe for human consumption when used to treat food commodities.

Nixtamalization which involves alkaline cooking has to be one of the most recently studied methods (Santiago-ramos *et al*., 2018) of reducing aflatoxin in cereals and legumes because of their effectiveness. Some studies have classified the nixtamalization technology under the physical method of reducing aflatoxin but in this review, it was classified under the chemical method due to the suggested mechanisms involved in the detoxification of aflatoxin by this technology. Although the mechanism involves in the detoxification process of aflatoxin by alkaline treatment has not been fully addressed but has been proposed to involve the opening of the lactone ring by alkaline hydrolysis resulting in a soluble water salt accompanied by decarboxylation (Temba *et al*., 2016). After the opening of the ring, reformation could occur, but in an acidic medium (Torres, *et al*., 2001; Temba *et al*., 2016). During the process of nixtamalization, the combination of heat and alkaline medium allows for the hydrolysis of the lactone ring of the aflatoxin to render it inactive.

According to Mao *et al*. (2016), using strong alkaline to chemically detoxify mycotoxin is effective and also a chemical measure. The combination of alkaline which is a base with a mycotoxin to detoxify it involves a chemical reaction (Karlovsky *et al*., 2016). During the nixtamalization process, a lot of chemical changes occur which contribute to the improvement of physicochemical properties of the nixtamal (Ménera-López *et al*., 2013). Food grade lime which has several names including pickling lime, builders

lime, hydrated lime, cal, and slack lime (Gopakumar & Treatment, 2017)**,** used in the nixtamalization process was termed as a food additive (Galvan-Ruiz *et al*., 2007) which undergo a chemical reaction making nixtamalization a chemical process. Although the application of heat was found to be ineffective in the reduction of aflatoxin (Karaca & Nas, 2008) because of the high temperature required for its degradation, nixtamalization on the other hand involve the use of mild heat and for that matter less temperature. It has been observed that a temperature of 30 to 40 ^{0}C during the traditional nixtamalization process was able to reduce the level of AFB_1 and AFM_1 by 94% and 90% in corn respectively (Elias-Orozco *et al*., 2002). At this temperature, most food items remain their nutritional contents and even improve as a result of the nixtamalization process. A study conducted by Owusu-kwarteng & Akabanda, (2013), indicated that the nixtamalization process was able to improve the crude protein and ash contents of millet dough from 11.8 to 15.2 and 1.8 to 2.5 respectively. There was also improvement in the physicochemical properties such as texture, colour, aroma, taste during the process of nixtamalization (Toro-Vazquez & Gómez-Aldapa, 2001). Due to the presence of calcium in the nixtamalization process, food commodities such as maize and millet treated with this nixtamalization technology have a higher level of calcium content (Bressani *et al*., 2002) which is beneficial to plant, animals and even humans for strong bone formation (Galvan-Ruiz *et al*., 2007). According to Galvan-Ruiz *et al*. (2007), the ideal intake of calcium varies from 400 to 1,500 mg/day and can be consumed safely up to 2,000 mg/day.

2.15 Origin of alkaline cooking (nixtamalization)

Nixtamalization originated in Mexico several years ago where maize was domesticated and supported life around villages (Guzm & Studies, 2016). As a result, this old process

is part of the culture of the Mexican passing from generation to generation. The product obtained from nixtamalization was soft corn dough which can be used to prepare a cake. The corn tortilla is a Mexican staple food that provides 38.8% of protein, 49.1% of calcium and 45.2% of calories (Guzm & Studies, 2016). There are four basic steps to follow in the traditional nixtamalization process which include; the boiling of the maize in lime and water, soaking of the mixture overnight, washing of the soaked maize, and finally grind the nixtamal to obtain dough which can be used to prepare several products like a *tortilla chip, tamales, tostadas, tacos, sopes, masa*, etc. (Carmen, 2015). Nixtamalization refers to the removal of pericarp from any grains using an alkaline process (Boniface & Gladys, 2011). The fundamental process starts by cooking the whole grain in water and lime and steep for 6-16hrs in a tank. The steeped grain is called nixtamal (Boniface & Gladys, 2011) and the cooked steep liquid rich in maize solid is called *nejayote* (Valderrama-Bravo *et al*., 2013). During the cooking period, there are a lot of physical and physical changes that occur in the grains (Owusu-Kwarteng & Akabanda, 2013). The kernels soften and their pericarp loosens causing the plant cell wall to become soluble, the grain becomes hydrated and absorb the alkali used from the cooking solution, while starch also swells and gelatinize and disperse into the liquid (Boniface & Gladys, 2011).

2.16 The nixtamalization (alkaline cooking) technology

The technology of nixtamalization (Lime cooking) has been found to have a significant effect on grains generated from this technology. Apart from the outer pericarp softening, surface material also dissolves partially which enhances the removal of the pericarp during washing. The diffusion of calcium ions in the pericarp, endocarp and germ during the nixtamalization process is governed by cooking time, steeping time,

temperature, the initial level of calcium ion and water content (Argun, & Guzm 2016). A lot of germs are reserved during the nixtamalization process and contribute to the overall nutritional composition of the product. Boiling in lime causes the removal of starch granules so that the soft endosperm is greatly altered, the starch arrangement becomes irregular, and some fibrils connect the dispersed starch granules where protein digestion occur and is gelatinized (Carmen, 2015). The role of lime is very crucial as it contributes to the rapid uptake and distribution throughout the grain and modifies the outer layer so that the pericarp fraction becomes gummy and sticky (Topete-Betancourt *et al*., 2019). The colour and intensity of nixtamalized products are related to carotenoid pigments, flavonoids, and PH (Andre, 2013). The development of colour during nixtamalization is very complex as alkaline reacts with different pigments. Flavour is enhanced by a reaction occurring between reducing sugars, peptides, and unsaturated fatty acids (Carmen, 2015).

2.17 Considering legume for nixtamalization

Legumes and cereals food products are part of the staple food crops consumed mostly in the world especially in African countries such as Ghana (Kaminski, Koroma, Iafrate, Division, & Division, 2013). This is as a result of consumers now been choosy in ready to eat food to avoid time wastage in food preparation. They have high protein content, fat, vitamin and other minerals which are obtained from an animal source (Erbersdobler, Barth, & Jahreis, 2017). Minerals and vitamins obtained from an animal could be replaced with those obtained from most legumes and cereals which could be expensive to purchase from an animal source (MapHosa & Jideani, 2017). The consumption and utilization of these crops sometimes are limited due to the infestation and the presence of toxic compounds (Kachapulula et al., 2017). Several methods have

been proposed to treat most of these leguminous crops to reduce some of the contaminants or compounds that pose risk to the consumption of these crops but did not avail (Sipos *et al*., 2021). Nixtamalization has been proven to be promising mostly in the treatment of cereals for the reduction of toxic (Schaarschmidt & Fauhl-Hassek, 2019) and other compounds not needed by the human body. This technology when incorporated into the treatment of leguminous crops could also have a significant contribution in terms of reduction of some of the toxic compounds present in it. Moreover, the nixtamalization technology has been studied to affect improving the nutritional composition (Morales & Zepeda, 2017) of the product being nixtamalized. There is no doubt that these technologies could improve the nutritional content of most of the leguminous crops consumed through the process of these technologies.

2.18 Types of nixtamalization methods

Generally, every food item being it cereals or legumes can be nixtamalized depending on the method or the process used. The sample to be used for the nixtamalization are mostly first prepared before the process in several ways including sorting, screening, washing drying, and storing. Cereals are mostly clean to remove debris and any other unwanted materials. For consumption purposes, the product to be nixtamalized passes through all these processes to enhance the hygienic condition of the final product.

2.18.1 Classic nixtamalization

The classic nixtamalization was one of the old methods used to process maize by using wood ash and has been agued to develop first before the subsequent methods (Escalante-aburto *et al*., 2019). The use of ash to cook or prepare corn for tortilla or masa production was predominant in the ancient days among the people of Mexico

before the introduction of lime (Mariscal *et al*., 2015). Different types of ashes made from different plant species could be used for the process with a level ranging from 2 to 25g. The replacement of the use of wood ash with that of lime was a result of the efficiency of the lime in terms of pericarp removal. Lime remove the seed coat of corn grain mere efficiently and provide a better texture of the masa and tortillas (Escalante-aburto *et al*., 2019). Moreover, the use of wood ash (Odukoya *et al*., 2021) was known to have wasting of a lot of water, delay the processing time, and creating polluted residues (Escalante-aburto *et al*., 2019). Notwithstanding this, it is important to point out the nutritional advantages of the classic nixtamalization since tortillas produced by this method contain a higher amount of functional components (Schaarschmidt & Fauhl-Hassek, 2019). In an experiment conducted by Maureen *et al*. (2020), 1% of ash solution was used to cook maize contaminated with aflatoxin which increases ash and niacin content and also reduces aflatoxin contents by 90%. Ash contents of the nixtamalized maize increase as a result of the intake of ash. It has been reported that wood ash contains micronutrients including iron, cupper, magnesium, potassium, and phosphorous (Jansone *et al.*, 2020) which are essential for humans and animals. The incorporation of some of these nutrients into the nixtamal product for human consumption through nixtamalization could help improve the intake of some of these nutrients. The increase in niacin in the nixtamal could also help prevents pellagra (Arif *et al*., 2018) when consumed. However, several studies have shown a general decrease in the protein contents of the classical nixtamalization process with an enhanced protein quality (Maureen *et al*., 2020). The decrease in the protein contents could be a result of cross-linking, protein degradation, and denaturation which may lead to reduce protein digestibility (Heck *et al*., 2013). Dry matter reduce in the process of nixtamalization based on the type of cooking method employed, the amount of heat supply, and the

leaching (Chang, 1987). Generally speaking, heat coupled with alkaline medium enhances easily penetration of the cooking medium into the grain thereby dissolving the contents of the grains to be leached into the medium. The use of wood ash solution in preparing maize to make masa or tortilla causes the pericarp or the seed coat to be removed at a minimal rate into the medium forming a solution known as nejayote (Pliego-Arreaga *et al*., 2013). The minimal removal of the pericarp and seed coat (Godwin *et al*., 2017) helps in maintaining dry matter of the grain and contribute to the increment of the fibre and starch content of the nixtamalized product. Moreover, the usage of ash in classical nixtamalization contribute significantly to the improvement in other minerals. A study conducted by Pappa, de Palomo, & Bressani (2010), found that wood ash used in classical nixtamalization provides more iron zinc, magnesium, and potassium as compared to lime processing. Mariscal Moreno *et al*. (2015), also observed a similar trend in a study conducted to evaluate tortillas made from different nixtamalization processes where they observed a higher value of 544.5mg/100g 907.70mg/100g of ash in classical nixtamalization for iron and magnesium as compared to traditional nixtamalization with the values of 4.3mg/100g, 138.3mg/100g for iron and magnesium respectively. It can be established that products/tortillas produced by classical nixtamalization have high iron content and therefore when consumed could increase the intake of iron in the body to help in blood and brain formation in humans. The use of ash in classical nixtamalization in the production of tortillas chips has been found to reduce acrylamide contents in the tortillas (Topete-Betancourt *et al*., 2019a). This was as a result of the cation presents in the wood ash used in the classical nixtamalization process (Rodrı & Morales-sa, 2019). Several studies (Mariscal Moreno *et al*., 2015; Santiago-Ramos *et al*., 2015; Mariscal-Moreno *et al*., 2017; Topete-Betancourt *et al*., 2019) have found that classical nixtamalization increases protein

content as compared to traditional and ecological nixtamalization due to several cations present in the wood ash used in the process.

2.18.2 Traditional nixtamalization

Traditional nixtamalization has been studied to have developed from a long time ago which is widely studied and known (Escalante-aburto *et al*., 2019). It is used by Mexican society to process a large quantity of maize for consumption. The process of traditional nixtamalization includes the addition of lime, rye or soda to achieve corn grain dehulling to obtain masa and tortilla (Santiago-Ramos, *et al*., 2018). In most studies, Ca $(OH)_2$ are used in the traditional nixtamalization process with an alkaline concentration ranging from 0.1 to 3% mostly to cook maize. The cooking time varies ranging from 5 to 60 minute depending on the type of food commodity used in the process. The process employed in the traditional nixtamalization process involves the selection of the alkaline medium, the gadget/apparatus/cooking device used in the cooking process, the method of cooking, the cooking time, cooking temperature, steeping time, and the washing of the nixtamal. The process has numerous advantages including rheological properties (Pappa *et al*., 2010; Santiago-ramos *et al*., 2018) which include elasticity, resistant to tearing and cracking (Carmen, 2015) and sensorial characteristics (Owusu-kwarteng & Akabanda, 2013). It also contributes to the improvement of the nutritional and microbiological properties which include the release of bound niacin, an increase of protein quality (Carmen, 2015), an increase of calcium content (Martı, 2006), and reduction of mycotoxin especially aflatoxin concentration (Guzm & Studies, 2016). A study conducted by Boniface & Gladys (2011), reveals a significant increment in the protein and carbohydrate content in sorghum with high water and oil absorption capacity. Generally speaking, the major setbacks of the

traditional nixtamalization are the loss of dry matter, long steeping time, and production of high amount of *Nejayote* with high PH around 9-12, which contains polluting residues (Escalante-aburto *et al*., 2019; Ramírez-Araujo & Reyes-Vega, 2019). A study conducted by Ramírez-Araujo *et al*. (2019), reveals that about 14,800 million litres of nejayote are produced yearly in Mexico contributing to economic losses through production cost and high labour force needed for the traditional nixtamalization process on a commercial production basis. Due to the high amount of water needed in the process on an industrial scale, more people are required for the production process which results in high labour costs. Moreover, the high energy demand during the cooking process is mostly not cost-effective and hence discourage people from practising the nixtamalization technology on a large scale. In most cases, the predominant alkaline medium used which mostly include Ca $(OH)_2$, and hydrated lime is reagents that are costly and are sometimes scarce especially the food-grade calcium hydroxide to buy as raw material for the process and at the end rendering the cost of the final product very high. The waste product produced as a result of traditional nixtamalization normally contains residues of phenols, dry matter, carbohydrate, protein, fat of which some are important component in food needed by the human body and other activities. Heat coupled with alkaline medium enhances easy penetration of the cooking medium into the grain to be leached into the cooking medium. Several studies (Maureen *et al*., 2020; Ramírez-Araujo *et al*., 2019; Guzm & Studies, 2016; Mariscal Moreno *et al*., 2015b; Ménera-López *et al*., 2013) have also revealed that other method use in the traditional nixtamalization including the separation of the corn (pericarp, germ and endosperm) all results in the loss of vital nutritional components. Not depicting the menace mentioned above, there is the need to improve upon the

traditional nixtamalization to make it more maintainable with addition of nutritive and sensorial benefits.

2.18.3 Ecological nixtamalization

Ecological nixtamalization was developed and proposed concerning the reduction of the contamination obtained by *nejayote* production (Campechano Carrera *et al*., 2012). In this process the use of water is reduced, the pH of the solution reduces which is less than that obtained from traditional nixtamalization. Salt (Calcium sulfate, calcium carbonate and calcium chloride) and weak acid have also been used in the ecological nixtamalization process (Bello-Perez *et al*., 2015; Rodr *et al*., 2013). The use of weak acid in the ecological nixtamalization is sometimes limited because the level of aflatoxin contamination in food commodities mostly could increase in an acidic medium. This method has also been proven to be promising in the reduction of mycotoxin especially aflatoxin. According to Sahai, (2014) and Arriola *et al*. (1988), concentrations ranging from 0.03-10% w/w of CaO employed in ecological nixtamalization were able to reduce aflatoxin levels in contaminated corn. The amount or concentration of salt used in ecological nixtamalization is a very crucial aspect of the process as this can greatly affect the final nixtamalized product by influencing the taste.

2.19 Factors influencing nixtamalization

2.19.1 Alkaline or lime concentration

The amount of alkaline used or lime concentration affects the nixtamalization process. Normally, food-grade limes such as calcium hydroxide, quicklime are used for traditional nixtamalization (Sahasrabudhe, 2015). These chemicals are not harmful to the human when consumed. Most alkaline cooking processes use a lime concentration

of 0.1 % to 3% w/v. An increase in lime concentration results in a higher uptake of water during soaking and coking (Laria *et al*. 2005). Water absorption during cooking leads to an increase in kernel weight and kernels swell to about 1.5 times their original size (Sahasrabudhe, 2015). It has been observed that the ash and moisture content of cooked corn increases in lime concentration up to 0.5% and dropped down as the concentration of lime increased to 1% (Sefa-Dedeh *et al*., 2004). A similar trend was observed by Escalante-aburto *et al*., (2019) where it was indicated that the amount of lime used could affect the ash content of the nixtamal. Given this, it is important to consider lime concentrations ranging from 0.1% to 5% depending on the alkaline medium used. The lime included in the nixtamalization process contributes to the changes in nutritional value, aroma, texture, colour and flavour of some cereal nixtamal (Sahasrabudhe, 2015).

2.19.2 Water

Water is an important component of the nixtamalization process. It contributes to the swelling of the kernel or grain during soaking and cooking as a result of water diffusion (Sahasrabudhe, 2015). Normally a 1:3 ratio of the food commodity to water is used for cooking (Sahasrabudhe, 2015). At the stage of cooking, lime is fairly solubilized in water. The uptake of water is controlled by physical changes in the corn or grain component which depend on endosperm type, pericarp thickness, and lime concentration (Laria *et al*. 2005). Water provides the enabling environment to enhance reaction during the nixtamalization process.

2.19.3 Alkaline cooking and steeping time

Alkaline cooking is one of the traditional ancient ways or methods used by the Mexicans to prepare tortillas which involves cooking the maize or corn in a lime

solution to produce a final product. The amount or concentration of alkaline use mostly varies from 0.1-3% for cereals depending on the type of alkaline used. Studies have indicated that alkaline concentration less than 2% has a greater effect on the quality of the nixtamal which includes reducing aflatoxin content in the final product. A study conducted by de Arriola *et al*., 1988 indicated that a lime concentration of more than 2% through the nixtamalization process was able to reduce aflatoxin B1 and B2 at 40% and 28% respectively only whiles at a concentration of 0.6% the reduction rate of B1 and B2 were 94% and 95% respectively. In a study conducted by Sefa-Dedeh (2004), different concentrations of lime were used for the nixtamalization process including 0.33%, 0.5%, and 1%. It was observed that the value obtained for viscosity and colour change during the nixtamalization process was different at a lime concentration of 1%. However, the ash and moisture content of the samples was increased at a lime concentration of 0.5% but decreased slightly at a concentration of 1%. The decreased moisture contents could be as a result of the sample imbibing or absorbing more solute thereby reducing the number of moisture contents in the nixtamal. In a study conducted by Owusu-Kwateng (2013), a lime concentration of 1% was used in the traditional nixtamalization of millet where higher crude protein contents were observed as compared to the non-nixtamalized samples. However, the fat content was reduced for the nixtamalized sample as compared to the non-nixtamalized. This may be as a result of the alkalinity removing excess fat from the sample thereby increasing the protein contents. It is important to indicate that in the nixtamalized product, the concentration of the lime is a very crucial factor in other for consumers to accept or reject the product. Excess lime concentration could lead to the rejection of the final product and likewise if it is less. According to Mendex-Albores, 2012, a lime concentration of 0.5% w/w of nixtamal has a pH value of 7.97 which maintain a good acceptable characteristic for

consumers. Heat also plays a significant role in achieving desire nixtamal. Several studies have indicated the use of mild heat with a longer cooking time or a higher heat with a shorter cooking time. In the nixtamalization process, the sample can be cooked at a temperature of 94 ^{0}C for 5munite or even at a temperature of 60 ^{0}C for 10-30 minutes depending on the type of product involved. The boiling point of water has been recorded to be 100 ^{0}C. Because of this the temperature requirements for most food commodities to boil fall within this range. Dues to this the temperature range for alkaline cooking for most cereals and legumes ranges from 1-100 ^{0}C. In many instances, nixtamalization does not only involves cooking but also soaking the product or commodity in an alkaline medium.

During nixtamalization, the most important issue is the absorption of the alkaline or lime into the product in the right amount or quantity. The cooking time required for pericarp to be soluble depends on the amount of alkaline used (Salinas *et al*., 2017). It has been observed in blue-purple maize grain during the nixtamalization procedure that the shortest time for the pericarp solubility was 5 minutes of cooking with 1% of alkaline concentration (Salinas Moreno *et al*., 2017). The cooking comprises of the rise in temperature time, cook time, and the time for the temperature to reduce. During the heating process, the rate of hydration begins to increase for about a temperature of 65^{0}C where the granule begins to gelatinized partially (Sahasrabudhe, 2015; Chen et al., 2015). The level of the grain cooked is based on the rate of stirring, the cooking time, the amount of alkaline used, temperature, the soaking time and the physical characteristics of the grain (Sahasrabudhe, 2015).

Steeping simply involve the deliberate introduction or soaking of a food commodity in a liquid or solution over time to allow absorption to take place in enhancing flavour and softening or to drain out some component from the grain. It is a common practice used

in nixtamalization and food processing. Ideal steeping and cooking times are determined based on the level of precise kernel softening/ gelatinization, pericarp removal, the uptake of water, and the overall appearance of the final nixtamalized product (Lusas and Rooney 2001; McDonough *et al*. 2001). Steeping also contributes to the overall uptake and increase of calcium contents in the final nixtamal.

In nixtamalization, the nixtamal is left to remain in the lime solution usually for 4-16 hours, during which a lot of physicochemical changes such as colour, texture taste aroma did occur (Schaarschmidt & Fauhl-Hassek, 2019). During this process, nutrients and other toxic substances are released into the cooking solvent. The colour of the solution and the viscosity depending on the amount of dry matter drained into it (Johnson & Ratnayake, *et al*., 2010). During the steeping stage, the pH of the nejayote becomes increased and there is saponification of free fatty acid and endosperm (Santiago-Ramos *et al*., 2018). Most studies have indicated that allowing the sample to steep for 4-16 hours is ideal. Accurate steeping has been reported to increase the rate of calcium intake into the germ corn (Fernandez-Munoz, *et al*., 2005). It is important to establish the fact that steeping and its effect varies for different food commodities and the type of nixtamalization method used. Argun and Dogan (2016) observed that, a significant difference in ash content of dent and flint corn at a steeping time of 6 hours. The major challenges during steeping time have to do with the labour force and the time is taken. The cooking and the steeping time economically are not cost-effective which prevents this type of method for commercial purposes.

2.20 Alkalinity during nixtamalization

Calcium diffusion through the pericarp is greater because it is the first component of the kernel to come into direct contact with the alkaline suspension (Martı, 2006).

Depending on the cooking temperature, the pericarp undergoes hydrolysis, losing components such as the hemicelluloses and other carbohydrates contained in the pericarp to leach out into the cooking solution, which can temporarily leach to a marked decrease in the calcium content of the pericarp (Sahai, 2014). Permeability of the pericarp to calcium ion, which is strongly determined by the cooking temperature, has a similar influence on the calcium content in the pericarp and the endosperm (Johnson, Ratnayake, *et al*., 2010). As a result of the physicochemical changes in the pericarp during the cooking stage, calcium diffusion into germ and endosperm is altered. Due to the high content of lipid and protein in the germ, calcium hydroxide can diffuse into it, saponifying the triglycerides, thus liberating the fatty acids (Greenwood *et al*., 2016). This reaction proceeds throughout the steeping process, leading to a gradual increase in calcium content in the germ (Martı, 2006).

2.21 Aflatoxin during nixtamalization

According to Schaarschmidt & Fauhl-Hassek (2019), the reduction in aflatoxin concentration and the transfer into the *nejayote* depend on the type of aflatoxin been managed The study further reported that aflatoxin G_1 and G_2 have a higher reduction rate of 75% as compare to aflatoxin B_1 and B_2 which have lower reduction rate ranging from 40% to 50% when treated with traditional nixtamalization process in nixtamal and masa. However, there has been a reduction of aflatoxin levels in raw maize of almost 100% in masa prepared through the process of nixtamalization (Arriola *et al*., 1988). Although aflatoxin B_1 was found to have a poor reduction rate, it has been observed that traditional nixtamalization was able to reduce the level of aflatoxin B_1 ranging from 75% to 100% in nixtamal, masa, and tortillas (Schaarschmidt & Fauhl-Hassek, 2019). Moreover, there was an 87% and 92% reduction of aflatoxin M_1 during the process of

nixtamalization with lime in dough and tortilla respectively (Elias-Orozco *et al*., 2002). The aflatoxin reduction rate of 90% was also observed in preparing maize through the nixtamalization process (Torres *et al*., 2001; Temba *et al*., 2016).

Generally, beyond the transfer of the aflatoxin into the alkaline media, nixtamalization can cause the lactone ring of aflatoxins to cleave and reduce the toxicity and mutagenicity (Vanhoutte *et al*., 2016).

2.22 Composition of wood ash

Wood ash is a mineral residue obtained from the burning of wood at a very high temperature. The high concentration of calcium in wood ash makes it similar to agricultural lime in terms of activity. It has been reported that ash contains some macronutrients in appreciable amounts, thus calcium (5-10%), potassium(25-40%), phosphorous (11%) and magnesium (Mathayo, 2020). Additionally, the presence of micronutrients has also been reported (Pappa and Palomo, 2016).

It is known that wood ash has alkaline properties that destroy the aflatoxin lactone ring when the toxins get in contact with alkaline (Kirui, 2016). A research carried out by Mathayo (2020) to assess the binding capacity of some materials confirmed the statement made by Kirui. Based on their experimental results, ash demonstrated a significant binding capacity owing to its high CEC (cation exchange capacity) values. Also, the ions Ca^{2+} and K^{+} in ash contribute significantly to aflatoxin binding but ions such as Si, Al and ion (Fe) have a negative impact on the cation exchange capacity values of some binding materials to aflatoxins. These ions increase the acidic nature of those binding materials thereby reducing their ability to bind. With this potential, wood ash has been a major component in the process of alkaline cooking of maize, thereby

making the treatment process very effective for the detoxification of aflatoxin (Kirui, 2016).

2.23 Nixtamalization using wood ash

Despite the positives of nixtamalization in terms of reduction of aflatoxin levels, there stands a chance of attaining very low levels if other measures are incorporated into the nixtamalization process (Eva Guadalupe *et al*., 1960). For instance, according to Schaarschmidt and Fauhl-Hassek (2019), poor mycotoxin reduction after rinsing and drying of samples cooked with ash could be owing to mycotoxins released from maize matrix components. Moreover, the existence of a waxy layer on maize could hinder water alkaline solution from being absorbed quickly (Vekiru *et al*., 2015). Despite all these drawbacks, aflatoxin-contaminated food can be reduced by 84% to 95% when cooked in an alkaline solution like ash (Saalia and PHillips, 2011; Torres *et al*., 2001). Therefore, nixtamalization using wood ash treatment has been reported by many researchers to be a potent process in aflatoxin reduction in grains.

In some South American countries, there has been the intense application of ashes (soda ash and wood ash) in the food processing industry. In addition, Kirui (2016) recorded a 91.6% aflatoxin reduction in maize samples when they were cooked with different ashes concentrations. It was further observed that when a small quantity of ash was used much time was needed for effective detoxification. Particularly in the nixtamalization of corn the reduction occurred as a result of the breakdown of the lactone ring of the aflatoxins by the alkaline activity of the ash (Schaarschmidt and Fauhl-Hassek, 2019). Also, Abbas *et al* (2009) reported the significant reduction of aflatoxins in maize grains when treated with ash solution. It has also been demonstrated by (Maureen *et al*., 2020) that aflatoxin levels can be lowered up to 46% after

nixtamalization with 1% of wood ash. Further analysis showed that aflatoxin levels in maize samples from some districts reduced by more than 90% when ash was used to detoxify contaminated maize. Other researches too have shown that a 50%-70% reduction in aflatoxin levels can be obtained when cooking, long period of steeping and washing of nixtamal is taken into consideration (Schaarschmidt and Fauhl-Hassek, 2019).

In a study by Mathayo (2020), ashes were found to bind efficiently to aflatoxins thereby reducing their levels. This was attributed to the relatively high cation exchange capacity (CEC) values which are known to promote binding. Additionally, potassium and calcium ions were in higher proportions which possibly rendered the ashes an efficient binding agent to the toxins. The possibility of significantly reducing aflatoxin levels through alkaline cooking of maize with wood ash is high due to the high levels of potassium, calcium, zinc and other relevant minerals (Mathayo, 2020). Therefore, moderate pH and a high concentration of these minerals are critical in the nixtamalization process to attain reduced levels of aflatoxins (Pappa & Palomo, 2016).

2.24 Composition and the usage of whitewash (calcium hydroxide or chalk calcium carbonate) and saltpetre (Potassium nitrate)

Whitewash also called slaked lime or chalk calcium carbonate is an inorganic compound that is formed from the reaction of calcium oxide and water together ($CaO + H2O \rightarrow Ca(OH)_2$) (Godbey & Mold, 2005). It could be used in preparing food in certain amounts especially the food grade. At room temperature, calcium hydroxide dissolves in pure water to produce an alkaline solution whose pH is about 10.7 (Bates, Bower, & Smith, 2011). Calcium hydroxide with is a molecular weight of about 74.09 is a compound that is whitish, odourless and powdery in appearance and whose

solubility in water decreases with temperature (De Mendonça Cavalcante *et al*., 2010). For instance, its solubility at 70 °C is said to be about half the solubility value when at 25 °C. It also can absorb CO_2 from the air to form calcium carbonate and has many uses. There are many ways in which calcium hydroxide is used both commercially and industrially. It could be used in water and sewage treatment and the preparation of ammonia gas. It is used in the food industry for the production of alcoholic beverages and soft drinks; as a replacement for baking soda (Lime *et al*., 2015), and also for nixtamalization of maize to improve its taste and digestibility (Carmen, 2015).

The history of saltpetre is a combination of chemistry, world trade, technology, politics, and warfare. Originally it was obtained from the dirt floors of stables, sheep pens, pigeon houses, caverns, and even peasants' cottages (Dennis *et al*, 2003*).* When these sources became inadequate to meet demand it was manufactured on saltpetre plantations, located in dry climates, where piles of dirt, limestone, and manure were allowed to stand for three to five years while soil microbes oxidized the nitrogen to nitrate (Dennis *et al*, 2003).it is used in many countries in food preparation and other purposes including cooking, preservation of meat, fireworks, and even gunpowder etc. *(*Dan *et al*, 2013*).* Potassium nitrate (saltpetre) is a white to dirty grey crystalline in nature, soluble in water and solid at room temperature *(*Helmenstine, 2019; Emily *et al*, 2018; Reddy, 2017*).*

2.25 Factors affecting aflatoxin levels in maize and groundnut during nixtamalization

Nixtamalization is a chemical method of reducing aflatoxins in maize and other foods. During this process, a lot of reactions take place before these toxins can be reduced and these reactions are facilitated by certain factors which include calcium diffusion, high

temperature as well as pH. High cation exchange capacity value and calcium diffusion are some of the major factors that promote the detoxification of aflatoxin-contaminated foods during nixtamalization. Calcium diffusion is proportional to the amount of steeping time (Vekiru, 2015; Fernández-Muñoz *et al*., 2004). When the temperature is raised during nixtamalization, the rate of calcium diffusion increases (Gonzalez *et al*., 2004). Ring-opening is also followed by decarboxylation at high temperatures and the process progress to degradation of methoxy group from the aromatic ring (Waliyar *et al*., 2000). Furthermore, phytate in some foods like groundnuts inhibits aflatoxin detoxification. Aflatoxins levels are generally higher in groundnut and maize seeds and it may be found specifically in groundnut skin or maize pericarp. Meanwhile, groundnut testa contains high levels of fibre, phytate and tannins which inhibit calcium absorption (Tariq *et al*., 2020). For instance, phytate in maize is usually found in the aleurone layer which makes it easy to degrade but in groundnuts, the presence of phytate close to proteins makes separation difficult. Because phytate has a negatively charged molecule, which forms compounds with positively charged ions like magnesium and calcium, lowering their bioavailability (Sinha and Khare, 2017). For decades phytate has been thought to be an anti-nutrient that can prevent the absorption of important minerals like calcium during the process of nixtamalization (Schlemmer, Frølich, Prieto, & Grases, 2009). Also, phytate is quite stable after been heated up to 100ºC thereby making its degradation difficult (Bullock et al., 1993). On the other hand, Muindia *et al* (1981) believe that alkaline cooking lowers phytate levels while increasing niacin availability. According to Hwang and Lee (2006), aflatoxin concentration, the magnitude of binding between the aflatoxin and food component, heat penetration, moisture content (groundnut), pH, strength, processing conditions and source of contamination all influence the effectiveness and degree of reduction. In addition, the amount of alkaline

solution (ash) and the time needed for effective detoxification is also considered. When the amount of ash is reduced in the solution, a longer time is needed for effective detoxification but when the amount of ash is increased, a lesser time is needed for detoxification (Kirui, 2016). Therefore, these factors have to be taken into serious consideration during the nixtamalization process.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Source of groundnut, maize and alkaline

Groundnut and maize samples were purchased from open markets in Tamale in the Northern region of Ghana. They were cleaned and kept in refrigerator (-4 ^{0}C) and aflatoxin concentrations determined. The samples were mixed and sorted manually based on sizes and appearance. This was done to help increase uniformity among the grains and to reduce the variations when taking samples for aflatoxin tests. In all, 200 kg of groundnut sample was sorted and used for the study. To allow for uniformity in sampling for aflatoxin test, 30 kg of groundnut sample was spread uniformly on a tarp and was divided into 12 blocks and 3 samples taken from different sections within each block. Each of the 3 samples taken was analyzed and aflatoxin levels were recorded. The same sampling procedure mentioned above was repeated for maize sample. Only groundnut stock with aflatoxin levels of 40 ppb and above were used for the study while for maize aflatoxin level of 80 ppb and above was used for the study.

Three natural alkaline sources (whitewash, wood ash and saltpetre) were used for the experiments. Whitewash and saltpetre were bought from the Tamale market while wood ash was collected from food vendors on UDS Nyankpala campus.

3.2 Determination of pH of whitewash, saltpetre, and ash solutions.

Tap water with known pH value was used to dissolve the wood ash, whitewash and saltpetre samples to prepare 0%, 1%, 5% and 10% (w/v). The pH of the solution was tested at 0 hour, 6 hours, 12 hours and 18 hours after preparation using a pH meter calibrated using buffer of pH 4.0 and 7.0.

3.2 Experimental design and sample preparation

The potential of natural alkaline sources to detoxify aflatoxin-contaminated groundnut and maize was carried out in two independents experiments. For each experiment, the initial aflatoxin levels in maize and groundnuts were determined before and after treatment with different concentrations of the natural alkaline.

3.2.1 First experiment (steeping groundnut in alkaline solution without cooking)

The first experiment was conducted in a completely randomized design with the concentrations of 0%, 1%, 5%, and 10% (w/v) and soaking time of 12 h, 18 h, and 24 h making 12 experimental units replicated three times. Here, groundnut samples (500 g) were steeped in saltpetre solutions with concentrations of 0%, 1%, 5%, and 10% (w/v) for 12 h, 18 h, and 24 h (Figure 3.1). Groundnut samples were then washed with distilled water, dried (dried in an oven for 6 hours at a temperature of 50 ^{0}C), and assessed for aflatoxins levels, proximate composition and consumer acceptability.

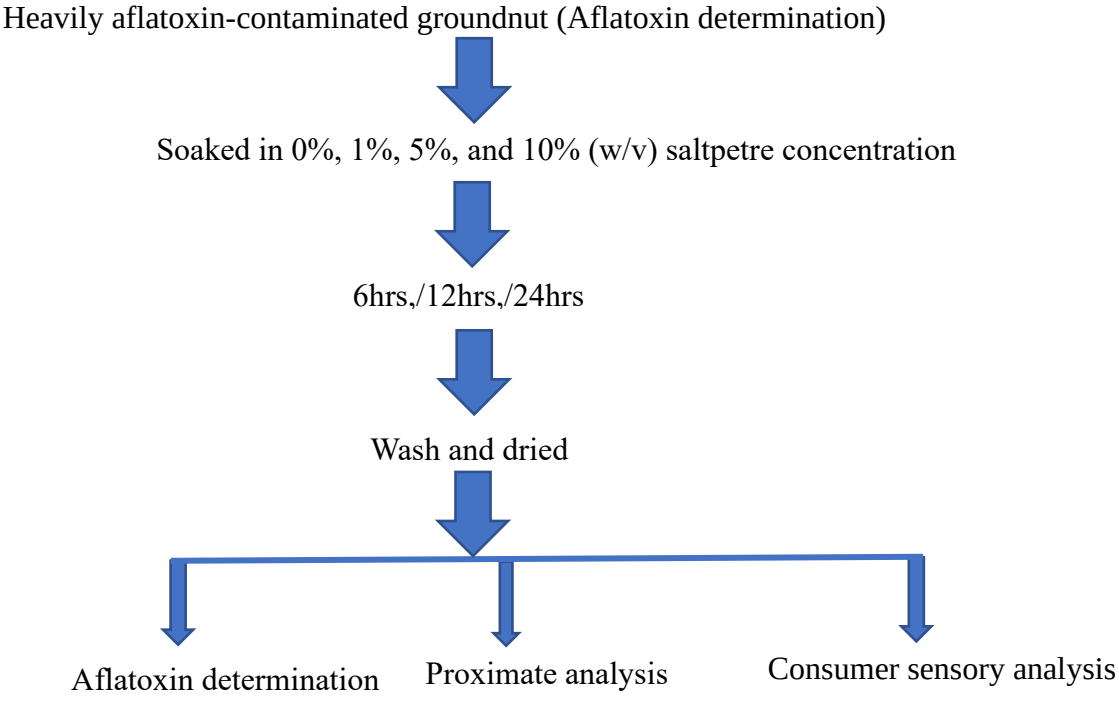

Figure 3. 1: Flow chart of the first experiment

3.2.2 Second experiment (stepping groundnut/maize in alkaline solution with cooking)

The second experiment was factorial in a completely randomized design with the concentrations of 0%, 1%, 5%, and 10% (w/v), cooking time of 5, 10, 15 minute, and steeping time of 0 h, 6 h, 12 h making 36 experimental units replicated three times. The raw groundnuts or maize (500 g for groundnut or maize) were cooked with the alkaline concentrations of 0%, 1%, 5%, and 10% (w/v) for 5, 10, 15 minutes and steeped for 0, 6, and 12 hours (Figure 3.2). Groundnut and maize samples were then washed with distilled water, dried (dried in an oven for 6 hours at a temperature of 50 ^{0}C), and assessed for aflatoxins levels, proximate composition and consumer acceptability (Figure 3.2). Total aflatoxin level was determined using Rapid Test Kit for a Quantitative Test with Mobile Diagnostic Reader (Mobile Assay Inc., Boulder, CO). The solution was heated to a temperature of 94 °C before transferring the samples into it for cooking.

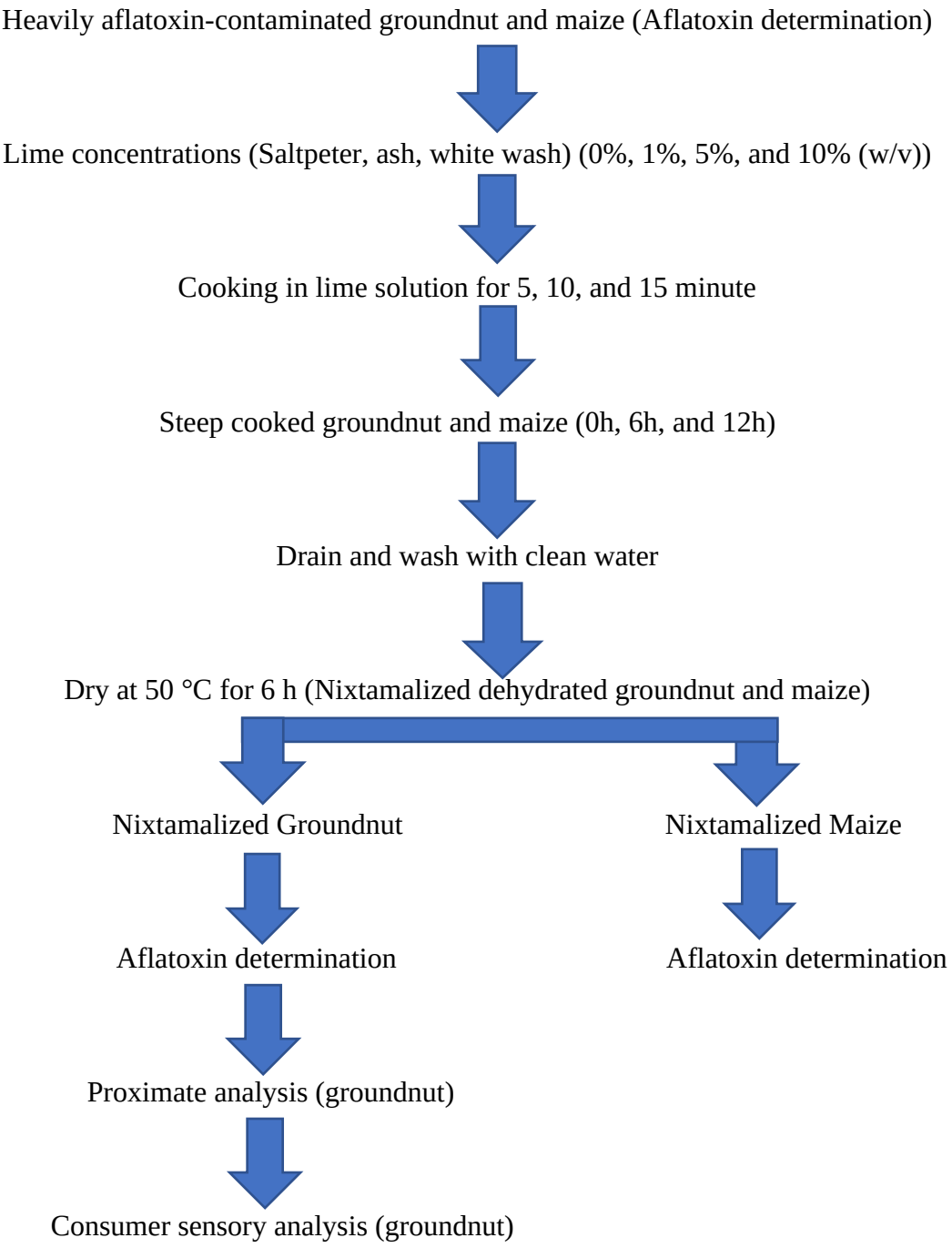

Figure 3. 2: Flow chart of the second experiment

3.5 Extraction and quantification of aflatoxin

Thirty (30) ml of 65% of ethanol solution was added to 10 g of maize or groundnut sample, shaken for 3 minute and filtered using whatman filter paper (Cat No 1001 150) into a glass bottle to obtain sample extract. Dilution cups were filled with 100 µl of

Phosphate-buffered saline solution (PBST). One hundred (100 µl) of the sample and PBST buffer were pipetted into the dilution cups and mixed thoroughly by pipetting up and down five times. A 100µl of this solution was transferred to a new dilution cup and a dropper was used to transfer 3 drops (equivalent to 100 µl) of solution into the specimen well of the test kit and timed for 3-5 minutes. The test strip was removed after development and tested using the mobile assay (reader). The test result of aflatoxin concentrations (ppb) was displayed on the reader. Before testing the samples, the reader was calibrated using two spiked groundnuts paste standards. The test was done using two different in-built calibration curves pre-designed in the reader for cereals and legumes.

3.7 Proximate analysis

Proximate composition on a dry matter basis was carried out to determine crude protein content, crude fat content, ash content, moisture content, and carbohydrate content of the nixtamalized groundnut sample using AOAC 945.39.

3.7.1 Moisture content determination

The moisture content of both nixtamalized and non-nixtamalized groundnut samples were determined using AOAC (2000) protocol. For every one of the samples, the aluminium dish was washed and dried in an oven for 15 minutes and cooled in a desiccator for about 10 minutes. The aluminium dish was weighed with a weighing scale and recorded. In all, 3 g of the sample was transferred into the aluminium dish and weighed. The sample was placed in an electronic oven for 7h at a temperature of 105 ^{o}C. The sample was removed after 7h and put into a desiccator for 30minute for cooling. After the cooling, the weight of the sample was recorded. The percentage

moisture content of the sample was calculated as the loss in weight of the sample using the formula, $\text{Moisture } \% = \frac{\text{Loss of weight} * 100}{\text{Sample weight}}$

3.7.2 Crude protein

Kjeldahl method AOAC (2000) protocol was used to measure the protein contents of each of the groundnut samples. In all, 2 g of the dry groundnut sample was weighed and transferred onto a piece of filter paper and placed in a digesting tube. The 15 ml of concentrated sulphuric acid and two tablets of Kjeldahl catalysts were added to the digestion tube and digested at 420 ^{o}C for two hours on a digestion block. The digested sample was allowed to cool in a rack and 50ml of deionized water was added to dilute the content in other to minimize the risk of explosion. Dilution was done on an automated unit for 9 minutes. The distillate from the samples was titrated against 0.1N HCL. A blank determination was done to provide a correctional factor for any extraneous nitrogen from other sources that might sum up to the nitrogen content of the samples. The titre values of the samples were recorded and used to calculate the percentage nitrogen which was converted to protein content using the conversion factor,

$$\text{Crude protein } \% = \frac{(\text{V2} - \text{V1}) * N * 1.4 * 6.25}{\text{W}}$$

3.7.3 Ash

Ash content of the groundnut sample was determined using AOAC (2005). In all, 2 g of the sample was weighed into the crucible and combusted in a muffle furnace at a temperature of 550 ^{o}C for 3 h. The sample was removed and cooled in a desiccator. The weight of the sample was taken and recorded. The loss in weight was calculated as percentage ash with the formula, $\text{Ash } \% = \frac{Weight\ of\ Ash * 100}{\text{Weight of sample}}$

3.7.4 Crude fat determination

2-3g of the groundnut sample was weighed onto a paper bag and placed in a thimble holder. 200ml of petroleum ether (40-60 ^{o}C) was added to a pre-weighed and dried round-bottom flask. Both the flask and the thimble holder were attached to the extraction unit along with a condenser. The solution was refluxed for six hours. The flask was removed and the solvent evaporated over a steam bath. The extracted fat was dried in an oven at 105 ^{o}C for 30 minutes, cooled in a desiccator and weighed. The crude fat was expressed as;

$$\text{Crude fat } \% = \frac{Fat\ weight}{\text{Sample weight}}$$

3.7.5 Carbohydrate

The total carbohydrate was determined by subtracting all the other proximate values from 100.

3.8 Consumer sensory analysis

3.8.1 Sensory Method

The final alkaline treated groundnut samples were served to 30 untrained panel. The panelists evaluated the final products for their sensory qualities (taste, colour, aroma, texture, and overall acceptability) using the Likert scale (1 to 5 representing extremely dislike to like extremely respectively). The panelists (n = 30) were from University for Development Studies and were recruited based on their age between 18 to 35 non-smokers, people without food allergies and people who consume groundnuts or groundnut products at least twice a week. For sample evaluation, 20g of the groundnut samples were placed into plastic cups with lids coded with 3-digit numbers and were served to panelists randomly during the test day. Panelists were instructed to taste the

samples and wash their mouths with water after evaluating each product to minimize any residual effect.

3.9 Data analysis

Data obtained were subjected to analysis of variance using the GenStat version 18 statistical package. The turkey's students range test was used to determine which of the means was significantly different at $p < 0.05$.

CHAPTER FOUR

RESULTS

4.1 The pH of the three natural alkaline materials

The highest pH of the wood ash was recorded at 10% (W/V) with the value of 11.35 while the least value was 10.03 when soaked for 18 hrs. Generally, the pH of wood ash decreases with increasing soaking time (Table 4.1).

Table 4. 1: pH of wood ash samples at different soaking period

Concentration (w/v)	**0 hr**	**6 hr**	**12 hr**	**18 hr**
1%	10.79 ± 0.09	10.02 ± 0.05	9.80 ± 0.14	9.86 ± 0.12
5%	10.97 ± 0.00	10.26 ± 0.50	10.03 ± 0.76	10.08 ± 0.11
10%	11.35 ± 0.35	10.64 ± 0.50	10.26 ± 0.49	10.03 ± 0.66
15%	11.05 ± 0.55	10.44 ± 0.35	10.02 ± 0.78	9.80 ± 0.70

The pH value recorded for saltpetre ranged from 9.84 to 10.38. However, the pH of the saltpetre increased with increasing soaking time (Table 4.2).

Table 4. 2: pH of saltpeter at different soaking periods

Concentration (w/v)	**0 hr**	**6 hr**	**12 hr**	**18 hr**
1%	10.25 ± 0.10	10.38 ± 0.10	10.27 ± 0.10	10.22 ± 0.11
5%	9.96 ± 0.00	10.08 ± 0.09	10.08 ± 0.10	10.11 ± 0.10
10%	9.84 ± 0.08	9.96 ± 0.64	9.94 ± 0.09	10.11 ± 0.10
15%	9.86 ± 0.50	9.87 ± 0.49	9.86 ± 0.49	9.90 ± 0.13

The pH values recorded for whitewash ranged from 12.44 to 12.59 indicating very strong alkalinity as compared to ash and saltpetre. However, the pH of whitewash was not affected by the soaking periods (Table 4.3).

Table 4. 3: pH of white wash at different soaking periods

Concentration (w/v)	**0 hr**	**6 hr**	**12 hr**	**18 hr**
1%	12.50 ± 0.06	12.55 ± 0.34	12.51 ± 0.48	12.52 ± 0.47
5%	12.53 ± 0.05	12.57 ± 0.11	12.55 ± 0.34	12.52 ± 0.05
10%	12.56 ± 0.03	12.56 ± 0.03	12.53 ± 0.16	12.57 ± 0.0
15%	12.55 ± 0.03	12.59 ± 0.00	12.55 ± 0.34	12.44 ± 0.05

4.2 Effect of saltpetre concentration and soaking time on aflatoxin levels in groundnut

Saltpetre concentrations and the soaking time significantly (P = 0.002) affected aflatoxin levels in the groundnut. When groundnut samples with initial aflatoxin concentrations of 50.8, 49.6 44.3ppb were treated, values reduced for some of the treatments while, surprisingly others increased. Obtained aflatoxin values after saltpeter treatment applications ranged between 2.4 and 55ppb (Table 4.4).

Table 4. 4: The effect of saltpetre concentration (w/v) and soaking time on aflatoxin in groundnut

	Soaking Time		
Treatment (Concentration)	12hrs	18hrs	24hrs
Raw Groundnut	49.60 ± 1.6	50.80 ± 1.61	44.3 ± 1.91
0%SP	53.30 ± 2.84	52.93 ± 4.59	55.3 ± 2.23
1%SP	47.90 ± 2.12	45.56 ± 1.33	49.56 ± 3.48
5%SP	2.40 ± 1.41	31.96 ± 10.58	25.53 ± 10.50
10%SP	3.90 ± 2.75	50.00± 3.7	53.96 ± 2.15
P-Value	0.002		
LSD	17.97		

Values are Mean ± Standard Error of triplicate determinations of aflatoxin.
SP = Saltpetre

The 5%SP and 10%SP concentration was able to cause about 83%% and 80% aflatoxin reduction respectively when the groundnut samples were soaked for 12 hr. Total aflatoxin reduction rate of 41% occurred when the groundnut sample was soaked with 5% SP for 24 hr (Figure 4.1) indicating that increasing the soaking time reduces the rate of aflatoxin reduction in the groundnut sample.

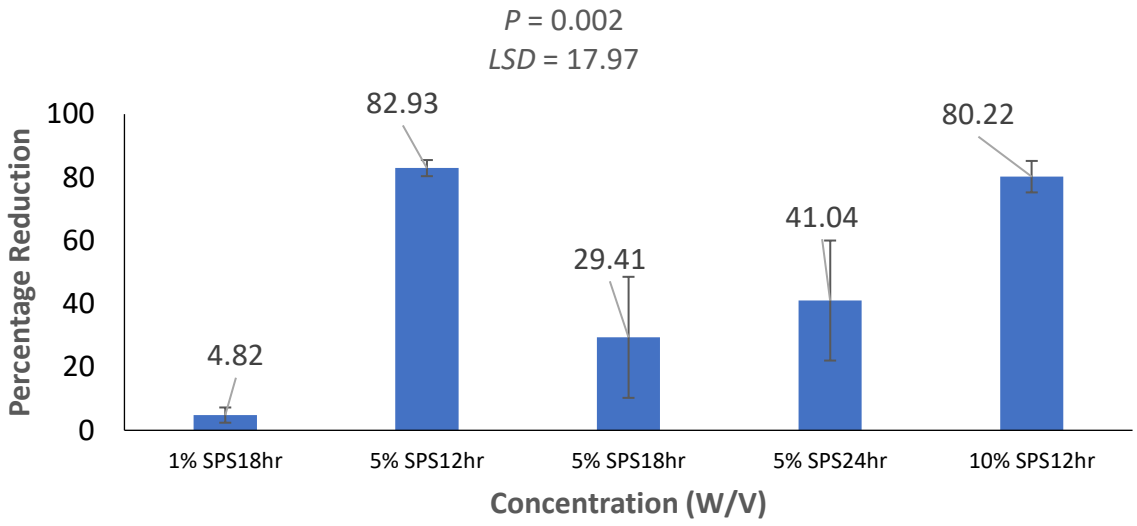

Figure 4. 1: Percentage reduction of total aflatoxin level in groundnut soaked with different concentrations (w/v) of saltpetre. Bars are ± Standard Error. SP = Saltpetre, hr = hours.

4.3 Effect of saltpetre concentration, cooking time and steeping time on aflatoxin levels in groundnut

For all the groundnut samples analyzed, 5% SP and 10% SP were able to reduce significant (p <.001), aflatoxin concentration from an initial of 49.60 ± 1.6 ppb to 5.23 ± 3.57 ppb and 5.80 ± 1.96 ppb respectively (Table 4.5).

Table 4. 5: The effect of saltpetre concentration (w/v), cooking time, and steeping time on aflatoxin detoxification in groundnut

		Steeping Time		
Concentration (W/V)	**Cooking Time**	**0 hr**	**6 hr**	**12 hr**
Initial Value	-	49.60 ± 1.6^{b}	50.80 ± 1.61^{b}	44.3 ± 1.91^{b}
0%SP	5m	56.30 ± 0.81^{b}	51.87 ± 1.39^{b}	54.90 ± 0.25^{b}
	10m	55.30 ± 1.79^{b}	55.50 ± 2.04^{b}	54.43 ± 1.91^{b}
	15m	54.57 ± 1.98^{b}	53.23 ± 1.57^{b}	51.57 ± 0.83^{b}
1%SP	5m	53.17 ± 0.54^{b}	53.87 ± 2.38^{b}	51.70 ± 1.76^{b}
	10m	55.37 ± 0.89^{b}	53.87 ± 2.64^{b}	53.87 ± 2.65^{b}
	15m	52.80 ± 2.51^{b}	44.97 ± 2.46^{b}	44.43 ± 2.94^{b}
5%SP	5m	5.23 ± 3.57^{a}	46.90 ± 2.42^{b}	58.43 ± 0.55^{b}
	10m	5.80 ± 1.96^{a}	58.43 ± 0.55^{b}	53.13 ± 3.69^{b}
	15m	48.33 ± 3.55^{b}	53.13 ± 3.69^{b}	44.53 ± 3.18^{b}
10%SP	5m	55.17 ± 1.71^{b}	49.70 ± 4.17^{b}	57.20 ± 0.49^{b}
	10m	49.00 ± 1.77^{b}	53.20 ± 2.04^{b}	50.07 ± 3.08^{b}
	15m	49.97 ± 4.01^{b}	48.50 ± 2.69^{b}	51.07 ± 3.39^{b}
P-Value	<.001			
LSD	8.36			

Where SP = Saltpetre

Values are Mean ± Standard Error
Values in the same column and row with different superscript letters are significantly different from each other ($p < 0.05$)

It is worth noting that, only one saltpetre concentrations with two different cooking times were able to cause a significant aflatoxin reduction in the groundnut sample with both resulting in percentage reduction above 85% (Figure 4.2).

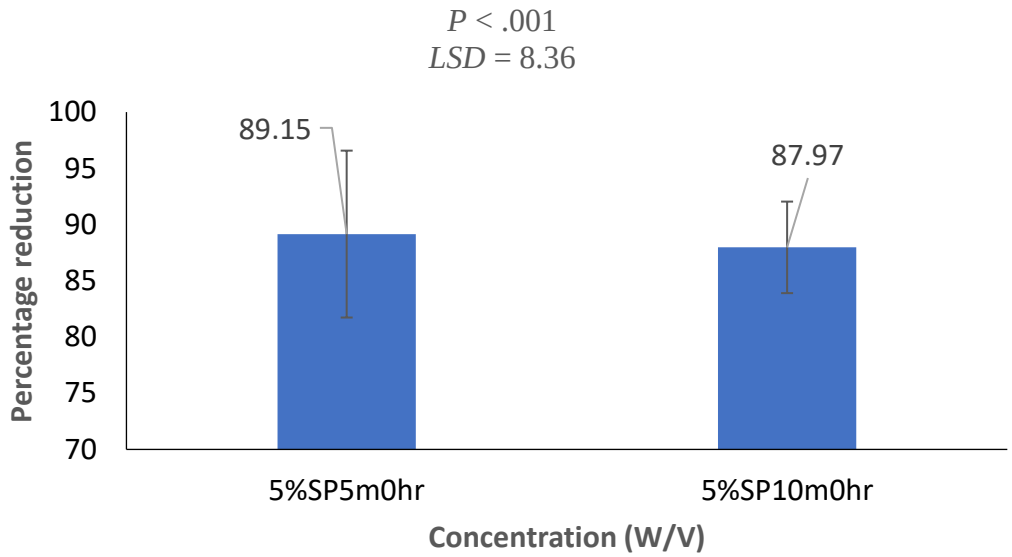

Figure 4. 2: Percentage reduction of total aflatoxin level in groundnut cooked with 5% saltpetre concentration (w/v) at different cooking times.
Bars are ± Standard Error. SP5m0hr = Saltpetre concentration cooked for 5 minutes and steeped for 0 hours, SP10m0hr = Saltpetre concentration cooked for 10 minutes and steeped for 0 hours.

4.4 Comparative effect of saltpetre on aflatoxin detoxification in groundnut and maize

The saltpetre concentrations were very effective in reducing the aflatoxin level in maize and groundnut. The highest aflatoxin reduction observed for maize and groundnut were samples treated with 5% saltpetre (Figure 4.3).

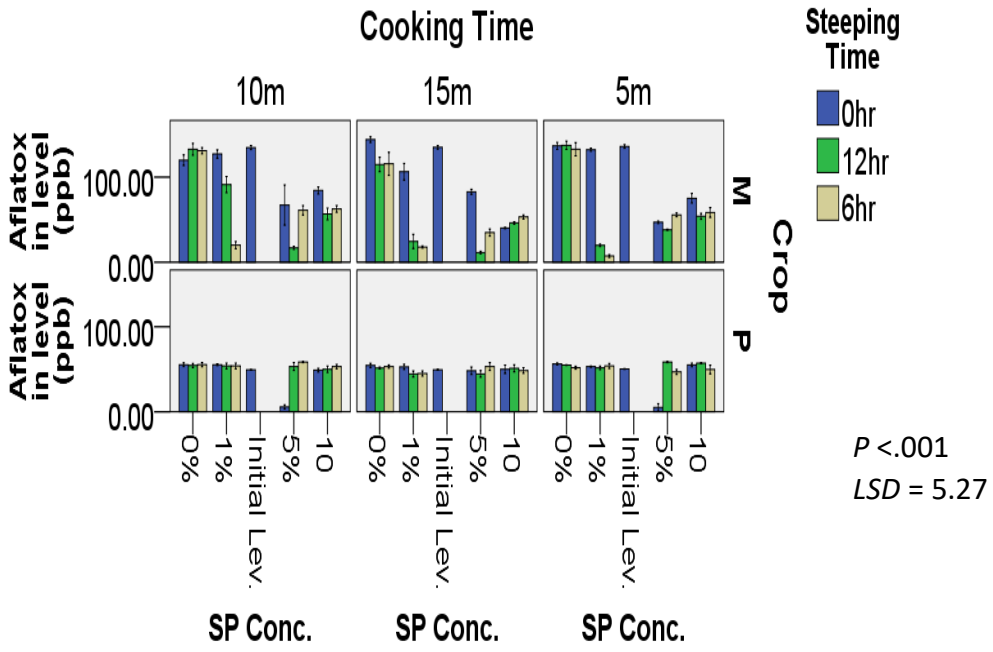

Figure 4. 3**:** The effect of saltpetre concentration, cooking time and steeping time on aflatoxin detoxification in groundnut and maize.
Bars are ± Standard Error.
P = peanut (Groundnut)
M = Maize

Saltpetre solution was able to cause over 80% and 90% in groundnut and maize respectively. However, all the treatement applied was very effective in reducing aflatoxin level in maize as compare to groundnut samples (Figure 4.4).

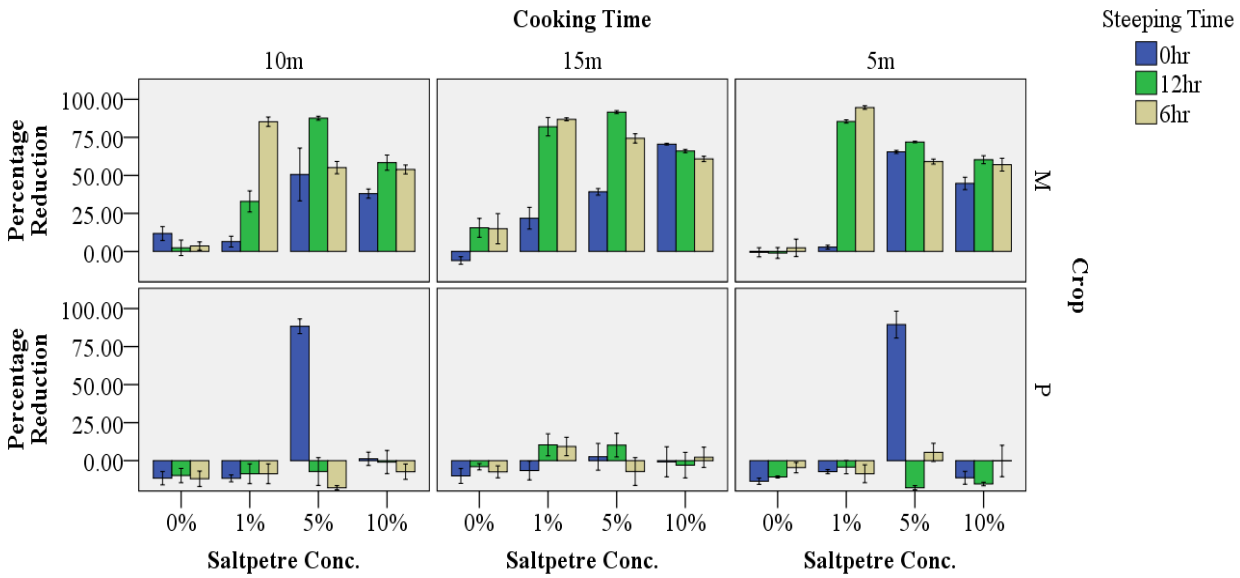

Figure 4. 4: Percentage reduction of saltpetre concentration, cooking time and steeping time on aflatoxin detoxification in groundnut and maize.
Bars are ± Standard Error.
P = peanut (Groundnut)
M = Maize

4.5 Effect of whitewash on aflatoxin level in maize and groundnut

The treatment worked differently in both maize and groundnut. There was a significant (P <.001) aflatoxin reduction in maize and groundnut. Maize samples treated with whitewash had the highest aflatoxin reduction as compare to groundnut samples (Figure 4.5).

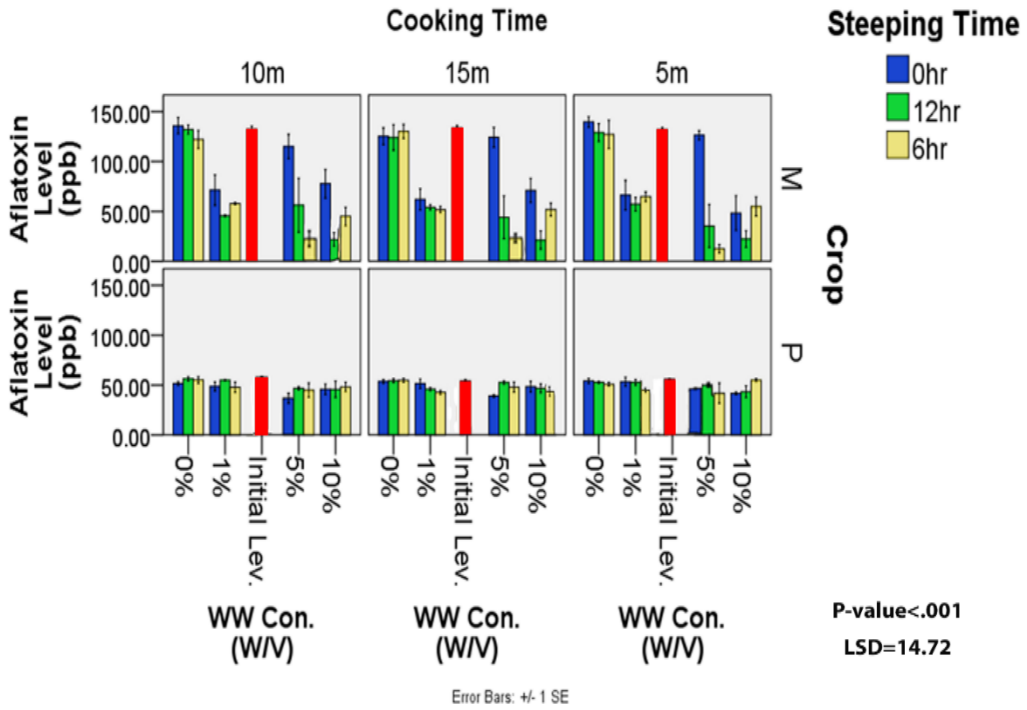

Figure 4. 5: Interactive effect of whitewash concentration, cooking time, and steeping time on aflatoxin level in maize and groundnut.
Bars are ± Standard Error.
P = peanut (Groundnut)
M = Maize
Where WW Con. = Whitewash concentration

The whitewash concentrations were very effective in reducing 94% total aflatoxin level in maize as compare to 20% in groundnut. Maize samples treated with 5% whitewash resulted in 82% total aflatoxin reduction while groundnut samples recorded 7% total aflatoxin reduction (Figure 4.6).

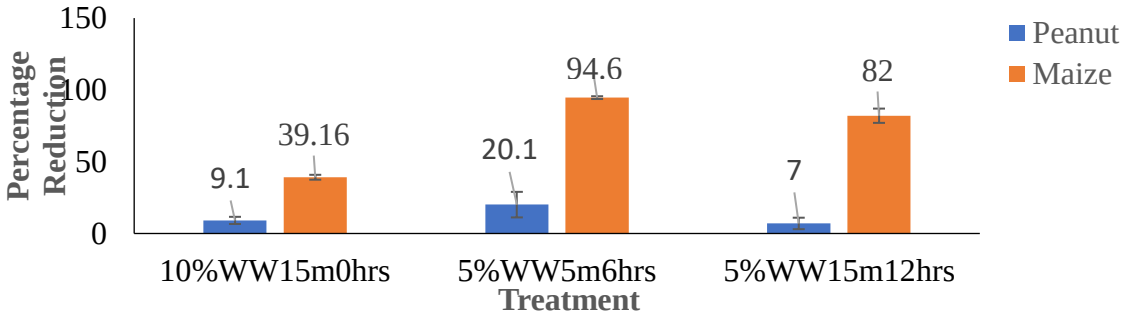

Figure 4. 6: Percentage reduction of total aflatoxin level in groundnut cooked with different concentrations (w/v) of whitewash for maize and groundnut.
Bars are ± Standard Error. Where WW5m0hrP = whitewash concentration cooked 5 minutes and steeped for zero hours for groundnut, WWW5m0hrM = whitewash concentration cooked 5 minutes and steeped for zero hours for Maize.

4.6 Effect of wood ash on aflatoxin level in maize and groundnut

Though there has been some amount of aflatoxin reduction in maize and groundnut, the treatment effectively worked in maize as compared to groundnuts. The highest aflatoxin reduction rate occurred at 10%w/v from 148 ppb to 4.73 ppb and 6.60 ppb for maize (Figure 4.7).

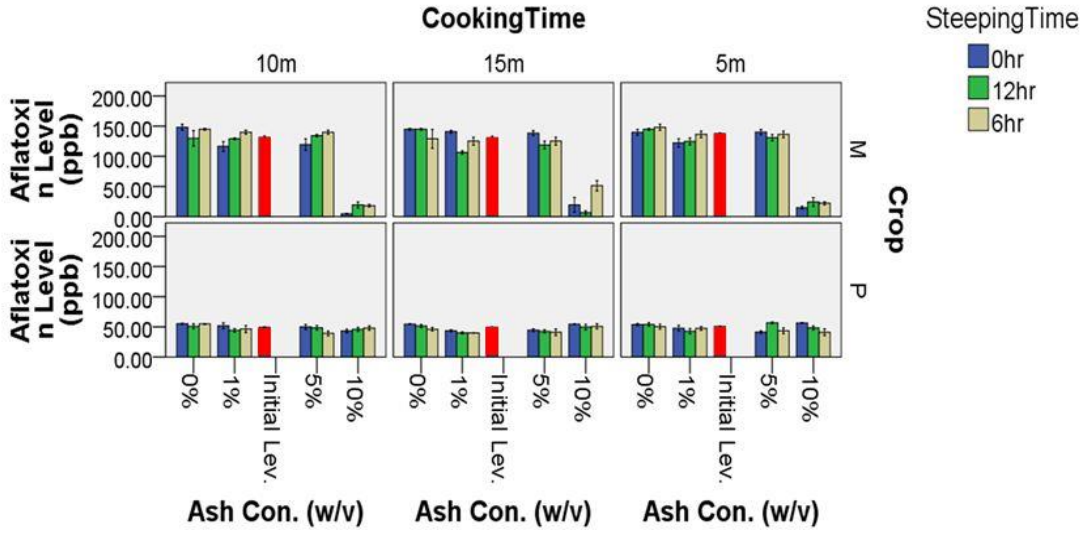

Figure 4. 7: Interactive effect of wood ash concentration (w/v), cooking time and steeping time on aflatoxin reduction in maize and groundnut (peanut).
Bars are ± Standard Error.
P = peanut (Groundnut)
M = Maize

Wood ash was able to cause almost 90% total aflatoxin reduction in maize and 28% in groundnut (Figure 4.8). For all the samples analysed, maize samples treated with 5% and 10% ash concentrations had the highest percentage of total aflatoxin reduction as compare to groundnut samples (Figure 4.8).

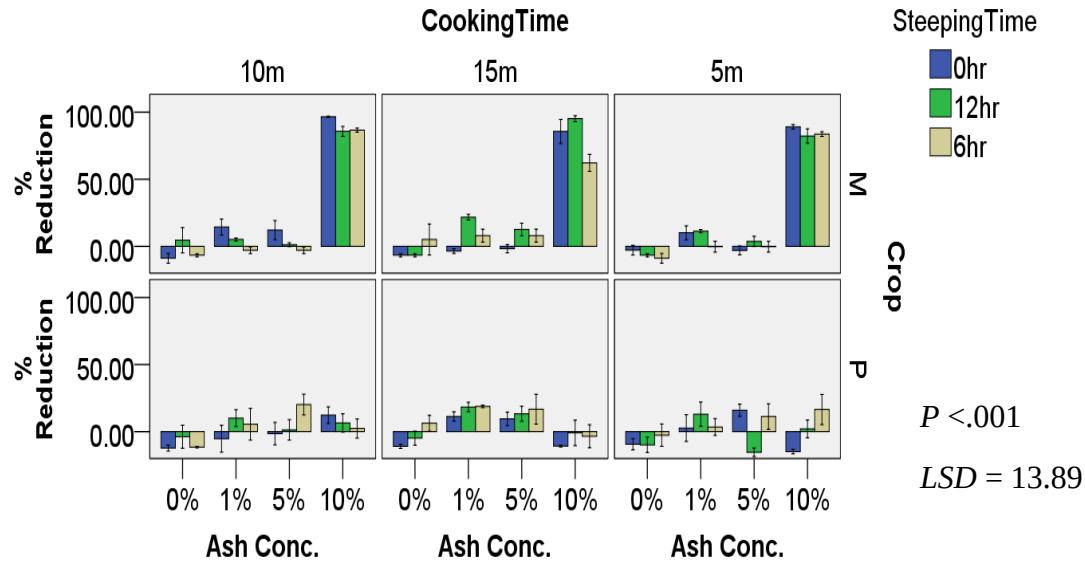

Figure 4. 8: Percentage reduction of total aflatoxin level in groundnut cooked with different concentrations (w/v) of wood ash for maize and groundnut.
M=maize; P=groundnut Bars are ± Standard Error of means.

4.7 The effect of ash concentration on split blanched (halved) grain of groundnut

Although ash concentration could not cause appreciable aflatoxin reduction in the whole groundnut grain, when kennels were split of halved there was a significant (*P* <. 001) aflatoxin reduction from 49.13 ppb to 33.8 ppb (Figure 4.9).

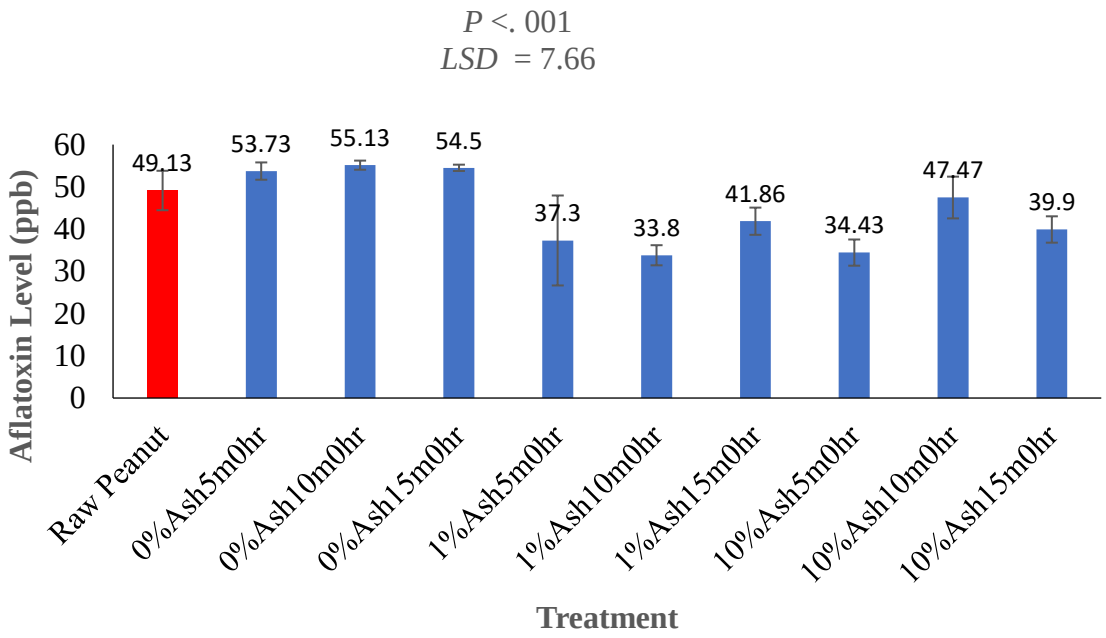

Figure 4. 9: Effect of ash concentration on split blanched grain (halved).
Where Ash5m0hr = Ash concentration cooked with groundnut for 5 minutes and steeped for zero hours, Ash10m0hr = Ash concentration cooked with groundnut for 10 minutes and steeped for zero hours, Ash15m0hr = Ash concentration cooked with groundnut for 15 minutes and steeped for zero hours. Bars are ± Standard Error.

The highest percentage reduction was observed when the groundnut grains were split of halved was 31.2% when treated with 1% (w/v) concentration of wood ash. A percentage reduction of 30% occurred at 10% (w/v) ash. The least total aflatoxin reduction occurred with samples cooked with 1% saltpetre for 15 minutes (Figure 4.10).

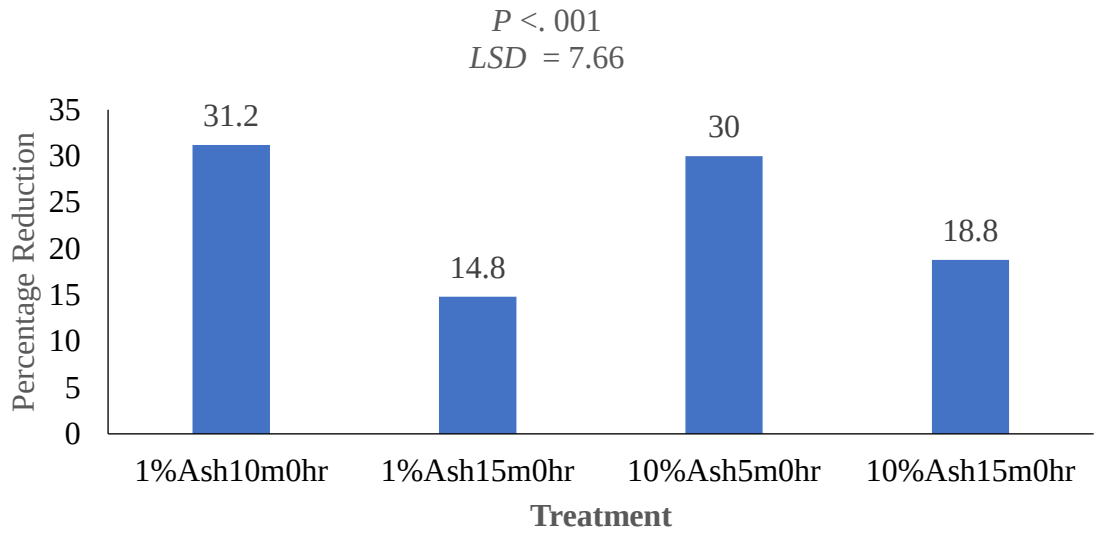

Figure 4. 10: Percentage reduction of total aflatoxin level in groundnut cooked with different concentrations (w/v) of ash on split blanched grain (halved) groundnut grain. *Where Ash5m0hr = Ash concentration cooked with groundnut for 5 minutes and steeped for zero hours, Ash10m0hr = Ash concentration cooked with groundnut for 10 minutes and steeped for zero hours, Ash15m0hr = Ash concentration cooked with groundnut for 15 minutes and steeped for zero hours. Bars are ± Standard Error*.

4.8 The effect of alkaline treatment on the proximate composition of groundnut

Samples soaked with 0%, 5% and 10% saltpetre had dry matter content range of 86.18 ± 0.58%, 88.77 ± 1.32% and 88.13 ± 0.95% respectively (Table 4.6). The moisture content of samples cooked with 0% and 5% saltpetre concentration ranged from 88.13 ± 0.95% to 5.58 ± 0.20% respectively whiles samples soaked in 0% and 5% concentrations had moisture content ranging from 13.82 ± 0.44% to 11.23 ± 0.57% respectively (Table 4.6). The samples soaked with 0%, 5% and 10% saltpetre concentrations had protein content ranging from 25.57 ± 0.30% to 23.13 ± 0.58% whiles protein contents ranging from 26.73 ± 0.54% to 24.65 ± 0.57% were recorded for samples cooked with 0% and 5% saltpetre concentration respectively (Table 4.6).

Table 4. 6: Proximate analysis of the treated groundnut sample

Treatment	Dry matter (%)	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Total CHO (%)
			Cooked			
0%SP5m0hr	94.22 ±1.15	5.78 ± 0.12	36.94 ± 0.57	26.73 ± 0.54	2.02 ± 0.09	29.26 ± 0.57
5%SP5m0hr	94.22 ± 1.98	5.58 ± 0.2	49.60 ± 1.00	24.65 ± 0.57	2.57 ± 0.12	17.4 ± 0.38
			Soaked			
0%SPSK12hrs	86.18 ± 0.58	13.82 ± 0.44	34.32 ± 0.17	25.57 ± 0.30	1.37 ± 0.11	24.92 ± 0.36
5%SPSK12hrs	88.77 ± 1.32	11.23 ± 0.57	46.77 ± 0.58	23.45 ± 0.56	3.18 ± 0.10	15.39 ± 0.51
10%SPSK12hrs	88.13 ± 0.95	11.89 ± 0.10	45.69 ± 1.15	23.13 ± 0.58	4.69 ± 0.57	14.6 ± 0.40
P-Value	<.001	<.001	<.001	0.020	<.001	<.001
Lsd	3.45	1.33	2.16	2.14	0.92	1.299

Where SP5m0hr = Saltpetre concentration cooked for 5 minutes and steeped for zero hours, SPSK12hrs = Saltpetre concentration soaked for 12 hours. Values in the same column with different superscript letters are significantly different from each other ($p < 0.05$).

Consumer sensory analysis

For colour, samples soaked with 0%SP(w/v) had the average score of 5.00 ± 0.98 as compared to 4.33 ± 1.06 recorded for samples soaked with 5%SP(w/v). For taste 5%SP(w/v) soaked for 12 hours and 5% cooked for 5 minutes have a mean value of 3.80 ± 0.62 and 4.03 ± 1.12 respectively (Table 4.7).

Table 4. 7: Consumer sensory analysis of the soaked and cooked groundnut sample

Sample	Colour	Taste	Texture	Aroma	Overall Acceptability
			Soaked		
0%SP12hr	5.00 ± 0.98^{b}	4.36 ± 0.99^{abc}	4.37 ± 0.99^{ab}	4.10 ± 0.99^{ab}	4.60 ± 1.00^{ab}
5%SP12hr	4.33 ± 1.06^{ab}	3.80 ± 0.62^{a}	4.40 ± 0.85^{ab}	3.96 ± 0.76^{a}	4.13 ± 0.90^{a}
10%SP12hr	4.03 ± 1.09^{a}	3.93 ± 1.04^{ab}	4.20 ± 0.92^{a}	4.06 ± 0.94^{ab}	4.03 ± 0.99^{a}
			Cooked		
0%SP5m	5.00 ± 0.58^{b}	4.83 ± 0.79^{c}	4.90 ± 0.54^{b}	4.80 ± 0.92^{b}	4.97 ± 0.80^{b}
5%SP5m	4.60 ± 1.13^{ab}	4.03 ± 1.12^{ab}	4.63 ± 0.92^{ab}	3.60 ± 1.22^{a}	4.40 ± 1.13^{ab}
5%SP10m	4.03 ± 1.03^{a}	4.60 ± 0.96^{bc}	4.56 ± 0.72^{ab}	4.17 ± 0.98^{ab}	4.40 ± 0.96^{ab}
P-Value	< 0.001	< 0.001	0.033	0.001	0.004

Values in the same column with different superscript letters are significantly different from each other ($p < 0.05$). SP = Saltpetre, SP5m = Saltpetre cooked for five minutes, SP10m = Saltpetre cooked for ten minutes, SP12hr = Saltpetre Soaked for twelve hours.

CHAPTER FIVE

DISCUSSION

Groundnut and maize are two crops that have gained attention due to their nutrient content and the rate of consumption in African countries including Ghana but are confronted with the risk of aflatoxin contamination. Treating aflatoxin-contaminated maize is not as difficult as that of groundnut due to so many factors such as the nature of the groundnut and the high oil content. The purpose of this work was to assess the detoxification potentials of natural alkaline sources in reducing aflatoxin levels in groundnut.

5.0 The pH of the three natural alkaline sources

Several studies have indicated that, a strong alkaline medium has the ability to reduce aflatoxin levels in food commodities such as maize, millet, and beans (Carmen, 2015; Guzm & Studies, 2016; Ramírez-Araujo *et al*., 2019) . Alkaline mediums that have been used successfully in the detoxification of aflatoxin include calcium hydroxide, ammonium hydroxide and sodium hydroxide with most of their pH ranging from 8 to 12 (Karlovsky *et al*., 2016). Notwithstanding this, the pH of whitewash, ash, and saltpetre were recorded to fall within the range of 9.84 to 12.59. The pH data from this study showed that the pH of wood ash, generally increases with increasing the concentration of ash (w/v) and decreases with soaking time (Table 4.1). Having determined the pH of whitewash, saltpetre, and ash, it was expected that the alkalinity of the medium used was strong enough to bring about detoxification of aflatoxin in peanuts and maize.

5.1 The impact of saltpetre on aflatoxin detoxification in groundnut

Several factors have been assessed by many researchers to determine the impact of alkaline cooking on aflatoxin detoxification especially in maize but have been limited in groundnut (Guzm & Studies, 2016; Schaarschmidt & Fauhl-Hassek, 2019; Escalante-aburto *et al*., 2019). Generally, the demand for these crops is very high not just in Ghana but at the international market as well. In Ghana for example, the high demand has been driven by limited agricultural land and poor yields (Fearon, 2000; Mengesha *et al*., 2008). Most of these grains are normally rejected in the international market due to aflatoxin contamination as a result of poor post and pre-harvesting practices (Guchi, 2015b; Pandey *et al*., 2019).

The current study reports that groundnuts contaminated with aflatoxin can be detoxified when soaked in different concentrations of alkaline solution. It is of interest to note that treatments (5% saltpetre, 10% saltpetre, 5% whitewash and 5% wood ash (w/v)) resulted in some level of aflatoxin decontamination. However, the most effective treatments were 5% saltpetre soaked for 12hr and 10% saltpetre soaked for 12hours which resulted in 82.93% and 80.22% whiles 5% saltpetre cooked for 5 minute and 5% saltpetre cooked for 10 minutes resulted in 89.15% and 87.97% aflatoxin reduction respectively. This result is of interest because, to the best of my knowledge this is the first report of such a significant aflatoxin reduction in groundnuts when treated with alkaline solution. The reduction rate observed could be as a result of chemical deactivation of the structure of the mycotoxin facilitating their ability to leach out from the groundnut grain. Moreover, during the process of alkaline treatment, there are a lot of physical and chemical reactions that occur (Sefa-Dedeh *et al*., 2004) which can also contribute to the detoxification of aflatoxins in the groundnut. The crucial aspect of alkaline treatment has to do with the absorption of the alkaline or lime into the

groundnut grain which contribute to the softening of the grains to help loosen bound mycotoxin. In maize for instance, the process of lime treatment result in making the kernel soluble and soft coupled with the removal of the pericarp which can be contaminated with mycotoxin. A number of researchers have reported on the effectiveness of alkaline treatment in reducing aflatoxin in maize (Juan de Dios Figueroa, 2002; Méndez-Albores *et al*., 2004; Mutungi *et al*., 2008; Kabak, 2009a; Pappa *et al*., 2010; Diedhiou et al., 2012; Moreno-Pedraza *et al*., 2015; Carmen, 2015; Mariscal Moreno *et al*., 2015b; Pappa & Palomo, 2016; Kirui, 2016; Schaarschmidt & Fauhl-Hassek, 2019; Escalante-aburto *et al*., 2019; Maureen *et al*., 2020; Odukoya *et al*., 2021).

During the process of alkaline cooking, the rate of hydration begins to increase around a temperature of 65^0C where the granule begins to gelatinized partially (Sahasrabudhe, 2015; Chen *et al*., 2015). Although the mechanism involved in the detoxification process of aflatoxin by alkaline treatment is not fully understood, it has been proposed to involve the opening of the lactone ring by alkaline hydrolysis accompanied by decarboxylation (Temba *et al*., 2016). Data from different works done on maize suggest that the actual pH (9-12), not just any alkaline solution is critical in the detoxification process. In maize a pH of about 10.78 seems to be very effective. In this study, the most effective alkaline treatments had a pH range of 9.96 to 10.08 which fell within the range reported for maize. The chemistry behind the effectiveness of different pH range in reducing aflatoxin level has not been fully comprehended. However, in the process of alkaline cooking, aflatoxin can be impacted in different ways including physical removal during the steeping and washing, degradation, modification or released by high pH (Schaarschmidt & Fauhl-Hassek, 2019). Generally, heat coupled with alkaline medium enhances easily penetration of the cooking medium into the grain thereby

dissolving the contents of the grains to be leached into the medium. During the heating process of alkaline treatment, nutrients and other toxic substances are released into the cooking solvent (Torres *et al*., 2001).

Tabata *et al*. (1994) found a different scenario when raw aflatoxin was treated with KNO_3 with a pH of 5.6 which was an acidic medium. The researcher found no aflatoxin reduction when potassium nitrate was used to treat raw aflatoxin. A study conducted by Temba *et al.* (2016) shows that reformation of aflatoxin could occur in an acidic medium which might have contributed to the failure of the detoxification process exhibited in the findings of Tabata *et al*. (1994). The pH of the saltpetre used in the present study ranged from 9.84 to 10.38, which indicates strong alkalinity. Mao *et al*. (2016), opined that strong alkaline can effectively detoxify mycotoxin in food commodities.

It has been reported by several researchers that, aflatoxin is heat stable (Pankaj *et al*., 2017; Kabak, 2009b; Kabak, 2009), especially in groundnuts. Under conventional cooking with no additives, a temperature of 269 ^{0}C is required for aflatoxin decomposition. It's only in few cases where heat or very high temperature had a slight effect on aflatoxin reduction (Pankaj *et al*., 2017) and most food commodities cannot withstand such high temperatures. This makes the ability of saltpeter to reduce aflatoxin in groundnuts to about 90% on just soaking even more remarkable and a promising opportunity as this could be used especially by local consumers readily. Saltpetre is readily available, and the treatment process does not require any additional processing such as cooking or heating to incur any additional processing cost.

Another one interesting observation made in this study was the effect of soaking time. When samples were steeped for longer periods (18hrs and 24hrs) at the same alkaline concentration, one would have expected a further aflatoxin reduction. However, there

was rather an increase in the aflatoxin concentrations in the samples. Although this is not properly understood, it could be speculated that the toxin may become loosely bound to the groundnut grains after the prolonged soaking but does not breakdown or go into solution and therefore result in more toxins becoming available for extraction and subsequent analysis. Samples cooked without saltpetre resulted in no significant aflatoxin reduction in aflatoxin content. Similar observation was made by Diedhiou *et al*. (2012) who found no aflatoxin reduction after boiling groundnut sample in normal water.

5.2 Comparative effect of saltpetre on aflatoxin detoxification in groundnut and maize

The saltpetre concentration worked well in reducing the aflatoxin level in maize and groundnut. For maize, all the treatments applied resulted in a significant reduction in aflatoxin content. Interestingly 5% (w/v) saltpetre solution was able to cause over 80% of aflatoxin reduction in groundnut and about 90% in maize. For maize it's not new as previous works (Kabak, 2009a; Diedhiou *et al*., 2012; Carmen, 2015; Mariscal Moreno *et al*., 2015b; Moreno-Pedraza *et al*., 2015; Schaarschmidt & Fauhl-Hassek, 2019) have made similar observations in treating aflatoxin contaminated maize with an alkaline medium.

The detoxification of aflatoxin in maize has been reported to involve the removal of the pericarp by incorporation of alkaline medium (Schaarschmidt & Fauhl-Hassek, 2019) either through the process of steeping or washing which might result in the washing of aflatoxin present in the maize (Torres *et al*., 2001). Moreover, other studies suggested that detoxification involves hydrolysis (Karlovsky *et al*., 2016). Aflatoxin detoxification in maize could have occurred as a result of alkaline hydrolysis. For

groundnut, it can be speculated that the saltpetre might have an active compound like potassium ion or nitrate ion which could be responsible for the detoxification process. It could also be that the saltpetre concentration used increased the solubility of the mycotoxin which then leached out into solution.

5.3 The effect of whitewash on aflatoxin reduction in groundnut and maize

The treatment applied to groundnut and maize behaved differently in each case. There was a drastic aflatoxin reduction of 94.6% and 82% when maize samples were treated with 5% whitewash at different cooking time and steeping times. This is because the presence of lime at various concentrations is very important in enhancing the breakdown and easy removal of the maize's seed coat (pericarp) during the washing stage (Pappa *et al*., 2010). The removal of the pericarp is essential to aflatoxin reduction. This seeks to suggest that the pericarp is major peripheral housing for aflatoxins. In groundnuts however, reduction was only 20%. This situation could have been caused by the difficulty in the removal of the groundnut testa (seed coat) during the washing stage of alkaline cooking process. Unlike the maize samples where the pericarps could easily peel off during washing after steeping, the groundnut testa was more strongly attached to its kernel. The nature of the seed coat found in the two crops could have been responsible for its easiness in removal in maize but not in groundnut. In determining the factors affecting the alkaline cooking performance of selected corn and sorghum hybrids, Johnson *et al*. (2010) reported that the properties of the pericarp such as its thickness, together with other factors could cause it to be resistant to the alkaline treatment. Zivoli *et al*. (2016) relatedly reported that blanching, a technology which is employed in the removal of the groundnut testa is effective in reducing the aflatoxin concentration of all types of groundnuts. This finding is further supported by

an experiment carried out by Siwela *et al*. (2011) which showed that the removal of the peanut skin before processing it into peanut butter resulted in about 27% aflatoxin reduction.

Another possible reason for the observed reduction in aflatoxin level could be because of the lime degradation and leaching of the aflatoxin into the alkaline medium. The presence of the alkaline condition enhances the thermal degradation of the aflatoxin by increasing its solubility (Mendoza and Bianchini, 2021). The removal of the seed coat enhanced an easy penetration of the lime into the corn (Gutiérrez-Cortez *et al*., 2010; Sahasrabudhe, 2015) to completely and effectively open up the lactone rings of the aflatoxin through hydrolysis, and destroy it (Guzmán-de-Peña, 2009). It can therefore be assumed that the inability of the calcium ion to penetrate the groundnut kernel is one important reason why there was no appreciable aflatoxin reduction rate observed in the groundnut. When the groundnut grains were split branched (halved) the aflatoxin reduction increased compared to when they were whole (Figure 4.10). This could be as a result of the groundnut grain having more surface area been exposed to the alkaline medium hence the reduction rate observed. This implies that opening the kernels increased the rate of absorption of alkaline medium, which in turn lead to the detoxification of the aflatoxin (Gutierrez *et al*., 2007).

5.4 Effect of wood ash on aflatoxin level in maize and groundnut

The ash treatment differently affected the aflatoxin level in maize and groundnut. The treatment was more effective in maize as compared to groundnuts. This is not an issue of concern because Kirui (2016) demonstrated a similar trend by observing a significant aflatoxin reduction rate in maize when boiled in ash solution.

The present study shows that wood ash has the potential to detoxify aflatoxin level especially in maize, however, its effectiveness could be enhanced if other measures are incorporated during the cooking process. The total aflatoxin reduction rate observed could be as a results of the high cation exchange capacity of ash that provides an excellent binding capacity to aflatoxins (Mathayo 2020). Generally, wood ash contains potassium ion and hydroxide ion which could also contribute to the binding capacity of the ash solution to the toxin through diffusion. It has been reported that wood ash contains micronutrients including iron, cupper, magnesium, potassium, and phosphorous (Jansone *et al*., 2020) which are essential for humans and animals. The incorporation of some of these nutrients into the nixtamal product for human consumption through nixtamalization could help improve the intake of some of these nutrients. The increase in niacin in the nixtamal could also help prevents pellagra (Arif *et al*., 2018) when consumed. The high alkalinity property of ash has been found to destroy the structure of aflatoxin thereby leaching into the alkaline solution (Hernández-Becerra *et al.,* 2016). It could also be that the increase in ash concentration which resulted in the increase of pH and the time provided for cooking and steeping lead to the reduction of aflatoxin (Kirui, 2016). Another contributing factor to the aflatoxin reduction is that the temperature at which the samples were treated was able to degrade the pericarp of the maize which acts as a barrier to the diffusion of the ash solution into the maize (Isela Rojas-molina *et al*., 2004). Gutierrez *et al.* (2007) found that, ion intake which contributes significantly to the binding of toxins does not occur at the same rate for all grains. This could also be another contributing factor to the variations in the detoxification of aflatoxin in the groundnut and maize.

For groundnut all the ash concentrations used could not reduce the aflatoxins concentration as much as in maize. The highest reduction recorded after the treatment

was 28%. Groundnut seed contains high levels of fibre, phytate and tannins which sometimes impede ion uptake. For example, phytate in maize is usually found in the aleurone layer which makes it easy to degrade but in groundnut, phytate gets in close proximity to proteins making its separation difficult to allow substances to diffuse into the seed. This is because phytate can forms compounds with other ions like magnesium and calcium, lowering their bioavailability for absorption (Sinha and Khare, 2017). Meanwhile, cation or the hydroxide ions are the most important factors that help in the detoxification of aflatoxins. An interesting observation was the increase in aflatoxin levels when groundnuts were treated with wood ash. This could be as a result of the toxin becoming loosely bound in the groundnut after cooking, making it more available for extraction.

5.5 Effect of alkaline treatment on the proximate and sensorial characteristics of the nixtamalized groundnut

The crude protein contents (24.65 ± 0.57%) of the sample cooked with saltpetre had a higher value as compared to soaked samples (23.45 ± 0.56%). Alkaline cooking led to the removal of soluble starch and thereby increasing the relative percentage of proteins (Owusu-Kwarteng, 2013). The value was not different from what was reported by USDA, National Nutrient Database for Standard Reference (2011) who reported protein contents of 23.68% in groundnut grain. Apart from the main protein foods such as egg and milk, groundnut is also another important source of protein with all the essential amino acids needed by the body for normal functioning (Kandala & Puppala, 2012) and lack of some of these amino acids may lead to disorder and abnormalities. The value recorded for the protein content reveals the presence of some essential amino acids which could be a crucial component of the human diet. However, there was a slight decrease which was not statistically different from the standard recorded by

USDA (2011) in protein contents for all the treatments containing saltpetre as compared to those without it. The decrease in the protein contents could be a result of cross-linking, protein degradation, and denaturation which may lead to reduce protein digestibility (Heck *et al*., 2013). The high moisture content recorded for samples soaked with saltpetre could be as a result of the groundnut molecules absorbing the medium in which it was soaked thereby increasing the moisture content (Carmen, 2015). The higher fat content recorded in the present study was not different from the value indicated by USDA, National Nutrient Database for Standard Reference (2011). Generally, the application of saltpetre increased the fat content of the nixtamalized groundnut. The treatment affected the total carbohydrate content of the final product though there was a decrease of the total carbohydrate content when compared to treatment without saltpetre concentration. The decrease is as a result of the soaking time and the leaching process (Chang, 1987). However, the value recorded for carbohydrate content fell within the range recorded by USDA (2011).

In this study, consumer sensory analysis was conducted to assess the differences in the sensorial characteristics of the final product. The treatment affected the overall acceptability of the final product. The level of colour preference decreased by increasing the saltpetre concentration for samples cooked with various concentrations of saltpetre. The development of colour during alkaline cooking is very complex as alkaline reacts with different pigments. The case was not different from what was observed for taste. The sample treated with saltpetre concentrations were accepted by the panelist. Flavour is enhanced by a reaction occurring between reducing sugars, peptides, and unsaturated fatty acids. The overall acceptability of texture, colour, taste and aroma for the treated groundnut were liked by the consumers. The study was not different from what was observed by Mendex-Albores (2012).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The study was carried out to assess the potentials of the different natural alkaline mediums in the detoxification of aflatoxin-contaminated groundnut and maize. This is the first study demonstrating the possibility of using natural alkaline medium in reducing aflatoxin levels in groundnut. At the end of the investigations, the following conclusions can be made:

i. Saltpetre solution (5% (w/v)) reduces aflatoxin level in groundnut to about 87 to 89% and over 82 to 90% in maize whiles wood ash solution (1% (w/v)) result in about 28% and 20% total aflatoxin reduction in groundnut and maize respectively.
ii. In maize however, 5% (w/v) whitewash and 10% (w/v) wood ash solutions were able to cause a total aflatoxin reduction rate of 94% and 91% respectively.
iii. The overall acceptability of texture, colour, taste and aroma for groundnut samples treated with 5% and 10% saltpetre solutions were liked by the consumers.
iv. Generally, saltpetre was the most effective in detoxifying aflatoxin and maintained nutritional and sensorial quality.

6.2 Recommendations

Based on the findings of this study, the following are recommended.

Further studies should be carried out to assess the process parameters (temperature, cooking time, steeping time) and its influence on aflatoxin in groundnut.

Further studies should be carried out to assess the mechanism or chemistry of the detoxification process of aflatoxin in the groundnut.

Toxicity studies should be carried out to check the toxicity of the nixtamal on human health and bioavailability of nutrients following alkaline treatment of groundnuts.

There should be an exploration of treating aflatoxin contaminated groundnut with saltpetre, taking into consideration the sensorial and nutritional characteristics.

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APPENDICES

QUESTIONNAIRE FOR SENSORY ANALYSIS

Demographic characteristics of Panelist

Age: …… Date:……

Sex: Female ☐ Male ☐

Please evaluate the samples using the scale below to describe your level of acceptability using;

1= Dislike extremely

2= Dislike

3= Neither like nor dislike

4= Like

5= Like extremely

Sample	Colour	Taste	Texture	Aroma	Overall Acceptability
0%SP12HrSK					
5%SP12HrSK					
10%SP12HrSK					
0%SP5m0hr					
5%SP5m0hr					
5%SP10m0hr					

<

Sample	Code
0%SP12HrSK	500
5%SP12HrSK	555
10%SP12HrSK	510
0%SP5mCK	501
5%SP5mCk	505

Groundnut samples

Sample cooking

Split branched groundnut nixtamal in an aluminum plate

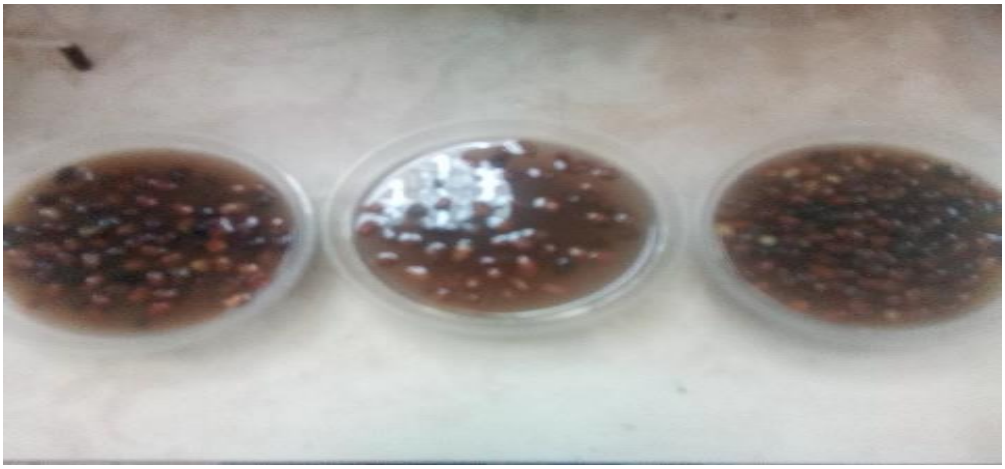
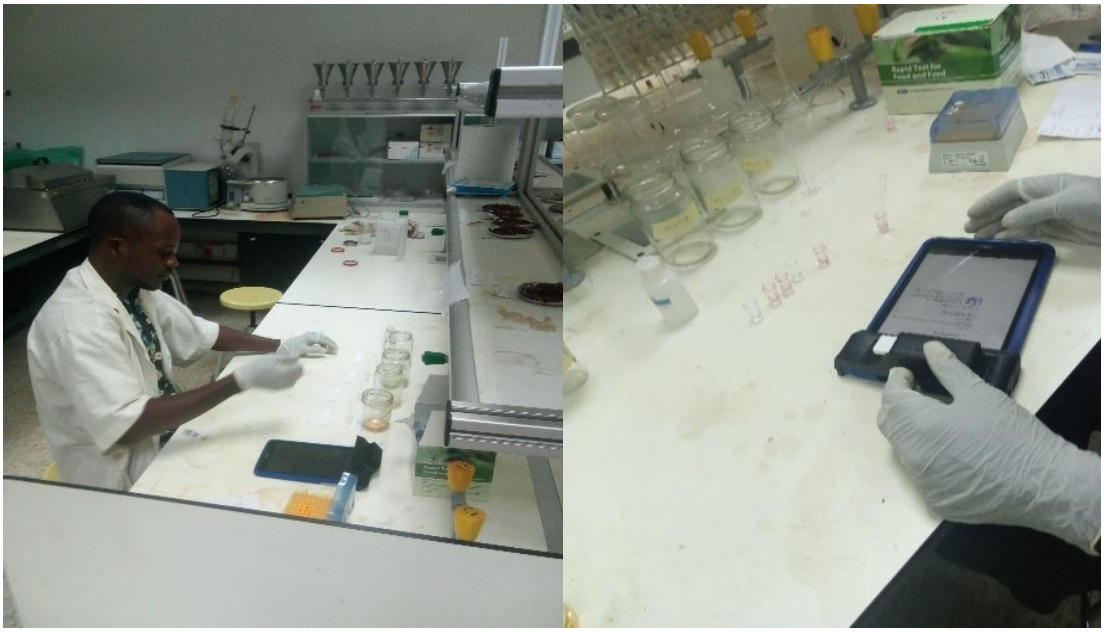
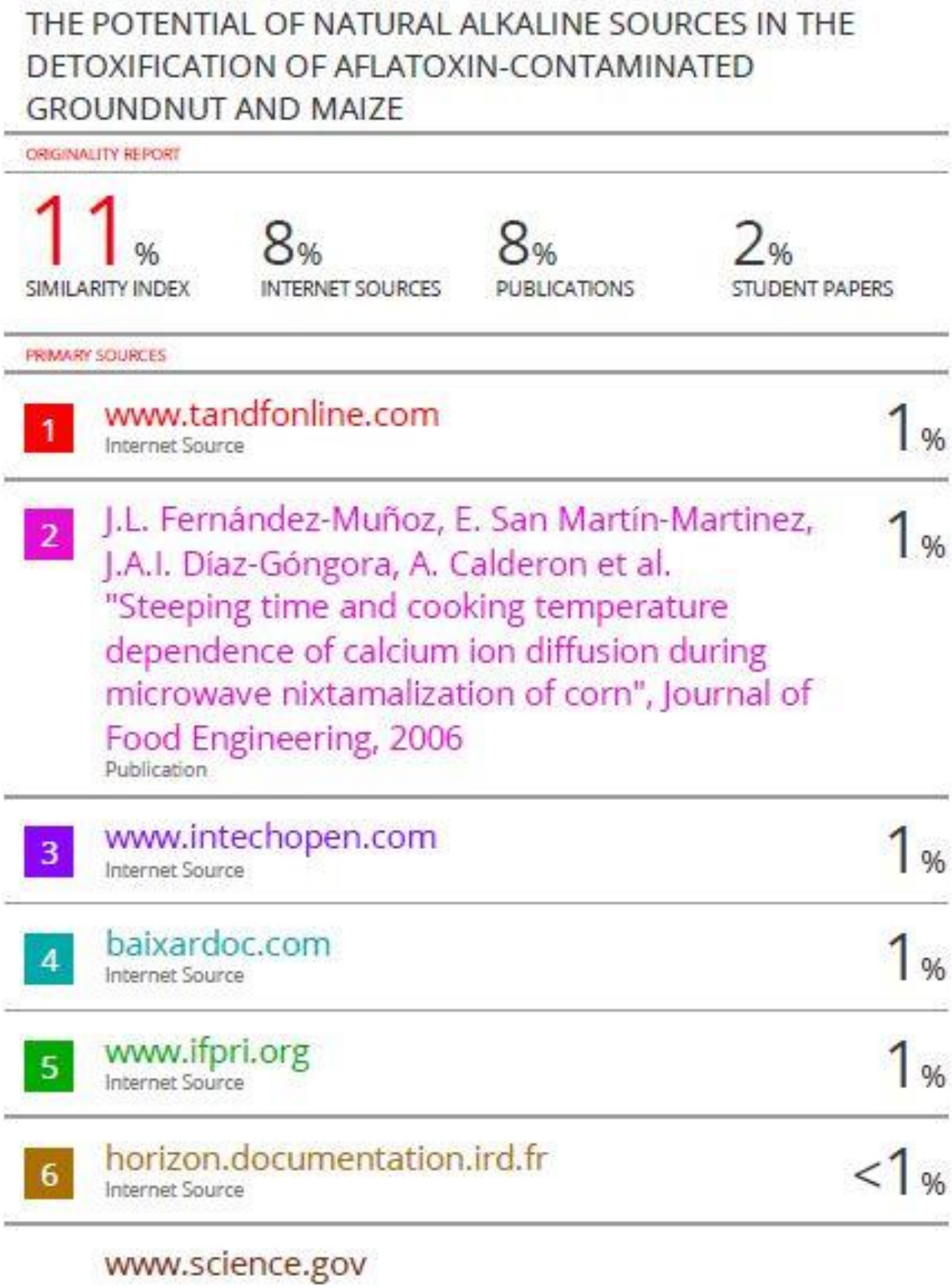


Plate 1: Steeped groundnut sample in a rubber container



Aflatoxin Analysis

Similarity Index



THE POTENTIAL OF NATURAL ALKALINE SOURCES IN THE DETOXIFICATION OF AFLATOXIN-CONTAMINATED GROUNDNUT AND MAIZE

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